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Study of Interactions between Relevant Organic Acids and Aluminium in Model Solutions Using HPLC and IC

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Abstract

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The interactions of different organic acids such as citric, malic, oxalic, and fulvic with aluminium were studied using ion-exchange chromatography (IC) and high performance liquid chromatography (HPLC). The experiments were carried out at low pH (1.5–3.1). The results of IC experiments on the interaction between Al and oxalate, citrate, and malate were compared with model chemical equilibrium calculations. The strongest effect on Al speciation was observed with oxalic acid and fulvic acid. These two ligands formed more than one type of complex with Al. In contrast, there was no significant effect of malic acid on Al speciation and a rather weak effect caused by citric acid. The studies show that the formed complexes are stable even at low pH.

Keywords: aluminium speciation; citric acid; fulvic acid; malic acid; oxalic acid

Aluminium (Al) occurs ubiquitously in the terrestrial environment. It is the most abundant metal in the lithosphere, comprising about 8% of the Earth's crust (Hendershot *et al.* 1996). Soil acidification may substantially mobilize and release Al into soil solution, ground and surface waters. This phenomenon is emphasized particularly in poorly buffered soils (Ščančar & Milačič 2006). The released mononuclear ionic Al species may undergo polymerization or may be complexed by available organic or inorganic ligands (Poléo 1995; Bi *et al*. 2004). In the soil solution of an unacidified forest soil, about 80% of Al in the upper horizons is organically bound (Dahlgren & Ugolini 1989; Lundström & Giesler 1995). Soil solution contains a wide variety of organic substances including organic acids with the ability of forming complexes with Al. Among these acids, high molecular mass (HMM) acids are fulvic acids (FA). However, low molecular mass (LMM) organic acids like oxalic and citric acids are also present (Tipping 2005).

Al speciation controls its mobility in the environment, bioavailability, and toxicity (e.g. Ma & Hiradate 2000). Al is recognized as a potentially toxic element for all living organisms including human beings (Exley & Korchazhkina 2001). In the case of toxicity towards plants it has been shown, by laboratory bioassay, that Al-organic binding can reduce or diminish Al toxicity (Alvarez *et al.* 1992). Moreover, organic acids play a key role in Al tolerance mechanisms. Some plants detoxify Al in the rhizosphere by releasing organic acids that chelate Al (Jones *et al.* 2001). Hue *et al.* (1986) found that organic acids with hydroxyl and carboxyl groups are able to form stable ring structures with Al^{3+} that consist of 5 or 6 bonds conferring the greatest protection from Al toxicity. The secretion of organic acids is highly specific to Al and the site of secretion is localized to the root apex (Ma & Hiradate 2000). The kind of organic acids secreted as well as secretion pattern differ among plant species. Also internal detoxification of Al in Al-accumulating plants is achieved

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by the formation of Al-organic acid complexes. For instance, the complex of Al-citrate (1:1) in hydrangea and Al-oxalate (1:3) in buckwheat has been identified (Shen *et al.* 2004).

Organic acid selection. LMM organic acids exist widely in soils and have been implicated in many soil processes such as mineral weathering, acquisition of plant nutrients (i.e. Fe, P), and Al detoxification (Li *et al.* 2000). They also act as the means of transport in the soil profile and contribute to the translocation of Al (Buurman & Van Reeuwijk 1984; Lundström & Giesler 1995). A number of organic acids have been found in soils, including oxalic, citric, formic, acetic, malic, succinic, malonic, tartaric, lactic, and fumaric acid (McKeague *et al.* 1971; van Hees *et al.* 1999). As an example the work done by Fox *et al.* (1990) can be mentioned; they found oxalic, formic, and citric acid to be present in forest floor and mineral soil extracts from slash pine, longleaf pine, and oak forest. The concentration of LMM organic acids in soil solution ranges from less than micromolar to more than millimolar, with seasonally elevated acid concentrations being attributed to microbial processes (Cromack *et al.* 1979; Roberts & Berthlein 1986) and root exudation (Stevenson 1994).

