



New operational method of testing colloid complexation with metals in natural waters

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ABSTRACT

A dialysis procedure was used to assess the distribution coefficients of ~50 major and trace elements (TEs) between colloidal (1 kDa–0.22 μm) and truly dissolved (<1 kDa) phases in Fe- and organic-rich boreal surface waters. These measurements allowed quantification of both TE partitioning coefficients and the proportion of colloidal forms as a function of solution pH (from 3 to 8). Two groups of elements can be distinguished according to their behaviour during dialysis: (i) elements which are strongly associated with colloids and exhibit significant increases of relative proportion of colloidal forms with pH increase (Al, Ba, Cd, Co, Cr, Cu, Fe, Ga, Hf, Mn, Ni, Pb, rare earth elements (REEs), Sr, Th, U, Y, Zn, Zr and dissolved organic C) and (ii) elements that are weakly associated with colloids and whose distribution coefficients between colloidal and truly dissolved phases are not significantly affected by solution pH (As, B, Ca, Cs, Ge, K, Li, Mg, Mo, Na, Nb, Rb, Sb, Si, Sn, Ti, V). Element speciation was assessed using the Visual MINTEQ computer code with an implemented NICA-Donnan humic ion binding model and database. The model reproduces quantitatively the pH-dependence of colloidal form proportion for alkaline-earth (Ba, Ca, Mg, Sr) and most divalent metals (Co, Cd, Mn, Ni, Pb, Zn) implying that the complexation of these metals with low molecular weight organic matter (<1 kDa fraction) is negligible. In contrast, model prediction of colloidal proportion (fraction of 1 kDa–0.22 μm) of Cu²⁺ and all trivalent and tetravalent metals is much higher than that measured in the experiment. This difference may be explained by (i) the presence of strong metal-binding organic ligands in the <1 kDa fraction whose stability constants are several orders of magnitude higher than those of colloidal humic and fulvic acids and/or (ii) coprecipitation of TE with Fe(Al) oxy(hydr)oxides in the colloidal fraction, whose dissolution and aggregation controls the pH-dependent pattern of TE partitioning. Quantitative modeling of metal – organic ligand complexation and empirical distribution coefficients corroborate the existence of two colloidal pools, formerly reported in boreal surface waters: “classic” fulvic or humic acids binding divalent transition metals and alkaline-earth elements and large-size organo-ferric colloids transporting insoluble trivalent and tetravalent elements.

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1. Introduction

Quantification of the association between trace elements (TEs) and colloids is one of the major issues for modelling TE migration and bioavailability (Buffle and van Leeuwen, 1992; Kretzschmar and Schafer, 2005). At present, there is little doubt that in continental waters, most insoluble TE except alkali and alkaline-earth metals, neutral species and oxyanions are not present in the form of ionic species but rather associated with organic and organo-mineral colloids (Doucet et al., 2007). This is especially true for

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boreal regions, where the presence of wetlands, abundant precipitation and sufficient plant production creates favourable conditions for formation of organic rich soils and provides high concentrations of organic matter (OM) in surface waters (Ingri et al., 2000). Quantitative modelling of migration and bioavailability of TE requires knowledge of stability constants for complexation reactions of trace metals with colloidal OM. The most powerful and precise method of assessing these constants is voltammetry and potentiometry (van Leeuwen et al., 1989; Gordon et al., 1996; Bakker and Pretsch, 2001; Buffle and Tercier-Waeber, 2005). However, the use of these techniques is restricted to a very low number of transition metals (Cd, Cu, Pb, Zn) and some rare earth elements (REEs) (Johannesson et al., 2004; Chang Chien et al., 2006). At present, a large number of TE, including trivalent and tetravalent elements, remains poorly explored and the

quantification of their stability constants with natural colloidal matter is very difficult. This hampers progress in applying quantitative physico-chemical models such as WHAM (Tipping, 1994; Tipping et al., 2002), WinHumicV based on WHAM model (Gustafsson, 1999) or NICA-Donnan (Benedetti et al., 1995; Milne et al., 2003) for assessing trace element speciation in continental waters.

Another difficulty in characterisation of TE speciation in natural waters is the extremely variable and multiple chemical nature of colloidal matter: usually, one can distinguish organic colloids such as humic and fulvic acids (HA and FA), microbial exudates, polysaccharides and organo-mineral entities such as Fe and Al hydroxides coated by OM (Gustafsson and Gschwend, 1997; Pokrovsky et al., 2005, 2006; Dahlqvist et al., 2007; Filella, 2007). Whereas the complexation of TE with organic colloids, notably with standard specially purified and thoroughly characterised HA and FA, is fairly well known, organo-mineral and mineral (Fe, Al) colloids stabilized by adsorbed OM do not exist in commercial ready-to-use form and experimental work with such synthetic or purified standard colloids is very limited. In this regard, direct experiments on natural water samples may shed new light on TE complexation with organo-mineral colloids. It has been shown previously (van Leeuwen et al., 2005, and references therein) that it is possible to measure *in situ* TE speciation and compare it to state of the art modelling in aquatic systems. For this, knowledge of the size fraction of studied colloids is crucial (Lead et al., 1997). Among the different size fractionation techniques, dialysis is a very efficient one, proven to be less subject to various artefacts of charge separation (Vasyukova et al., 2010; Pokrovsky et al., 2011, 2012). Combined with the usual 0.22 μm (0.45 μm) filtration, equilibrium dialysis through a 1 kDa (~ 1 nm) membrane allows straightforward assessment of colloidal entities which are, by definition, between 1 nm and 0.45 μm (Buffle and van Leeuwen, 1992; Sigg et al., 2006). As a result, dialysis is frequently applied to quantify the colloidal fraction of metals both in natural waters and in laboratory conditions (Magaritz et al., 1989; Alfaro-De la Torre et al., 2000; Gimpel et al., 2003) and it has been used previously to isolate small size fractions of metals (Beneš and Steinnes, 1974; Jansen et al., 2001), to sample and assess the speciation of metals in lake waters, groundwaters and in sediments (Carignan et al., 1985; Vesely et al., 2001; Huerta-Diaz et al., 2007) and to remove small particles of Fe oxy(hydr)oxide from solution (Lofts et al., 2008). In this work, a first attempt was made to conduct dialysis at various artificially modified solution pHs.

Natural water samples from the boreal and subarctic zone were chosen for this work, because these regions are among the most fragile zones in the world due to their vulnerability to industrial impact, low productivity of terrestrial biota and limited biological activity. At the same time, the effect of global warming consisting not only in rising surface temperature but also acidification of water reservoirs is expected to be mostly pronounced in the Arctic (Reuss et al., 1987). Acidification is likely to change the speciation of many TE, and the proportion of labile and biologically available forms and thus could influence pollutant migration and bioavailability. Quantitative prediction of these phenomena requires rigorous knowledge of the proportion of colloidal forms of TE in solution as a function of pH. Equilibrium dialysis through a 1-kDa membrane, comparable with the pore size of biological membranes, should allow tracing these changes in almost *in situ* conditions using relatively inexpensive and straightforward techniques.

To summarise, the goals of the present study were the following: (1) to test a simple *in situ* method for characterisation of colloidal status of TE in natural waters depending on solution pH; (2) to acquire apparent distribution coefficients of TE between truly dissolved (<1 kDa) and the colloidal fraction (1 kDa–0.22 μm) as a function of solution pH in several typical boreal

waters, and (3) to apply existing thermodynamic models for description of TE speciation as a function of pH in solutions containing complex organo-mineral colloids of poorly defined structure. It is anticipated that achieving these goals will allow better understanding of the TE speciation and, consequently, their bioavailability in pristine natural waters.

2. Materials and methods

2.1. Sampling and storage

Surface waters were collected during the summer base flow period (July and August) from typical small creeks, medium size rivers and peatland zones belonging to watersheds of various lithology of the White Sea basin and located in NW Russia (Fig. SI-1 Of Supporting information). Detailed geographical, geological and climate descriptions of the region are given elsewhere (Pokrovsky and Schott, 2002; Zakharova et al., 2007; Pokrovsky et al., 2010; Vasyukova et al., 2010). Brief descriptions of samples and the chemical composition of water are given in Table SI-2. Three groups of samples corresponding to different lithology, pH, Dissolved Organic C (DOC) and Fe concentration were considered. Samples No. 12 (Ruiga river draining basalts in southern Karelia) and M-1 (Palajoki river draining glacial deposits and basic rocks in northern Karelia) correspond to permanently flowing rivers with a watershed of ~ 20 –50 km^2 . Sample No. 15 is a small creek draining basic and ultramafic rocks within an ombrotrophic bog and feeding the Ruiga river (No. 12); K-43 and K-23 are surface peatland waters (N Karelia) draining glacial till covering gabbro-norites, located at the lake coast and within the riparian zone of the Palajoki river, respectively. Sample S-32 (Peschanaya river) corresponds to a small temporary coastal creek originating in the wetland zone and discharging into the sea, and sample S-40 (peat water) is groundwater from a soil pit in the wetland coastal zone of the North White Sea (Arkhangelsk region) underlain by sedimentary clay and carbonate deposits. This range of the samples covers most representative surface waters of the region including those forming the discharge to the Arctic Ocean. The studied waters are essentially neutral with pH varying from 6 to 7.7, except swamp creeks (samples No. 15, S-32 and S-40) that have pH between 4 and 5. All studied samples exhibit high concentration of DOC, from 15 to 46 mg/L, and dissolved Fe concentration from 0.35 to 5.5 mg/L, which is typical for waters draining peatland areas of the European subarctic (Ingri et al., 2000; Dahlqvist et al., 2007). In accord with literature data on Fe status in other organic-rich neutral surface waters (Rose et al., 1998; Olivé-Lauquet et al., 1999; Allard et al., 2004; Benedetti et al., 2003) and as supported by quantitative modelling (Tipping et al., 2002; Lofts et al., 2008) it is assumed that most conventionally dissolved Fe (<0.22 μm) was present in a trivalent state. In the field, several litres of water were collected in sterile acid-washed containers. Samples were filtered on site within 1 h of sampling using 0.22 or 0.45 μm cellulose acetate filters and placed in sterile acid-washed dark polypropylene Nalgene bottles, stored at 4–10 °C in the darkness and transported to the laboratory within a week of sampling, where they were stored in the refrigerator. The storage temperature was reduced to decrease coagulation and bacterial production in the experimental samples (Chen and Buffle, 1996; Wilkinson et al., 1997).

