Complexation of Dissolved Organic Matter with Trace Metal Ions in Natural Waters

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1 Introduction

Complexation of the metal ions (M) with dissolved organic matter (DOM), i.e. M-DOM formation is of fundamental importance in metal ion chemistry and can control the occurrence of free toxic metals, the transport or migration of metals, acid–base balance and solubility in water, occurrence of the photo-Fenton reaction in surface water, biological effects, the bioavailability and toxicity to organisms in water, sediment and soil environments. DOM in natural waters can either enhance or decrease metal transport and affect the bioavailability of metals depending on its composition. DOM is a complex mixture of organic compounds of allochthonous

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and autochthonous origin. Allochthonous organic substances are generally derived from terrestrial plant material in soil ecosystems (and may reach the aquatic environmental because of soil leaching and runoff), while autochthonous organic substances are produced mostly from algae and phytoplankton within the water column. The contribution of fulvic and humic acids (terrestrial humic substances) accounts for the most of the dissolved organic carbon (DOC), approximately 20–85 % in rivers and 14–90 % in lakes (Moran et al. 1991; Moran and Hodson 1994; Malcolm 1985; Hedges et al. 1997; Ma et al. 2001; Mostofa et al. 2009a). In contrast, they account for a relatively minor fraction in oceans, approximately 1–35 % except the shelf (see also chapter "Dissolved Organic Matter in Natural Waters") (Moran et al. 1991; Moran and Hodson 1994; Malcolm 1985; Hedges et al. 1997; Ma et al. 2001; Mostofa et al. 2009a). They play an important role on DOM physical, chemical and biological characteristics in the aquatic environments.

On the other hand, autochthonous DOM of algal, phytoplankton or bacterial origin is generally composed of autochthonous fulvic acids, carbohydrates, amino acids, proteins, lipids, organic acids and so on (Mostofa et al. 2009a; Coble 1996; Parlanti et al. 2000; Tanoue 2000; Jennings and Steinberg 1994; Yamashita and Tanoue 2003; Wada et al. 2007; Hulatt et al. 2009; Mostofa and Sakugawa 2009; Zhang et al.2009). Phytoplankton is capable of releasing 10–60 % of the carbon and 15–50 % of the nitrogen assimilated during photosynthesis in natural waters (Sundh 1992; Bronk et al. 1994; Braven et al. 1995; MalinskyRushansky and Legrand 1996; Slawyk et al. 1998, 2000). The autochthonous DOM in the surface waters is significantly high, by approximately 0–102 % in lakes and 0–194 % in oceans estimated compared to the deeper waters during the summer stratification period (Mostofa et al. 2009a; see also DOM chapter). Therefore, autochthonous DOM plays an important role in M-DOM complexation, particularly in the surface layer of lakes, estuaries, wetlands and oceans. Extracellular polymeric substances (EPS) are produced by many microorganisms in natural waters and are mainly composed of polysaccharides, proteins, uronic acids, lipids and so on (Beech and Sunner 2004; Quiroz et al. 2006; Pal and Paul 2008; Merroun and Selenska-Pobell 2008; Zhang et al. 2008, 2010).

Most of the DOM components, such as allochthonous fulvic and humic acids of vascular plant origin, autochthonous fulvic acids of algal or phytoplankton origin, proteins, aromatic amino acids (tryptophan, tyrosine and phenylalanine), extracellular polymeric substances (EPS) and so on, show fluorescence properties and can simultaneously exhibit complexing properties (Saar and Weber 1980; Ryan and Weber 1982a; Cabaniss 1992; daSilva et al. 1996, 1998a; Smith and Kramer 1998, 1999; Mounier et al. 1999; Wu and Tanoue 2001a; Wu et al. 2004a; Dudal et al. 2006; Manciulea et al. 2009, 2011). DOM components are composed of diverse functional groups (including fluorophores or chromophores) in their molecular structures, which have strong binding capacity with metals in water (Malcolm 1985; Mostofa et al. 2009a; Saar and Weber 1980; Senesi 1990; Morel and Hering 1993; Morra et al. 1997; Xia et al. 1998; Leenheer et al. 1998; Bloom et al. 2001; Steelink 2002; Leenheer and Croue 2003; Stenson et al. 2003; Schwartz et al. 2004; Klinck et al. 2005). Therefore, it has been possible to assess the complexing properties of fluorescent DOM from the fluorescence quenching upon complexation between various DOM components and metal ions.

The M-DOM interaction is generally estimated by determining the conditional stability constant, which significantly depends on several important factors in aqueous solution such as contents, nature and molecular size of DOM (Nair and Chander 1983; Kim et al. 1990; Wu et al. 2004b; Sonke and Salters 2006; Shcherbina et al. 2007; Konstantinou et al. 2009; Vidali et al. 2009); effect of pH (Zhang et al. 2009, 2010; Ryan and Weber 1982a; Sonke and Salters 2006; Shcherbina et al. 2007; Christoforidis et al. 2010; Iskrenova-Tchoukova et al. 2010; Liu and Cai 2010); effect of ions (cations and anions) and ionic strength (Sonke and Salters 2006; Christoforidis et al. 2010; Iskrenova-Tchoukova et al. 2010; Smith and Martell 1987; Cabaniss and Shuman 1988; Fu et al. 2007); effect of photoinduced processes (Zhang et al. 2009; Wu et al. 2004a; Bergquist and Blum 2007; Brooks et al. 2007; Yang and Sturgeon 2009; Sanchez-Marin et al. 2010; Vidali et al. 2010; Yin et al. 2010); effect of microbial processes (Bergquist and Blum 2007; Yin et al. 2010; Tabak et al. 2005; Francis and Dodge 2008; Fletcher et al. 2010), and finally effects of freshwater and seawater (Wu et al. 2004a, c; Fu et al. 2007; Harper et al. 2008). M-DOM complexation plays a vital role in the toxicity and bioavailability of heavy metals in the environment (Christoforidis et al. 2010; Winner 1985; Stackhouse and Benson 1988; Jiang et al. 2009). Optically darker DOM (or deep lake and marine DOM) has higher metal binding capability and typically tends to decrease metal toxicity to fish and algae in natural waters (Schwartz et al. 2004; Winch et al. 2002; Luider et al. 2004). Ternary complexes between organic ligands and trace metal ions and their relative stability constants may pave the way to find out high variations in conditional stability constants of binary complexes of DOM in aqueous solution (Martin and Prados 1974; Khalil and Radalla 1998; Khalil and Attia 1999, 2000; Khalil 2000a, b; Khalil and Taha 2004; Khalil and Fazary 2004; Radalla 2010; Rosas et al. 2010). A review by Hays et al. (2004) on the determination of conditional stability constants and ligand concentrations of fulvic acid with metal ions covers the Stern–Volmer equation for non-linear relationship, the differences between linear and non-linear relationship of formed complex and fluorescence quenching, and finally the Modified Multisite Stern–Volmer Equation. A review by Mostofa et al. (2011) summarizes the studies into metal ion complexation with various organic substances, and discusses about the possible mechanisms behind the quenching or enhancement in metal-DOM complexation in waters.

This chapter will give a general overview on the complexation of metal ions (M) with DOM (M-DOM), fluorescence characteristics of M-DOM complexes, theories for binary and ternary complexes as well as their conditional stability constants in aqueous solution, and finally the binding sites (or functional groups or flurophores or chromophores) in various DOM components. A mechanism will be provided on M-DOM complexation, with use of tryptophan, a well known molecularly characterized fluorescent DOM component, as a model to understand the complexing properties. This review will discuss the key factors affecting the M-DOM complexation and the importance of the M-DOM complexation in waters.

