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Coordination Chemistry Reviews 228 (2002) 205–225

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Spectroscopic approaches to the study of the interaction of aluminum with humic substances

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Received 14 August 2001; accepted 12 February 2002

Contents

Abstract

Aluminum ion (Al^{3+}) in the 'free' (aquo) state is becoming increasingly prevalent in environmental waters, especially fresh waters, as a consequence of acid rain and other environmental processes. As Al^{3+} ion is known to affect markedly a wide range of biological systems, and since the presence of Al^{3+} in humans has been linked to a number of human diseases, it is important to understand the speciation of Al^{3+} ion in natural waters. Since some of the most important complexation agents for Al^{3+} in both fresh and sea waters are members of the complex humic substances group, it is important to understand the manner in which Al^{3+} interacts with this class of molecules, especially since binding of Al^{3+} to these molecules can effectively increase the bioavailability of this toxic metal ion to biological systems. The objective of this review is to present the current state of our understanding of aqueous aluminum complexation with the most acidic members (and therefore the most likely candidates for serving as Al^{3+} complexing agents) of the humic substances group, the fulvic acids. Much of the current knowledge has been revealed by comprehensive fluorescence titration analyses. Some additional information has come from other experimental approaches, including infrared spectroscopy, nuclear magnetic resonance spectroscopy, and a variety of electrochemical approaches. In this review, we also report on the results of our recent fluorescence and IR spectroscopy survey of the interaction of metals from of all three Nieboer and Richardson categories of environmental metals (Class A, Class B and Intermediate Class) with the fulvic acid sub-group of the humic substances. This has proven helpful in understanding some of the unique spectral behaviors of the Al^{3+}–fulvic acid complex vis-a-vis fulvic acid complexes with many other metal ions. The results of our fluorescence and IR experiments with the model compounds, such as salicylic and phthalic acids, have allowed confirmation of the important roles played by both salicylic acid-like sites and phthalic acid-like sites in the unique complexation of Al^{3+} to humic substances, and help to explain some of the observed spectroscopic changes associated with Al^{3+} ion complexation to humic material. From the current work, it seems clear that major sources of the deviation in spectral properties between Al^{3+} and many other metal ions (across all three Nieboer and Richardson categories) are the unusually high value of its charge density *and* relatively low propensity for involvement in covalent bonding interactions (i.e. a very high ionic index *combined* with a relatively low covalent index in the Nieboer and Richardson classification of environmental metals), as well as affinity for certain functional groups. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Aluminum; Humic substances; Fulvic acid; Formation constants

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PII: S0010-8545(02)00040-1

1. Introduction

Humic substances are the organic material that remains after dead plant tissue, dead organisms and the excrements of living organisms have been degraded by micro-organisms and other processes. They are produced by the oxidative decomposition of complex organic molecules including carbohydrates, proteins, lignins, lignans, and fats. [1–3]. They are found in soil and water and constitute a major component of terrestrial (soil organic matter) and aquatic (dissolved organic matter) carbon pool [4]. The elementary composition of humic material generally is within the range: C, 45–55%; H, 3–6%; N, 1–5%; O, 30–45%; S, 0–1%. Humic material is commonly subdivided into three categories: humin (insoluble in water at all pH values), humic acid (insoluble at pH values less than 2, but soluble at higher pH), and fulvic acid (soluble in aqueous solutions over the entire pH range). The differential solubility exhibited by the humic substances has proven very useful in the separation of these materials [1,3]. The fulvic acids, which are the central focus of the research reported in this paper, are the lowest molecular weight substances with the highest oxygen content in the complex humic group [1]. They range in molecular weight from about 500 to 2000 Da [1,5]. Fulvic acids are a complex organic mixture; however, there is general agreement about some of the more common functionalities present. Fig. 1 presents a hypothetical molecule containing many of the functionalities believed to be prevalent in a sample of fulvic acid [6]. The functionalities include aliphatic carboxyl, aromatic carboxyl, aliphatic dicarboxyl, aromatic dicarboxyl, phenolic hydroxyl, alcoholic hydroxyl, carbonyl, hydroxyacid, and salicylic acid. Approximate ranges (in units of milliequivalents per gram of acid): total acidity, 12–14; carboxyl, 8–9; phenolic hydroxyl, 3–6; alcoholic hydroxyl, 3–5; and carbonyl, 1–3 [7].

Humic material, including the fulvic acids, are of current environmental importance for a number of reasons. Humics present in an aqueous environment can easily be oxidized by oxygen present in water, causing oxygen depletion which can have adverse effects on aquatic life forms and can ultimately lead to anoxic conditions and low pE (reducing) states. Humic material also can participate in a wide range of interactions with small organic molecules. Several interaction mechanisms are possible, including ionic bonding, hydrogen bonding, and weak van der Waals interactions. Positively-charged organics, such as triazine class herbicide *atrazine*, can interact with negatively-charged humics via favorable electrostatic interactions, while uncharged polar organics, such as the insecticide *carbaryl*, can interact with humic material via multiple hydrogen bonding interactions. Non-polar organics, such as dichlorodiphenyltrichloroethane (DDT), bind to humics through hydrophobic interactions. Of particular relevance to this review is the long known fact that humic material, especially the low molecular weight, highly soluble fulvic acid, have long been known to serve as complexing agents for metal ions leached in the environment [8]. Complexation of metal ions to humic and fulvic acids would be expected to play a major role in maintaining many toxic metals in a bioavailable state in environmental waters. Iron and Al^{3+} are known to bind very strongly to humic substances, whereas ions such as Mg^{2+} bind weakly, and many common metal ions, such as Ni^{2+}, Pb^{2+}, Ca^{2+}, and Zn^{2+} have intermediate binding affinities [7]. [The strong aluminum complexation to humic substances (log $K_f \sim 5–6$ in the pH 4–5 range) is of particular concern with the increasing acidification of many natural water courses as a consequence of 'acid rain'. The increased acidity levels lead directly to increased levels of 'free' Al^{3+}, readily available for interaction with dissolved humic substances. Fig. 2 presents Al^{3+} species distribution plots associated with 'pure' water (salinity = 0) and sea water

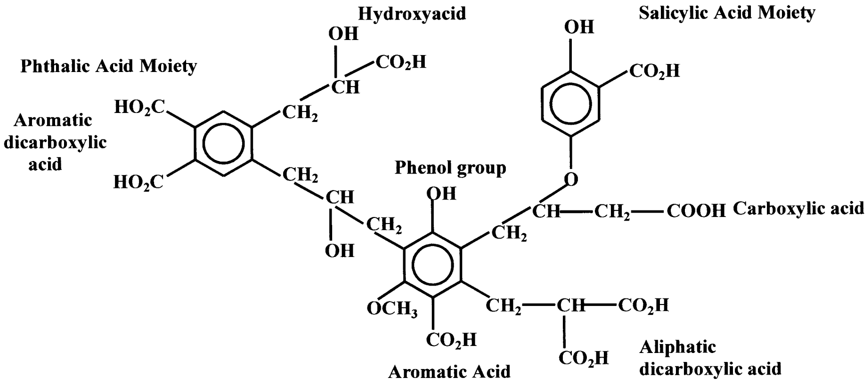


Fig. 1. Examples of humic material functional groups available for complexation with metal ions. Phthalic and salicylic acids moieties are thought to play particularly important roles in metal chelate formation processes. Redrawn after Stumm and Morgan (Ref. [6]). Reprinted by permission of John Wiley and Sons, Inc.

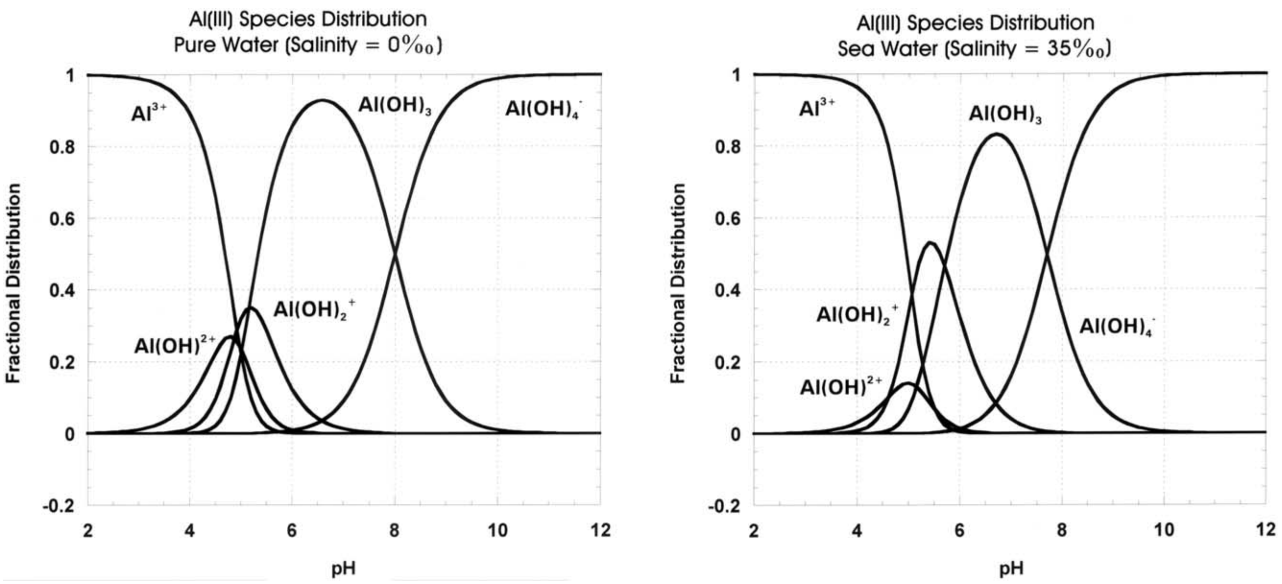


Fig. 2. Aluminum species distribution plots calculated as a function of pH for ‘pure’ water (salinity = 0) and ‘sea’ water (salinity = 35). Aluminum hydrolysis constants appropriate for pure and sea waters were obtained from the tabulation of Stumm and Morgan (Appendix 2, Ref. [6]).

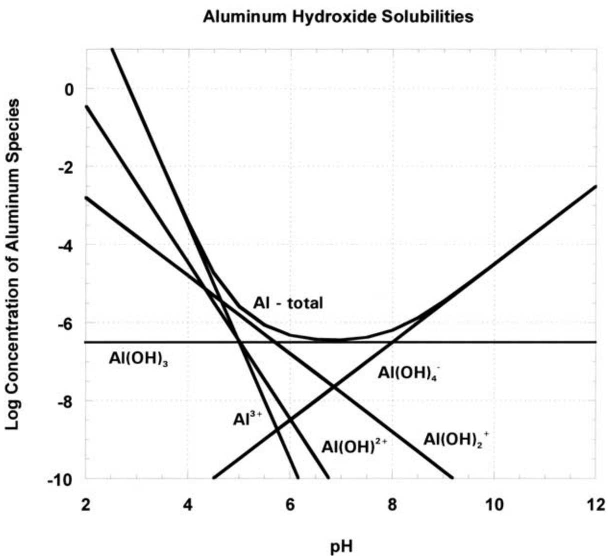


Fig. 3. The effect of hydrolysis on the solubility of aluminum hydroxides. Recalculated, using the data of Baes and Mesmer (Ref. [5]), by permission of Krieger Publishing Co.

(salinity = 35). In both pure water and sea water, at pH 4.0, nearly all of the aluminum exists as the free Al^{3+} ion while at pH 6.0, the precipitated $Al(OH)_3$ species predominates. Fig. 3 presents a plot of calculated aluminum hydroxide solubilities as a function of pH.] Finally, metal ions bound to humic acids can also serve as ‘bridges’ to facilitate the binding of *negatively*-charged organics, such as the phenoxyacetic acid pesticides, to the humic material, and in so doing potentially increasing the bioavailability of these toxic *organic* species.