It is known that the removal of a volume of water from a natural system isolates it from potential metal sources and sinks inevitably changing the distribution of element between different colloidal and dissolved fractions (Evans et al., 2005). In this work, 1–2 L of filtered river and soil water in were kept isolated in a refrigerator for 6–12 months before the laboratory dialysis procedure. During

this period of storage, only a little or no coagulation occurred as proven by periodic sampling, filtration and analysis for DOC and TE. Thus, it is believed that the colloidal fraction is stable during storage of filtered water. Furthermore, in methodological experiments of bacteria culturing on rich nutrient media it was demonstrated that both 0.22 μm and 0.45 μm filtered organic-rich waters (in particular, samples M-1, K-23 and K-43) remained sterile during transport and storage, since no detectable heterotrophic bacteria growth was evidenced. Therefore, strong microbial degradation of organic matter was not anticipated during storage. The colloidal distribution of elements separated via 1-kDa dialysis *in situ* (in the field, Vasyukova et al., 2010) and *ex situ* (in the laboratory, see Section 3.1) for the same sample (No. 15) yielded similar results, with the proportion of the colloidal fraction for Fe, Al, DOC and TE separated *in situ* being only 10–30% higher than those obtained by *ex situ* dialysis. Overall it is believed that the long-term storage of filtered samples before the experiment resulted in greater stability of organo-mineral colloids, their internal re-equilibration between different colloidal and truly dissolved pools and thus identical initial conditions for all samples before the experimental pH adjustment. Concerning the stability of experimental solutions in the full pH-range during dialysis procedure, it has been argued that OM begins to precipitate below pH 3 during flocculation experiments of river water/sea water mixing (Gimpel et al., 2003). However, in the present study the apparent colour of the solution remained uniform, DOC concentration was stable (see Section 2) and no flocs were observed after several days of exposure at different pH during dialysis.

2.2. Dialysis procedure

An equilibrium dialysis procedure was used to assess the distribution coefficients of major and trace elements between formally defined colloidal or high molecular weight (1 kDa–0.22 μm) and truly dissolved low molecular weight (<1 kDa) forms. In essence, performing the dialysis procedure as a function of pH is equivalent to measuring the pH-dependent adsorption edge of TE on natural colloids (1 kDa–0.22 μm). Unlike the usual adsorption procedure for natural solid sorbents, when the separation of phases is performed by filtration, in this case, the separation of two phases is conducted via a 1 kDa membrane. For this, EDTA-cleaned, trace-metal pure Spectra Pore 7[®] dialysis membranes composed of regenerated cellulose and having 1-kDa pore

size were thoroughly washed in 0.1 M bi-distilled HNO_3 and ultrapure water, filled with 10 mL ultrapure MQ deionised water (18 MOhm Element[®]) and placed in 250 mL acid-cleaned polypropylene containers with natural filtered water (Fig. 1). All manipulations were carried out in clean bench rooms class A 10,000. Sample pH was varied between 3 and 8 via addition of ultra-pure HNO_3 and NaOH. The pH of water was measured both before and after the dialysis procedure using a combined Schott-Geräte electrode calibrated against NIST buffer solutions (pH = 4.00 and 6.86 at 25 °C). It remained stable within ± 0.2 pH units during the full duration (72–160 h) of the dialysis procedure. Pokrovsky et al. (2005) showed in laboratory experiments that, for 1-kDa membranes, equilibrium distribution is achieved after 12 h, in agreement with specifications given by the manufacturer. *In situ* dialysis for organic-rich boreal lake waters showed that 48–72 h is sufficient to achieve dialysis equilibrium (Shirokova et al., 2010; Pokrovsky et al., 2011, 2012).

Concentrations of DOC, major and trace elements were measured both inside the dialysis bag and in the external solution (inner and outer compartments). The external solution was filtered through sterile, single-used filter units Minisart[®] (Sartorius, acetate cellulose filter) having a diameter of 25 mm and pore size 0.22 μm . Each filter was washed in MQ water before the experiment and used only once. The first 20–30 mL of the filtrate was systematically rejected. DOC was measured using a Shimadzu TOC 6000 with an uncertainty of 5%. Major cations and trace elements were measured in 2% HNO_3 by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500, with In and Re as internal standards and a precision better than $\pm 5\%$). The international geostandard SLRS-4 (Riverine Water Reference Material for Trace Metals certified by the National Research Council of Canada) was used to check the validity and reproducibility of each analysis. A good agreement between the replicated measurements of SLRS-4 and the certified values was obtained (relative difference <10%).

The efficiency of the dialysis procedure was evaluated by comparison of the concentration of major anions (i.e., Cl^- , SO_4^{2-}) or neutral species (H_4SiO_4^0) not associated with colloids in the dialysis bag and in the external solution. At the end of the dialysis procedure, these concentrations were always identical within $\pm 10\%$ suggesting equilibrium distribution of truly dissolved components. In addition the mass balance was calculated between the external solution (<0.22 μm) and dialysate (<1 kDa) for OC, major elements and some trace elements (Al, Ba, Ce, Fe, La, Mn, Sr, Th and Y). The total recovery was better than 90% except for several samples for Cu, Pb and Zn and when clear contamination was observed. These elements were not used for modelling and interpretation.

The stability of the initial filtered solution with respect to colloid coagulation during the dialysis experiment was tested via measurement of DOC and Fe concentrations as a function of pH. The concentrations of organic C (OC) and Fe in 0.22 μm fractions of all seven natural waters measured after 2 days exposure at different pH are plotted in Fig. 2. Dissolved Fe and OC concentrations remained stable for 5 of the 7 samples. The less stable samples K-43 and No. 12 probably have some amount of divalent Fe which undergoes oxidation in circumneutral and neutral solutions thus leading to a decrease of $\sim 70\%$ to 80% of Fe (<0.22 μm) concentration with pH increase. The enrichment of Fe at pH < pH_{natural} in these two samples stems from the filtration of acidified solutions. Indeed, large amounts of Fe-oxyhydroxide mineral colloids present in these samples at original natural water pH partially coagulated before the acidification and filtration through 0.22 μm . These particulates were subsequently dissolved in acidic solutions producing Fe^{3+} aqueous species ($\text{Fe}(\text{OH})_2^+(\text{aq})$) at pH = 3–4 that enriches the filtrates in Fe compared to OC.

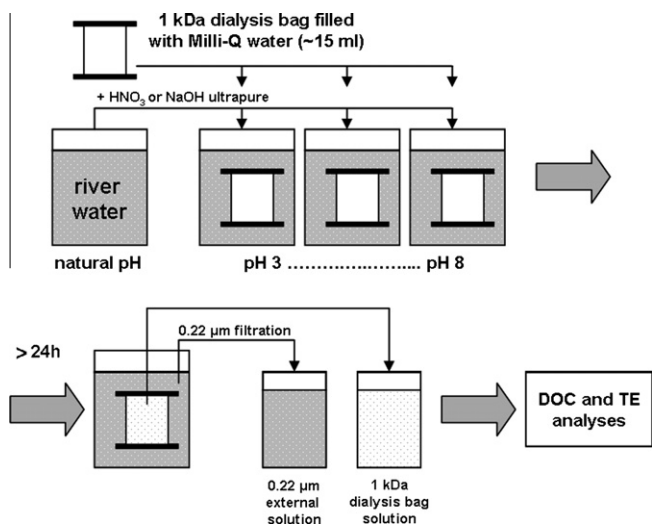


Fig. 1. Schematic representation of dialysis experimental procedure.

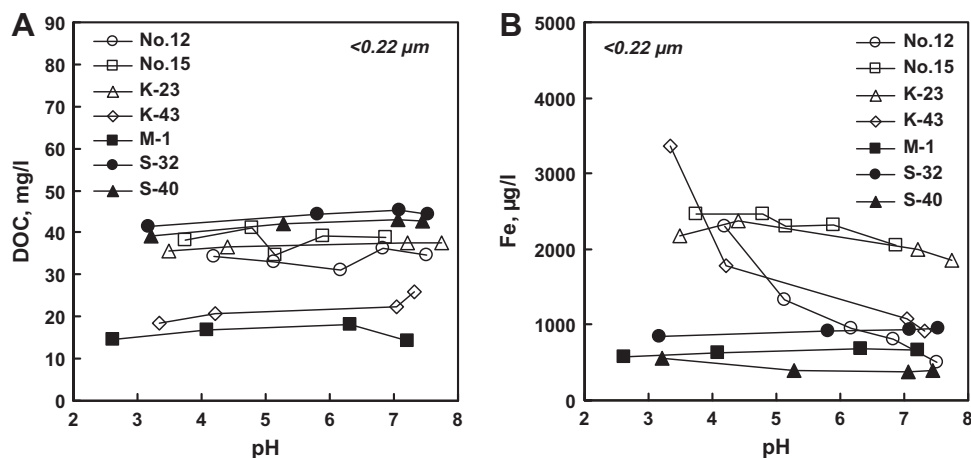


Fig. 2. DOC (A) and Fe (B) concentrations in <0.22 μm fraction as a function of pH for seven natural waters. Concentrations of DOC and Fe of all samples were measured after 2 days of exposure at different pH.

3. Results

Measured major and trace element concentrations in filtrates (<0.22 μm) and dialysates (<1 kDa) are reported in Table SI-3 of Supporting information 3. In the following section, the behaviour of elements depending on pH during dialysis experiments will be discussed considering mainly the average values of all seven representative natural water samples. Hence, the average value represents typical boreal surface water which may or may not eventually prove a universal pattern of TE distribution between colloidal and LMW (<1 kDa) fractions as a function of pH.