From the group of the LMM organic acids, major importance is ascribed to oxalic, citric, malonic, malic, tartaric, and lactic acids (Stevenson 1994). Using size exclusion chromatography with UV, inductively coupled plasma atomic emission spectrometry, and electro-thermal atomic absorption spectrometry detection, Mitrovič and Milačič (2000) found that 40–55% of total water soluble Al in soil extracts exist in the form of low molecular mass complexes (Al-citrate, Al-oxalate, and AlF^{2+}).

Based on the above stated facts, the three acids (oxalic, citric, and malic) were selected as major representatives of the entire soil LMM organic acids group. They were found to be present in significant quantities; moreover, they are major plant exudates. Their exudation is meant to shield the plants from toxic polyvalent cations and they have been known to have significant influence on Al chemistry (Strobel 2001).

In addition, fulvic acid was selected for ion-exchange chromatography (IC) experiments as this acid is also an important natural soil constituent. A possible similarity in its behaviour with LMM organic acids was explored. As it is known, fulvic acids provide multiple and natural chemical reactions in the soil, instigating positive influence on metabolic processes of plants (Thurman & Malcolm 1981). Fulvic acid is especially active in dissolving minerals and metals when in solution with water. The metallic minerals simply dissolve into ionic form, and disappear into the fulvic structure becoming bio-chemically reactive and mobile (Hayes & Swift 1978).

The objectives of the present work are: (1) to select the low molecule mass organic acids of highest interest from the acidic soil chemistry point of view, (2) to check the possibility of determination of organic acids in the presence of excess free Al by means of high performance liquid chromatography (HPLC), and (3) to study the influence of the selected organic acids on Al speciation in low pH environment.

MATERIAL AND METHODS

The first step was the organic acid selection based on literature survey and also selection of the analytical technique suitable and commonly available for their determination.

The experiments were carried out at low pH (1.5–3.1) with the same concentration of Al for HPLC and IC experiments (7.41×10^{-5}M, 3.70×10^{-4}M, and 7.41×10^{-4}M). The same concentrations also of various organic acids were used for both HPLC and IC studies (0.1×10^{-3}M, 1×10^{-3}M, and 100×10^{-3}M). Aluminium solutions of different concentrations were prepared from 99.999% Al stock solution in 5% HCl acid (Analytika, Prague, Czech Republic). Different concentrations of oxalic, malic, and citric acids where prepared from analytical grade pure acids (Fluka, Seelze, Germany). Fulvic acid was extracted from the O horizon of Spodosol soil (Jizerské hory Mts., locality Smědava) using the method based on the solubility of humic substances in alkali solutions and further acidification and purification of the extract (Piccolo *et al.* 2002). Two concentrations of fulvic acid, 20 and 200 mg/l, were used for the IC experiment.

The pH of created solutions was left at a "natural" value and was determined potentiometrically (UB-5; Denver Instruments, Göttingen, Germany). All the experiments were run in triplicates; no significant variations between replicates were observed.

All solutions were equilibrated for 12 h prior to the analysis. The changes of achieved speciation were also checked after 24 and 48 h; however, these changes were not significant.

All experiments were compared to two types of blank solutions, that means solutions of Al with no organic acids and solutions of organic acids with no Al addition.

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The techniques HPLC and IC have been the commonly used instrumentations for determination of LMM carboxylic acids (Ström *et al.* 1994; Krzyszowska *et al.* 1996; Tani & Higashi 1999; van Hees *et al.* 1999). An investigation was done in order to find out if there is a possibility to detect and determine the malic, oxalic, and citric acids in the presence of Al; HPLC technique with UV detection was used. The HPLC SUMMIT instrumentation (automated sample injector ASI-100; thermostatted column compartment TCC-100; dual gradient pump P680 and photodiode array detector PDA-100) were manufactured by Dionex (Germering, Germany) and operated by Chromeleon software (Dionex). The HPLC column used was Luna 3μ C18 (2) 100A by Phenomenex (Torrance, USA). The pre-column was a HPLC Guard cartridge C18 (Phenomenex). The mobile phase for HPLC was 0.05M KH_2PO_4 adjusted to pH 2.75 using 85% phosphoric acid. The detection was carried out at the wavelength of 210 nm.