Al^{3+} ion *interaction* with humic acids is of particular environmental interest because of its increasing prevalence in environmental waters, its tight binding to humic materials (which are also abundant in both fresh and sea waters), and its toxicity (information about which is currently coming to light with increasing frequency). The nature of metal ion (including Al^{3+} ion) interaction with of humic materials has been investigated by a large number of experimental approaches, including fluorescence spectroscopy [9–30], infrared spectroscopy [25,30–36], ion-selective electrode techniques [37–39], gas-phase hydrogen/deuterium exchange and electrospray Fourier transform cycloelectron mass spectrometry (ESI-ICR) [40], ^{113}Cd-NMR [41], ^{1}H- and ^{13}C-NMR [35,36], cathode stripping voltammetry (CSV) and anode stripping voltammetry (ASV) [42], ^{13}C MAS-NMR [43] and atomic force spectroscopy (AFM) [44]. A number of observations made in these studies, especially in the fluorescence studies (to be reviewed below), has suggested that Al^{3+} interacts with humic material in a manner that is considerably different than most other metal ions. An important objective of our research is to examine the apparent ‘uniqueness’ of the Al^{3+} interaction with humic acids, vis-a-vis other metal ions, and, if this ‘uniqueness’ tag can be imparted to Al^{3+}, to try to understand how the unique set of properties associated with the Al^{3+} ion relate to some of the spectroscopic behavior observed in solutions containing Al^{3+} and humic acids. In our own spectroscopic studies, in which we compare the spectroscopic response in solutions of fulvic acid and Al^{3+} to that obtained in solutions of fulvic acid and other metal ions, we have selected the comparative metals based on their position in the

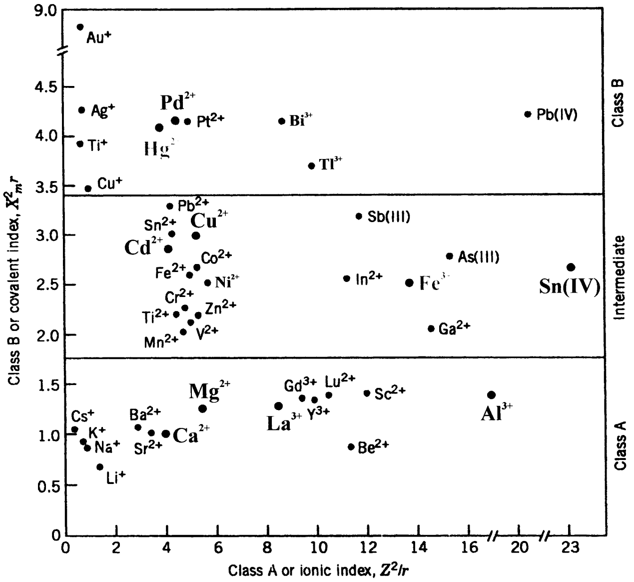


Fig. 4. Separation of metal ions and the metalloid ion As(III) and Sb(III) into three categories: Class A (oxygen-seeking), Class B (nitrogen/sulfur seeking) and Borderline (intermediate or ambivalent). The Class B or covalent-bonding index $(\chi_m)^2r$ is plotted for each ion against the Class A or ionic-bonding index z^2/r. In these expressions, χ_m is the metal-ion Pauling electronegativity, r is the ionic radius corresponding to the most common coordination number and z is the formal charge on the ion. Oxidation states given by Roman numerals imply that simple cations do not exist, even in acidic solutions. Metals highlighted in bold have been utilized for our fluorescence studies. Reprinted from Environmental Pollution, vol. 1, Nieboer and Richardson, "The Replacement of the Nondescript Term 'Heavy Metals' by a Biologically and Chemically Significant Classification of Metal Ions", pp. 3–26, 1980, with permission from Elsevier Science (Ref. [45]).

Nieboer and Richardson [45] classification of metals. Nieboer and Richardson suggested back in 1980 that important environmental metals can be categorized into three classes, Class A, Class B and Intermediate Class, and that this classification system might be particularly applicable for use in research on aspects of environmental pollution. As will be seen, this classification scheme allows the prediction of certain spectroscopic properties related to the interaction of various metal ions with humic substances. Fig. 4 presents the Nieboer and Richardson classification scheme. In this classification metal ions and the metalloid ions (As(III) and Sb(III)) are separated into three categories: Class A (oxygen-seeking, 'hard' acids), Class B (nitrogen/sulfur seeking, 'soft' acids) and Borderline (intermediate or ambivalent). In the Nieboer and Richardson plot (Fig. 4), the Class B or covalent index, $(\chi_m)^2r$, is plotted against the Class A or ionic index, z^2/r. χ_m is the Pauling electronegativity, r is the ionic radius corresponding to the most common coordination number, and z is the formal charge on the ion. Examination of Fig. 4 reveals that Al^{3+} does, in fact, occupy a somewhat unique position in the Nieboer and Richardson classification system. Its covalent index–ionic index coordinate places it at the lower right hand corner of the plot, a very high ionic index *combined* with a very low covalent index. Nieboer et al. [46] point out that generally, for a fixed value of the ionic radius, toxicity increases with increasing covalent index, and conversely, for a fixed value of covalent index, toxicity increases with increasing ionic index. A large number of applications in environmental and toxicological studies of the Nieboer and Richardson classification scheme are summarized in the work of Nieboer et al. [46]. In this work, we report on the interaction of Suwannee River fulvic acid (SRFA) with Class A metals (Mg^{2+}, Ca^{2+}, La^{3+}, Tb^{3+}, Yb^{3+}, Al^{3+}), Class B metals (Pd^{2+}, Hg^{2+}) and Intermediate metals (Cd^{2+}, Cu^{2+}, Co^{2+}, Fe^{3+}, and Sn^{4+}).

Of the spectroscopic approaches to the study of fulvic acid interaction with metal ions, fluorescence spectroscopy, has proven to be one of the most productive [9–30], and it is the primary approach used in our own studies of the binding of Al^{3+} (and comparative metals from the three major Nieboer and Richardson categories of metals) to SRFA. As is evident in Fig. 1, the fulvic acids contain a large collection of oxygen-containing functional groups (aliphatic and aromatic carboxylates, phthalates, phenols, aliphatic alcohols, salicylates and others) that typically give rise to broad and featureless fluorescence spectra. Moreover, as pointed out by Elkins and Nelson [30], the main acidic functionalities responsible for the acidic nature of the humic and fulvic acids (the phthalates and the salicylates) are prime targets for metal ion complexation as well as prime fluorophores. The interaction of many metal ions, including Al^{3+}, with fulvic acids has been examined by the fluorescence approach [9–30]. Certain metal ions (Cu^{2+}, Co^{2+}, Cr^{3+}, Cr^{4+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, Pb^{2+}) [9,10] quench fulvic acid fluorescence whereas other metal ions (such as Al^{3+}) enhance the fluorescence [9]. Whether or not a metal quenches fulvic acid fluorescence depends strongly on the precise conditions of the experiment (i.e. solution pH, ionic strength, concentration of fulvic acid, metal ion-to-fulvic acid ratio, presence or absence of unpaired electrons on the metal ion) [9,10]. Paramagnetic ions *usually* quench fluorescence, while diamagnetic metal ions, such as Al^{3+}, can quench, enhance or leave the fluorescence virtually unchanged [9]. Ryan et al. [9], in their study of Al^{3+} ion complexation to *soil* fulvic acid material, demonstrated both quenching and enhancement behavior 'depending primarily on the *wavelength* used and to some extent on pH. Ryan et al. [9] have reviewed many of the early studies which document the very complex fluorescence behavior associated with Al^{3+} complexation to humic substances. Much of the earlier work has recently been expanded upon to include studies of Al^{3+} induced aggregation on humic substance fluorescence [20], multi-site Al^{3+} ion binding, and speciation with natural organic matter [21,22]. Fluorescence polarization has also been used to probe the structure and aluminum complexation of various molecular weight fractions of soil fulvic acid [26] and factor analysis of synchronous fluorescence spectra has been successfully applied to further examine fulvic acid–Al^{3+} ion complexation [28]. In a very recent study, the interaction of three Class A metal ions (Ca^{2+}, Tb^{3+}, and Al^{3+}), which have very similar covalent indices ($(\chi_m)^2r$), but very different ionic indices (z^2/r), with SRFA was examined by fluorescence spectroscopy [30]. In this study, the fluorescence excitation–emission behavior exhibited by the Al^{3+}–fulvic acid complex was substantially different from that exhibited by the fulvic acid complex with Ca^{2+} ion or the complex formed with the similarly-charged metal ion of lower ionic index (i.e. Tb^{3+}). Fluorescence experiments performed with model compounds pointed to a unique interaction with salicylic acid like moieties not present in fulvic acid complexes with the two other Class A metal ions. The current work extends the scope of this previous study to include a more systematic survey of SRFA complexes with representatives spanning all three Nieboer and Richardson classes of metal ions. It attempts to address the question: Can the fluorescence behavior (i.e. values for $\lambda_{\text{excitation, max}}$, $\lambda_{\text{emission, max}}$, relative emission intensity at $\lambda_{\text{emission, max}}$) exhibited by Al^{3+} complexes with fulvic acid be explained, at least in part, by the truly unique $\{(\chi_m)^2r{:}z^2/r\}$coordinates possessed by Al^{3+}?

Infrared spectroscopy also has been successfully employed to study humic substances, although to a much lesser extent than fluorescence spectroscopy [25,30–36]. The conventional KBr pellet approach has been applied to natural humic material as well as on model systems in the absence and presence of added metal ion. IR bands arising from these complex molecules are broad, making band assignment challenging, nonetheless, useful information can obtained from this approach [1,30]. Elkins and Nelson [30] have recently applied the KBr FT-IR approach to investigate fulvic acid complexes with the three Class A metal ions, Ca^{2+}, Tb^{3+}, and Al^{3+}. The IR spectral comparisons indicated unique IR bands in the fulvic acid–Al^{3+} spectra, bands not found in fulvic acid complexes with either Ca^{2+} ion of trivalent Tb^{3+}. FT-IR experiments performed with model compounds pointed to a unique interaction with phthalic acid like moieties not present in fulvic acid complexes with the two other Class A metal ions. Marley et al. has compared the KBr IR approach with some of the more recent IR approaches, including cylindrical internal reflectance (CIR), which allows study of the complex humic group of substances in the solution phase [34].

One focus of this review is to present the current state of our understanding of aqueous aluminum complexation with the most acidic members (and therefore the most likely candidates for serving as Al^{3+} complexing agents) of the humic substances group, the fulvic acids. In this context the results obtained by fluorescence spectroscopy will receive special emphasis, as much of the current knowledge of Al^{3+}–fulvic acid complexes derives from application of this experimental approach. Another focus, utilizing recent results obtained in our own laboratory, is to compare Al^{3+} ion complexation to *river* fulvic acid material to complexes formed with selected representative of all three Nieboer and Richardson classes of metal ions, using fluorescence and IR spectroscopy. Class A metals examined, other than Al^{3+}, include Mg^{2+}, Ca^{2+}, La^{3+}, Tb^{3+}, and Yb^{3+}. Intermediate metals examined include Cd^{2+}, Co^{2+}, Cu^{2+}, and Sn^{4+}. Class B metals examined includes Pd^{2+} and Hg^{2+}. Most of the interpretive emphasis is placed on data collected at pH 4.0, conditions under which the Al^{3+} is predominantly in the 'free' (aquo) state; however, data also are presented and discussed for experiments carried out at other pH values. A major objective of our recent work is to explore of the linkage between *both* the covalent index, $(\chi_m)^2r$, and the ionic index, z^2/r, of metal ions and fluorescence and IR spectral features associated with metal ion–fulvic acid complexation, to better understand the unique spectral features associated with the aluminum–fulvic acid complex. Spectroscopic results obtained on model compounds, mainly salicylic and phthalic acids, will be utilized in the analysis. Where data permit (i.e. when significant metal ion-induced fulvic acid fluorescence enhancement or quenching is observed) low pH formation constants are determined. Representative fluorescence and IR spectroscopic data obtained on fulvic acid complexes with metals from all three Nieboer and Richardson classes will be presented for comparative purposes for further investigating the functionalities involved in the complexation of these metal ions to fulvic acid material.