3.1. The pH-dependency of dissolved trace elements

In all samples, between 30% and 55% of OC is concentrated in the LMW <1 kDa fraction (except K-43 having 15% and S-32 having 77% of OC in <1 kDa fraction) consistent with the presence of small-size polymers of fulvic nature (Pokrovsky and Schott, 2002). The average percentage of colloidal Fe and OC are depicted as a function of pH in Fig. 3. It can be seen that the proportion of colloidal OC increases with pH increase to a smaller degree than Fe. It was shown previously that Fe is often present in large-size colloids (10 kDa–0.22 μm , Pokrovsky and Schott, 2002) and 5–40 nm large size inorganic Fe oxy(hydr)oxides (e.g., Stolpe and Hasselöv, 2010; Stolpe et al., 2010). As a result, Fe is almost completely removed from solution before passing through a 1 kDa

membrane at natural pH. This is corroborated by Fig. 4 where the molar ratio of [Fe] to [Organic C] in 0.22 μm filtrates and 1 kDa dialysates are plotted for different samples. In filtrates, the [Fe]/[OC] ratio barely depend on pH except for the Ruiga river No. 12 and swamp water K-43 where the proportion of Fe decreases with pH increase (Fig. 4a). In contrast, in the <1 kDa fraction of all the studied samples a gradual decrease of the [Fe]/[OC] ratio is observed with the pH increase indicating the highest proportion of Fe colloids in circumneutral and neutral solutions (Fig. 4b). The increase of [Fe] at lower pH in the <1 kDa fraction could be explained by the dissolution of Fe oxyhydroxide colloids.

The TE distribution between truly dissolved (<1 kDa) and colloidal (1 kDa–0.22 μm) pools for the seven studied waters at different pH values are presented as average values in a stack diagram (Fig. 5). Based on these results, one can distinguish two groups of elements whose behaviour is illustrated in Figs. 6 and 7, respectively:

- (1) Elements whose colloidal state is strongly dependent on solution pH with 40–100% being in the LMW fraction at pH 3–3.5 and only 0–40% being in the fraction of <1 kDa at pH 7–7.5: Al, Ba, Cd, Co, Cr, Cu, Fe, Ga, Hf, Mn, Ni, Pb, rare earth elements (REEs), Sr, Th, U, Y, Zn, Zr and dissolved organic C (Fig. 6). Most of these elements exhibit good correlation between their percentage content in the colloidal fraction and that of Fe and OC ($r^2 = 0.6\text{--}0.9$ for both).

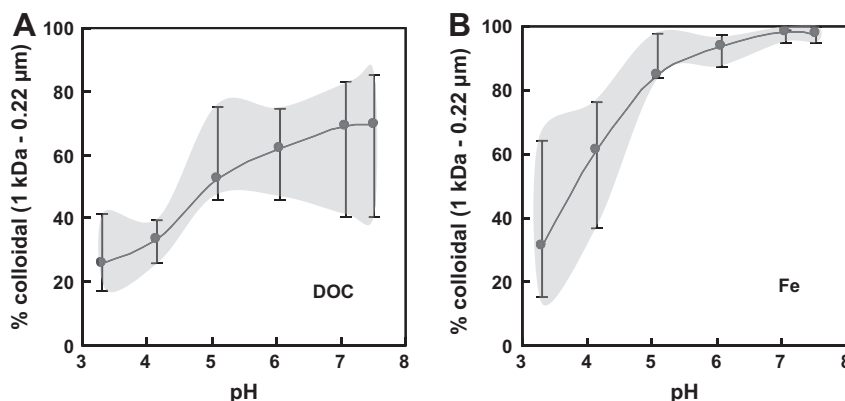


Fig. 3. Dependence of colloidal organic carbon (A) and Fe (B) on pH in all studied samples. The solid line is for guiding purposes. Symbols correspond to the median and the error bars reflecting minimum and maximum obtained values of all samples (shaded area). Solid line is for guide purposes.

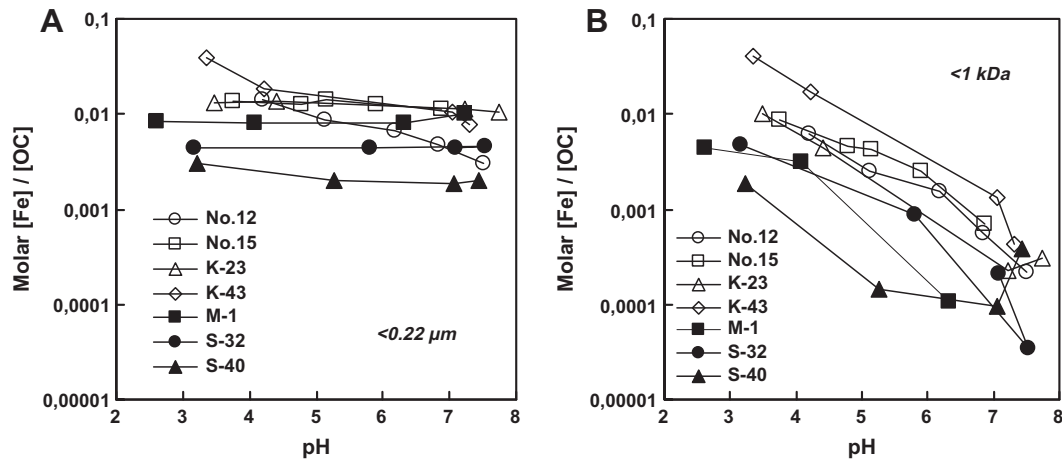


Fig. 4. Molar [Fe] to [organic carbon] ratio in <0.22 μm filtrates and <1 kDa dialysates for studied samples. In <0.22 μm filtrates (A), the [Fe]/[OC] ratio shows very little dependence on pH except for the Ruiga river No. 12 and swamp water K-43 where the proportion of Fe decreases with pH increase. In the <1 kDa fraction (B) of all studied samples a gradual decrease of the [Fe]/[OC] ratio was observed with the pH increase indicating that the highest proportion of Fe colloids are in the circumneutral and neutral solutions.

- (2) Elements whose colloidal status is not subjected to pH variation: As, B, Ca, Cs, Ge, K, Li, Mg, Mo, Na, Nb, Rb, Sb, Si, Sn, Ti, Tl, V, W (Fig. 7). Their percentage in dissolved form remains high (>50%) and varies within ± 10 –15% at pH 3–7. These elements do not correlate with Fe or with OC in the colloidal fraction ($r^2 < 0.4$).

3.2. Thermodynamic modelling of metal complexation with dissolved organic matter

The weak dependence of organic C concentrations on pH in 0.22 μm filtrates (Fig. 2a) implies that the concentrations of fulvic and humic organic acids – main ligands that complex dissolved metals – remain constant over the full pH range. It is known that separation through 1-kDa membrane is used to assess the proportion of colloidal (i.e., >1 kDa) forms compared to total dissolved content of trace metals (Buffle and van Leeuwen, 1992; Marsac et al., 2010). Note that because fulvic acids are just a few nm in size (e.g., 1.5–2.5 nm, Lead et al., 2000), a significant part of soil-derived organic matter can be found in the 1-kDa dialysate. All samples were checked by HPLC analysis and the concentration of low molecular weight organic acids (formic, acetic acids etc.) was negligible compared to total DOC concentration. Therefore, complexation of metals with humic (HA) and fulvic acids (FAs) assessed via available speciation codes can be compared with TE speciation in colloids measured by dialysis in the laboratory. For this comparison, only colloidal (humic and fulvic) complexes were taken into account and it was assumed that metal complexation with LMW (<1 kDa) organic matter does not occur. This is equivalent to testing the hypothesis that the important binding ligands are solely in the 1 kDa–0.22 μm fraction.

The geochemical program Visual MINTEQ (Gustafsson, 1999), version 2.52 for Windows, a recent adaption of the original code written by Allison et al. (1991) (see Unsworth et al., 2006; for a vMINTEQ application example) was used in conjunction with a database and the NICA-Donnan humic ion binding model (Benedetti et al., 1995; Milne et al., 2003). Speciation calculations were performed for divalent metals Ba, Ca, Cd, Co, Cu, Mg, Mn, Ni, Pb, Sr, Zn, and Al, Fe^{III}, Th^{IV}, and U^{VI}O₂ for all seven samples at pH 3–8 at first without modification of the default database (Milne et al., 2003). Note that a significant proportion of data used to create this database represents soil humic and fulvic acids, and all aquatic FA and HA originate from temperate rivers and lakes which may be quite different from boreal subarctic waters studied in this work.

Results of speciation calculation are presented in Supporting information (Fig. SI-4) and described below according to different groups of elements. For divalent alkaline-earth elements and heavy metals (Ba, Ca, Cd, Co, Mg, Mn, Ni, Pb, Sr, Zn), the model predicts a gradual increase of colloidal (1 kDa–0.22 μm) forms proportional with pH increase in all samples. For most samples, the agreement between the experimental proportion of the colloidal form and predicted percentage of HA, FA-complexed metal is remarkable given (i) the varying chemical and physical nature of soil and temperate aquatic HA and FA of the database and aquatic boreal waters in this study and (ii) the totally different methods used to measure Me-HA(FA) stability constants for reactions available in the vMINTEQ database (titration, voltammetry or potentiometry) and Me-colloid association by size fractionation (dialysis) in this study. Adequate description of experimental colloidal form proportions for Cu could not be achieved, and significant modification of stability constants, almost a 4 order magnitude decrease, was necessary to approach the experimental data. At the same time, using the Stockholm Humic model (SHM of the vMINTEQ) instead of the Nica-Donnan approach allowed reproducing the percentage of colloidal Cu as a function of pH (tested for samples No. 15 and K-43). A disagreement between the results of SHM and the Nica Donnan formalism is observed only for Cu; the other divalent ions demonstrate reasonable accord between the two models. Another possible description of metal – OM complexation in the vMINTEQ code, Gaussian DOM Model, yields results somewhat intermediate between SHM and Nica Donnan. The most likely causes for such a discrepancy between different models are stability constants of databases and electrostatic vs non electrostatic description of metal – colloid interaction (see Dudal and Gérard, 2004; for a critical review on existing models). Reproducing the experimental data for Al, Fe^{III}, Th^{IV} and U^{VI} using the NICA-Donnan model of vMINTEQ codes with the implemented database was not possible.