The IC instrumentation was used to study the influence of selected organic acids on Al speciation. The instrument consisted of a GP50 gradient pump and PC pneumatic controller for post column derivatization (Dionex), a UV/VIS detector Deltachrom UVD200 (Watrex, Prague, Czech Republic), and an automated sampler Triathlon (Sparks Ltd., Emmen, the Netherlands). The IC column used was an Alltech cation R (Alltech, Deerfield, USA), with the use of an old column as a guard column. The mobile phase used in IC was 100mM Na_2SO_4 (pH 2.40) in 7.5mM H_2SO_4. For post-column derivatization, an agent 3×10^{-4}M tiron in 1M ammonium acetate (pH 7.05) was used. Detection of created Al-tiron complexes was done at 310 nm. This IC method (Drábek *et al.* 2003) enables separation of Al forms into three different groups, mainly according to their charge: $Al(X)^{\leq 1+}$ ($Al(OH_2^+)$,$Al(SO_4)^+$, AlF_2^+, $Al(org.)^{\leq 1+}$, etc.); $Al(Y)^{2+}$ ($(Al(OH)^{2+}$, $(AlF)^{2+}$, etc.) and Al^{3+} (Al^{3+} and transformed hydroxyl Al polymers).

The PHREEQC-2 speciation-solubility code Version 2.15 for MS Windows and the Visual MINTEQ Version 2.61 were used to determine the chemical speciation of Al in the experimental solutions (Parkhurst & Appelo 1999). The databases were supplemented with stability constants according to the International Union of Pure and Applied Chemistry (IUPAC) stability constant database, Version 5.83.

RESULTS AND DISCUSSION

Experiments with reversed phase HPLC

The mixture of acids in the case of zero Al addition was well separated and acids were eluted in this order: oxalic acid, malic acid, and citric acid. However, after Al addition, strong effect of Al complexation was observed. The signals of free acids significantly decreased and there was a big peak cluster co-eluting with free oxalic acid. The peak cluster had an irregular shape and presumably consists of a mixture of oxalate, malate, and citrate complexes with Al. That is why different chromatography column (Acclaim OA by Dionex) with different mobile phase (100mM Na_2SO_4, pH 2.65) was also tested. Nevertheless, the separation did not improve and the same peak cluster co-eluting with free oxalic acid was observed. Thus, we can conclude that the determination of free oxalic acid under such conditions was not possible due to the presence of stable Al complexes co-eluting with it. Similar problems were reported by Hue *et al.* (1986), Fox *et al.* (1990), and Pohlman and McColl (1988). They used ion exclusion chromatography columns in combination with UV detection. Although this type of column offered good selectivity, the determination was interfered by unretained substances which cause a large void peak with tailing. For these reasons interactions of each acid with Al were studied separately. These separated studies confirmed possibility of determination of free citric and malic acids in the presence of Al by means of HPLC. However, problems with co-elution of Al-oxalate complexes with free oxalic acids were not resolved. Also Al-citrate and malate complexes were proved to elute in retention time closely similar to the one for free oxalic acid. Our observations are in agreement with the work done by Ma and Hiradate (2000).

According to Lundström and Öhman (1990), Al and oxalic acid form complexes in the ratio between 1:3 and 1:2. This explains why even at lower concentration of Al and relatively high concentrations of oxalic acid, the peak for oxalic acid is greatly diminished. The free oxalic peak sometimes elutes only like a tail of Al-oxalate complexes peak. This fact is not mentioned by chromatography column producers in their application manuals and might lead to serious misinterpretations of chromatography data.

Experiments with ion-exchange chromatography

Oxalic acid. Oxalic acid generally diminishes the appearance of the Al^{3+} species peak. Even with the lowest concentration of oxalic acid, the Al^{3+} signal was diminished as compared with the signal from Al without oxalic acid. In the IC analyses, at least two types of complexes appear to be formed. One which is relatively weakly bound and is able to react