2 Complexation of Metal Ions (M) With DOM (M-DOM) in Natural Waters

Dissolved organic matter (DOM) is composed of a variety of organic compounds, which are the most important sources of organic ligands that can form complexes with trace metal ions in natural surface water, soil, sediment pore water and groundwater (Wu et al. 2004a, b, c; Christoforidis et al. 2010; Liu and Cai 2010; Lu and Allen 2002; Filella 2008; Yamashita and Jaffe 2008; Schmeide and Bernhard 2009; Reiller and Brevet 2010; Sachs et al. 2010; Weng et al. 2010; Tserenpil and Liu 2011). The most important organic ligands in DOM that can form complexes with metal ions are allochthonous fulvic acids (Sonke and Salters 2006; Wu et al. 2002a, b, 2004c; Schmeide and Bernhard 2009; Reiller and Brevet 2010; Vlassopoulos et al. 1990; Mandal et al. 1999; 2000; Sekaly et al. 1999, 2003; Shin et al. 2001; Haitzer et al. 2003; Cao et al. 2004; Fujii et al. 2008); allochthonous humic acids (Sonke and Salters 2006; Shcherbina et al. 2007; Christoforidis et al. 2010; Liu and Cai 2010; Wu et al. 2004c; Reiller and Brevet 2010; Sachs et al. 2010; Tserenpil and Liu 2011; Haitzer et al. 2003; Cao et al. 2004; Takahashi et al. 1997; Buschmann and Sigg 2004; Filella and May 2005; Lippold and Lippmann-Pipke 2009; Pourret and Martinez 2009; Tella and Pokrovski 2009; Kim and Czerwinski 1996); hydrophobic acids extracted from natural waters (Haitzer et al. 2002, 2003); hydrophylic acids extracted from olive cake (Konstantinou et al. 2009); autochthonous DOM (ca. autochthonous fulvic acids or marine fulvic acids) of algal origin (see also chapters "Dissolved Organic Matter in Natural Waters" and "Fluorescent Dissolved Organic Matter in Natural Waters") (daSilva et al. 1996; Xue and Sigg 1993; Xue et al. 1995); tryptophan amino acid (Wu and Tanoue 2001a, b); glycine and other amino acid having O- and N-functional groups (carboxyl, alcoholic hydroxyl, phenolic hydroxyl and amine) (Tella and Pokrovski 2009); cysteine, histidine, aspartic acid, glutamic acid (Rosas et al. 2010; Santana-Casiano et al. 2000; Shoukry 2005; Shiozawa et al. 2011); picolinic acid and dipicolinic acid (Rosas et al. 2010; Shiozawa et al. 2011; Lubes et al. 2010; Da Costa et al. 2011); protein, peptide and selenoprotein P (Shoukry 2005; Sidenius et al. 1999; Motson et al. 2004); glycoprotein exopolymer produced by *Pseudoalteromonas* sp. strain (Gutierrez et al. 2008); extracellular polymeric substances (Zhang et al.2009; Harper et al. 2008; Guibaud et al. 2004, 2006; Comte et al. 2008; d'Abzac et al. 2010); melanin (Felix et al. 1978); 2,4-diiodo-6-(((2- pyridinylmethyl)amino) methyl)phenol (Frezza et al. 2009); indolo[3,2-*c*]quinolines (Primik et al. 2010); 2-[4-dimethylaminocinnamalamino]-benzoic acid (Yalcin et al. 1998); 8-hydroxyquinoline (oxine) (Xia et al. 1996; Sarmiento et al. 2010); thenoyltrifluoroacetone (HTTA) (Xia et al. 1996); bipyridyl and 2,2′-bipyridine (Sarmiento et al. 2010; Bhattacharyya et al. 2010); 1,10-phenanthroline (Xia et al. 1996; Sarmiento et al. 2010; Bhattacharyya et al. 2010); phenols (e.g., catechol, hydroquinone, resorcinol) and polyphenols (Shcherbina et al. 2007; Tella and Pokrovski 2009; Thakur et al. 2006); quinonoid compounds (Shcherbina et al. 2007); benzylmalonic acid and n-hexadecylmalonic acid (Palmer et al. 1998); bis (2,4,4-trimethylpentyl) dithiophosphinic acid (Bhattacharyya et al. 2010); 2-(aminomethyl)-benzimidazole (El-Sherif 2010); α-isosaccharinic acid and α-isosaccharinate (Vercammen et al. 2001; van Loon et al. 2004; Warwick et al. 2003, 2004); citrate (Fujii et al. 2008); acetate (Saito et al. 2010); phthalic and salicylic acids (Panak et al. 1995); salicylate and thiosalicylate (Vlassopoulos et al. 1990); ethylenediaminetetraacetic acid (EDTA) (Fu et al. 2007; Fujii et al. 2008); galacturonic acid (Harper et al. 2008); gluconic acid (Warwick et al. 2003, 2004); aminopolycarboxylic acids (Smith and Martell 1987); and finally O-bearing organic compounds such as carboxylic acids (acetic, adipic, succinic, malic, malonic, maleic, lactic, oxalic, tartaric, citric and ortophosphoric acids) (Francis and Dodge 2008; Radalla 2010; Filella and May 2005; Tella and Pokrovski 2009; Shoukry 2005; Da Costa et al. 2011; El-Sherif 2010). Besides these organic ligands, the gill membrane in fish can form complexes with metal ions such as Ag^+, Ca^{2+}, Cu^{2+}, Cd^{2+}, and Co^{2+} (Playle et al. 1993; Janes and Playle 1995; Richards and Playle 1998; Playle 1998; Tao et al. 2002). In addition, algae can complex or uptake trace metals either directly or in the presence of humic acid that can enhance the metals uptake substantially (Zhou and Wangersky 1985, 1989; Xue and Sigg 1990; Koukal et al. 2003; Mylon et al. 2003; Lamelas and Slaveykova 2007; Lamelas et al. 2009).

The studied trace metal ions that form M-DOM complexes are the transition metals [Sc^{3+}, Y^{3+}, V^{2+} or $VO_2{}^+$, Cr^{2+} or Cr^{3+}, Mn^{2+}, Fe (Fe^{2+} or Fe^{3+}), Co^{2+}, Ni^{2+}, Cu^{2+}, Au^+, Mo^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}]; lanthanides [Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu, hereafter Ln^{3+}]; actinides [Th^{4+}, U^{4+}, $UO_2{}^{2+}$, Np (Np^{4+}, Np^{5+}), $NpO_2{}^+$, Pu^{3+}, Am^{3+}, Cm^{3+}]; metal/metalloid [Al^{3+}, Ga^{3+}, As^{3+}, Sb^{3+}, Sb^{5+}, Tl^{3+}, Sn^{2+}, Pb^{2+}], as well as the alkali/alkaline earth elements [H^+, Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}] (Nair and Chander 1983; Cabaniss and Shuman 1988; Vlassopoulos et al. 1990; Cabaniss 1992; Panak et al. 1995; Xia et al. 1996; Bidoglio et al. 1997; Takahashi et al. 1997; Kaiser 1998; Murphy et al. 1999; Sekaly et al. 1999; Wu et al. 2004a, b; Kautenburger et al. 2006; Sonke and Salters 2006; de Zarruk et al. 2007; Fu et al. 2007; Reszat and Hendry 2007; Shcherbina et al. 2007; Lippold and Lippmann-Pipke 2009; Pourret and Martinez 2009; Schmeide and Bernhard 2009; Vidali et al. 2009; Bhattacharyya et al. 2010; Christoforidis et al. 2010; Reiller and Brevet 2010; Saito et al. 2010; Tserenpil and Liu 2011). Any given metal ion in the natural environment may potentially be found in many diverse forms, namely, 'free' (hydrated), complexed by 'simple' inorganic or organic ligands, complexed by ligand atoms which are part of the structure of naturally occurring macromolecules or colloids, and adsorbed on suspended organic or inorganic particles or living organisms (Huber et al. 2002; Filella et al. 2007).

Heavy metals (e.g. Cd^{2+}, Pb^{2+}, and Sr^{2+}) show strong interaction with humic acids (HA) in forming M-DOM complexes, leading to formation of covalent bonds with the radicals of humic acids (Christoforidis et al. 2010). Metal partitioning between colloidal (1 μm–1 kDa) and truly dissolved (<1 kDa) fractions is detected to match a decrease of metal toxicity (for Cd and Zn ions) in the presence of humic acid, but not in the presence of Suwannee River Fulvic Acid (Koukal et al. 2003). This suggest that metal-HA complexes are of high molecular weight and relatively stable with regard to metal-exchange reactions, thus the cited metals (Cd and Zn ions) are less bioavailable (Koukal et al. 2003). Chemodynamic modeling suggests that the enhancement of the metal uptake flux in the presence of HA originates from an increasing amount of metal bound to the internalization sites, through ternary complex formation between metal—HA complex and internalization sites (Lamelas et al. 2009). Cell wall speciation calculations indicate that the metal—humic acid complex is the predominant species in the cell wall layer in algae, while for some other metals [e.g. Cu(II) and Cd(II)] the binding to the internalization (Cu) and adsorption (Cd) sites significantly dominates over the M−HA complexes (Lamelas et al. 2009).