It is important to note that all experimental solutions become oversaturated with respect to Fe hydroxide at pH ≥ 3.4 and to Al hydroxide at pH ≥ 5.2 . This could explain the disagreement between the model description of the percentage of OM-bound forms and experimental proportion of colloids. Iron hydroxides may form colloidal precipitates that will be retained by 1-kDa membrane. The discrepancy between the model and the measurements can also be observed from the Fig. 8 where the logarithm of activity of Fe³⁺ calculated by vMINTEQ in <1 kDa dialysates is plotted as a function of pH for the seven considered samples. Literature data reported by Byrne and Luo (2000) and Byrne et al. (2005) are also presented as

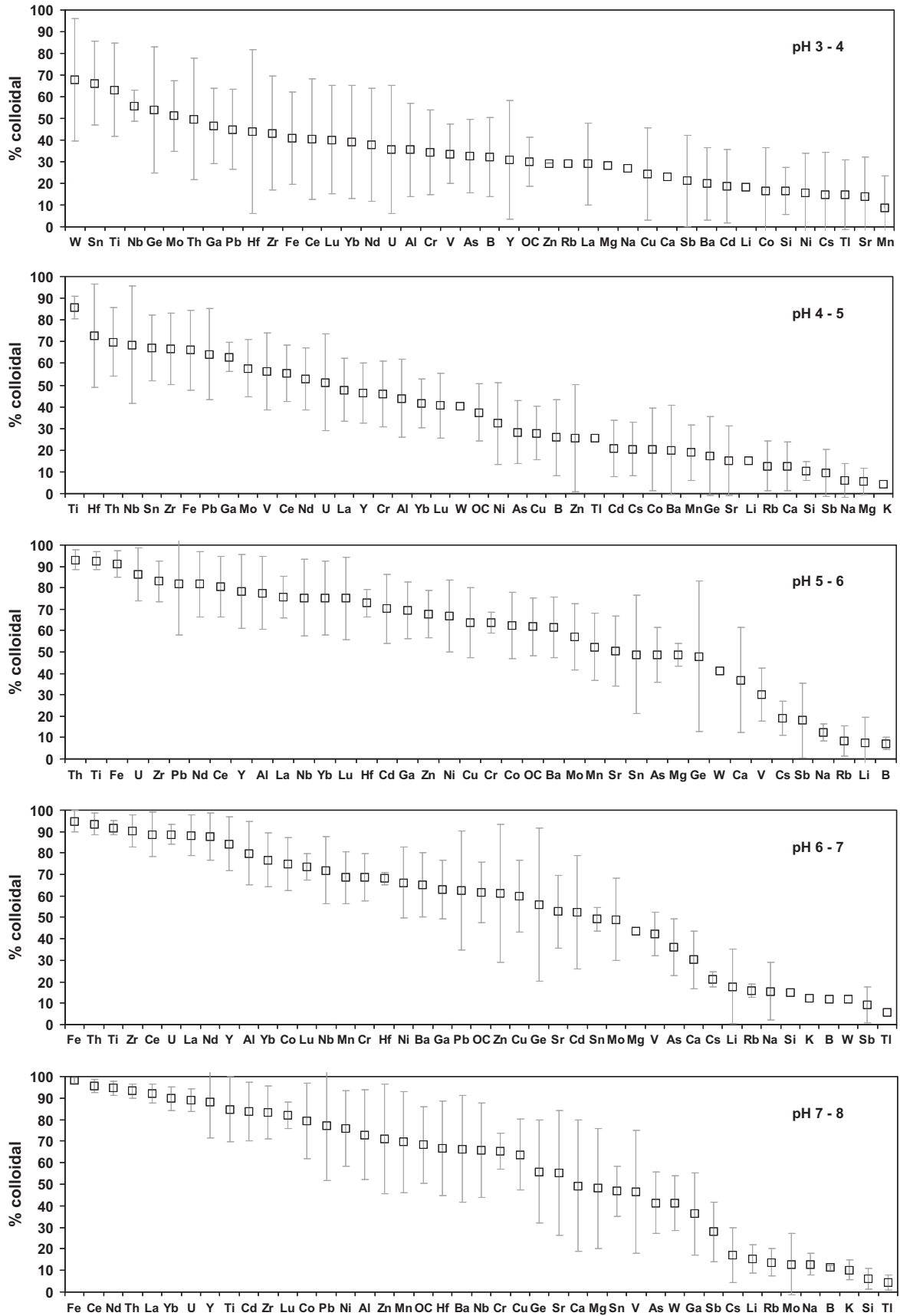


Fig. 5. Percentage of colloidal forms (1 kDa–0.22 μm) in all samples (average). Error bars represent the 2σ uncertainty.

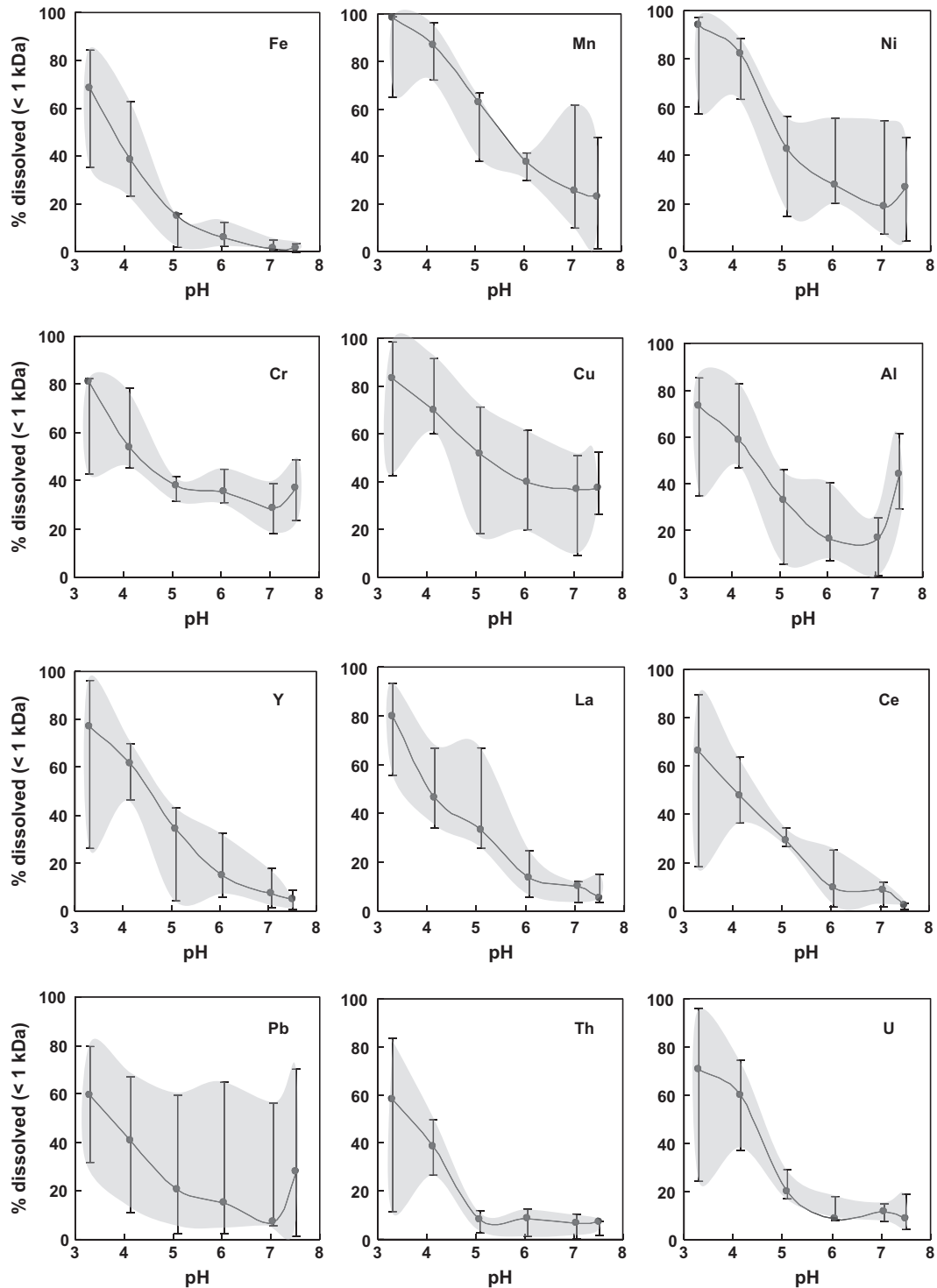


Fig. 6. Dependence of Fe, Mn, Ni, Cr, Cu, Al, Y, La, Ce, Pb, Th and U in truly dissolved (<1 kDa) form on pH in seven studied samples. The diagrams are representative for the first group of elements whose colloidal state is strongly dependent on solution pH with 40–100% being in truly dissolved form at pH = 3–3.5 and only 0–10% being in the fraction of <1 kDa at pH = 7–7.5. The solid line is for guide purposes. Symbols correspond to the median and the error bars reflect minimum and maximum obtained values among all samples (shaded areas).

dashed and double-dotted lines, respectively, which have a slope of -3 on the figure and correspond to equilibrium with precipitated Fe hydroxide. The slope of the line for the $\log a_{\text{Fe}^{3+}}$ given by modelling data of this study is close to -7 (Fig. 8A) and at pH > 4 calculated activity of $\text{Fe}^{3+}(\text{aq})$ is several orders of magnitude below the value in equilibrium with Fe hydroxide. This apparent contradiction between the experiments on Fe oxide solubility by Byrne et al.