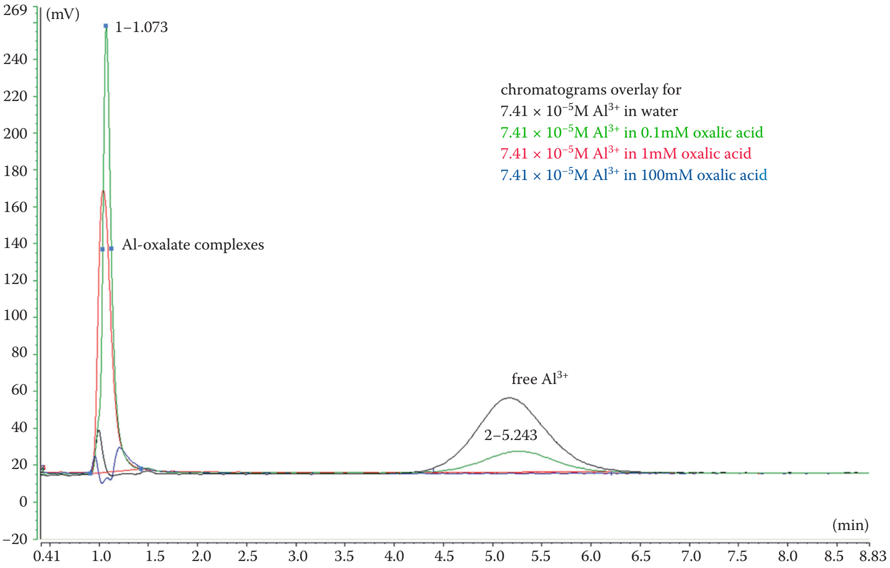

Figure 1. Overlay of chromatograms (IC) of Al^{3+} with oxalic acid addition; blank solution of 7.41×10^{-5}M Al in water creates only one peak assigned as Al^{3+}; in the solution of 7.41×10^{-5}M Al in 0.1×10^{-3}M oxalic acid smaller peak of Al^{3+} and new peak of $Al(X)^{1+}$ belonging to Al oxalate complexes are observed; as the concentration of oxalic acid increases to 1×10^{-3}M, Al^{3+} peak completely disappears and the peak of $Al(X)^{1+}$ is significantly diminished; in the case of 7.41×10^{-5}M Al in 100×10^{-3}M oxalic acid solution almost all signals of Al disappear; only small and shifted peak of $Al(X)^{1+}$ can be noted

with the tiron, and another which is strong and not able to react with the tiron (see Figure 1). The peaks representing the formation of Al-oxalic acid complexes are evident and appear to be stable, despite severe pH conditions in the column. It has to be noted that Al^{3+} disappears with the highest concentration of oxalic acid. Thus, the majority of Al is strongly bound and that leads to the so called "masking" of Al. This means that we observed only a small and slightly shifted peak of $Al(X)^{1+}$ in the chromatograms. The same behaviour is shown in experiments with the other higher concentrations of oxalic acid. These findings are in agreement with statements of Lundström and Öhman (1990) and Nordstrom and May (1996), who suggested that oxalic acid can form complexes with Al in the ratio 1:1, 1:2, and 1:3 depending on the concentration of Al. However, the three ratios will have different stability constants.

Citric acid. It can be generally stated that we did not observe presence of any $Al(Y)^{2+}$ or $Al(X)^{+}$ forms in the chromatograms. However, presence of citric acid in solution caused diminishing of signal of Al^{3+} (Figure 2). These facts lead to the suggestions that some stable Al-citrate complexes are created and as they do not react with the tiron, they become IC-undetectable. The peak of Al^{3+} also became distorted and broadened by a not fully separated left shoulder. The broadening of the peak forming the shoulder suggests that there are some other Al-citrate species that either break up during the chromatography process or they have similar elution characteristics with Al^{3+}.

From the results of the mathematical modelling discussed later, the peaks (the missing $Al(X)^{+}$ and $Al(Y)^{2+}$ forms) could represent $AlH(citrate)^{+}$ and/or $Al(citrate)^{0}$ complexes with the former being the more predominant. Because it is known that the Al-citrate complex is very stable, it therefore means that it probably does not react with the tiron and that is the reason why we do not see clear complex formation. Nevertheless, it is also possible that these predicted $AlH(citrate)^{+}$ or $Al(citrate)^{0}$ complexes cause the observed changes in Al^{3+} peak symmetry. The higher the concentration of citric acid, the higher the effectivity in diminishing Al^{3+} signals. In contrast, with the increasing concentration of Al the described behaviour was less pronounced.