2.1 Fluorescence Characteristics of the M-DOM Complexation

Allochthonous fulvic acids of vascular plant origin are generally composed of two fluorescence excitation-emission (Ex/Em) peaks (or maxima) such as the peak C at the peak C-region (280–400/380–550 nm) and the peak A at the peak A-region (215–280/380–550 nm) (see chapter "Fluorescent Dissolved Organic Matter in Natural Waters" for detailed description) (Fig. 1a) (Mostofa et al. 2005, 2009a; Coble 1996; Mostofa and Sakugawa 2009; Mounier et al. 2011; Yamashita and Jaffe 2008; Coble 2007). In contrast, allochthonous humic acids of vascular plant origin are composed of three or more fluorescence Ex/Em peaks such as the peak C at the peak C-region (280–400/380–550 nm) and the peak A at the peak A-region (215–280/380–550 nm) (Fig. 1b; see also chapter "Fluorescent Dissolved Organic Matter in Natural Waters") (Mostofa et al. 2005, 2009a; Mostofa and Sakugawa 2009; Yamashita and Jaffe 2008). Similarly, the fluorescence Ex/Em peaks of autochthonous fulvic acids of algal origin resemble those of allochthonous fulvic acids (Fig. 1c, d) (Parlanti et al. 2000; Mostofa and Sakugawa 2009; Zhang et al. 2009; Stedmon et al. 2007). The tryptophan (or protein-like) component shows two fluorescence peaks such as the peak T at the peak T-region (260–285/290–380 nm) and the peak T_{UV} at the peak T_{UV}-region (215–260/280–380 nm) (Fig. 1e; see chapter "Fluorescent Dissolved Organic Matter in Natural Waters"). The fluorophores or functional groups in fluorescent DOM are susceptible to show their fluorescent properties as well as to interact with metals via complex formation. Therefore, the fluorescence intensity of M-DOM is either enhanced or quenched compared to the original fluorescent DOM (Saar and Weber 1980; Ryan and Weber 1982a; Cabaniss and Shuman 1988; Grimm et al. 1991; Cabaniss 1992; dasilva et al. 1995, 1996, 1997, 1998; Smith and Kramer 1998; Lu and Jaffe 2001; Wu and Tanoue 2001a; Wu et al. 2004a; b; Dudal et al. 2006; Plaza et al. 2006; Fu et al. 2007; Antunes et al. 2007; Ohno et al. 2008; Manciulea et al. 2009, 2011).

Many studies show that complexation of DOM with trace elements often decreases the overall fluorescence intensity of DOM (peak C, peak A, peak T and peak T_{UV}), after addition of metal ions and with increasing their concentration. On the other hand, elements such as Al^{3+}, Be^{3+}, actinides (ca. Cm^{3+}), Ca^{2+} and Mg^{2+} are responsible for an enhancement of the fluorescence intensity of organic ligands (fulvic acid, humic

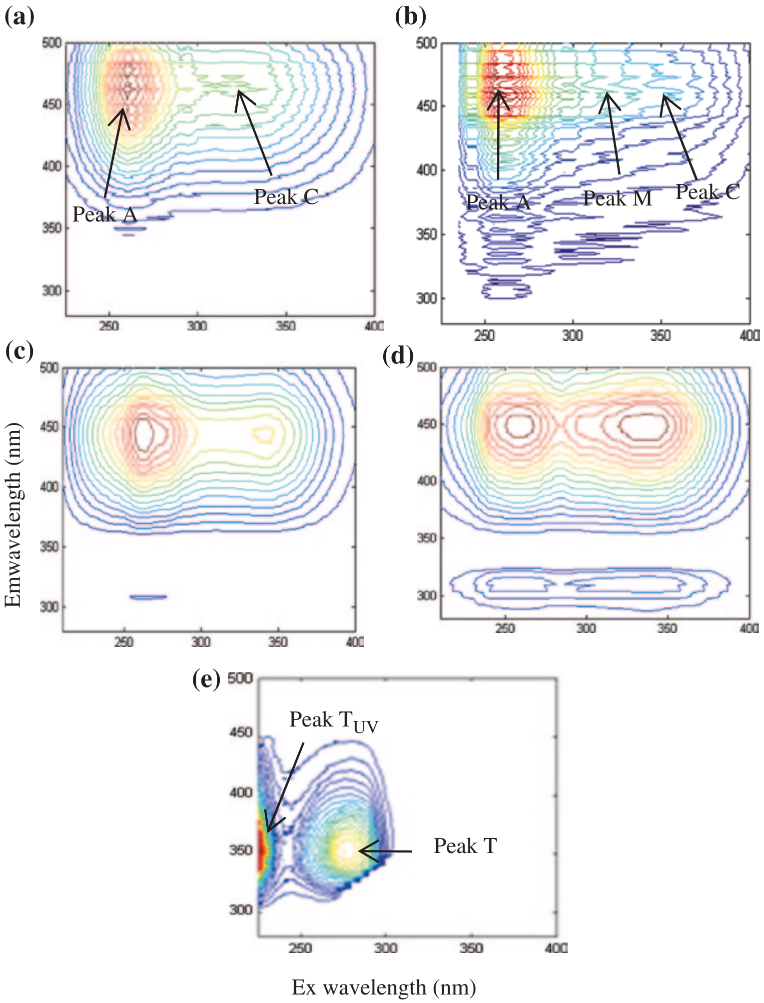

Fig. 1 The fluorescent components of allochthonous Suwannee River fulvic acid standard (C-like, **a**); allochthonous Suwannee River humic acid standard (C-like, **b**), autochthonous fulvic acid (C-like) of microbial assimilation (**c**) and photoinduced assimilation (**d**) of lake algae in aqueous samples (Milli-Q and river waters), and tryptophan standard in Milli-Q waters (**e**) identified using PARAFAC modeling. *Data source* Mostofa and Sakugawa (2009); Mostofa et al. (2009b)

acid, 5-sulfosalicylic acid, and other organic compounds) that is linked with complexation (Cabaniss 1992; Smith and Kramer 1998, 1999; Cabaniss and Shuman 1988; Fu et al. 2007; Panak et al. 1995; dasilva et al. 1995; Ohno et al. 2008; Silva et al. 1994; Lakshman et al. 1993, 1996; Seritti et al. 1994). The increase in fluorescence intensity upon complexation of Cm^{3+} with 5-sulfosalicylic acid, fulvic acid, and humic acid suggests that the salicylic acid-like functional group may present in the molecular structure in allochthonous fulvic and humic acids (Panak et al. 1995). The fluorophores in fluorescent DOM (or functional groups or chromophores) are likely responsible for the formation of complexes with trace elements.

The key fluorophores in allochthonous fulvic and humic acids, tryptophan amino acid and protein in natural waters are composed of Schiff-base derivatives (–N = C–C = C–N–), –COOH, –$COOCH_3$, –OH, –OCH_3, –CH = O, –C = O, –NH_2, –NH–, –SH, –CH = CH–COOH, –OCH_3, –CH_2–(NH_2)CH–COOH, S-, O- or N-containing aromatic compounds, and so on (Malcolm 1985; Mostofa et al. 2009a; Mostofa and Sakugawa 2009; Senesi 1990; Steelink 2002; Leenheer and Croue 2003; Fu et al. 2007; Corin et al. 1996; Peña-Méndez et al. 2005; Seitzinger et al. 2005; Zhang et al. 2005). The complexation of trace elements with fluorescent substances also affects the fluorescence peak position of the respective fluorophore. Usually, both excitation and emission wavelengths of the respective peak position gradually shift toward the longer wavelength with increase in the reaction time (Wu et al. 2004a, c; Plaza et al. 2006). It has also been found that comparison of the EEM spectra before and after binding in metal-DOM complexes shows that the fast binding site in fulvic acid is responsible for 71–87 % of the total fluorescence decrease, while the remainder is associated with the slow binding site (Wu et al. 2004c).