2. Fluorescence analysis of fulvic acid complexes with aluminum and comparison metals at low pH and determination of conditional formation constants

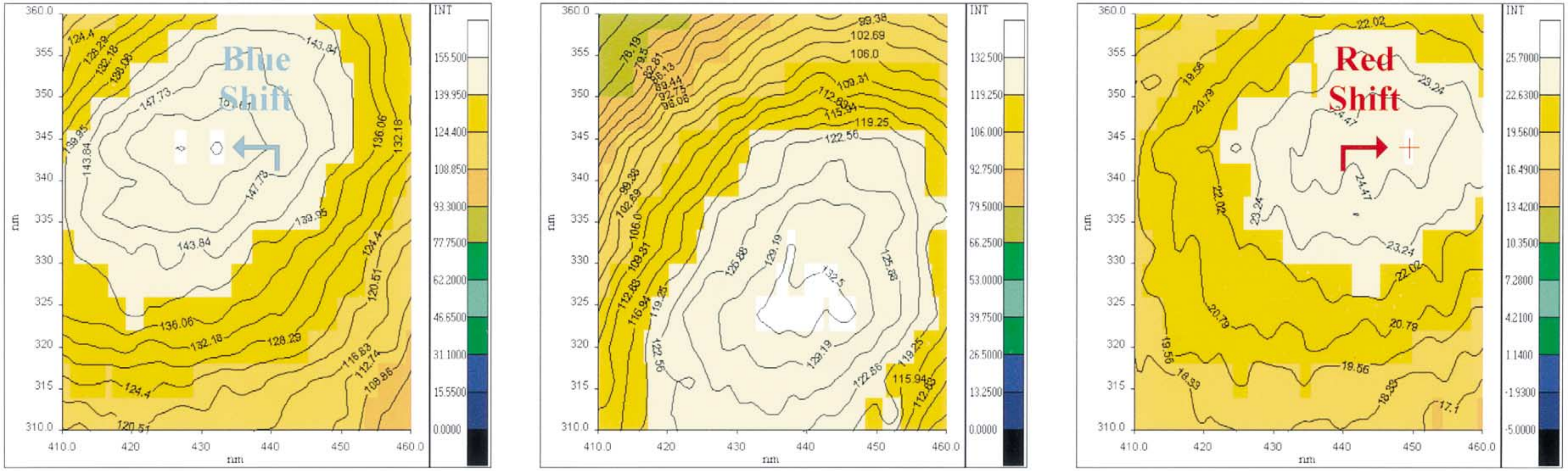
As indicated in Section 1, fluorescence spectroscopy has proven to be a particularly productive approach to the study of metal ion complexation to humic material. This approach has been utilized in our own laboratory to investigate the binding of Al^{3+} (and many other metal ions) to SRFA, and some representative data, illustrating the sensitivity of the fluorescence approach to these systems, will be presented in this section. To monitor the effect that added metal ion has on the fulvic acid fluorescence response, we typically add metal ion to a fulvic acid solution at a concentration of 15 mg l^{-1}. This concentration has been chosen for the following reasons: (1) there is significant intensity and minimal contributions from inner-filter effects at this concentration; (2) there is a precedent in the literature for using this concentration, facilitating comparisons between the results of our studies of *river* fulvic acid with those of other investigators who have studied the interaction of metal ions (in particular Al^{3+}) with *soil* fulvic acid [9,39]; and (3) it has been estimated that *average* US river water contains 10–13 mg l^{-1} (i.e. 10–13 ppm) of dissolved humic substances [47] so the experiments will be environmentally relevant.

The central panel of Fig. 5 presents the excitation–emission matrix (EEM) plot for SRFA (at 15 mg l^{-1} in 0.1 M $NaClO_4$, pH 4.0) in the absence of any added metal ions. The $\lambda_{emission}$ range spans 410–460 nm and the $\lambda_{excitation}$ range spans 310–360 nm. This is the region that has been analyzed by other investigators in fluorescence studies of metal ion interactions with fulvic acids [9,11]. Fluorescence in this region, referred to as ‘region C’ by Luster et al. [48] and centered at about $\lambda_{excitation} = 323$ nm/$\lambda_{emission} = 448$ nm, has been ‘ascribed mainly to simple phenolic compounds’ [48–50]. The other panels in Fig. 5 present EEM plots for SRFA, in the presence (at 300 μM concentration) of the Class A metal, Al^{3+} (left panel), and the Class B metal, Pd^{2+} (right panel). Note that addition of either Al^{3+} (electronic configuration = [Ne]) or Pd^{2+} ([Kr]$4d^8$) to the fulvic acid solution causes a *very significant change* in the EEM plot. The fluorescence excitation–emission behavior is clearly very sensitive to the complexation of these very different metal ions to the fulvic acid material. Yet, these two metals do not behave identically. The Al^{3+} addition results in a very significant *increase* (‘red shift’) in the wavelength of maximum excitation (from ca. 324 up to ca. 344 nm) and a significant *decrease* (‘blue shift’) in the wavelength of maximum emission (from ca. 440 down to ca. 424 nm). Pd^{2+} addition also results in a $\lambda_{excitation,\ max}$ red shift virtually identical to that observed with Al^{3+} addition (from ca. 324 up to ca. 344 nm), but, in contrast to Al^{3+}, a significant *increase* (‘red shift’) in the wavelength of maximum emission (from ca. 440 down to ca. 424 nm). The very significant shifts observed in both $\lambda_{excitation,\ max}$ and $\lambda_{emission,\ max}$ (associated with the addition of both metal ions) in the EEM plot region being examined are entirely consistent with the involvement of phenolic moieties (especially salicylic acid groups) in the complexation process [30,48–50]. (Addition of Al^{3+} to a salicylic acid solution was shown to produce a $\lambda_{excitation,\ max}$ red shift and a $\lambda_{emission,\ max}$ blue shift in the salicylic acid EEM peak comparable to that observed when Al^{3+} is added to a SRFA solution under identical conditions of pH and ionic strength [30].) Relative fluorescence emission intensity also is significantly altered upon addition of these two metals to the fulvic acid solution, with marked enhancement with addition of diamagnetic Al^{3+}, and very substantial quenching upon addition of paramagnetic Pd^{2+} (consistent with expectations). Ryan et al. has interpreted the observed Al^{3+} induced enhancement of fulvic acid fluorescence to originate in the increased rigidity of the fulvic acid structure as a direct consequence of the binding of the small, highly charged Al^{3+} ion, thus increasing the fluorescence quantum yield by reducing the probability of non-radiative energy dissipation processes [9].

Fluorescence titration experiments have proven to be most useful in providing data necessary to estimate conditional formation constants for the binding of various metal ions to fulvic acids [8,9,11,22,26,27,29,30,48]. In our own laboratory, SRFA fluorescence titration experiments have been performed at pH 4.0 and 5.0, for the purpose of obtaining conditional formation constant and binding site capacity information for Al^{3+} ion and comparison metal ions selected from all three Nieboer and Richardson classes of metal ions [45,46]. Fluorescence measurements were made in solutions containing 15 mg SRFA per liter in 0.1 M $NaClO_4$, pH 4.0, with $\lambda_{excitation}$ set to 344 nm. In Fig. 6, the results of the SRFA–Al^{3+} titrations (top panel) are compared to those of titrations of Intermediate Class metals Cd^{2+} and Cu^{2+} (middle panel) and Class B metal Pd^{2+} (bottom panel). Quantitation was not attempted at pH values greater than 5.0 to avoid complications that would result from the formation of insoluble $Al(OH)_3$ (see Fig. 2). Moreover, the results reported for the pH 4.0 titration should be considered more reliable than that reported for the pH 5.0 titration (Fig. 7) as the ‘free’ (aquo) Al^{3+} species is the predominant species by far at the lower pH value. In Fig. 6, the experimentally determined relative fluorescence intensity (with $\lambda_{ex} = 344$ nm) is plotted against concentration for the pH 4.0 titrations of each metal Al^{3+} ($\lambda_{em} = 424$ nm), Cd^{2+} ($\lambda_{em} = 448$ nm), Cu^{2+} ($\lambda_{em} = 448$ nm), and Pd^{2+} ($\lambda_{em} = 438$ nm). (Titration curves for these same metals at pH 5.0 are presented in Fig. 7. Note the significant increase in the ability of Cu^{2+} to quench SRFA fluorescence at the higher pH value. Also note the greatly increased initial slope for the Al^{3+} titration, consistent with a higher K_f' value at the higher pH value.) Also presented in Fig. 6 are best fit curves for the two metals (Al^{3+} and Pd^{2+}) which exhibit saturation (or near-saturation) behavior, generated by analyzing our experimental results with the non-linear fluorescence quenching/enhancement data treatment model of Ryan and Weber [8,11]. This model is based on the basic relationship linking fluorescence quenching/enhancement to complexation of metal ion, M, to the organic ligand, L, in the M + L = ML equilibrium system:

$$[ML]/c_L = (I_0 - I)/(I_0 - I_{RES})$$

where [ML] is the concentration of metal–ligand com-



Fulvic Acid
+
Al(III)
(Class A Metal)

Fulvic Acid

Fulvic Acid
+
Pd(II)
(Class B Metal)

Fig. 5. 3D fluorescence EEM plots are shown for SRFA (center panel), SRFA-Al^{3+} (left panel), and SRFA-Pd^{2+} (right panel) in 0.1 M $NaClO_4$ (pH 4.0). The concentration of fulvic acid and metal in each sample is 15 mg l^{-1} and 300 μM, respectively. Emission wavelength (nm) is plotted on the x-axis and excitation wavelength (nm) on the y-axis. The intensities have been adjusted separately in these EEM plots to best display the excitation–emission peaks. The EEM peaks are characterized as follows: SRFA alone, $\lambda_{\text{excitation, maxima}} = 324$ nm, $\lambda_{\text{emission, maxima}} = 439.5$ nm, 133 relative intensity units; SRFA-Al^{3+}, $\lambda_{\text{excitation, maxima}} = 344$ nm, $\lambda_{\text{emission, maxima}} = 424$ nm, 156 relative intensity units; SRFA-Pd^{2+}, $\lambda_{\text{excitation, maxima}} = 344$ nm, $\lambda_{\text{emission, maxima}} = 449$ nm, 28 relative intensity units. Note that Al^{3+} addition causes a significant blue shift in $\lambda_{\text{emission, maxima}}$, while that Pd^{2+} addition causes a significant red shift in $\lambda_{\text{emission, maxima}}$. Addition of both metals causes virtually the same significant red shift in $\lambda_{\text{excitation, maxima}}$. All fluorescence data were collected on a Perkin–Elmer LS-50B Luminescence Spectrometer.