It has been stated in literature that citric acid is far more effective in complexing Al compared to oxalic acid (Lundström & Öhman 1990). However, our experiments show opposite. Moreover, the experimental results of our work can be confirmed by the literature constants for oxalate (Grasso *et al.* 1980; Jackson & Cosgrove 1982). Preliminary calculations, using equilibration simulation on the system oxalate-citrate-Al^{3+} (data not shown), lead to observing an absolute dominance of oxalate

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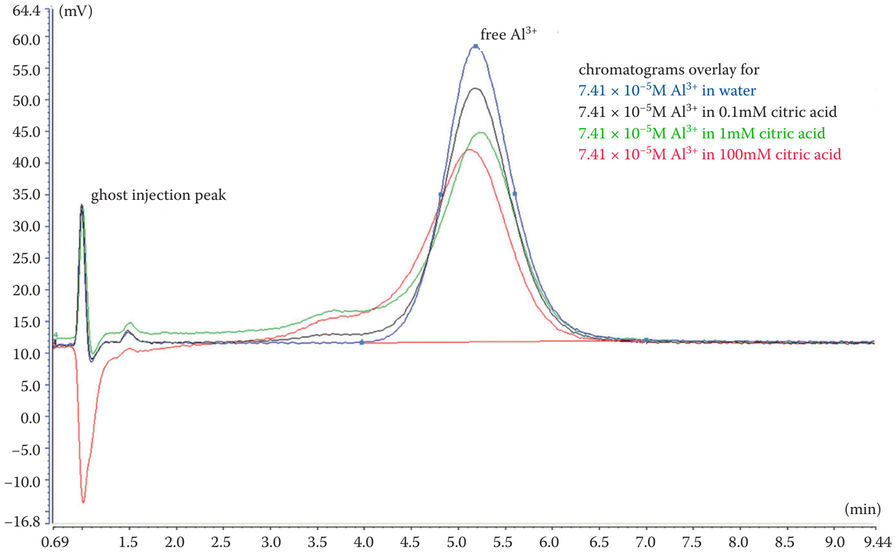

Figure 2. Overlay of chromatograms (IC) of Al^{3+} with citric acid addition; blank solution of 7.41×10^{-5}M Al in water creates only one peak assigned as Al^{3+}; in the solution of 7.41×10^{-5}M Al in 0.1×10^{-3}M citric acid smaller peak of Al^{3+} and no peak of $Al(X)^{1+}$ is observed; as the concentration of citric acid increases to 1×10^{-3}M, Al^{3+} peak is further depressed and distorted by apparent left shoulder; in the case of 7.41×10^{-5}M Al in 100×10^{-3}M citric acid solution the peak of Al^{3+} is even more depressed and distorted by apparent left shoulder; no peak of $Al(X)^{1+}$ is observed in any experiments

complexes. Therefore the effectivity of citric acid is more applicable in the soil environment where other pedological factors like pH heterogeneity and other anions play part (Lundström & Öhman 1990).

Malic acid. The HPLC experiment clearly demonstrated that complexes of malic acid and Al do exist even in extremely low pH conditions. Unfortunately, the existence of these complexes was not confirmed by IC analysis. In detail, insignificant reduction of Al^{3+} signal was observed, compared with Al^{3+} without malic acid. It shows that there was indeed a reaction which took some Al^{3+} from the solution, creating strong complexes that do not react with tiron.

A majority of Al-malate complexes seems not to be able to resist the conditions in the column. There is a very low pH of 2.40 of the mobile phase. Also presence of SO_4^{2-} in the mobile phase might break up the Al-malic acid complexes through competition for Al. It is also possible that the Al-malate complexes might have the same retention characteristics as free Al because the Al^{3+} peak is quite broad and the peak for the Al-malate could be hidden within the peak of free Al^{3+}. This means that we do not detect any Al-malate complexes by means of IC.