(2005) and this study of short-term dialyses of natural samples at different pH may stem from the following. First, it is possible that the equilibrium of the <1 kDa fraction with colloidal (1 kDa–0.22 μm) Fe(III) oxy(hydr)oxides coated by organic matter was not attained during the ≤ 7 days of exposure in this work. Second, it is very likely that the LMW fraction of organic C controls the speciation of Fe^{3+} in the <1 kDa fraction notably in circumneutral solutions at

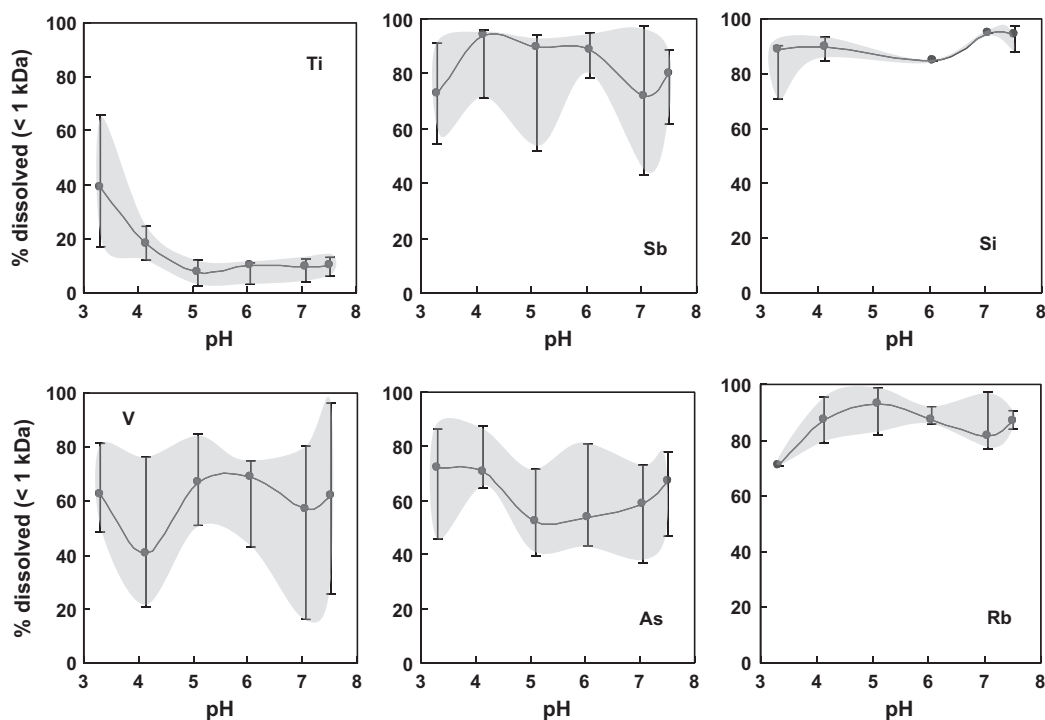


Fig. 7. Dependence of Ti, Sb, Si, V, As and Rb in truly dissolved (<1 kDa) form on pH in seven studied samples. The percentage of the dissolved form depends weakly on pH. The solid line is for guide purposes. Symbols correspond to the median and the error bars reflect minimum and maximum obtained values for all samples (shaded areas).

6 < pH < 8. For this reason, the Fe^{3+} activity in the <1 kDa and <0.22 μm fractions may not be the same, regardless of whether it is under equilibrium control or not. Indeed, the vMINTEQ calculation of $\text{Fe}^{3+}(\text{aq})$ activity in the 1-kDa dialysates performed assuming no DOC present (Fig. 8B) yields values much closer to the theoretical curve of Fe oxy(hydr)oxide solubility, also compatible with modelling of natural dialysates (Lofts et al., 2008). Calculation of $\text{Fe}^{3+}(\text{aq})$ activity in the <0.22 μm fraction demonstrated significant scatter in Fe^{3+} activity among different samples, most likely linked to various concentrations and nature of DOM (Fig. 8C). At the same time, the agreement of model prediction with available solubility data for samples No. 15, K-23, K-43 and M-1 and No. 12 (at natural pH) is quite reasonable. These samples exhibit very similar Fe^{3+} activity between the organic-free 1 kDa and 0.22 μm fractions (compare Fig. 8B and C). As such, it is possible that the main reason for difference between this study and that of Lofts et al. (2008) stems from consideration or not of dissolved organic C in the LMW (<1 kDa) fraction and the differences in the databases of NICA Donnan and WHAM, respectively, for Fe^{3+} complexes with the <1 kDa organic ligands and 1 kDa–0.22 μm organic colloidal fraction. Note that if the data are modeled with WHAM in the presence of DOC represented by fulvic acid (i.e., using the same assumptions as was done in Fig. 8A), then: (1) a slope of $\log a_{\text{Fe}^{3+}}$ against pH of approximately -3 is obtained, and (2) the WHAM-predicted Fe^{3+} activities are close to the solubility equations of Byrne and Luo (2000) and Byrne et al. (2005) presented in Fig. 8. Therefore, given that the two models give very different predictions, drawing definite conclusions regarding the chemistry of the system solely from vMINTEQ should be done with care.

4. Discussion

All studied samples, despite significant differences in their environmental context and chemical composition, exhibit similar

patterns of colloidal TE concentration dependence on pH. This characteristics resembles the one proposed for element association with soil organic colloids (Pédrot et al., 2008) and is also similar to the pattern of TE association with colloids based on results of ultra-filtration of boreal waters and soil solutions (Pokrovsky et al., 2005, 2006). The most likely cause for this partitioning is the relative affinity of the chemical element for hydroxyl groups of Fe oxy(-hydr)oxides and carboxylates of dissolved organics, as has been discussed widely in the literature.

For divalent alkaline-earth (Ba, Ca, Mg, Sr), transition metals (Co, Mn, Ni, Zn), and Cd (for some samples), the model prediction of colloidal fraction as a function of pH agrees reasonably well with the experiments (Fig. SI-4 of Supporting information). This strongly suggests that the majority of these nine metals are bound to humic and fulvic acids of the colloidal (1 kDa–0.22 μm) fraction and the complexation of metals with the LMW (<1 kDa) fraction of organic C is negligible. If the complexation of divalent metals with the LMW fraction was significant, then the model would strongly overestimate the proportion of colloidal metals because the <1 kDa fraction of OC would act as an additional source of ligands that complex metal. At the same time, Pb represents an exception because the model yields ca. 10–30% higher estimation of the percentage of colloidal material. As such, a non-negligible amount of Pb is likely to be bound with LMW in <1 kDa fraction, not accounted for in the vMINTEQ model.

In contrast, significant adjustment of metal-OM stability constants (up to 4 orders of magnitude) was necessary to reproduce Cu partitioning between the LMW (<1 kDa) and colloidal fractions (Fig. SI-4). For Fe and Al, even a 4–8 logK unit decrease of OM-metal complexes stability constants does not approach the experimental pH-dependence of the colloidal proportion (not shown). There are two possible explanations for such a discrepancy between the results of the Nica-Donnan model and experimental data for Cu^{2+} and trivalent elements. Most of the available constants are ‘conditional’ or ‘apparent’ stability constants since they

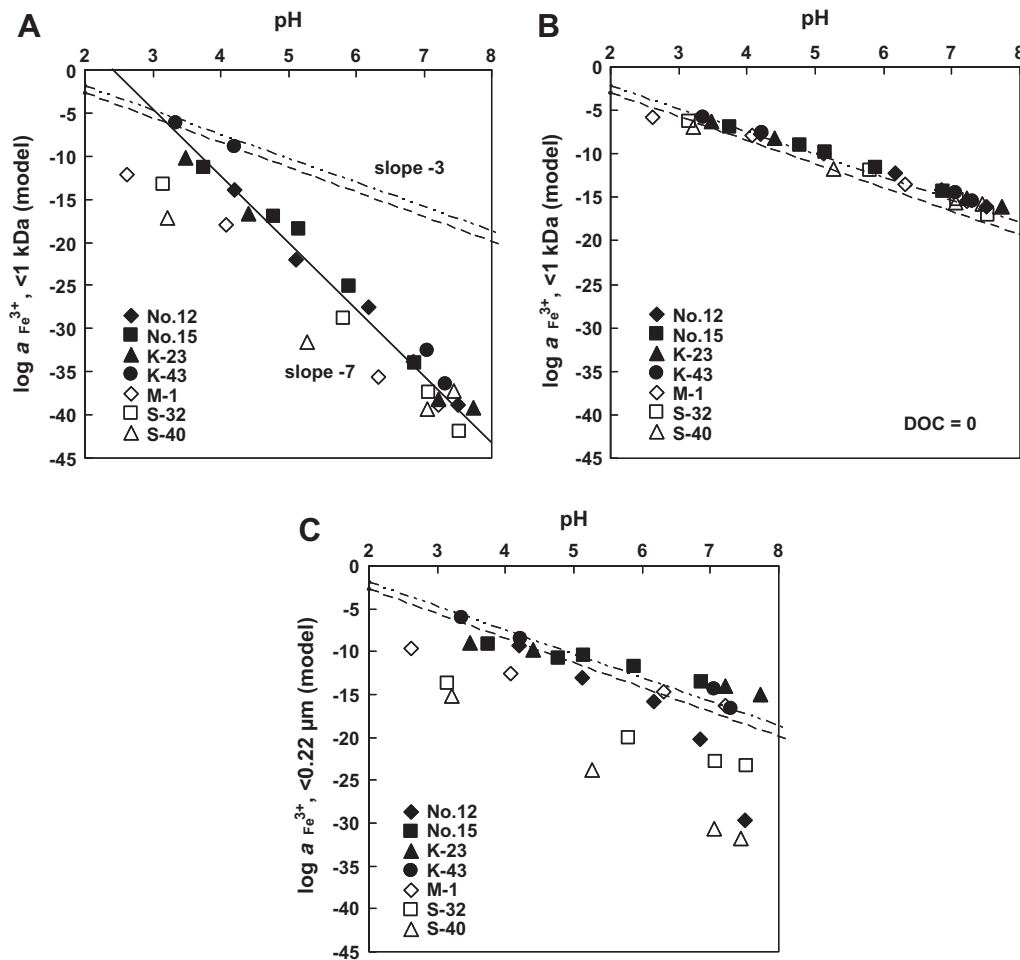


Fig. 8. Logarithm of Fe^{3+} activity (calculated by the vMINTEQ NICA Donnan model) plotted versus pH in <1 kDa dialysates for seven samples: with real measured DOC concentrations in samples (A) and assuming that no DOC is present (B). (C) Logarithm of activity of Fe^{3+} (vMINTEQ calculations with measured DOC concentrations in samples) plotted versus pH in 0.22 μm filtrates. Dashed and double-dotted lines stand for the $\log a_{\text{Fe}^{3+}}$ reported by Byrne and Luo (2000) and Byrne et al. (2005), respectively, assuming equilibrium with Fe oxy(hydr)oxides.