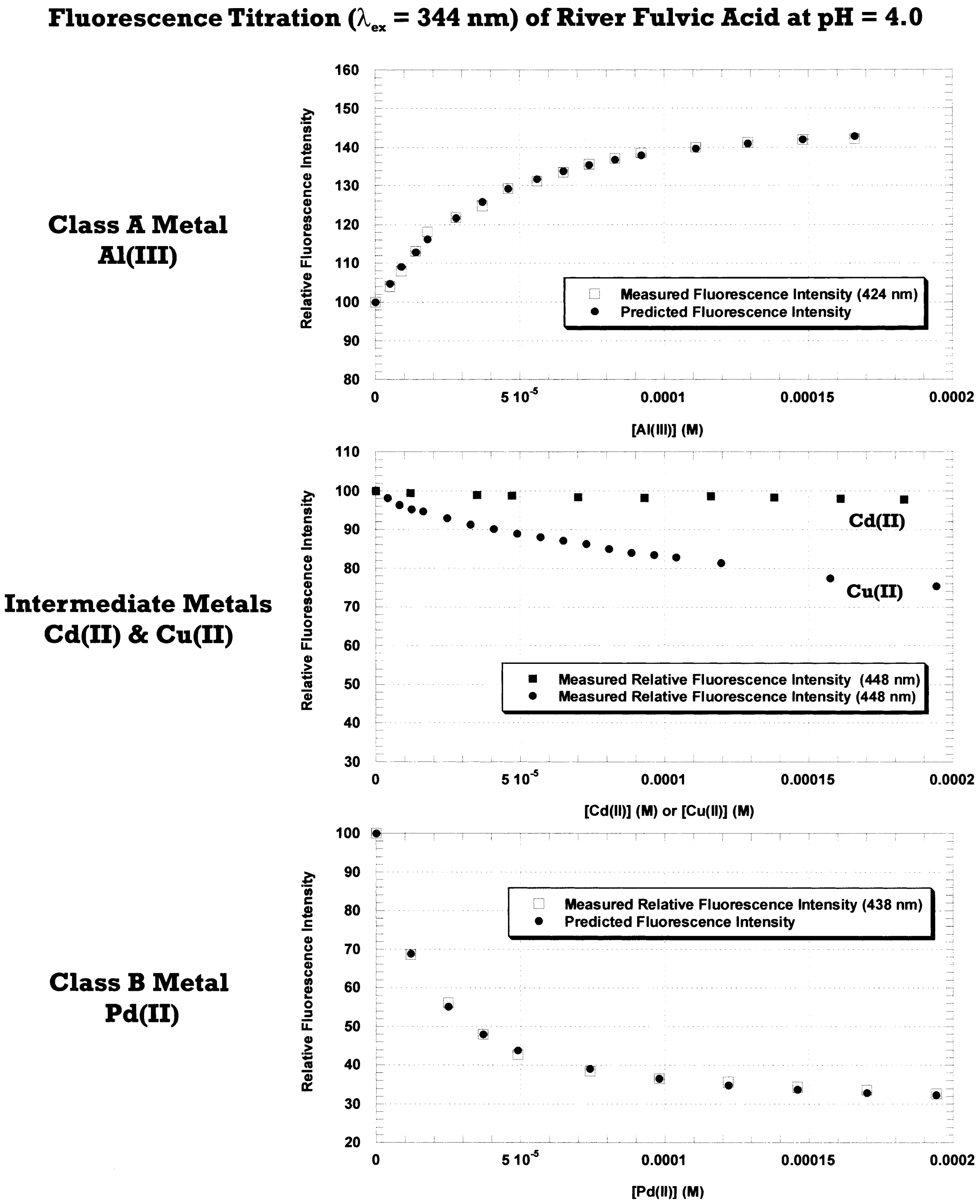


Fig. 6. The fluorescence titration data at pH 4.0 for the formation of fulvic acid–metal ion complexes are shown. Each metal ion was added to 15 mg l^{-1} SRFA in 0.1 M $NaClO_4$ (pH 4.0). All data were collected at $\lambda_{excitation} = 344$ nm and at $\lambda_{emission}$ values associated with the emission peak. The relative fluorescence intensity is plotted against the emission wavelength. The fluorescence intensity associated with SRFA prior to the addition of metal ion was normalized to 100 intensity units. The top panel presents data for the titration of SRFA with the Class A metal Al^{3+}, while the bottom panel presents data for the Class B metal Pd^{2+}. The middle panel presents titration results for the two intermediate metal ions, Cd^{2+} and Cu^{2+}. For the two metals exhibiting saturation or near saturation responses (Al^{3+} and Pd^{2+}), the data were analyzed by the non-linear fluorescence data enhancement model of Ryan and Weber (Ref. [11]) and the fit points (open symbols) produced by the model are shown along side the experimental data (closed symbols).

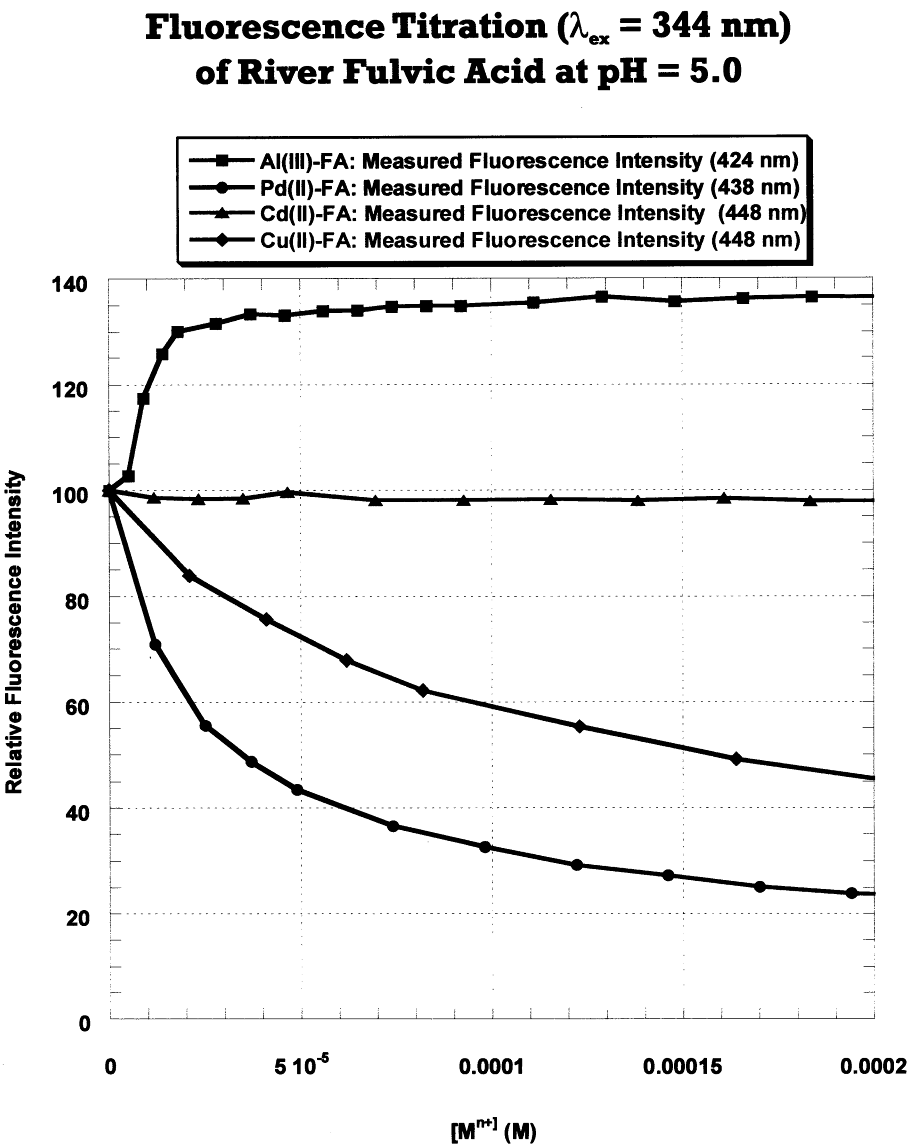


Fig. 7. The fluorescence titration data at pH 5.0 for the formation of fulvic acid–metal ion complexes are shown. Each metal ion (Al^{3+}, Pd^{2+}, Cd^{2+}, and Cu^{2+}) was added to 15 mg l^{-1} SRFA in 0.1 M $NaClO_4$ (pH 5.0). All data were collected at $\lambda_{\mathrm{excitation}} = 344$ nm and at $\lambda_{\mathrm{emission}}$ values associated with the emission peak. The relative fluorescence intensity is plotted against the emission wavelength. The fluorescence intensity associated with SRFA prior to the addition of metal ion was normalized to 100 intensity units. Note the greatly enhanced ability of Cu^{2+} to quench SRFA fluorescence, relative to that observed at pH 4.0.

plex, c_L is the concentration of ligand sites, I_0 is the initial intensity value from the experimental data (before any metal ion has been added to the sample of fulvic acid in our case) and I_{RES} is the residual intensity (the final predicted plateau in the fluorescence response). Algebraic manipulation of the conditional formation constant, K'_f (where $K'_f = [\mathrm{ML}]/[\mathrm{M}][\mathrm{L}]$), expression and mass balance equations for the $\mathrm{M}+\mathrm{L}=\mathrm{ML}$ equilibrium system, leads to the Ryan and Weber equation for fluorescence intensity as a function of total metal concentration, c_M (where $c_M = [\mathrm{M}]+[\mathrm{ML}]$), and total ligand concentration, c_L (where $c_L = [\mathrm{L}]+[\mathrm{ML}]$):

$$I = ((I_{\mathrm{RES}} - I_0)/2K'_f c_L)\ \{K'_f c_L + K'_f c_M + 1) - [K'_f c_L + K'_f c_M + 1)^2 - 4K'^2_f c_L]^{0.5}\} + I_0$$

In this equation, K'_f, c_L and I_{RES} are parameters to be fit by the program. Of particular interest are the values for K'_f and c_L. The program NLREG was utilized to carry out the non-linear regression analysis for data collected at both pH values examined [51]. Fig. 6 shows that the predicted fluorescence values correlate very well with the experimentally determined values for both the Al^{3+} and Pd^{2+} titrations. All values seem reasonable and were obtained with good statistics. The K'_f value (M^{-1}) is

Table 1
Fulvic acid–Al^{3+} complexation

Fulvic acid source	pH	Log K_f' (M^{-1})	Model [a]	Reference
Northern coniferous forest soil (< 500 Da)	3.0	4.9	One-site	[26]
Northern coniferous forest soil (< 500 Da)	3.5	5	One-site	[26]
Northern coniferous forest soil (< 500 Da)	3.5	4.4 (type 1) 4.5 (type 2) 4.9 (type 3)	Multi-site	[5]
Northern coniferous forest soil (< 500–1000 Da)	3.5	4.6 (type 1) 5.0 (type 2) 5.3 (type 3)	Multi-site	[5]
Northern coniferous forest soil (< 1000–10,000 Da)	3.5	5.1 (type 1) 5.2 (type 2) 5.5 (type 3)	Multi-site	[5]
Northern coniferous forest soil (all MW)	3.5	4.6 (type 1) 5.0 (type 2) 5.3 (type 3)	Multi-site	[5]
Northern coniferous forest soil (< 500 Da)	4.0	5.2	One-site	[26]
Suwannee River	4.0	4.67	One-site	[30]
Coastal marine	4.0	5.1	One-site	[29]
Podzol soil	4.00	5.79	One-site	[9]
Suwannee River	4.36	6.8 (class 2) 5.0 (class 4) 4.9 (class 5) 5.0 (class 6) 5.1 (class 7)	Multi-site	[22]
Suwannee River	5.0	6.46	One-site	[30]
Juniper leaf litter extract	5	8.05 (type 1) 5.79 (type 2)	One-site (applied multiple times)	[18]

[a] One-site model values were calculated as described by Ryan and Weber [11] and multi-site values as described within the representative publications.

lower for binding at pH 4 than at 5. It is probable that the increase at pH 5 is due to additional carboxyl group ionization over that present at pH 4. The K_f' values measured in our study, ranging from 4.67×10^4 (pH 4.0) to 2.87×10^6 M^{-1} (pH 5.0) for the aluminum binding to river fulvic acid material, are comparable to the values determined independently by many groups. These are tabulated as log K_f' values in Table 1. Ryan et al. measured 6.2×10^5 M^{-1} for the K_f at pH 4.0 for soil fulvic acid (15.0 mg l^{-1}) [9]. Smith and Kramer [22] used a multi-site model to fit Al^{3+} binding to SRFA at pH 4.36 and determined log K_f' values ranging from 4.9 to 6.8, depending on the class of fluorescence analyzed. These agree well with the log K_f' values we determined at pH 4.0 (4.67) and 5.0 (6.46). Other sources of fulvic acid examined include the Northern Coniferous Forest Soil fulvic acids, which at pH 3.5 had log K_f' values for Al^{3+} binding ranging from 4.4 to 5.5 for type 1, 2, and 3 sites as fit to a multi-site model [27]. A Coastal Marine fulvic acid studied by da Silva and Machado [29] for its Al^{3+} complexation properties yielded a log K_f' of 5.1 as fit by a single-site model. A Juniper Leaf Litter Extract of fulvic acids at pH 5 had a K_f' of 8.05 for a type 1 site and 5.79 for a type 2 site as determined using a one-site model (applied twice) when Al^{3+} complexation was examined [48]. The c_L values measured here, ranging from 18.6×10^{-6} (pH 4.0) to 20.0×10^{-6} M (pH 5.0), for the river fulvic acid material, are also comparable to the value (50.6×10^{-6} M) measured by Ryan et al. at pH 4.0 for soil fulvic acid [9]. The pH dependence of Al^{3+} binding, as noted in the values reported above, are consistent with the trends noted by Browne and Driscoll [52] in their work on SRFA.

The Ryan and Weber model makes the assumption that there is only one type of site. Although this assumption is not strictly correct, due to the complexity and heterogeneity of the fulvic acid material, the experimental data fit very well to this model (as was previously demonstrated by Ryan an coworkers in their work on soil fulvic acid material and the other groups [29,48] that have used this model to determine K_f' values for Al^{3+} binding to other fulvic acids). The high quality fit suggests that although the sites are not exactly the same, they are very similar and thus the near-perfect fit of our data to the model. There are other approaches that could have been employed to fit our fluorescence data than the Ryan and Weber single-site model, including the factor analysis approach of Silva et al.

[28], and various multi-site model approaches, as presented by Luster et al. [48] and Smith and Kramer [21,22]. We chose the single-site model to facilitate direct comparison of our formation constant results on SRFA with those of Ryan et al. [9] (since we use exactly the same conditions: ionic strength, pH, concentration and temperature).