EPSs show two fluorescence peaks (peak T and T_{UV}) in the absence and presence of trace elements such as Hg^{2+}, with the maximal fluorescence intensity at neutral pH (Fig. 2) (Zhang et al. 2010). The excitation-emission matrix (EEM)

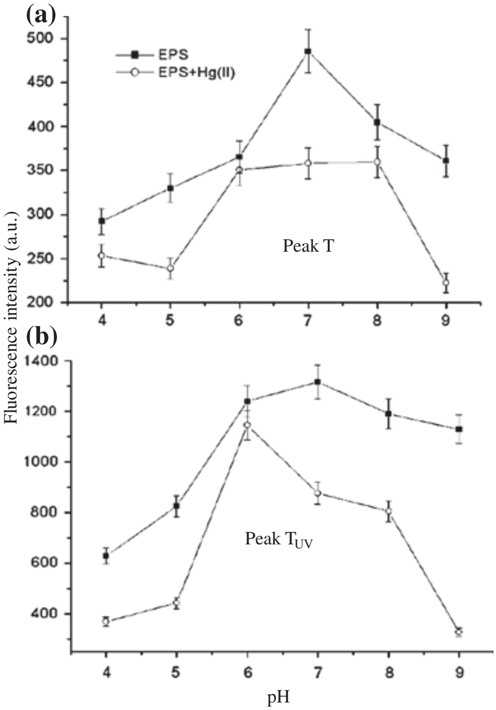

Fig. 2 Changes in the fluorescence intensities of peak T (**a**) and peak T_{UV} (**b**) for extracellular polymeric substances (EPS) with solution pH in the absence and presence of 3.0 mg L^{-1} Hg(II). The error bar indicates the standard deviation of three independent measurements. *Data source* Zhang et al. (2010)

spectra of EPS show that the fluorescence intensity of the peak T_{UV}-region is higher than the peak T-region, which is a similar behavior as tryptophan (Fig. 1e) (Mostofa et al. 2009a; Zhang et al. 2010). The fluorescence intensity of EPS and its complexation with Hg^{2+} significantly varies with pH, and is highest under neutral conditions (Fig. 2; Zhang et al. (2010)).

3 Complexation Theorem

The complexation theorem depends on the linear or non-linear relationship between formed complex and fluorescence quenching (Ryan and Weber 1982a; Cabaniss and Shuman 1988; Hays et al. 2003, 2004; Ryan and Ventry 1990; Cook and Langford 1995). The nonlinearity depends on several factors (Hays et al. 2004; Cook and Langford 1995), which can be distinguished as (i) Sample type, pretreatment, and age of the DOM; (ii) Overlap of fluorophores that typify chemically different complexation sites; (iii) The wavelengths (or scanning method) at which the fluorescence measurements are acquired; (iv) The varied physicochemical microenvironments of the fulvic acid structure as characterized by either a single fluorophore or fluorophore set; (v) Cooperational and molecular configuration changes due to metal loading or ionization state; and (vi) Shielding of binding sites or fluorescence (i.e., controlling photophysical effects). In addition (vii) the fluorescence intensity of fulvic and humic acids either at peak C-region or peak A-region might be the result of various types of fluorophores (functional groups) associated with those substances (Malcolm 1985; Senesi 1990; Steelink 2002). Sequential decomposition of the fluorophores bound at peak C- and A-regions in fulvic and humic acids by photoinduced degradation decreases the fluorescence intensity at the cited peaks during the transport from rivers to lakes and oceans (Mostofa et al. 2005a; b, 2007, 2010; Amador et al. 1989; Malcolm 1990; Allard et al. 1994; Moran et al. 2000; Xie et al. 2004; Li and Crittenden 2009; Minakata et al. 2009). Such changes may affect the nature and contents of fluorophores bound to fulvic and humic acids in various types of natural waters. Such changes are responsible for the variation of the binding capacity of metal ions, thereby exerting a significantly effect on the cited linear or non-linear relationship. The complexation theorem can therefore be expressed in two ways: (1) Theory of metal–ligand complexation based on linearity; and (2) Theory of modified Multisite Stern–Volmer (MSV) equation based on nonlinearity.

3.1 Theory of the M-DOM Complexation Based on Linearity

The fluorescence quenching of an organic ligand (e.g. fulvic acid, termed as L) by complexation with a metal ion (e.g. Cu^{2+}, termed as M) assuming a simple 1:1 metal ligand coordination ratio (Fig. 3) can be expressed in terms of the individual fluorescence of each species, as depicted by Ryan and Weber (1982a, b). The

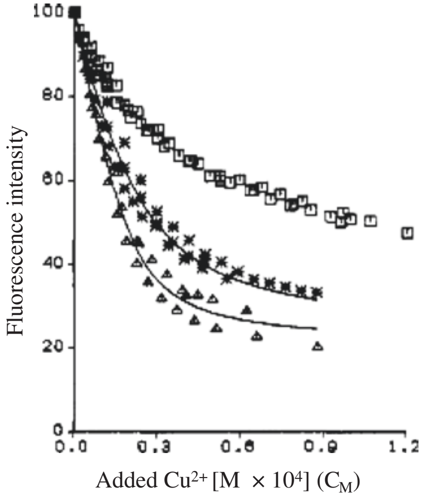

Fig. 3 Fluorescence quenching curves for 10 mg L^{-1} soil fulvic acid with Cu^{2+} in 0.1 M KNO_3 at 25 °C: (*squares*) pH 5, four replicates; (*asterisk*) pH 6, three replicates; (*triangles*) pH 7, three replicates. *Data source* Ryan and Weber (1982a)

concentration of bound metal ion with an organic ligand (ML) is the difference between total and free metal ion concentration. A titration of naturally occurring fulvic acid ligand with a metal ion can allow determination of the stability constant (K) and of the complexing capacity (C_L) of the ligand if a stoichiometry is considered (Hart 1981).

3.2 Theory of Multisite Stern–Volmer Equation for Determination of M-DOM Complexation

The multisite Stern–Volmer (MSV) equation is modified, simulated, validated for predictive capability with a suitable model compound set by Hays and his colleagues (2004), and applied to fluorescence titration data in the complexation of fulvic acid with Cu^{2+} (Fig. 4). The MSV approach assumes a simple 1:1 coordination ratio between Cu^{2+} and the fluorescent ligand components, giving the following reversible solution equilibria: $M + L1 \leftrightarrows ML1$ and $M + L2 \leftrightarrows ML2$ where L1 and L2 are the free ligand species (all forms of metal-free ligand) at sites 1 and 2, respectively, M is free metal, and ML1 and ML2 are the metal-bound species at these sites. Other reaction stoichiometries are possible, which rely on metal loading. For example, at low metal loadings, chelation (2:1 = ligand:metal) may be induced, contorting the fulvic acid shape and affecting fluorescence. Evidences for the effect of these molecular conformation changes on fluorescence are scarce, inconclusive, and not easily quantified.

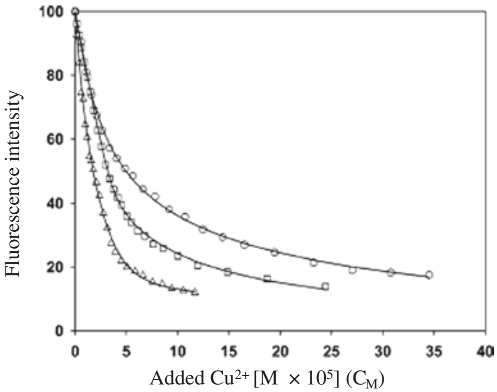

Fig. 4 Multisite Stern–Volmer (MSV)-fitted fluorescence quenching data of soil fulvic acid (15 mg L^{-1}) titrated with Cu^{2+} at 0.1 M ionic strength: (*circles*) pH 5 data; (*squares*) pH 6 data; (*triangles*) pH 7 data; (*solid lines*) MSV predicted intensity values. *Data source* Ryan and Weber (1982a)

3.3 Theory for Conditional Stability Constant of M-DOM Complexation

The conditional stability constant of a M-DOM complex is operationally defined as the binding efficiency of the newly formed bond between the functional group of the DOM component (acting as an organic ligand) and the trace metal ion M, when they are mixed up under specific conditions in aqueous media. Conditional stability constants of a M-DOM complex can be useful to characterize the formed complex, to apply the strong binding capacity of organic substances to control speciation, toxicity, bioavailability and fate of toxic metals used e.g. in industries, and for predicting biological effects of metals in natural water, sediment and soil environments (Shcherbina et al. 2007; Mostofa et al. 2011; Sekaly et al. 2003; Huber et al. 2002; Filella et al. 2007; Hörnström et al. 1984; Hughes et al. 1995; Markich 2002; Matsumoto et al. 2005; Martel and Motekaitis 1988).

A conditional stability constant has been determined by Midorikawa and Tanoue (Appendix A) (Midorikawa and Tanoue 1998), adopting the relationship between measured fluorescence intensity and complexation for a divalent metal ion (M, ca. Cu^{2+}) with organic ligands, and assuming a 1:1 stoichiometry (Ryan and Weber 1982a). The complexing reactions that fit the experimental data can be described by the linear regression program. The relationship between measured fluorescence intensity and complexation can be described as follows (Eq. 3.1) (Ryan and Weber 1982a):

$$X = \frac{[\mathrm{ML}]}{C_\mathrm{L}} = \frac{F_0 - F}{F_0 - F_\mathrm{end}} \tag{3.1}$$

where the quantity [ML]/C is the fraction of the ligand bound to the metal to form the complex ML. Such a fraction can be expressed in terms of the measured fluorescence intensity, F. F_0 and F_end are the limiting intensities before and after metal titration. They correspond to the intensities when all ligands are entirely free and bound, respectively.