were determined at given pH and ionic strength. These constants were measured using different experimental techniques (potentiometric titration, ion exchange equilibrium, cation exchange resin) and with HA and FA of different nature (extracted from soil or river). As a result, values reported in the literature are often inconsistent, and, for example, the Cu^{2+} -humate stability constants vary by several orders of magnitude (Morel and Hering, 1983). The stability constant values for Fe^{3+} have often been approximated from a linear correlation between the logarithm of the metal-humate stability constant and the logarithm of the first hydrolysis constant available for other elements. Thus, Fe^{III} -humate/fulvate stability constants used in the database stem from a very limited set of soil organic matter experiments conducted at a pH of 1.0–2.5 (see discussion in Milne et al. (2003)).

Recently, it has been demonstrated that metal speciation in freshwaters assessed using various *in situ* techniques and model predictions of free ion activities generally do not agree with measurements (Sigg et al., 2006; Unsworth et al., 2006). In the case of the boreal surface waters analysed in the present work, the main cause of the discrepancy between the model prediction and the experimental proportion of colloidal Cu^{2+} and insoluble trivalent metals may be significant binding of some metals in the non-colloidal dialysis fraction (<1 kDa) given significant concentration of organic C in this fraction (from 70% to 20%, Fig. 3a). As a result of such a strong competition between <1 kDa and 1 kDa–0.22 μm or-

ganic ligands, significant amounts of Me will be in truly dissolved form, which is not considered in the model because of the lack of stability constants for non-colloidal, LMW < 1 kDa organic fraction. This is especially true for Cu, notoriously known, compared to other transition metals, for its high affinity for strongly-bound organic ligands less than 1 kDa molecular size (e.g., Gordon et al., 1996). Such small-size strong ligands for Cu in freshwaters are most likely related to phytoplankton (e.g., Xue and Sigg, 1993; Wu and Tanoue, 2001).

At the same time, the present data do not evidence that the strength of metal binding in the <1 kDa fraction is significantly greater than that of colloidal humic and fulvic acids. Indeed, given that the molecular weight of organic ligands decreases exponentially with the decrease of the poresize (Alekhin et al., 2010), the smallest fraction of <1 kDa, having only 20–30% of total $\text{DOC}_{<0.22\mu\text{m}}$ and exhibiting metal-ligand stability constants comparable with those of humic and fulvic acids in colloidal fraction, will be able to bind significant proportion of metals due to an elevated ligand to metal ratio.

Another possible reason for the observed discrepancy between the experimental proportion of colloidal form and the degree of calculated Metal-OM complexation for these elements is that, in addition and in competition to HA and FA, the important carriers of many trivalent and tetravalent elements (Al, Hf, REEs, Th, Ti, Y, Zr) and U in boreal waters are Fe-rich, OM-stabilized ferric colloids

(Ingri et al., 2000; Pokrovsky and Schott, 2002; Allard et al., 2004; Lyvén et al., 2003; Johannesson et al., 2004; Stolpe and Hassellöv, 2010; Stolpe et al., 2010). Indeed, there is a positive correlation ($r^2 = 0.4\text{--}0.9$) between the relative proportion of Fe and TE such as Al, Hf, REEs, Th, Ti, Y, U in the colloidal fraction of the studied natural waters over the full pH range (Fig. S1-5 of Supporting information). These correlations can be explained by (i) release of TE incorporated in the bulk of mineral colloids in circumneutral and acidic solution due to the dissolution of Fe oxyhydroxides and/or (ii) desorption of TE from the surface of colloidal Fe oxy(hydr)oxides. The second process is less likely to occur since the calculated adsorption capacity of large-size Fe-rich colloids of boreal waters is normally not sufficient to accommodate all the associated TE (cf. Pokrovsky and Schott, 2002). In addition, the localisation of TE on the surface of Fe colloids suggests metal-organic matter complexation as ternary surface complexes. These complexes would greatly increase the model predicted degree of TE binding to colloids compared to experimental data, thus increasing the discrepancy between the experimental data and the model prediction. Therefore, since Fe is one of the major constituents of the studied colloids, it is suggested that a significant part of the trivalent and tetravalent elements and U may be located in the bulk of the Fe-rich colloids stabilized (covered) by OM as it has been reported previously (Dia et al., 2000; Ingri et al., 2000; Andersson et al., 2006).

For the quantitative assessment of TE distribution in the bulk of colloids, a Fe-normalised TE partition coefficient between truly dissolved (<1 kDa) and colloidal (1 kDa–0.22 μm) fractions can be defined as:

$$K_d = (TE/Fe)_{\text{colloidal}} / (TE/Fe)_{\text{dissolved}}$$

The average values for all seven samples range from 0.2 to 3.0 (see Fig. S1-6 of Supporting information). There is a decrease of K_d with pH increase for Ti and Th, but no systematic variations for REEs and U. Note that the distribution coefficients calculated from the data for U, Pb and REE are close to those of co-precipitation of these elements with solid $\text{Fe}(\text{OH})_3$ in seawater: average K_d is 0.58, 0.87 and 0.44 for U, Pb and REEs, respectively (Savenko, 1996a, 2001), whereas the K_d of Al, Cr, V and Th are only two to three times lower than those reported for seawater (Savenko, 1996b; Savenko and Pokrovsky, 2007). Although a single cut-off dialysis technique cannot reveal a generic scheme of distribution of many elements over species above and below 1 kDa, it allows a simple and straightforward procedure for quantification of element partitioning between truly dissolved LMW (<1 kDa) and colloidal (1 kDa–0.22 μm) pools.

To sum up, the results of the present study confirm the presence of two distinct colloidal pools responsible for element migration in surficial boreal aquatic environments, also shown in several previous works: small-size organic-rich colloids and large-size Fe-rich colloids (Baalousha et al., 2006; Stolpe and Hassellöv, 2007, 2010; Stolpe et al., 2010). As represented in the graphical abstract, there is a main colloidal fraction containing large aggregates of Fe(III) oxy(hydro)oxides stabilized by organic C in the form of a coating. The LMW (<1 kDa) fraction essentially contains organic ligands with a much smaller proportion of Fe(III) aggregates. Concerning complexation with trace metals, C-rich and LMW organic ligands are capable of binding Fe(III) and a number of divalent metals such as Zn, Mn, Ni, Co, Cd, Pb and even alkaline-earth metals like Ca^{2+} (Dahlqvist et al., 2004). On the other hand, large size organo-ferric colloids bind a number of insoluble “immobile” metals (Pokrovsky et al., 2011, 2012). Trace metals, notably trivalent and tetravalent elements, and U associated with these organo-mineral colloids may be present in the following three forms: (i) complexed with organic ligands of the organic part of the colloids, (ii) adsorbed at the surface of colloidal particles, and (iii) incorporated

in the bulk of Fe(Al)-rich colloids stabilized (coated) by OM as reported in previous studies (Ingri et al., 2000; Dia et al., 2000; Andersson et al., 2006).

The experimentally measured proportion of colloidal divalent metals (Ca, Mg, Mn, Co, Ni, Zn, Sr, Cd, Ba) as a function of solution pH can be satisfactorily approximated by assuming that all complexation of metals with organic matter occurs in the colloidal (1 kDa–0.22 μm) fraction with a negligible amount of binding in the LMW (<1 kDa) fraction. At the same time, considering the Fe(oxy)hydroxide interaction for these divalent elements is unwarranted. In this regard, the present work offers a simple and relatively straightforward technique of assessment of bonding strength of divalent metals with organic ligands in truly dissolved (<1 kDa) and colloidal (1 kDa–0.22 μm) fractions as a function of pH.

The method of estimation the proportion of colloidal forms proposed in this study can be used to assess the possible change in metal speciation induced by environmental changes such as abrupt or gradual acidification of surface waters which is one of the major issues of concern (Reuss et al., 1987; Borg et al., 1989; Skjellkvåle et al., 2001; Davies et al., 2005; Neal et al., 2008). According to the present results, a decrease of pH from 6 to 5 will bring about a 2- to 3-fold increase in the proportion of non-colloidal (<1 kDa) forms of most potentially toxic metals (Al, Cd, Co, Cu, Ni, Pb, Th, U; see Fig. 6). These truly dissolved species are potentially bioavailable since the pore sizes of cell wall transport channels (10–30 Å in bacteria, 35–50 Å in plant cells, Carpita et al., 1979; Trias et al., 1992; and that of 1 kDa dialysis membrane, 1–2 nm) are comparable. Therefore, a significant increase in the bioavailability of trace metals may occur upon acidification of natural waters. On the other hand, an increase of DOC concentration in surface waters due to global warming as is observed in Nordic Countries, the British Isles, and Northern and Eastern USA (by approx. 10% over 10 a, Evans et al., 2005) may as well increase the proportion of metals bound to colloidal OM thus compensating for the effect of acidification. In perspective, the study of natural colloidal samples subjected to different treatment (H_2O_2 to remove the OM, Fe^{3+} or Al^{3+} addition to bind possible binding sites of organics) will be necessary to better quantify the relative role of organic and mineral colloidal constituents in TE complexation.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.apgeochem.2012.02.026](https://doi.org/10.1016/j.apgeochem.2012.02.026).