In the middle panel of Fig. 6, the results of titrating SRFA with Cd^{2+} and Cu^{2+} are shown. Cd^{2+} does not quench, while Cu^{2+} measurably quenches fulvic acid fluorescence. Although, we do not report K_f' values derived from Cu^{2+} and Cd^{2+} SRFA fluorescence quenching, these values have been reported by other groups [11,41]. As is evident in the bottom panel of Fig. 6, Pd^{2+} is by far the best quencher of fulvic acid fluorescence that has been observed by any group. The determined K_f' of the titration of Pd^{2+} to SRFA was 7.97×10^4 M^{-1} and the concentration of ligand sites 6.53×10^{-6} M. Ryan and Weber [11] have in their work on the Podzol soil fulvic acids (10 mg l^{-1}) reported a $K_f'=2.08\times10^5$ (pH 5), 9.26×10^4 (pH 6), and 3.52×10^4 M^{-1} (pH 7) and $c_L=22.1$ (pH 5), 19.7 (pH 6), and 19.6 (pH 7) for Cu^{2+} binding. Otto et al. [41] determined K_f' values (pH 4.0) for Cd^{2+} binding to for Suwannee River FA (2.6×10^3 M^{-1}), Wakarusa River FA (1.9×10^3 M^{-1}), Clinton Lake FA (1.2×10^3 M^{-1}) and Laurentian soil FA (3.1×10^3 M^{-1}). Saar and Weber [47] have reported comparable K_f' values (pH 4.0) for Cd^{2+} binding to soil fulvic acid (1.4×10^3 M^{-1}) and water fulvic acid (1.7×10^3 M^{-1}).

Fig. 5 compared the effect of adding *one* Class A metal, Al^{3+}, versus *one* Class B metal, Pd^{2+}, on the fluorescence EEM behavior of SRFA. Significant (and quite different) effects were observed for both of these metal ions. What fluorescence behavior might we expect from other Class A, Intermediate and Class B metal ions? Fig. 8 presents 3D EEM plots obtained at pH 4.0 for fulvic acid in the presence of 300 μM of Class A metals La^{3+}, Ca^{2+}, and Mg^{2+} in comparison to Al^{3+}. Note the Al^{3+} ion-induced $\lambda_{\text{excitation, max}}$ red shift already discussed in the context of Fig. 5, and that neither La^{3+}, Ca^{2+}, nor Mg^{2+} ion give rise to this effect. (Note: The ability of other trivalent metal ions (for example, Tb^{3+} and Yb^{3+}) to perturb the fluorescence behavior of fulvic acid has also been examined with effects similar to that observed for La^{3+} [30].) The La^{3+} results are particularly relevant since it is a *diamagnetic* species (like Al^{3+}), while Tb^{3+} and Yb^{3+} are both *paramagnetic*. Thus, the Al^{3+} fluorescence effects differ substantially from all of the other Class A metal ions thus far examined in our system, irrespective if they are divalent or trivalent, irrespective if they are diamagnetic or paramagnetic. The $\lambda_{\text{max, excitation}}$ red shift observed for the fulvic acid–Al^{3+} complex was also evident in EEM data collected at pH 6.0 (data not shown), although relative intensities varied from that observed at pH 4.0. As fluorescence is strongly pH dependent, this is not surprising [23]. The 'red-shifting' of the fulvic acid fluorescence excitation (and concomitant 'blue-shifting' of the emission) provides clear evidence of a strong interaction between the river fulvic acid material and Al^{3+} ion, as these shifts are indicative of a significant alteration in the electronic structure of the fulvic acid, shifts that are not observed with any of the other Class A metal ions tested. Fig. 9 presents the results of SRFA titrations (at pH 4.0) with the same four Class A metals (Al^{3+}, La^{3+}, Ca^{2+}, and Mg^{2+}). In all cases $\lambda_{ex}=344$ nm and the relative fluorescence intensity was recorded over increasing concentrations of metal added at the emission peak associated with each metal added. Note that *only* Al^{3+} enhances the SRFA fluorescence intensity, the other metals do not measurably change the fluorescence. Recall that all of these metal ions, being in Class A, are oxygen-seeking hard acids. La^{3+} has the same charge as Al^{3+} and both La^{3+} and Al^{3+} are diamagnetic. They differ dramatically, however, in their Nieboer and Richardson ionic index (z^2/r) (see Fig. 4). The data presently available might suggest that the high value for the ionic index possessed by Al^{3+} ion is important in dictating the observed fluorescence behavior in the fulvic acid–Al^{3+} complex. Will other ions which possess even higher values for the ionic index, such as Sn^{4+}, give rise to the Al^{3+}-like fluorescence behavior?

In Fig. 10, 3D fluorescence EEM plots of some representative Intermediate Class metals (Cu^{2+}–[Ar]$3d^9$, Cd^{2+}–[Kr]$4d^{10}$ and Sn^{4+}–[Kr]$4d^{10}$) are compared to the Class A metal ion, Al^{3+}. As was seen in the EEM plot comparisons for the Class A metals (Fig. 8), none of the Intermediate Class metal ions show the Al^{3+}-like perturbations present in the SRFA–Al^{3+} system. The excitation wavelengths of these Intermediate Class metals *do not* shift to the 'red' and the emission wavelengths *do not* shift to the 'blue' as is observed when Al^{3+} ion is added to the fulvic acid solution. The 'null' result observed in the fulvic acid–Sn^{4+} system is particularly noteworthy, since Sn^{4+}, with its 4+ charge, has a Nieboer and Richardson index substantially higher than the Al^{3+} ion. Therefore, the ionic index *alone* cannot be responsible for dictating the Al^{3+} behavior. EEM plots also have been generated for the Intermediate Class metals Co^{2+} ([Ar]$3d^7$) and Fe^{3+} ([Ar]$3d^5$), however, like Cu^{2+}, Cd^{2+}, and Sn^{4+}, no significant Al^{3+}-like perturbations of fulvic acid EEM pattern were observed in the presence of these metal ions (data not shown).

Two Class B metals have been examined in our laboratory thus far: Pd^{2+} ([Kr]$4d^8$) and Hg^{2+} ([Xe]$4f^{14}5d^{10}$). Pd^{2+} has already been discussed in the context of Fig. 5. Recall that Pd^{2+}, like Al^{3+}, produced a $\lambda_{\text{max, excitation}}$ red shift in the EEM plot for SRFA. Unlike Al^{3+} however, Pd^{2+} produced a red-shift in

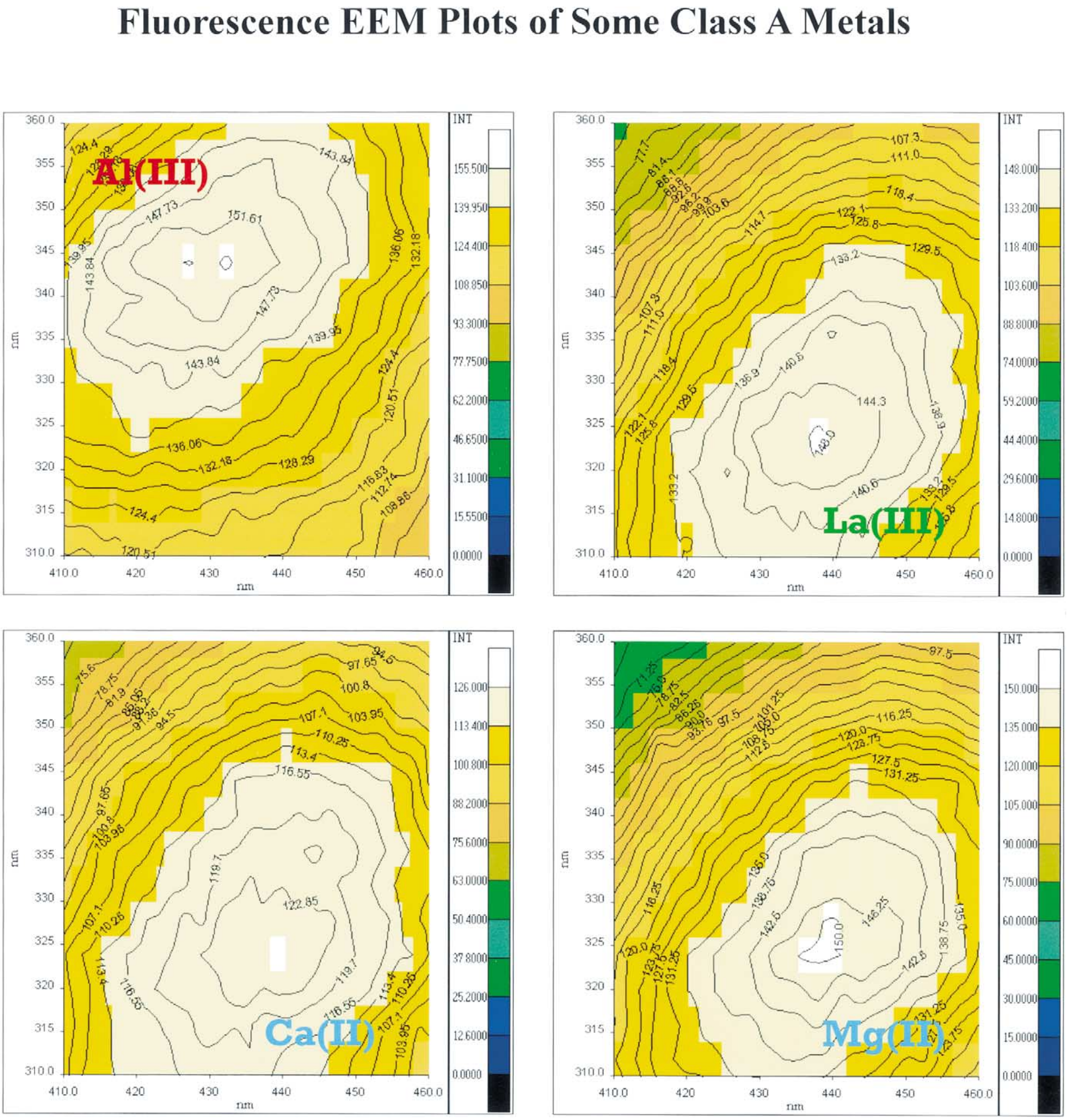

Fluorescence EEM Plots of Some Class A Metals

Fig. 8. 3D fluorescence EEM plots are shown for SRFA in the presence of four different Class A metal ions of increasing ionic index (Ca^{2+}, Mg^{2+}, La^{3+}, and Al^{3+}) in 0.1 M $NaClO_4$ (pH 4.0). The concentration of fulvic acid and metal in each sample is 15 mg l^{-1} and 300 μM, respectively. Emission wavelength (nm) is plotted on the x-axis and excitation wavelength (nm) on the y-axis. The intensities have been adjusted separately in these EEM plots to best display the excitation–emission peaks. Note that Al^{3+} is the only metal in this group that gives rise to a significant blue shift in $\lambda_{\text{excitation, maxima}}$ and red shift in $\lambda_{\text{emission, maxima}}$, relative to that observed for SRFA in the absence of metal ion under the same conditions of pH and ionic strength.