Fulvic acid. The whole experiment result is clearly described by the Figure 3. Addition of fulvic acid (20 mg/l) to the Al^{3+} solution leads to a slight Al^{3+} signal reduction and creation of a small peak of $Al(X)^{1+}$. At the higher concentration of fulvic acid (200 mg/l), the Al^{3+} peak is significantly reduced and $Al(X)^{1+}$ and $Al(Y)^{2+}$ peaks are observed with the $Al(Y)^{2+}$ appearing as a tail on the $Al(X)^{1+}$ peak. With the increasing concentration of Al in the solution, the effect of fulvic acid on final speciation is diminished.

Significance of fulvic acid, in regards to Al binding, was confirmed also by Mitrović and Milačič (2000) who found in soil extracts 30–40% of total water-soluble Al bound to humic substances.

Study using equilibrium model simulation

For the purpose of this study, analyses were done on the oxalic, citric, and malic acids and Al reactions previously analyzed by IC. The speciation analysis for fulvic acid was not done because of lack of the reliable stability constants for their complexes.

Tables 1–3 show the predominant calculated species related to the experimental solution composition and its pH. There is an evident effect of pH and composition of solution on speciation of the final solution. It is hard to describe the influence of each factor separately because they seem to influence the speciation simultaneously. There is also a likelihood of interferences by Cl^- anions which were present in the Al solution and were not taken into account in the chemical equilibrium model.

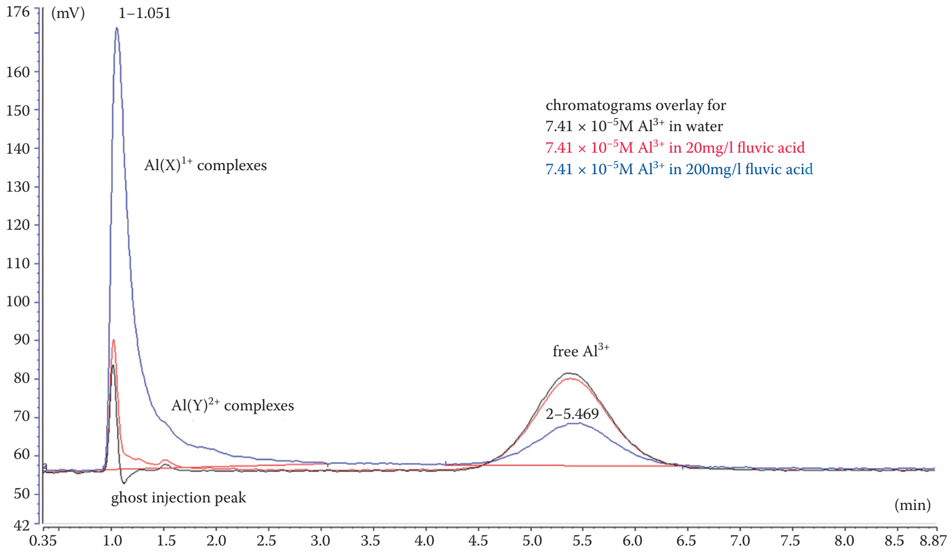

Figure 3. Overlay of chromatograms (IC) of Al^{3+} with fulvic acid addition; blank solution of 7.41×10^{-5}M Al in water creates only one peak assigned as Al^{3+}; in the solution of 7.41×10^{-5}M Al in 20 mg/l fulvic acid slightly smaller peak of Al^{3+} and sign of new $Al(X)^{1+}$, rising over ghost injection peak, are observed; as the concentration of fulvic acid increases to 200 mg/l, Al^{3+} peak is significantly decreased; in the same time large peak of $Al(X)^{1+}$ with a small sign of $Al(Y)^{2+}$ peak can be observed. The $Al(X)^{1+}$ and $Al(Y)^{2+}$ peaks belong to Al fulvic acid complexes

Aluminium and oxalic acid. The results of equilibrium model simulations (Table 1) are in full agreement with the results of IC experiments (see Figure 1). Generally, oxalic acid significantly diminishes the amount of free Al^{3+}. It corresponds to the decreasing the Al^{3+} peak area. When the $Al(oxalate)^{+}$ species is predicted by equilibrium simulations, the $Al(X)^{1+}$ form is observed by IC. When $Al(oxalate)_2^{-}$ species is calculated, the peak of $Al(X)^{1+}$ is decreasing and Al^{3+} species is neither detected nor calculated. The dominance of calculated $Al(oxalate)_3^{3-}$ corresponds to the so called Al "masking" discussed earlier.