The estimated fraction [ML]/C_L is applied to the approach of Ruzic (1982). The conditional stability constants (K'_{ML}) for complex formation between M and the ligand, L, assuming simple 1:1 equilibrium can be written as follows (Eq. 3.2):

$$K'_{\mathrm{ML}} = \frac{[\mathrm{ML}]}{[M] \cdot [\mathrm{L}']} = \frac{[\mathrm{ML} \cdot \alpha_{\mathrm{M}}]}{(C_{\mathrm{M}} - [\mathrm{ML}])\,(C_{\mathrm{L}} - [\mathrm{ML}])} \tag{3.2}$$

where K'_{ML} is the constant with regard to the concentration of free metal ion, [M]; [M′] and [L′] are the total concentrations of all inorganic forms of M and of L unbound to M, respectively; α_{M} is the inorganic side-reaction coefficient for M that is estimated to be $\alpha_{\mathrm{M}} = [\mathrm{M}']/[\mathrm{M}] = 11$ at pH 8.15 under the same conditions (Midorikawa et al. 1990).

Substituting for $[\mathrm{ML}] = C_{\mathrm{L}}\,X$, Eq. (3.2) can be rewritten as (Eq. 3.3)

$$C_{\mathrm{M}} \cdot \left(\frac{1-X}{X}\right) = C_{\mathrm{L}} \cdot (1 - X) + \frac{\alpha_{\mathrm{M}}}{K'_{\mathrm{ML}}} \tag{3.3}$$

By plotting of $C_{\mathrm{M}}\,(1-X)/X$ versus. $(1 - X)$, a linear regression is observed by the least-squares analysis that will give the best-fit values of C_{L} from the slope, and the conditional stability constant K'_{ML} from the intercept. For the nonlinear diagram, two 1:1 complexes by two discrete ligand classes with different stability constants are assumed, which can be treated by another model (van Den Berg 1984).

3.4 Theory for Protonation Constants of DOM in M-DOM Complexation

The protonation constants of organic ligands are estimated from the changes in fluorescence according to the changes in pH with regard to single protonation (Midorikawa and Tanoue 1998). The fluorescence (*F*) of the ligand (L) during the acid–base titration can be expressed by the concentration of each species of the ligand by the molar fluorescence coefficient (ε) as follows (Eqs. 3.4–3.6):

$$F_{\mathrm{H-pH}} = \varepsilon_{\mathrm{L}} C_{\mathrm{L}} \quad \text{at high pH} \tag{3.4}$$

$$F_{\mathrm{L-pH}} = \varepsilon_{\mathrm{HL}} C_{\mathrm{L}} \quad \text{at low pH} \tag{3.5}$$

$$F = \varepsilon_{\mathrm{HL}}[\mathrm{L}] + \varepsilon_{\mathrm{HL}}\,[\mathrm{HL}] \quad \text{at middle pH} \tag{3.6}$$

where the quantities $F_{\text{H-pH}}$ and $F_{\text{L-pH}}$ are the limiting fluorescence intensities at either extreme of the titration: $F_{\text{H-pH}}$ is for the free ligand (L) that is dissociated at high pH; and $F_{\text{L-pH}}$ is for HL that is protonated at low pH.

From the mass balance of the ligand, $C_{\mathrm{L}} = [\mathrm{L}] + [\mathrm{HL}]$, the above equations can be rewritten as follows:

$$F_{\mathrm{H-pH}} - F = (\varepsilon_{\mathrm{L}} - \varepsilon_{\mathrm{HL}})\,[\mathrm{HL}] \tag{3.7}$$

$$F - F_{\mathrm{L-pH}} = (\varepsilon_{\mathrm{L}} - \varepsilon_{\mathrm{HL}})\,[\mathrm{L}] \tag{3.8}$$

From Eqs. (3.7) and (3.8), the fluorescence of the ligand can be expressed in terms of the protonation constant and the proton concentration as follows:

$$\frac{F_{\mathrm{H-pH}} - F}{F - F_{\mathrm{L-pH}}} = \frac{[\mathrm{HL}]}{\mathrm{L}} = K'_{\mathrm{HL}} \cdot [\mathrm{H}] \tag{3.9}$$

Using the logarithm, Eq. (3.9) becomes

$$\log \frac{F_{\mathrm{H-pH}} - F}{F - F_{\mathrm{L-pH}}} = \log K'_{\mathrm{HL}} - \mathrm{pH} \tag{3.10}$$

The plot of $\log(F_{\text{H-pH}}–F)/(F–F_{\text{L-pH}})$ versus pH gives the values of $\log K'_{\mathrm{HL}}$ from the intercept. For the occurrence of a further protonation at lower pH, two protonation constants are operational that can be treated by another model (van Den Berg 1984).

3.5 *Kinetics of M-Fulvic Acid Complexation*

It has been shown that allochthonous fulvic acids are the main DOM components studied in natural waters (Moran et al. 1991; Malcolm 1990; Ma et al. 2001; Mostofa et al. 2009a). Therefore, it is vital to know how they form complexes with metal ions. Changes in the full fluorescence spectral kinetic, i.e., in both Ex/Em wavelengths of the fluorescence maxima occur in the EEM spectra of fulvic acid or DOM during their complexation with trace elements (Wu et al. 2004a, c). The complexation of a fulvic acid (extracted from Cavan Bog, Canada) with several metals (Cu^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+} and Ca^{2+}) at pH 7 shows that the fulvic acid can react rapidly with all metals studied (Wu et al. 2004c). The result of pseudo-first order kinetic plots demonstrates that fulvic acid has two major kinetically distinguishable binding sites, ‘fast’ and ‘slow’, having reaction half-lives of 1.3–3.9 and 34.7–69.3 s, respectively (Wu et al. 2004c). The binding of copper to fulvic acid is found to be fairly rapid, and the reaction is virtually at equilibrium after approximately 20–30 s (Lin et al. 1995). Another study demonstrates that the three lifetimes and emission wavelength maxima for three fluorophores in fulvic acid are as follows: ~50 ps (392 nm), ~430 ps (465 nm), and 4.2 ns (512 nm) (Cook and Langford 1995). Kinetic changes of excitation-emission wavelengths of the fluorescence maxima also suggest the presence of two major binding sites. For the fast-reacting binding site, the rate constant and the site relative contribution are in the order $Cu^{2+} > Ni^{2+} > Co^{2+} > Cd^{2+} > Ca^{2+}$, which agrees with the Irving-Williams series, indicating affinity dependence of complexation kinetics (Wu et al. 2004c). For the fast-reacting binding site in fulvic acid, proteins and other organic ligands, the relative contribution of rate constant for bivalent metal complexes follows the same order (Wu et al. 2002a, 2004c; Sidenius et al. 1999; Irving and Williams 1953; Winzerling et al. 1992). This result implies that metal ions react initially with the fast reacting and strong binding site on fulvic acid; after the fast binding site is entirely occupied, the slow reacting and weak binding site starts to bind with the metal ions (Wu et al. 2004c). In each phase, the red-shifted excitation-emission spectra suggest physical structural changes (ca. molecular conformation or rigidness) of the fulvic acid-metal complex (Wu et al. 2004c). Changes in the molecular conformation and formation of aggregates of fulvic acid upon complexation are also supported by other studies (Hays et al. 2004; Filella 2008; Chakraborty et al. 2007).

Within each kinetic phase, both excitation-emission wavelengths of fluorescence maxima gradually increase with reaction time (Wu et al. 2004a, c). Such red-shifted phenomena are also observed in humic acid extracted from sewerage sludge (Plaza et al. 2006).The kinetics of metal-fulvic acid complexation depends on the concentration; chemical nature and sources of DOM components; temperature; pH; molecular size; occurrence of anions and cations; salinity; and metal affinity (da Silva et al. 1998a; Wu and Tanoue 2001a, b; Wu et al. 2004b, c; Fu et al. 2007; Mostofa et al. 2011; Lu and Jaffe 2001; Lin et al. 1995; Plankey and Patterson 1987). Recent studies show that all protonated and unprotonated forms of both the ligand and the hydrated central metal ion are involved in the formation of the precursor inner- and outer-sphere complexes, which control the kinetics of complex formation/dissociation (van Leeuwen et al. 2007; van Leeuwen and Town 2009a, b). It has also been shown that stronger the affinity of the metal, the greater proportion of strong and fast reacting fulvic acid binding sites are involved in the complexation process. This finding indicates that metal affinity affects both the thermodynamic equilibrium and the reaction kinetics (Wu et al. 2004c).