$\lambda_{\text{max, emission}}$. Fulvic acid EEM plots produced in the presence of the other Class B metal, Hg^{2+}, did not show any significant shifting of $\lambda_{\text{max, excitation}}$ or $\lambda_{\text{max, emission}}$ (data not shown). As the covalent and ionic indices for these two metal ions are very similar, the different fluorescence response is clearly linked to the outer, unfilled 4d electronic shell present in the Pd^{2+} ion, in contrast to the closed electronic configuration of the Hg^{2+} ion. Neither metal ion influences fulvic acid fluorescence behavior in a manner reminiscent of Al^{3+}

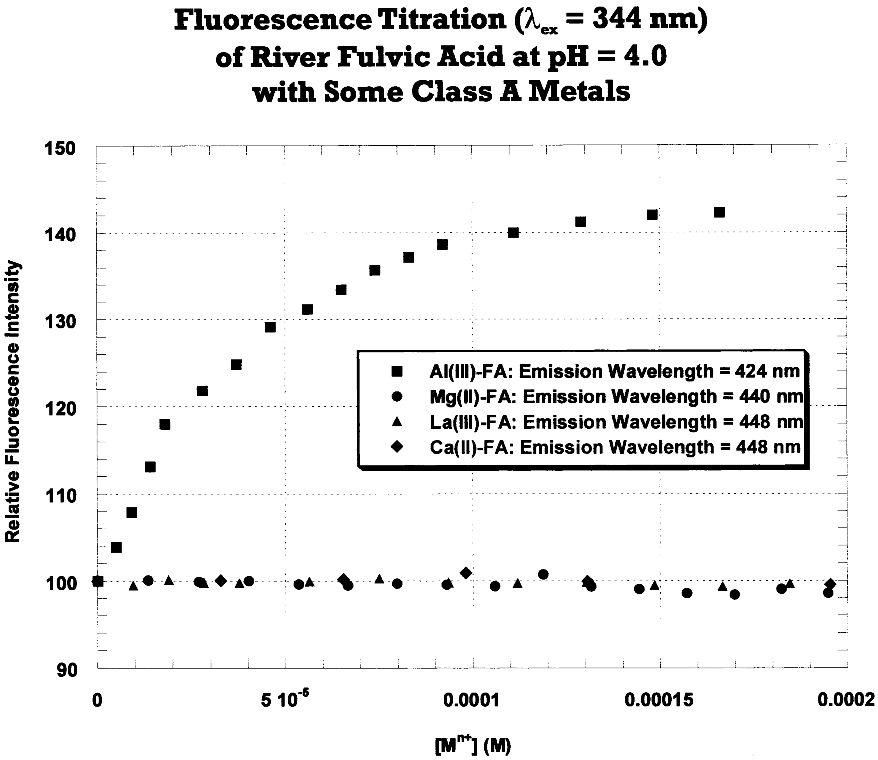


Fig. 9. The fluorescence titration data for the formation of fulvic acid–metal ion complexes with some Class A metal ions of increasing ionic index (Ca^{2+}, Mg^{2+}, La^{3+}, and Al^{3+}). Each metal ion was added to 15 mg l^{-1} SRFA in 0.1 M $NaClO_4$ (pH 4.0). All data were collected at $\lambda_{excitation} = 344$ nm and at $\lambda_{emission}$ values associated with the emission peak. The relative fluorescence intensity is plotted against the emission wavelength. The fluorescence intensity associated with SRFA prior to the addition of metal ion was normalized to 100 intensity units. Note that Al^{3+} is the only metal in this group that gives rise to a significant change (in this case, an *enhancement*) in the fluorescence of the SRFA solution, consistent with the behavior observed for Al^{3+} in Fig. 8.

ion. Perhaps this is not surprising, as Pd^{2+} and Hg^{2+} occupy positions in the Nieboer and Richardson metal ion classification scheme that are diametrically opposite from Al^{3+}. Pd^{2+} and Hg^{2+} have very large values for the covalent index and very small values for the ionic index, whereas Al^{3+} has a very small covalent index coupled to a very large ionic index. It may well be the precise *combination* of the two values possessed by Al^{3+} (i.e. low $(\chi_m)^2$, high z^2/r) that is critical in dictating (at least some of the) spectroscopic properties of this important metal ion. Further experiments are now under way, focusing on the interaction of additional metal ions from the three Nieboer and Richardson categories with humic materials, to better understand the nature of the Al^{3+}-induced spectral changes in Al^{3+} complexes with humic materials.

3. FT-IR analysis of fulvic acid complexes with aluminum and comparison metals at low pH

Infrared spectroscopy of various types has been successfully utilized to characterize a wide variety of humic material [31–34]; however, there have been relatively few IR studies on metal ion complexes with humic material [25,34,35], especially those performed on Al^{3+} complexes with fulvic acid [25,30]. In a recent paper, we presented FT-IR (KBr pellet) data on SRFA complexes with three Class A metal ions. In the order of increasing ionic index, the metals examined were Ca^{2+}, Tb^{3+}, and Al^{3+} [30]. In this earlier study, as well as in the new results reported here, metal ion was added to aqueous solutions of SRFA to obtain a *ratio* of fulvic acid to metal ion comparable to that typically used in the generation of the fluorescence EEM plots (5.0 mg fulvic acid and 2.0×10^{-2} M metal ion) in a total volume of 5.0 ml. The pH was adjusted to 4.0 with NaOH or HCl. For the model compound phthalic acids a concentration of 0.30 mM was used with 4.0 mM metal ion. Prior to spectroscopy, all samples were lyophilized from pH 4.0 in solution to produce a fine powder. (Additional experimental details are given in the legend to Fig. 11.) The KBr pellet IR approach applied to humic substances generally gives rise to broad bands and severe band overlap, as is clearly evident in our data. Nonetheless, rather significant changes have been observed in our earlier experiments as well as in the results presented here. Considering the large spectral perturbation in the FT-IR spectrum of fulvic acid caused by the addition of selected metal ions, we feel

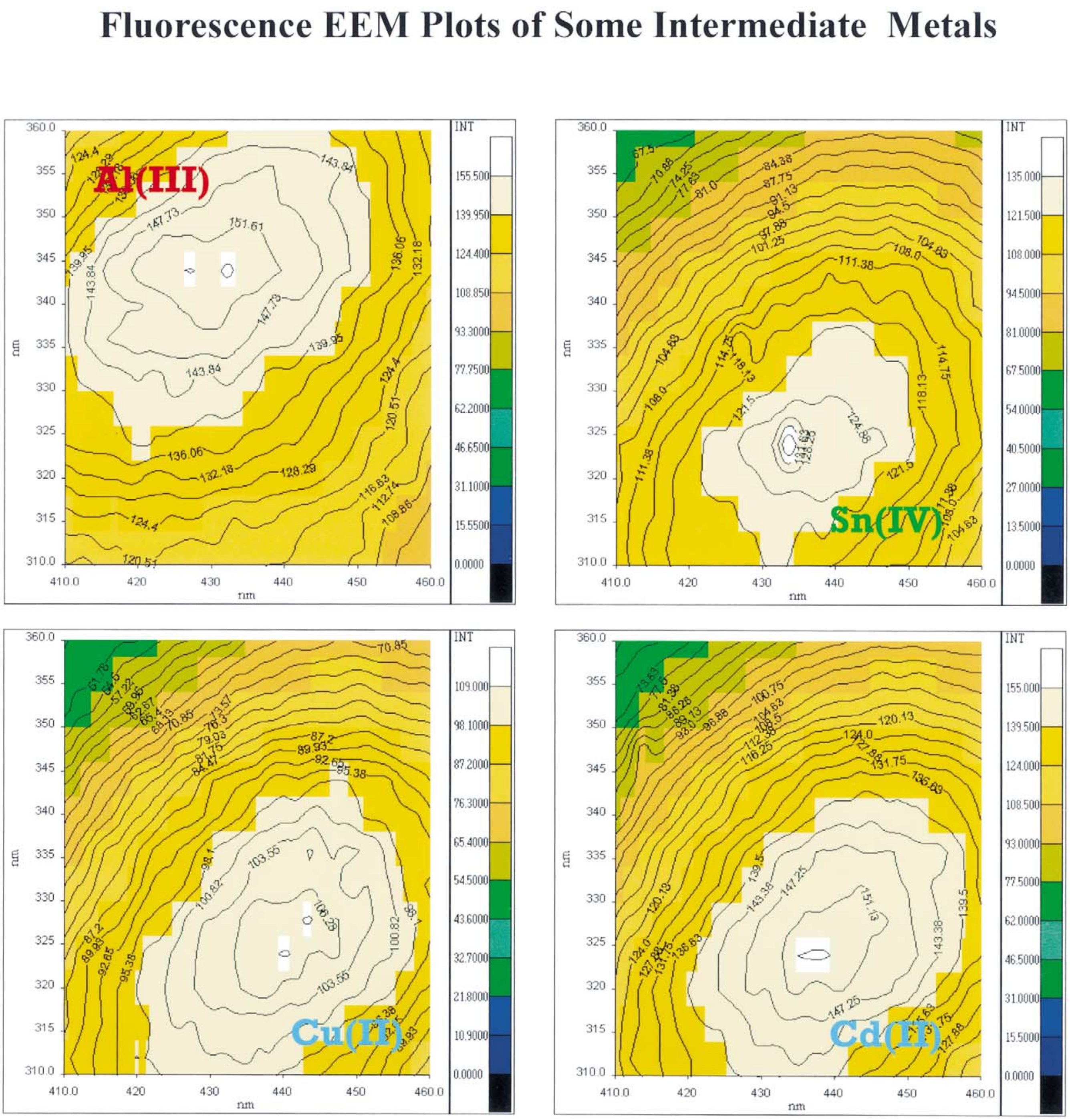


Fig. 10. 3D fluorescence EEM plots are shown for SRFA in the presence of Al^{3+} and three different 'Intermediate Class' metal ions of increasing ionic index (Cd^{2+}, Cu^{2+}, and Sn^{4+}) in 0.1 M $NaClO_4$ (pH 4.0). The concentration of fulvic acid and metal in each sample is 15 mg l^{-1} and 300 μM, respectively. Emission wavelength (nm) is plotted on the x-axis and excitation wavelength (nm) on the y-axis. The intensities have been adjusted separately in these EEM plots to best display the excitation–emission peaks. Note that Al^{3+} is the only metal in this group that gives rise to a significant blue shift in $\lambda_{\text{excitation, maxima}}$ and red shift in $\lambda_{\text{emission, maxima}}$, relative to that observed for SRFA in the absence of metal ion under the same conditions of pH and ionic strength. Sn^{4+}, even though it has a significantly higher 'ionic index' that Al^{3+}, does not give rise to the $\lambda_{\text{excitation, maxima}}$ and $\lambda_{\text{emission, maxima}}$ shifts observed when Al^{3+} is added to solutions of SRFA.

that the IR data are worthy of interpretation, despite the broad bands which are evident in these spectra.

Fig. 11 presents FT-IR data on SRFA (in the absence and presence of Al^{3+}, La^{3+}, and Pd^{2+}) focusing on the 2000–1000 cm^{-1} IR spectral region, rich in COOH stretch and COO^{-1} asymmetric and symmetric stretch bands. The most prominent IR band in the fulvic acid spectrum is that appearing at 1720 cm^{-1}, and is

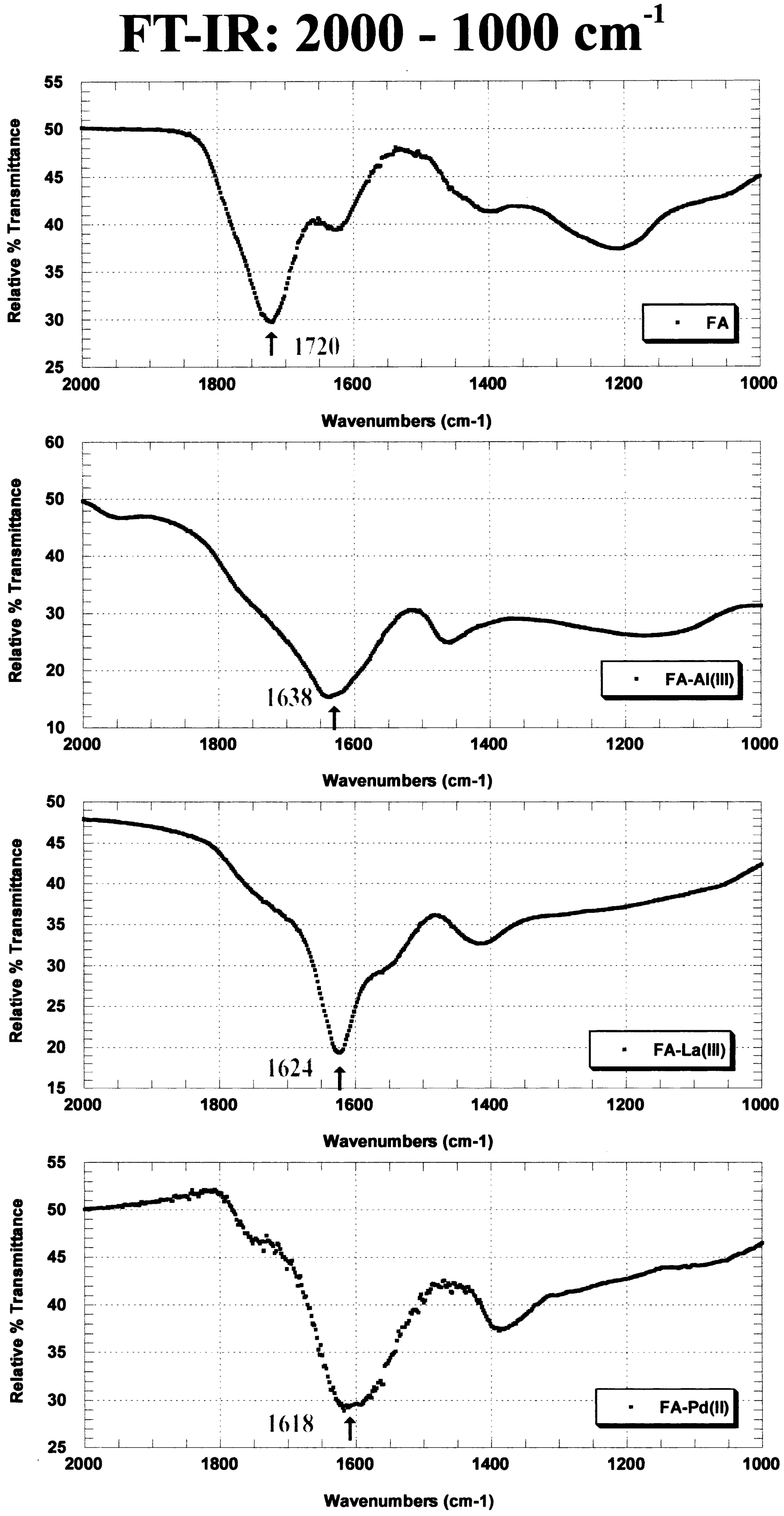


Fig. 11 (*Continued*)