Aluminium and citric acid. With increasing amount of citric acid in solution, $AlH(citrate)^{+}$ becomes more abundant. At the same time, pH rises and drops again with the exception of the lowest concentration of Al. No matter what the predominant species by calculation was, IC clearly identified free Al (Al^{3+}) as the predominant form in all solutions. Therefore, these facts lead to the conclusion that the observed Al speciation with IC is not in full agreement with the predicted speciation done by mathematical modelling. Even though, a similar trend was seen; e.g., increasing Al-citrate complexes lead to the decrease in Al^{3+} peak in the chromatograms.

Table 1. Dominant Al species in the presence of oxalate (results of PHREEQC/ MINTEQ model)

Experiment No.	Composition	pH	Dominant predicted species (% of total Al)
1	7.41×10^{-5}M Al + 0.1×10^{-3}M oxalic acid	1.90	$Al(oxalate)^{+}$ (73.1%), Al^{3+} (21.8%)
2	7.41×10^{-5}M Al + 1×10^{-3}M oxalic acid	2.53	$Al(oxalate)_2^{-}$ (80.0%), $Al(oxalate)^{+}$ (12.0%)
3	7.41×10^{-5}M Al + 100×10^{-3}M oxalic acid	2.63	$Al(oxalate)_3^{3-}$ (97.7%)
4	3.7×10^{-4}M Al + 0.1×10^{-3}M oxalic acid	1.35	Al^{3+} (80.0%), $Al(oxalate)^{+}$ (12.0%)
5	3.7×10^{-4}M Al + 1×10^{-3}M oxalic acid	1.40	$Al(oxalate)^{+}$ (72.2%), $Al(oxalate)_2^{-}$ (15.4%)
6	3.7×10^{-4}M Al + 100×10^{-3}M oxalic acid	1.38	$Al(oxalate)_3^{3-}$ (61.6%), $Al(oxalate)_2^{-}$(37.2%)
7	7.41×10^{-4}M Al + 0.1×10^{-3}M oxalic acid	2.16	Al^{3+} (86.6%), $Al(oxalate)^{+}$ (13.2%)
8	7.41×10^{-4}M Al + 1×10^{-3}M oxalic acid	2.65	$Al(oxalate)^{+}$ (67.1%), $Al(oxalate)_2^{-}$ (30.5%)
9	7.41×10^{-4}M Al + 100×10^{-3}M oxalic acid	2.25	$Al(oxalate)_3^{3-}$ (94.4%)

log K $Al(oxalate)_3^{3-}$ = 17.09; log K $Al(oxalate)_2^{-}$ = 13.41; log K $Al(oxalate)^{+}$ = 7.73; log K $AlH(oxalate)^{2+}$ = 6.84; IUPAC stability constant database, Version 5.83

Table 2. Dominant Al species in the presence of citrate (results of PHREEQC/MINTEQ model)

Experiment No.	Composition	pH	Dominant predicted species (% of total Al)
10	7.41×10^{-5}M Al + 0.1×10^{-3}M citric acid	1.84	Al^{3+} (99.1%)
11	7.41×10^{-5}M Al + 1×10^{-3}M citric acid	2.11	Al^{3+} (73.8%), AlH(citrate)$^{+}$ (22.5%)
12	7.41×10^{-5}M Al + 100×10^{-3}M citric acid	2.40	AlH(citrate)$^{+}$ (76.0%), Al(citrate)^{0} (23.1%)
13	3.7×10^{-4}M Al + 0.1×10^{-3}M citric acid	1.72	Al^{3+} (99.6%)
14	3.7×10^{-4}M Al + 1×10^{-3}M citric acid	2.05	Al^{3+} (81.3%)
15	3.7×10^{-4}M Al + 100×10^{-3}M citric acid	1.87	AlH(citrate)$^{+}$ (82.4%)
16	7.41×10^{-4}M Al + 0.1×10^{-3}M citric acid	2.11	Al^{3+} (97.5%)
17	7.41×10^{-4}M Al + 1×10^{-3}M citric acid	2.62	AlH(citrate)$^{+}$ (45.4%), Al^{3+} (30.4%), Al(citrate)^{0} (24.1%)
18	7.41×10^{-4}M Al + 100×10^{-3}M citric acid	2.13	AlH(citrate)$^{+}$ (83.2%), Al(citrate)^{0} (13.7%)

log K Al(citrate)^{0} = 9.98; log K AlH(citrate)$^{+}$ = 12.85; log K Al(citrate)$_2^{3-}$ = 14.83; IUPAC stability constant database, Version 5.83