3.6 *Conditional Stability Constants ($\log_{10}K$) for M-DOM Complexation in Water*

Conditional stability constants ($\log_{10} K$) for M-DOM complexation are presented for various DOM components in water (Table 1) (Zhang et al. 2010; Wu et al. 2001, 2004a; Nair and Chander 1983; Kim et al. 1990; Sonke and Salters 2006; Shcherbina et al. 2007; Konstantinou et al. 2009; Vidali et al. 2009; Liu and Cai 2010; Fu et al. 2007; Brooks et al. 2007; Hays et al. 2004; Cao et al. 2004; Takahashi et al. 1997; Haitzer et al. 2002; Xue and Sigg 1993; Xue et al. 1995; Wu and Tanoue 2001b; Guibaud et al. 2004, 2006; Thakur et al. 2006; van Loon et al. 2004; Plaza et al. 2006; Antunes et al. 2007; Midorikawa and Tanoue 1998; Tipping 1994; Breault et al. 1996; Mcknight et al. 1983; van Den Berg et al. 1987; Sunda and Huntsman 1991; Sunda and Hanson 1987; Sunda and Ferguson 1983; Moffett et al. 1990; Coale and Bruland 1988, 1990; van Den Berg 1984; Midorikawa and Tanoue 1996; Benoit et al. 2001; Buckau et al. 1992; Comte et al. 2006; Drexel et al. 2002; Gasper et al. 2007; Hsu and Sedlak 2003; Kim et al. 1991; Sander et al. 2005; Shank et al. 2006; Smith 1974). Fulvic acids (FA) of different origin can exhibit two major binding sites in the

Table 1 Conditional stability constants ($\log_{10} K$) of the M-DOM complexes in aqueous solution

Elements or groups	Conditional stability constants ($\log_{10} K$)					pH	References
	Fulvic acid[a]	Humic acid[a]	Tryptophan or protein or EPS[a]	Autochthonou DOM or hydrophilic or hydrophobic[a]	Other organic ligands[a]		
Model ligands							
Cu^{2+} with COOH and OH group	–	–	–	–	3.0–6.0	8.15	Martell and Smith (1974)
Cu^{2+} with NH_2 (NH_3)	–	–	–	–	2.8	8.15	Martell and Smith (1974)
Cu^{2+} with ethanol amine	–	–	–	–	4.4	8.15	Martell and Smith (1974)
Cu^{2+} with glycine (NH_2–CH_2–COOH or NH_3^+–CH_2–COO^-)	–	–	–	–	6.6	8.15	Martell and Smith (1974)
Cu^{2+} with ethylene diamine (NH2–CH_2–CH_2–NH_2)	–	–	–	–	8.5	8.15	Martell and Smith (1974)
Cu^{2+} with aspartic acid [HOOC–CH(NH_2)–CH_2–COOH]	–	–	–	–	7.2	8.15	Martell and Smith (1974)
Cu^{2+} with iminodiaacetic acid (HOOC–CH_2–NH_2–CH_2–COOH)	–	–	–	–	9.4	8.15	Martell and Smith (1974)
Cu^{2+} with diaminopropanol	–	–	–	–	8.3	8.15	Martell and Smith (1974)
Cu^{2+} with ethylenediamine-N-acetic acid	–	–	–	–	11.4	8.15	Martell and Smith (1974)
Cu^{2+} with diethylenetriamine	–	–	–	–	13.0	8.15	Martell and Smith (1974)
Cu^{2+} with fulvic acid (modeled)	9.64 (K_1); 3.26 (K_2)	–	–	–	–	?	Tipping (1994)
Cu^{2+} with fulvic acid (modeled)	4.37 (K_2)	–	–	–	–	6	Antunes et al. (2007)
Cu^{2+} with fulvic acid (extraced from soil)	5.30 (K_1)	–	–	–	–	4	Cao et al. (2004)

(continued)

Table 1 (continued)

Elements or groups	Conditional stability constants ($\log_{10} K$)					pH	References
	Fulvic acid[a]	Humic acid[a]	Tryptophan or protein or EPS[a]	Autochthonou DOM or hydrophilic or hydrophobic[a]	Other organic ligands[a]		
Cu^{2+} with fulvic acid (extracted from soil) (Ex/Em = 335/350 nm)	4.78–5.70 (K_1)	–	–	–	–	5.0–7.0	Hays et al. (2004)
Fe^{3+} with fulvic acid (modeled)	5.66 (K_1)	–	–	–	–	4	Antunes et al. (2007)
Hg^{2+} with fulvic acid (modeled)	4.44 (K_1)	–	–	–	–	6	Antunes et al. (2007)
UO_2^{2+} with fulvic acid (modeled)	5.46 (K_1)	–	–	–	–	3.5	Antunes et al. (2007)
Cu^{2+} with humic acid (modeled)	–	4.83 (K_1)	–	–	–	6	Antunes et al. (2007)
Cu^{2+} with humic acid (modeled)	–	8.55 (K_1); 4.02 (K_2)	–	–	–	?	Tipping (1994)
Cu^{2+} with humic acid (extraced from soil)	–	3.99–4.49 (K_1)	–	–	–	4	Cao et al. (2004)
Fe^{3+} with humic acid (modeled)	–	6.79 (K_1)	–	–	–	4	Antunes et al. (2007)
Hg^{2+} with humic acid (modeled)	–	5.50 (K_1)	–	–	–	6	Antunes et al. (2007)
UO_2^{2+} with humic acid (modeled)	–	4.27 (K_1)	–	–	–	3.5	Antunes et al. (2007)
As^{3+} with Aldrich humic acid (modeled)	–	5.8–7.2 (K_1); 4.5–5.3 (K_2)	–	–	–	5.2–9.3	Liu and Cai (2010)
Cu^{2+} with tryptophan (modeled) (Ex/Em = 285/360 nm)	–	–	4.88–4.90 (K_1)	–	–	6	Hays et al. (2004)
Cu^{2+} with glycyl-tryptophan (modeled) ($C_{13}H_{15}N_3O_3$) (Ex/Em = 285/360 nm)	–	–	5.81–6.02 (K_1)	–	–	6	Hays et al. (2004)
Acid/alkali/alkaline earth elements	–	–	–	–	–		

(continued)

Table 1 (continued)

Elements or groups	Conditional stability constants ($\log_{10} K$)					pH	References
	Fulvic acid[a]	Humic acid[a]	Tryptophan or protein or EPS[a]	Autochthonou DOM or hydrophilic or hydrophobic[a]	Other organic ligands[a]		
H^+ with humic acid of various sources	–	7.15–9.80 (K_1); 2.80–4.94 (K_2)	–	–	–		Vidali et al. (2009)
Be^{2+}	–	4.0	–	–	–	7	Takahashi et al. (1997)
Ca^{2+}	–	1.0	–	–	–	7	Takahashi et al. (1997)
Ca^{2+} with alpha-isosaccharinate	–	–	–	–	1.70–1.80		van Loon et al. (2004)
Sr^{2+}	–	0.5	–	–	–	7	Takahashi et al. (1997)
Ba^{2+}	–	0.5	–	–	–	7	Takahashi et al. (1997)
Ga^{3+}	–	10.0	–	–	–	7	Takahashi et al. (1997)
Transition metals							
Cu^{2+} with river DOM/humic substances	7.0–8.1 (K_1); 5.4–6.1 (K_2)	–	–	–	–	?	Breault et al. (1996)
Cu^{2+} with river and canal DOM/humic substances	8.3–8.5 (K_1); 6.0–6.6 (K_2)	–	–	–	–	?	Mcknight et al. (1983)
Cu^{2+} with river fulvic acid (Ex/Em = 315–330/426–434 nm)	7.21–7.31 (K_1)	–	–	–	–	?	Wu et al. (2001)
Cu^{2+}	5.3	–	–	–	–	6	Konstantinou et al. 2009
Cu^{2+} with lake autochthonous fulvic acid (M-like: 310–320/376–386 nm) at 2.5 m	7.84–7.96 (K_1)	–	–	–	–	?	Wu et al. (2001)
Cu^{2+} with lake autochthonous fulvic acid (M-like: 250/414–438 nm) at 2.5 m	7.06–7.67 (K_2)	–	–	–	–	?	Wu et al. (2001)
Cu^{2+} with lake autochthonous fulvic acid (M-like: 310–320/376–386 nm) at 70 m	9.23 (K_1)	–	–	–	–	?	Wu et al. (2001)