References

- Alekhnin, Yu.V., Ilina, S.M., Lapitsky, S.A., Sitnikova, M.V., 2010. Results of a study of co-migration of trace elements and organic matter in a river flow in a boreal zone. *Moscow Univ. Bull.* 65, 380–386. <http://dx.doi.org/10.3103/S0145875210060050>.
- Alfaro-De la Torre, M.C., Beaulieu, P.Y., Tessier, A.T., 2000. In situ measurement of trace metals in lakewater using the dialysis and DGT techniques. *Anal. Chim. Acta* 418, 53–68.
- Allard, T., Menguy, N., Salomon, J., Calligaro, T., Weber, T., Calas, G., Benedetti, M.F., 2004. Revealing forms of iron in river-borne material from major tropical rivers of the Amazon Basin (Brazil). *Geochim. Cosmochim. Acta* 68, 3079–3094.

- Allison, J.D., Brown, D.S., Novo-Gradac, K.J., 1991. MINTEQA2/PRODEFA2. A Geochemical Assessment Model for Environmental Systems: Version 3.0 User's Manual. EPA/600/3-91/021.
- Andersson, K., Dahlgvist, R., Turner, D., Stolpe, B., Larsson, T., Ingri, J., Andersson, P., 2006. Colloidal rare earth elements in a boreal river: changing sources and distributions during the spring flood. *Geochim. Cosmochim. Acta* 70, 3261–3274.
- Baalousha, M., Kammer, F.V.D., Motelica-Heino, M., Babrowski, M., Hofmeister, C., Le Coustumer, P., 2006. Size-based speciation of natural colloidal particles by flow field flow fractionation, inductively coupled plasma-mass spectroscopy, and transmission electron microscopy/X-ray energy dispersive spectroscopy: colloid-trace element interaction. *Environ. Sci. Technol.* 40, 2156–2162.
- Bakker, E., Pretsch, E., 2001. Potentiometry at trace levels. *Trends Anal. Chem.* 20, 11–19.
- Benedetti, M.F., Milne, C., Kinniburgh, D., van Riemsdijk, W., Koopal, L., 1995. Metal ion binding to humic substances: application of the non ideal competitive adsorption model. *Environ. Sci. Technol.* 29, 446–457.
- Benedetti, M.F., Ranville, J.F., Allard, T., Bednar, A.J., Menguy, N., 2003. The iron status in colloidal matter from the Rio Negro, Brasil. *Colloids Surf. A* 217, 1–9.
- Beneš, P., Steinnes, E., 1974. *In situ* dialysis for the determination of the state of trace elements in natural waters. *Water Res.* 8, 947–953.
- Borg, H., Andersson, P., Johansson, K., 1989. Influence of acidification on metal fluxes in Swedish forest lakes. *Sci. Total Environ.* 87 (88), 241–253.
- Buffle, J., Tercier-Waeber, M.-L., 2005. Voltammetric environmental trace-metal analysis and speciation: from laboratory to in situ measurements (review). *Trends Anal. Chem.* 24, 172–191.
- Buffle, J., van Leeuwen, H.P., 1992. *Environmental Particles*. Lewis Publishers.
- Byrne, R.H., Luo, Y.R., 2000. Direct observations of nonintegral hydrous ferric oxide solubility products: $K'_{50} = [\text{Fe}^{3+}][\text{H}^+]^{-2.86}$. *Geochim. Cosmochim. Acta* 64, 1873–1877.
- Byrne, R.H., Yao, W., Luo, Y.-R., Wang, B., 2005. The dependence of Fe(III) hydrolysis on ionic strength in NaCl solutions. *Mar. Chem.* 97, 34–48.
- Carignan, R., Rapin, F., Tessier, A., 1985. Sediment porewater sampling for metal analysis: a comparison of techniques. *Geochim. Cosmochim. Acta* 49, 2493–2497.
- Carpita, N., Sabularse, D., Montezinos, D., Delmer, D., 1979. Determination of the pore size of cell walls of living plant cells. *Science* 205, 1144–1147.
- Chang Chien, S., Wang, M., Huang, C., 2006. Reactions of compost-derived humic substances with lead, copper, cadmium, and zinc. *Chemosphere* 64, 1353–1361.
- Chen, Y.-W., Buffle, J., 1996. Conditions for the physico-chemical and microbial preservation of colloid characteristics of water samples. II. Physicochemical and microbial evolution. *Water Res.* 30, 2185–2192.
- Dahlgvist, R., Benedetti, M.F., Andersson, K., Turner, D., Larsson, T., Stolpe, B., Ingri, J., 2004. Association of calcium with colloidal particles and speciation of calcium in the Kalix and Amazon rivers. *Geochim. Cosmochim. Acta* 68, 4059–4075.
- Dahlgvist, R., Andersson, K., Ingri, J., Larsson, T., Stolpe, B., Turner, D., 2007. Temporal variations of colloidal carrier phases and associated trace elements in a boreal river. *Geochim. Cosmochim. Acta* 71, 5339–5354.
- Davies, J., Jenkins, A., Monteith, D., Evans, C., Cooper, D., 2005. Trends in surface water chemistry of acidified UK Freshwaters, 1988–2002. *Environ. Pollut.* 137, 27–39.
- Dia, A., Gruau, G., Olivé-Lauquet, G., Riou, C., Molénat, J., Curmi, P., 2000. The distribution of rare earth elements in groundwaters: assessing the role of source-rock composition, redox changes and colloidal particles. *Geochim. Cosmochim. Acta* 64, 4131–4151.
- Doucet, F.J., Lead, J.R., Santschi, P.H., 2007. Colloid-trace element interactions in aquatic systems. In: Wilkinson, K.J., Lead, J.R. (Eds.), *Environmental Colloids and Particles. Behaviour, separation and characterization*. J. Wiley and Sons Ltd., pp. 95–157.
- Dudal, Y., Gérard, F., 2004. Accounting for natural organic matter in aqueous chemical equilibrium models: a review of the theories and applications. *Earth-Sci. Rev.* 66, 199–216.
- Evans, C., Monteith, D., Cooper, D., 2005. Long-term increases in surface water dissolved organic carbon: observations, possible causes and environmental impacts. *Environ. Pollut.* 137, 55–71.
- Filella, M., 2007. Colloidal properties of submicron particles in natural waters. In: Wilkinson, K.J., Lead, J.R. (Eds.), *Environmental Colloids and Particles. Behaviour, separation and characterization*. J. Wiley and Sons Ltd., pp. 17–93.
- Gimpel, J., Zhang, H., Davison, W., Edwards, A., 2003. In situ trace metal speciation in lake surface waters using DGT, dialysis, and filtration. *Environ. Sci. Technol.* 37, 138–146.
- Gordon, A.S., Dyer, B.J., Kango, R.A., Donat, J.R., 1996. Copper ligands isolated from estuarine waters by immobilized metal affinity chromatography: temporal variability and partial characterization. *Mar. Chem.* 53, 163–172.
- Gustafsson, J. WinHumicV for Win95/98/NT, 1999. <<http://amov.ce.kth.se/people/gustafjp/winhumicv.htm>>. A Windows Version of MINTEQA2 Website, Gustafsson, J.; <<http://www.lwr.kth.se/English/OurSoftware/vminaq/index.htm>>.
- Gustafsson, O., Gschwend, P.M., 1997. Aquatic colloids: concepts, definitions, and current challenges. *Limnol. Oceanogr.* 42, 519–528.
- Huerta-Diaz, M.A., Rivera-Duarte, I., Sañudo-Wilhelmy, S.A., Flegler, R., 2007. Comparative distributions of size fractionated metals in pore waters sampled by in situ dialysis and whole-core sediment squeezing: implications for diffusive flux calculations. *Appl. Geochem.* 22, 2509–2525.
- Ingri, J., Widerlund, A., Land, M., Gustafsson, O., Andersson, P.S., Öhlander, B., 2000. Temporal variations in the fractionation of the rare earth elements in a boreal river; the role of colloidal particles. *Chem. Geol.* 166, 23–45.
- Jansen, B., Kotte, M.C., van Wijk, A.J., Verstraten, J.M., 2001. Comparison of diffusive gradients in thin films and equilibrium dialysis for the determination of Al, Fe(III) and Zn complexed with dissolved organic matter. *Sci. Total Environ.* 277, 45–55.
- Johannesson, K.H., Tang, J., Daniels, J.M., Bounds, W.J., Burdige, D.J., 2004. Rare earth element concentrations and speciation in organic-rich blackwaters of the Great Dismal Swamp, Virginia, USA. *Chem. Geol.* 209, 271–294.
- Kretzschmar, R., Schafer, T., 2005. Metal retention and transport on colloidal particles in the environment. *Elements* 1, 205–210.
- Lead, J.R., Davison, W., Hamilton-Taylor, J., Buffle, J., 1997. Characterizing colloidal material in natural waters. *Aquat. Geochem.* 3, 213–232.
- Lead, J.R., Wilkinson, K.J., Balnois, E., Cutak, B.J., Larive, C.K., Beckett, R., 2000. Diffusion coefficients and polydispersities of the Suwannee River Fulvic Acid: comparison of fluorescence correlation spectroscopy, pulsed-field gradient nuclear magnetic resonance, and flow field-flow fractionation. *Environ. Sci. Technol.* 34, 3508–3513.
- Lofts, S., Tipping, E., Hamilton-Taylor, J., 2008. The chemical speciation of Fe(III) in freshwaters. *Aquat. Geochem.* 14, 337–358.
- Lyvén, B., Hasselöv, M., Turner, D.R., Haraldsson, C., Andersson, K., 2003. Competition between iron- and carbon-based colloidal carriers for trace metals in a freshwater assessed using flow field-flow fractionation coupled to ICPMS. *Geochim. Cosmochim. Acta* 67, 3791–3802.
- Magaritz, M., Wells, M., Amiel, A.J., Ronen, D., 1989. Application of a multi-layer sampler based on the dialysis cell technique for the study of trace metals in groundwater. *Appl. Geochem.* 4, 617–624.
- Marsac, R., Davranche, M., Gruau, G., Dia, A., 2010. Metal loading effect on rare earth element binding to humic acid: experimental and modelling evidence. *Geochim. Cosmochim. Acta* 74, 1749–1761.
- Milne, C.J., Kinniburgh, D.G., van Riemsdijk, W.H., Tipping, E., 2003. Generic NICA-donnan model parameters for metal-ion binding by humic substances. *Environ. Sci. Technol.* 37, 958–971.
- Morel, F.M.M., Hering, J.G., 1983. *Principles and Applications of Aquatic Chemistry*. Wiley Interscience.
- Neal, C., Lofts, S., Evans, C.D., Reynolds, B., Tipping, E., Neal, M., 2008. Increasing iron concentrations in UK upland waters. *Aquat. Geochem.* 14, 263–288.
- Olivé-Lauquet, G., Allard, T., Benedetti, M., Muller, J.-P., 1999. Chemical distribution of trivalent iron in riverine material from a tropical ecosystem: a quantitative EPR study. *Water Res.* 33, 2726–2734.
- Pédrot, M., Dia, A., Davranche, M., Bouhnik-Le Coz, M., Henin, O., Gruau, G., 2008. Insights into colloid-mediated trace element release at the soil/water interface. *J. Colloid Interf. Sci.* 325, 187–197.
- Pokrovsky, O., Schott, J., 2002. Iron colloids/organic matter associated transport of major and trace elements in small boreal rivers and their estuaries (NW Russia). *Chem. Geol.* 190, 141–179.
- Pokrovsky, O.S., Dupré, B., Schott, J., 2005. Fe–Al–organic colloids control of trace elements in peat soil solutions. *Aquat. Geochem.* 11, 241–278.
- Pokrovsky, O.S., Schott, J., Dupré, B., 2006. Trace element fractionation and transport in boreal rivers and soil porewaters of permafrost-dominated basaltic terrain in Central Siberia. *Geochim. Cosmochim. Acta* 70, 3239–3260.
- Pokrovsky, O.S., Viers, J., Shirokova, L.S., Shevchenko, V.P., Filipov, A.S., Dupré, B., 2010. Dissolved, suspended, and colloidal fluxes of organic carbon, major and trace elements in Severnaya Dvina River and its tributary. *Chem. Geol.* 273, 136–149.
- Pokrovsky, O.S., Shirokova, L.S., Kirpotin, S.N., Audry, S., Viers, J., Dupré, B., 2011. Effect of permafrost thawing on the organic carbon and metal speciation in thermokarst lakes of western Siberia. *Biogeosciences* 8, 565–583.
- Pokrovsky, O.S., Shirokova, L.S., Zabelina, S.A., Vorobieva, T.Y., Moreva, O.Y., Klimov, S.I., Chupakov, A.V., Shorina, N.V., Kokryatskaya, N.M., Audry, S., Viers, J., Zoutien, C., Freydier, R., 2012. Size fractionation of trace elements in a seasonally stratified boreal lake: control of organic matter and iron colloids. *Aquat. Geochem.* 18, 115–139.
- Reuss, J., Cosby, B., Wright, R., 1987. Chemical processes governing soil and water acidification. *Nature* 329, 27–32.
- Rose, J., Vilge, A., Olivé-Lauquet, G., Masion, A., Frechou, C., Bottero, J.-Y., 1998. Iron speciation in natural organic matter colloids. *Colloid. Surf. A: Physicochem. Eng. Asp.* 136, 11–19.
- Savenko, A.V., 1996a. Coprecipitation of uranium with iron(III) hydroxide formed in sea water by oxidation of iron (II). *Geochem. Int.* 33 (10), 1–9.
- Savenko, A.V., 1996b. Aluminium behaviour in mixing submarine hydrothermal and seawater solutions: results of experimental modelling. *Oceanology* 36, 692–697.
- Savenko, A.V., 2001. Coprecipitation of manganese, copper, zinc, lead and cadmium with iron hydroxide in hydrothermal plumes (by the data of laboratory modeling). *Oceanology* 41, 502–507.
- Savenko, A.V., Pokrovsky, O.S., 2007. Coprecipitation of hydrolyzate elements with iron (III) hydroxide in submarine hydrothermal plumes. In: *Proceedings of XVII International Scientific Conference on Marine Geology "Geology of Seas and Oceans"*, vol. II. GEOS, Moscow; pp. 74–76.
- Shirokova, L.S., Pokrovsky, O.S., Viers, J., Klimov, S.I., Moreva, O.Y., Zabelina, S.A., Vorobieva, T.Ya., Dupré, B., 2010. Diurnal variations of trace elements and heterotrophic bacterioplankton concentration in a small boreal lake of the White Sea basin. *Ann. Limnol. Int. J. Limnol.* 46, 67–75. <http://dx.doi.org/10.1051/limn/2010011>.
- Sigg, L., Black, F., Buffle, J., Cao, J., Cleven, R., Davison, W., Galceran, J., Gunkel, P., Kalis, E., Kistler, D., Martin, M., Noël, S., Nur, Y., Odzak, N., Puy, J., Van Riemsdijk, W., Temminghoff, E., Tercier-Waeber, M.L., Toepferwien, S., Town, R.M., Unsworth, E., Warnken, K.W., Weng, L., Xue, H., Zhang, H., 2006. Comparison