Table 3. Dominant Al species in the presence of malate (results of PHREEQC/MINTEQ model)

Experiment No.	Composition	pH	Dominant predicted species (% of total Al)
19	7.41×10^{-5}M Al + 0.1×10^{-3}M malic acid	2.11	Al(malate)$^{+}$ (53.4%), Al^{3+} (46.6%)
20	7.41×10^{-5}M Al + 1×10^{-3}M malic acid	2.40	Al(malate)$^{+}$ (94.8%), Al^{3+} (5.2%)
21	7.41×10^{-5}M Al + 100×10^{-3}M malic acid	2.93	Al(malate)$^{+}$ (99.4%)
22	3.7×10^{-4}M Al + 0.1×10^{-3}M malic acid	2.08	Al^{3+} (77.5%), Al(malate)$^{+}$ (22.4%)
23	3.7×10^{-4}M Al + 1×10^{-3}M malic acid	2.33	Al(malate)$^{+}$ (92.8%), Al^{3+} (7.2%)
24	3.7×10^{-4}M Al + 100×10^{-3}M malic acid	2.18	Al(malate)$^{+}$ (99.4%)
25	7.41×10^{-4}M Al + 0.1×10^{-3}M malic acid	2.41	Al^{3+} (87.4%), Al(malate)$^{+}$ (12.4%)
26	7.41×10^{-4}M Al + 1×10^{-3}M malic acid	3.04	Al(malate)$^{+}$ (89.4%), Al^{3+} (10.5%)
27	7.41×10^{-4}M Al + 100×10^{-3}M malic acid	2.42	Al(malate)$^{+}$ (99.4%)

log K Al(malate)$^{+}$ = 4.65; IUPAC stability constant database, Version 5.83

Aluminium and malic acid. From the equilibrium simulations it is obvious that Al(malate)$^{+}$ is the predominant species in the solution, with the exception of experiments No. 22 and 26 (two highest Al and lowest malic acid concentrations). However, this species was not detected by IC; moreover we have not detected significant reduction of Al^{3+} peak. It seems that Al(malate)$^{+}$ complex is created easily but it is not detected by IC. This fact leads to slight overestimation of presence of Al^{3+} in real soil extracts or water samples if malic acid is present and conditions are favourable for the creation of Al(malate)$^{+}$.

General remark

From above stated facts it is obvious that the use of some hyphenated techniques combining different chromatography and atomic spectroscopy methods (Borrmann & Seubert 1999; Drábek *et al.* 2005; Ščančar & Milačič 2006) is more adequate for Al speciation in the environment rich in organic ligands. Nevertheless, the use of the Al speciation method proposed by Drábek *et al.* (2003) is still useful for environment studies and routine soil analysis as it was proved by our previous works performed on acidified Spodsols and Cambisols originating from Czech mountains – the Jizerské, Krkonoše, and Novodhradské hory Mts. (Drábek *et al.* 2005, 2007; Dlouhá *et al.* 2009; Tejnecký *et al.* 2010).

CONCLUSION

The HPLC determination of free oxalic acid under studied conditions was not possible due to the presence of stable Al complexes co-eluting with it. In the IC experiments, strong and well pronounced effect of oxalic and fulvic acids on the speciation of Al was observed, where more than one type of complexes with Al was created. There was no significant effect by malic acid on Al speciation observed by IC; however, we have proved the existence of such a complex by the HPLC experiments. The observed effect caused by citric acid on Al speciation was rather weak. The studies show that the complexes formed are stable even at a very low pH. Therefore, organic acids can form different types of Al complexes with different stabilities and chromatographic characteristics. Further research of these processes is necessary for soil Al chemistry as well as for Al toxicity assessment.

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