(continued)

Table 1 (continued)

Elements or groups	Conditional stability constants ($\log_{10} K$)					pH	References
	Fulvic acid[a]	Humic acid[a]	Tryptophan or protein or EPS[a]	Autochthonou DOM or hydrophilic or hydrophobic[a]	Other organic ligands[a]		
Cu^{2+} with lake autochthonous fulvic acid (M-like: 250/414–438 nm) at 70 m	8.69 (K_2)	–	–	–	–	?	Wu et al. (2001)
Cu^{2+} with lake DOM/humic substances	7.0 (K_1); 5.4 (K_2)	–	–	–	–	?	Mcknight et al. (1983)
Cu^{2+} with fulvic acid (lake)	7.05–8.78 (K_1)	–	–	–	–	?	Wu and Tanoue (2001b)
Cu^{2+} with humic acid, sewage sludge (Ex/Em = 340/438 nm)	–	4.65	–	–	–	8.1	Plaza et al. (2006)
Cu^{2+} with humic acid, soil (Ex/Em = 440/510 nm)	–	5.55	–	–	–	8.1	Plaza et al. (2006)
Cu^{2+} with humic acid, amended soil + sewage sludge (Ex/Em = 440/510 nm)	–	5.36	–	–	–	8.1	Plaza et al. (2006)
Zn^{2+} with autochthonous DOM (Lake Greifen)	–	–	–	7.8–9.6	–	8.0	Xue et al. (1995)
Cu^{2+} with autochthonous DOM (Lake Greifen)	–	–	–	13.9–14.9 (K_1); 11.8–12.9 (K_2)	–	7.8	Xue and Sigg (1993)
Cu^{2+} with autochthonous DOM (Scheldt estuary)	–	–	–	13.0–14.9 (K_1); 11.5–12.8 (K_2)	–	7.7	van Den Berg et al. (1987)
Cu^{2+} with DOM (Shelf water)	–	–	–	13.2; 10.0	–	8.1	Sunda and Huntsman (1991)
Cu^{2+} with DOM (Mississippi River plume)	–	–	–	11.1; 8.9	–	8.1	Sunda and Ferguson (1983)

(continued)

Table 1 (continued)

Elements or groups	Conditional stability constants ($\log_{10} K$)					pH	References
	Fulvic acid[a]	Humic acid[a]	Tryptophan or protein or EPS[a]	Autochthonou DOM or hydrophilic or hydrophobic[a]	Other organic ligands[a]		
Cu^{2+} with DOM (Southeastern Gulf of Mexico)	–	–	–	≥12; 9.8	–	8.2	Sunda and Ferguson (1983)
Cu^{2+} with DOM (Coast of Peru)	–	–	–	12.3; 9.2	–	8.2	Sunda and Hanson (1987)
Cu^{2+} with DOM (Sargasso Sea)	–	–	–	13.2; 9.7	–	?	Moffett et al. (1990)
Cu^{2+} with DOM (North Pacific)	–	–	–	13.0; 10.0	–	?	Coale and Bruland (1988)
Cu^{2+} with DOM (South Atlantic)	–	–	–	12.2; 10.2	–	7.7	van Den Berg (1984)
Cu^{2+} with DOM (equatorial Pacific)	–	–	–	8.92–9.26 (K_1); 6.87–7.44 (K_2)	–	8.15	Midorikawa and Tanoue (1996, 1998)
Cu^{2+} (groundwater)	–	5.60	–	–	–		Kim et al. (1990)
Hg^{2+} with isolated hydrophobic fraction of DOM (Florida Everglades surface waters)	–	–	–	11.6–12.0 (K_1); 10.5–10.9 (K_2)	–		Benoit et al. (2001)
Mn^{2+}	–	0.40	–	–	–	7	Takahashi et al. (1997)
Co^{2+}	–	0.50	–	–	–	7	Takahashi et al. (1997)
Zn^{2+} with humic acid, sewage sludge (Ex/Em = 340/438 nm)	–	4.08	–	–	–	8.1	Plaza et al. (2006)
Zn^{2+} with humic acid, soil (Ex/Em = 440/510 nm)	–	4.43	–	–	–	8.1	Plaza et al. (2006)
Zn^{2+} with humic acid, amended soil + sewage sludge (Ex/Em = 440/510 nm)	–	4.31	–	–	–	8.1	Plaza et al. (2006)

(continued)

Table 1 (continued)

Elements or groups	Conditional stability constants ($\log_{10} K$)					pH	References
	Fulvic acid[a]	Humic acid[a]	Tryptophan or protein or EPS[a]	Autochthonou DOM or hydrophilic or hydrophobic[a]	Other organic ligands[a]		
Zn^{2+}	–	0.50	–	–	–	7	Takahashi et al. (1997)
Cr^{3+}	–	6.00	–	–	–	7	Takahashi et al. (1997)
Fe^{3+}	–	10.0	–	–	–	7	Takahashi et al. (1997)
Sc^{3+}	–	15.0	–	–	–	7	Takahashi et al. (1997)
VO_2^+	–	2.1	–	–	–	7	Takahashi et al. (1997)
Hg^{2+} with stream humic substances (peak C: 340–355/430–455 nm)	4.34–5.20	–	–	–	–	7.92	Fu et al. (2007)
Hg^{2+} with river fulvic acid (peak A: 245/432–438 nm)	5.62–5.72		–	–	–	7.92	Fu et al. (2007)
Hg^{2+} with river fulvic acid (peak C: 330–335/426–434 nm)	5.01–5.55	–	–	–	–	7.92	Fu et al. (2007)
Hg^{2+} with stream DOM	4.34–5.2	–	–		–	4.37–7.01	Wu et al. (2004)
Hg^{2+} with surface waters	–	–	–	21.2–30.2	–	7	Hsu and Sedlak (2003)
Hg^{2+} with hydrophobic acid extracted from Florida Everglades surface waters	–	–	–	26–31	–	7	Gasper et al. (2007)
Hg^{2+} with hydrophobic acid	–	–	–	25.5	–	7	Haitzer et al. (2002)
Hg^{2+} with hydrophobic acid	–	–	–	19.8	–	6	Benoit et al. (2001)
Hg^{2+} with peat DOC (Florida Everglades peats)	–	–	–	23.2	–	6	Drexel et al. (2002)
Hg^{2+} with tryptophan (peak T: 275/340–346 nm)	–	–	4.99–5.33		–	7.92	Fu et al. (2007)

(continued)

Table 1 (continued)

Elements or groups	Conditional stability constants ($\log_{10} K$)					pH	References
	Fulvic acid[a]	Humic acid[a]	Tryptophan or protein or EPS[a]	Autochthonou DOM or hydrophilic or hydrophobic[a]	Other organic ligands[a]		
Cu^{2+} with tryptophan-like or protein-like component	–	–	7.82–9.56		–	?	Wu and Tanoue (2001b)
Pb^{2+} with humic acid, sewage sludge (Ex/Em = 340/438 nm)	–	4.95	–	–	–	8.1	Plaza et al. (2006)
Pb^{2+} with humic acid, soil (Ex/Em = 440/510 nm)	–	5.81	–	–	–	8.1	Plaza et al. (2006)
Pb^{2+} with humic acid, amended soil + sewage sludge (Ex/Em = 440/510 nm)	–	5.53	–	–	–	8.1	Plaza et al. (2006)
Cd^{2+} with humic acid, sewage sludge (Ex/Em = 340/438 nm)	–	4.24	–	–	–	8.1	Plaza et al. (2006)
Cd^{2+} with humic acid, soil (Ex/Em = 440/510 nm)	–	4.63	–	–	–	8.1	Plaza et al. (2006)
Cd^{2+} with humic acid, amended soil + sewage sludge (Ex/Em = 440/510 nm)	–	4.47	–	–	–	8.1	Plaza et al. (2006)
Hg^{2+} with extracellular polymeric substances (EPS) (peak T: 275–280/428–334 nm)	–	–	3.28–4.12 (K_1)	–	–	4.0–8.0	Zhang et al. (2010)
Hg^{2+} with EPS (peak T_{UV}: 220–230/422–336 nm)	–	–	4.28–4.49 (K_2)	–	–	4.0–8.0	Zhang et al. (2010)
Pb^{2+} with EPS	–	–	3.1–4.9 (K_1); 3.2–4.6 (K_2)	–	–	7	Comte et al. (2006)
Pb^{2+} with EPS	–	–	0.45–1.28	–	–	7	Guibaud et al. (2006)