- of analytical techniques for dynamic trace metal speciation in natural freshwaters. *Environ. Sci. Technol.* 40, 1934–1941.
- Skjelkvåle, B.L., Stoddard, J.L., Andersen, T., 2001. Trends in surface water acidification in Europe and North America (1989–1998). *Water Air Soil Pollut.* 130, 787–792.
- Stolpe, B., Hassellöv, M., 2007. Changes in size distribution of fresh water nanoscale colloidal matter and associated elements on mixing with seawater. *Geochim. Cosmochim. Acta.* 71, 3292–3301.
- Stolpe, B., Hassellöv, M., 2010. Nanofibrils and other colloidal biopolymers binding trace elements in coastal seawater: Significance for variations in element size distributions. *Limnol. Oceanogr.* 55, 187–202.
- Stolpe, B., Guo, L., Shiller, A.M., Hassellöv, M., 2010. Size and composition of colloidal organic matter and trace elements in the Mississippi River, Pearl River and the northern Gulf of Mexico, as characterized by flow field-flow fractionation. *Mar. Chem.* 118, 119–128.
- Tipping, E., 1994. WHAM – a chemical equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site/electrostatic model of ion-binding by humic substances. *Comput. Geosci.* 20, 973–1023.
- Tipping, E., Rey-Castro, C., Bryan, S.E., Hamilton-Taylor, J., 2002. Al(III) and Fe(III) binding by humic substances in freshwaters, and implications for trace metal speciation. *Geochim. Cosmochim. Acta* 66, 3211–3224.
- Trias, J., Jarlier, V., Benz, R., 1992. Porins in the cell wall of mycobacteria. *Science* 258, 1479–1481.
- Unsworth, E.R., Warnken, K.W., Zhang, H., Davison, W., Black, F., et al., 2006. Model predictions of metal speciation in freshwaters compared to measurements by in situ techniques. *Environ. Sci. Technol.* 40, 1942–1949.
- van Leeuwen, H., Cleven, R., Buffle, J., 1989. Voltammetric techniques for complexation measurements in natural aquatic media: role of the size of macromolecular ligands and dissociation kinetics of complexes. *Pure Appl. Chem.* 61, 255–274.
- van Leeuwen, H.P., Town, R.M., Buffle, J., Cleven, R.F.M.J., Davison, W., Puy, J., van Riemsdijk, W.H., Sigg, L., 2005. Dynamic speciation analysis, and bioavailability of metals in aquatic systems. *Environ. Sci. Technol.* 39, 8545–8556.
- Vasyukova, E., Pokrovsky, O.S., Viers, J., Oliva, P., Dupré, B., Martin, F., Candaudap, F., 2010. Trace elements in organic- and iron-rich surficial fluids of the boreal zone: assessing colloidal forms via dialysis and ultrafiltration. *Geochim. Cosmochim. Acta* 74, 449–468.
- Vesely, J., Majer, V., Kučera, J., Havránek, V., 2001. Solid–water partitioning of elements in Czech freshwaters. *Appl. Geochem.* 16, 437–450.
- Wilkinson, K.J., Joz-Roland, A., Buffle, J., 1997. Different roles of pedogenic fulvic acids and aquagenic biopolymers on colloid aggregation and stability in freshwaters. *Limnol. Oceanogr.* 42, 1714–1724.
- Wu, F., Tanoue, E., 2001. Isolation and partial characterization of dissolved copper-complexing ligands in streamwaters. *Environ. Sci. Technol.* 35, 3646–3652.
- Xue, H.B., Sigg, L., 1993. Free cupric ion concentration and Cu(II) speciation in a eutrophic lake. *Limnol. Oceanogr.* 38, 1200–1213.
- Zakharova, E., Pokrovsky, O.S., Dupré, B., Gaillardet, J., Efimova, L., 2007. Chemical weathering of silicate rocks in Karelia region and Kola peninsula, NW Russia: assessing the effect of rock composition, wetlands and vegetation. *Chem. Geol.* 242, 255–277.