attributable to the C=O asymmetric stretch from neutral –COOH groups. Examination of this region in the IR spectra obtained from fulvic acid–Al^{3+}, –La^{3+}, and –Pd^{2+} mixtures clearly shows that the 1720 cm^{-1} band is completely gone. A new band emerges for the metals at higher energy: 1638 cm^{-1} for Al^{3+}, 1624 cm^{-1} for La^{3+}, and 1618 cm^{-1} for Pd^{2+}. Since the 1720 cm^{-1} band has essentially disappeared in the Al^{3+}, La^{3+}, and Pd^{2+} spectra, and since the new peaks (ca. 1618–1638 cm^{-1}) are consistent with a –COO–M asymmetric stretch [34], the data provide clear evidence that in these lyophilized samples, prepared from pH 4.0 solutions of river fulvic acid and the metals, these metals are complexed (i.e. the prominent 1720 cm^{-1} band in fulvic acid alone is consistent with the pH 4.0, –COOH, environment, and the clear shift to higher energy with fulvic acid plus added metals is consistent with metal ion coordination). While other (unknown) species may come into play in our lyophilized samples, it is clear that the metals (Al^{3+}, La^{3+}, and Pd^{2+}) are still bound. Note that the 1624 cm^{-1} band in the La^{3+} spectrum, albeit broad, is *considerably* sharper than the 1638 cm^{-1} band in the Al^{3+} spectrum. The broadness in the Al^{3+} spectrum may be due to the unique interaction of water molecules tightly bound to Al^{3+} associated with the fulvic acid material. The features present in the 2000–1000 cm^{-1} spectral region of the fulvic acid–La^{3+}complex are virtually identical to those observed previously in the fulvic acid–Tb^{3+} complex [30].

Fig. 12 presents FT-IR data on fulvic acid (in the absence and presence of Al^{3+}, La^{3+}, and Pd^{2+}) focusing in on the 4000–2000 cm^{-1} IR spectral region, abundant in H-bonded OH stretch and aromatic and aliphatic C–H stretch bands. The most prominent IR feature in all four spectra presented is the very broad band between ca. 3500 and 3000 cm^{-1}. This broad band arises predominantly from hydrogen-bond water –OH stretching modes. In the fulvic acid spectrum, the peak associated with this very broad feature appears at 3426 cm^{-1}, close to the 3400 cm^{-1} value usually associated with this hydrogen-bond –OH stretching mode. In the La^{3+} spectrum, the peak shifts to slightly higher energy (3386 cm^{-1}), while the Al^{3+} spectrum shows a shift to considerably higher energy (3043 cm^{-1}). By comparison, the Pd^{2+} spectrum shifts to slightly lower energy (3436 cm^{-1}). The vibrational frequency observed in the Al^{3+} spectrum is consistent with an aromatic –CH stretch, but the shape and, even more importantly, the intensity is inconsistent with this assignment [30]. Intensity considerations also rule out vibrational modes associated with nitrogen, as the low level of nitrogen in the river fulvic acid material (analyzed at $<0.75\%$) would be insufficient to produce such a large feature. The 3043 cm^{-1} feature is most likely attributable to –OH stretching vibrations associated with hydrated waters tightly associated with the fulvic acid-bound Al^{3+}. The most unusual IR band, which appears *only* in the Al^{3+} spectrum is the feature at 2407 cm^{-1}. (Note that this feature was not observed in IR spectra of fulvic acid complexes with other Class A metal ions Tb^{3+} and Ca^{2+} [30], and has not been observed with any of the Intermediate Class metals (Cd^{2+}, Cu^{2+}) or Class B metals (Pd^{2+}, Hg^{2+}) examined thus far in our laboratory (data not shown).) Two possible sources for the 2407 cm^{-1} feature were considered in our earlier study [30], based on Al^{3+} complexation to two prevalent fulvic acid functionalities: salicylic and phthalic acids. There is a broad band of low intensity, centered at about 2600 cm^{-1}, in the SRFA spectrum (Figure, top spectrum). This same feature is present in the IR spectrum of the compound salicylic acid [53]. This 2600 cm^{-1} band likely derives from the hydrogen-bonded six-membered ring formed between the phenolic hydrogen and the carbonyl oxygen of the carboxylic acid group. Note that the 2600 cm^{-1} is absent in the fulvic acid plus Al^{3+} IR spectrum, as the new band appears at higher energy (2407 cm^{-1}). When Al^{3+} is added to fulvic acid, a very different six-membered ring will likely form, in which the Al^{3+} will be coordinated to the phenolic oxygen and to the (deprotonated) oxygen atom of the carboxylic acid (see Fig. 14). The carbonyl oxygen atom of the carboxylic acid is not involved in the coordination of Al^{3+}. The phenolic OH moiety in this complex was considered a possible source of the 2407 cm^{-1} band, but the FT-IR spectrum obtained on salicylic acid plus Al^{3+} sample, prepared identically to the fulvic acid plus Al^{3+} sample, *did not* show this 2407 cm^{-1} feature [30]. The second possible source of the 2407 cm^{-1} band, Al^{3+} complexed to phthalic acid-like functionalities, was explored through the use of model compound phthalic acid. The 2407.33 cm^{-1} feature is *not* present in the IR spectrum of pure phthalic acid [53], in agreement with our IR measurements on the lyophilized pure compound [30]. Fig. 13 presents the complete IR spectrum obtained on the lyophilized complex of phthalic acid and Al^{3+}, prepared

Fig. 11. The 2000–1000 cm^{-1} region of the Fourier transform infrared spectra of SRFA and SRFA complexes with Al^{3+}, La^{3+}, and Pd^{2+}. For all spectra, the relative percent transmittance is plotted against wavenumber (reciprocal centimeters). In the preparation of the IR samples, metal ion was added to aqueous solutions of fulvic acid to obtain a *ratio* of fulvic acid to metal ion comparable to that used in the generation of the fluorescence EEM plots (5.0 mg fulvic acid and 2.0×10^{-2} M metal ion) in a total volume of 5.0 ml. The pH was adjusted to 4.0 with NaOH or HCl. Prior to spectroscopy, all samples were lyophilized from pH 4.0 in solution to produce a fine powder. The KBr matrix was created using 1 mg of lyophilized sample to 100 mg KBr. Fourier transform infrared spectra were taken on a Perkin–Elmer FT-IR Paragon 500 spectrophotometer and processed using the Perkin–Elmer Spectrum for Windows version 1.5 software package. The spectrometer was blanked with KBr prior to use and calibrated with a polystyrene film.

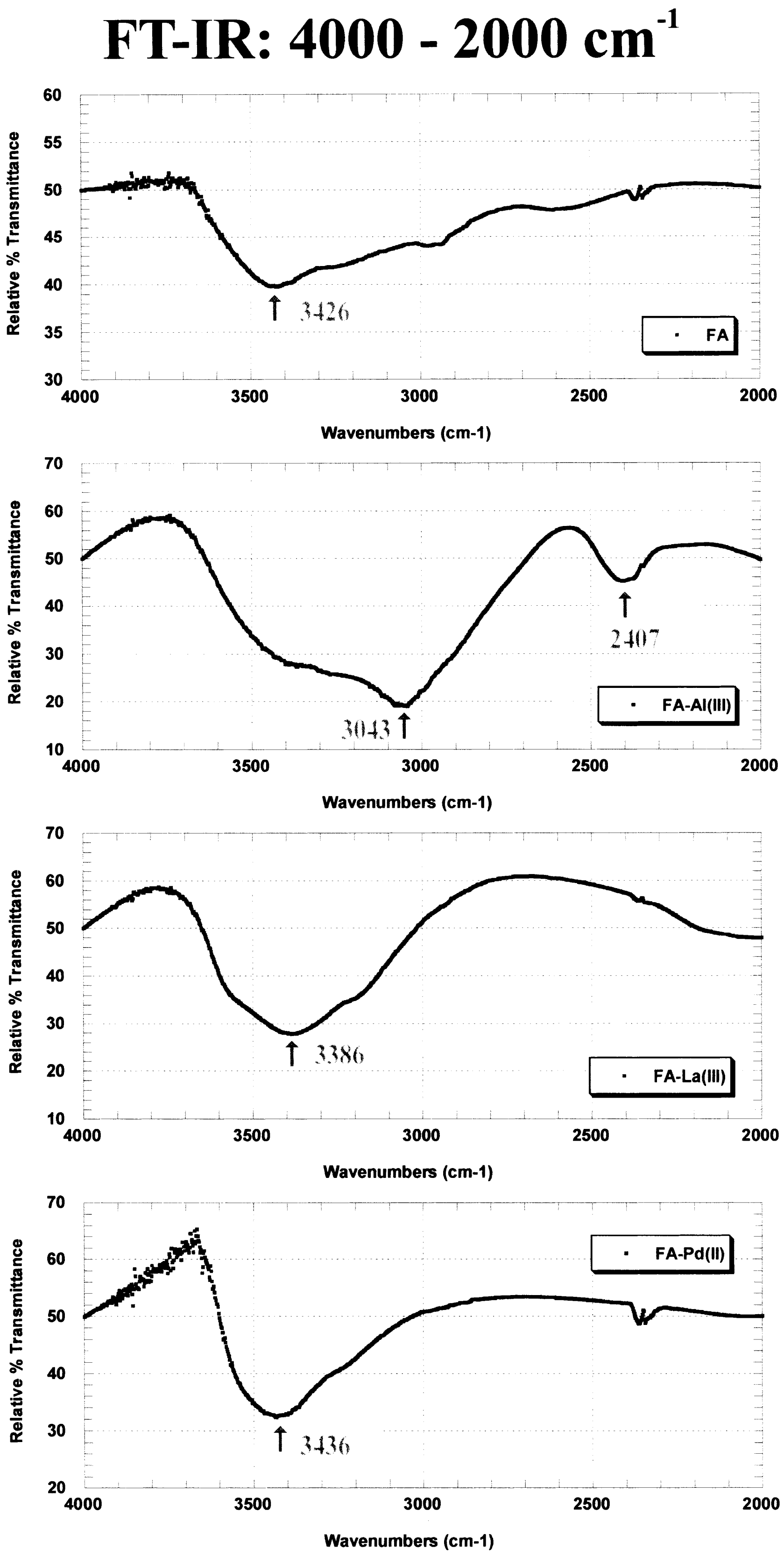


Fig. 12. The 4000–2000 cm^{-1} region of the Fourier transform infrared spectra of SRFA and SRFA complexes with Al^{3+}, La^{3+}, and Pd^{2+}. For all spectra, the relative percent transmittance is plotted against wavenumber (reciprocal centimeters). FT-IR samples were prepared as described in Fig. 11.

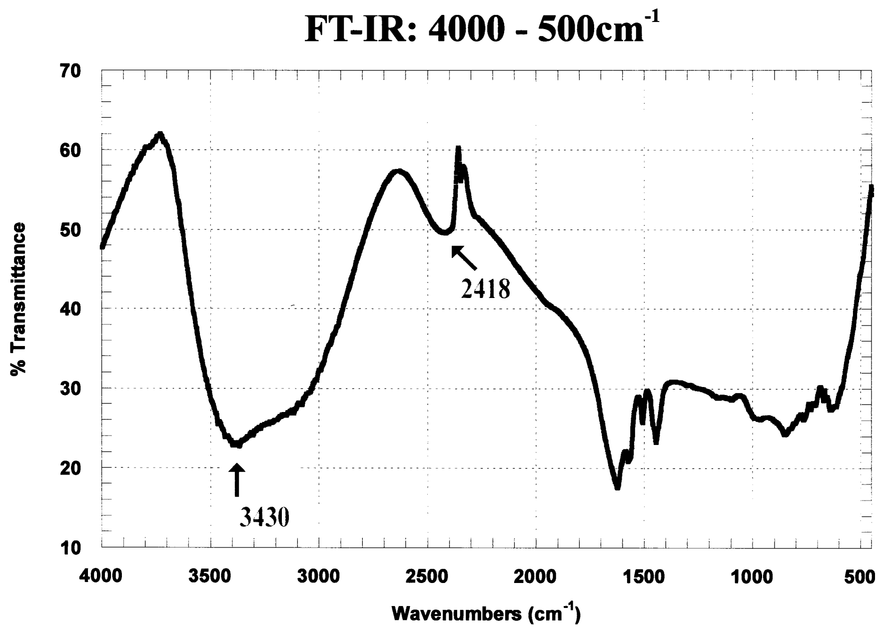


Fig. 13. The 4000–500 cm^{-1} region of the Fourier transform infrared spectra of the phthalic acid aluminum complex. The FT-IR sample was prepared as described in Fig. 11.

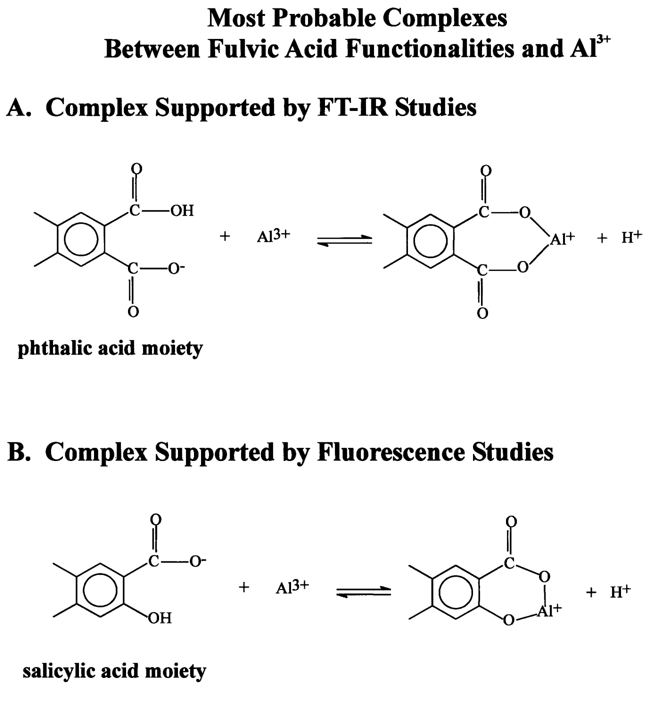


Fig. 14. Likely modes of complexation of Al^{3+} to salicylic and phthalic acids moieties in SRFA.

in a manner identical to that used for the fulvic acid–Al^{3+} sample. The spectrum clearly reveals a band (at ca. 2418 cm^{-1}) having a shape, intensity and position almost identical to that observed in the IR spectrum of SRFA complexed to Al^{3+} (Fig. 12, the fulvic acid–Al^{3+} spectrum), consistent with a preliminary attribution of this band to phthalic acid-like moieties [30]. Fig. 14 shows the most probable complexes between fulvic acid functionalities (phthalic acid-like, Fig. 14A, and salicylic acid-like, Fig. 14B) and Al^{3+}. In Fig. 14A, the phthalic acid moiety which forms a seven-membered ring complex upon binding Al^{3+} is diagrammed. This complex is clearly supported by the results of our FT-IR studies. The possibility of finding a seven-membered ring phthalic acid–metal ion structure has been documented in the work of Mercê et al. [54], who investigated binding of various metals ions, including Fe^{3+}, to models for nitrohumic acids by electrochemical (potentiometric titrations, cyclic voltammetry) and spectroscopic (UV–vis) methods. Although, more spectrocopic work needs to be done with model compound metal ion complexes, it appears (from our model studies) that IR spectroscopy is particularly sensitive/useful in detecting Al^{3+} complexation to aromatic di-carboxylate (phthalic acid-like) functionalities in humic substances, whereas fluorescence spectroscopy is considerably more sensitive to Al^{3+} complexation to salicylic acid-like moieties (i.e. the low fluorescence quantum yield for the phthalic acid moiety precludes convenient detection of this functionality in the presence of stronger fluorescing salicylic acid moieties).

4. Conclusion

In this review, we have discussed our previous and current work on the interaction of Al^{3+} (and other metal ions) with river fulvic acid, utilizing fluorescence and FT-IR spectroscopy. The discussion has been placed in the context of work done by other investigators on related systems, using a wide variety of experimental approaches, including the widely used fluorescence approaches, but also other approaches, such as ISE, ESI-ICR, NMR, CSV/ASV, and AFM. Much recent work has been directed at the determination of conditional (low pH) formation constants for Al^{3+} binding to humic materials. Many of these studies have exploited sensitive fluorescence approaches for this purpose (although other approaches, for example NMR, have also been successfully used). Our own determination of K'_f for Al^{3+} binding to SRFA at pH 4.0 (0.1 M ionic strength), which yielded a value of 4.7×10^4 M^{-1}, is in good general agreement with other K'_f values for other fulvic acid materials at similar pH values (as summarized in Table 1). The K'_f values for Al^{3+} binding to soluble river fulvic acids are relatively high, indicating that these stable, biological decay products are important in sequestering and maintaining Al^{3+} ion in the soluble (bio-available) state in natural waters for long periods of time.

In this review, we also report on the results of our recent fluorescence and IR spectroscopy survey of the interaction of *selected* metals from all three Nieboer and Richardson categories (Class A, Class B and Intermediate Class, see Fig. 4) with SRFA. The Class A metal ions included: Al^{3+}, Ca^{2+}, Mg^{2+}, La^{3+}, Tb^{3+}, and Yb^{3+}; the Intermediate Class metal ions included: Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, and Sn^{4+}; and the Class B metal ions examined included: Hg^{2+} and Pd^{2+}. Fig. 15 presents bar graphs on just those metal ions examined in the current study, comparing values for critical Nieboer and Richardson electronic properties: Pauling ionic radii, ionic index (z^2/r), and covalent index ($(\chi_m)^2 r$). In our survey, the point of reference is Al^{3+}. How does Al^{3+} ion differ (in its spectroscopic response) from all of the other metals in its interaction with fulvic acid material? Most of the experiments were performed at low pH (pH 4.0) and at environmental levels of fulvic acid (10–15 mg l^{-1}) and low concentrations of metal ion (300 μM), conditions under which the 'free' (aquo) ions predominate and the formation of insoluble metal hydroxides and insoluble metal ion–fulvic acid complexes are minimized. The results obtained from our survey have aided our understanding of some of the unique spectral behaviors (*under our experimental conditions*) of the Al^{3+}–fulvic acid complex vis-a-vis fulvic acid complexes with many other metal ions. One of the unique fluorescence spectral features associated with Al^{3+} binding to river fulvic acid related to the observed red shift in $\lambda_{max,\ excitation}$ coupled to the blue shift in $\lambda_{max,\ emission}$, not observed for any of the other metal ions examined, from any class. Results of fluorescence experiments with the model compounds salicylic and phthalic acids, strongly point to salicylic acid moiety complexation with Al^{3+} as the source of the $\lambda_{max,\ excitation}/\lambda_{max,\ emission}$ shifts observed in the river fulvic acid–Al^{3+} system. One of the unique FT-IR spectral features associated with Al^{3+} binding to river fulvic acid related to the 2407 cm^{-1} band, not observed for any of the other metal ions examined, from any class. Results of FT-IR experiments with the model compounds salicylic and phthalic acids, strongly point to phthalic acid moiety complexation with Al^{3+} as the source of the 2407 cm^{-1} band observed in the river fulvic acid–Al^{3+} system. Examination of the bar graphs in Fig. 15 is informative. There is no obvious trend displayed in graph A (Pauling ionic radius); however,

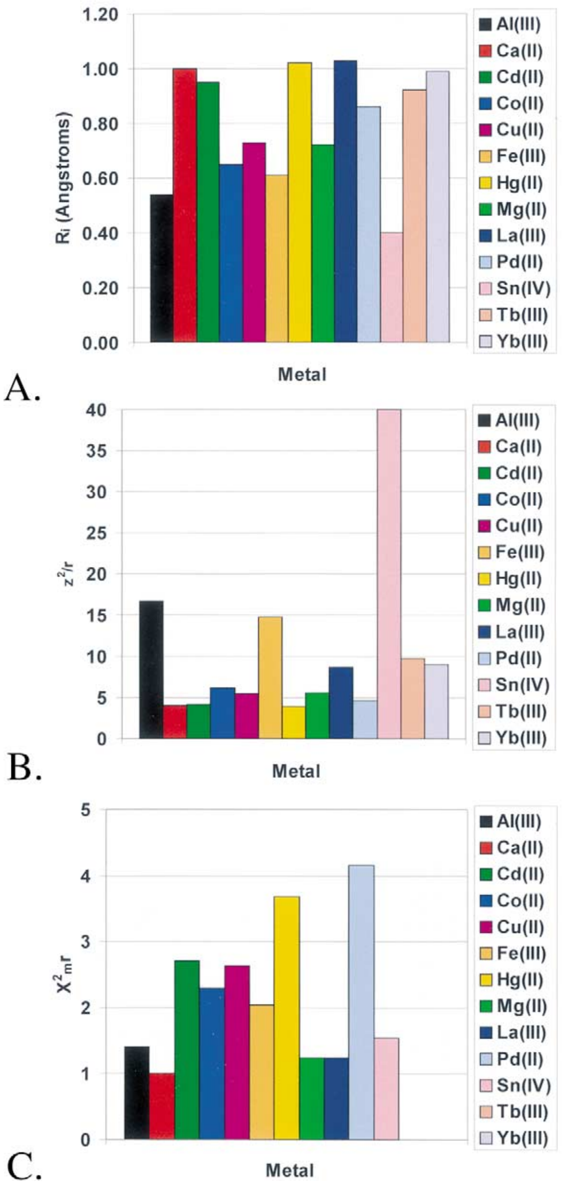


Fig. 15. Bar graphs comparing electronic properties of metal ions examined in this study. (A) Pauling ionic radii (r, in Å). (B) Nieboer and Richardson [45] ionic index (z^2/r), where z, formal charge. (C) Nieboer and Richardson [45] covalent index ($(\chi_m)^2 r$, where χ_m, Pauling electronegativity. (Six-coordinate ionic radii and Pauling electronegativity values were obtained from CRC Handbook of Chemistry and Physics, 79th ed., D.R. Lide (Ed.), Boca Raton, CRC Press LLC, 1998.)

graph B (which tracks the Nieboer and Richardson ionic index) reveals that three metals depart from the rest (Al^{3+}, Fe^{3+}, and Sn^{4+}). Sn^{4+} has an unusually high ionic index because of the 4+ formal charge. Al^{3+} and Fe^{3+} have similar ionic indices, consistent with fact that these two metal ions share similar chemical properties (although our fluorescence work indicates clear differences in the ability of these two metal ions to perturb fulvic acid fluorescence). Bar graph C (which tracks the Nieboer and Richardson covalent index) indicates that of the metal ions examined, Al^{3+} has one of the lowest values for the covalent index. In conclusion, it should be emphasized that our work, as well as the work of others, has not been exhaustive. Many metals from the various Nieboer and Richardson categories have not yet been thoroughly examined. The 'unique' spectral properties discussed above may prove not to be unique at all. Assuming, however, that the 'uniqueness' tag for Al^{3+} complexation with fulvic acid material is valid, the question remains: why is Al^{3+} unique among the metals in its interaction with certain ligands? From the current work, it seems clear that major sources of the deviation in spectral properties between Al^{3+} and many other metal ions (across all three Nieboer and Richardson categories) is the unusually high value of its charge density *and* relatively low propensity for involvement in covalent bonding interactions (i.e. a very high ionic index *combined* with a relatively low covalent index in the Nieboer and Richardson classification of environmental metals), as well as affinity for certain functional groups.

Acknowledgements

The authors wish to thank Dr Mark M. Turnbull (Clark University) for many helpful discussions related to the FT-IR phase of research. This work was supported by instrument grants to DJN from the National Science Foundation and the W.M. Keck Foundation. KME is a recipient of a Clare Booth Luce Fellowship and is the winner of the 2000 Glorine Tuohey National Scholarship from the Steven Bufton Memorial Education Fund of the American Business Women's Association.

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