(continued)

Table 1 (continued)

Elements or groups	Conditional stability constants ($\log_{10} K$)					pH	References
	Fulvic acid[a]	Humic acid[a]	Tryptophan or protein or EPS[a]	Autochthonou DOM or hydrophilic or hydrophobic[a]	Other organic ligands[a]		
Cd^{2+} with EPS	–	–	3.0–3.5 (K_1); 2.7–3.8 (K_2)	–	–	7	Comte et al. (2006)
Cd^{2+} with EPS	–	–	1.54–3.35	–	–	7	Guibaud et al. (2006)
Cu^{2+} with EPS	–	–	3.0–4.4	–	–	7	Guibaud et al. (2004)
Ni^{2+} with EPS	–	–	2.6–3.0	–	–	7	Guibaud et al. (2004)
Sc^{3+}	17.57	17.54–20.47	–	–	–	7–9.	Sonke and Salters (2006)
Y^{3+}	9.21–13.67	10.95–14.94	–	–	–	6–9.	Sonke and Salters (2006)
Nanthnides (14 elements)							
La^{3+}	9.15–11.65	10.64–13.29	–	–	–	6.0 –9.0	Sonke and Salters (2006)
Ce^{3+}	8.90–12.09	10.56–13.55	–	–	–	6.0 –9.0	Sonke and Salters (2006)
Pr^{3+}	8.93–12.36	10.39–13.84	–	–	–	6.0 –9.0	Sonke and Salters (2006)
Nd^{3+}	9.07–12.54	10.34–13.96	–	–	–	6.0 –9.0	Sonke and Salters (2006)
Pm^{3+}	–	–	–	–	–	6.0 –9.0	Sonke and Salters (2006)
Sm^{3+}	9.56–12.65	10.58–14.38	–	–	–	6.0 –9.0	Sonke and Salters (2006)
Eu^{3+}	9.36–11.52	10.71–14.30	–	–	–	6.0 –9.0	Sonke and Salters (2006)
Gd^{3+}	9.14–11.39	10.75–14.20	–	–	–	6.0 –9.0	Sonke and Salters (2006)

(continued)

Table 1 (continued)

Elements or groups	Conditional stability constants ($\log_{10} K$)					pH	References
	Fulvic acid[a]	Humic acid[a]	Tryptophan or protein or EPS[a]	Autochthonou DOM or hydrophilic or hydrophobic[a]	Other organic ligands[a]		
Tb^{3+}	9.39–11.75	11.19–14.80	–	–	–	6.0 –9.0	Sonke and Salters (2006)
Dy^{3+}	9.47–13.64	11.31–15.30	–	–	–	6.0 –9.0	Sonke and Salters (2006)
Ho^{3+}	9.67–13.91	11.44–15.49	–	–	–	6.0 –9.0	Sonke and Salters (2006)
Er^{3+}	9.97–14.29	12.85–15.78	–	–	–	6.0 –9.0	Sonke and Salters (2006)
Tm^{3+}	10.25–14.45	11.89–16.20	–	–	–	6.0 –9.0	Sonke and Salters (2006)
Yb^{3+}	10.34–14.32	13.26–16.23	–	–	–	6.0 –9.0	Sonke and Salters (2006)
Lu^{3+}	10.44–14.58	12.25–16.50	–	–	–	6.0 –9.0	Sonke and Salters (2006)
Eu^{3+}	–	–	–	6.3	–	6	Konstantinou et al. (2009)
Eu^{3+} with 5-Sulfosalicylic acid	–	–	–	–	6.27; 11.76	?	Nair and Chander (1983)
Actinides							
Np^{5+}	–	3.3–3.7 (2.5–3.2)	–	–	–	7.4 (4.7)	Shcherbina et al. (2007)
Cm^{3+}	5.9	6.22	–	–	–	?	Buckau et al. (1992); Kim et al. (1991)
Cm^{3+} with 5-sulfosalicylic acid	–	–	–	–	6.44; 11.99	?	Nair and Chander (1983)
Am^{3+} with 5-sulfosalicylic acid	–	–	–	–	8.06; 15.34	?	Nair and Chander (1983)

(continued)

Table 1 (continued)

Elements or groups	Conditional stability constants ($\log_{10} K$)					pH	References
	Fulvic acid[a]	Humic acid[a]	Tryptophan or protein or EPS[a]	Autochthonou DOM or hydrophilic or hydrophobic[a]	Other organic ligands[a]		
Pu^{3+} with 5-sulfosalicylic acid	–	–	–	–	8.56; 17.51	?	Nair and Chander (1983)
Th^{4+} with catechol	–	–	–	–	59.65; 14.06	?	Thakur et al. (2006)
Th^{4+} with hydroquinone	–	–	–	–	48.51; 64.86	?	Thakur et al. (2006)
Th^{4+} with resorcinol	–	–	–	–	16.98; 46.46; 59.65	?	Thakur et al. (2006)
Irradiation effect on metal-DOM complexation	–	–	–	–	–	?	
Hg^{2+} with stream DOM (before irradiation)	4.9 (K_1)	–	–	–	–	?	Wu et al. (2004)
Hg^{2+} with stream DOM (after irradiation: 1–8 d)	4.6 (1 d); 4.1 (8 d)	–	–	–	–	?	Wu et al. (2004)
Cu^{2+} with river DOM (before irradiation)	7.59 (K_1); 5.83 (K_2)	–	–	–	–	?	Brooks et al. (2007)
Cu^{2+} with river DOM (after irradiation: 72 h)	7.63 (K_1); 5.88 (K_2)	–	–	–	–	?	Brooks et al. (2007)
Cu^{2+} with wetland DOM (before irradiation)	–	–	–	–	8.02 (K_1); 6.31 (K_2)	?	Brooks et al. (2007)
Cu^{2+} with wetland DOM (after irradiation: 72 h)	–	–	–	–	7.61 (K_1); 5.85 (K_2)	?	Brooks et al. (2007)
Cu^{2+} with lake DOM (before irradiation)	–	–	–	–	16.3 (K_1)	?	Sander et al. (2005)
Cu^{2+} with lake DOM (after irradiation: 30 h and 175 h)	–	–	–	–	16.7 (30 h); 16.8 (175 h)	?	Sander et al. (2005)

(continued)

Table 1 (continued)

Elements or groups	Conditional stability constants ($\log_{10} K$)					pH	References
	Fulvic acid[a]	Humic acid[a]	Tryptophan or protein or EPS[a]	Autochthonou DOM or hydrophilic or hydrophobic[a]	Other organic ligands[a]		
Cu^{2+} with autochthonous DOM (Estuary: before irradiation)	–	–	–	–	13.5	?	Shank et al. (2006)
Cu^{2+} with autochthonous DOM (Estuary: after irradiation: 1.5 and 14.5 d)	–	–	–	–	13.5	?	Shank et al. (2006)

[a]indicates the components which are extracted or standard or under natural condition used
pH values in parentheses indicates the conditional stability constant in parentheses
Standard deviations do not mention in the results to avoid the complexiety
K_1 and K_2 values for two binding sites are mentioned using semicolon (;)
h = hours; d = days

complexation of different trace elements (Wu et al. 2004a; c; Plaza et al. 2006; Cook and Langford 1995; Kumke et al. 1998; Mcgown et al. 1995)· The conditional stability constants ($\log_{10} K_1$ and $\log_{10} K_2$) for the complexation of two major binding sites of fulvic acid with metal ions are 3.26–6.66 ($\log_{10} K_1$) and 7.0–9.64 ($\log_{10} K_2$) for Cu^{2+}-FA; 5.66 for Fe^{3+}-FA; 4.34–5.70 for Hg^{2+}-FA; 5.46 for $UO_2{}^{2+}$-FA; and 5.9 ($\log_{10} K_1$) for Cm^{3+}-FA, determined in aqueous solution except for lanthanides (Table 1) (Wu et al. 2002a, 2004; Fu et al. 2007; Hays et al. 2004; Cao et al. 2004; Sidenius et al. 1999; Antunes et al. 2007; Winzerling et al. 1992; Tipping 1994; Breault et al. 1996; Mcknight et al. 1983; Buckau et al. 1992; Kim et al. 1991). Lanthanides (Ln) are strong complexing agents for Suwannee River Fulvic Acid and show relatively high conditional stability constants that range from 8.90 to 14.58 for Ln-SRFA (Suwannee River Fulvic Acid) complexes at pH 6−9 in aqueous solution. The values obtained for SRFA are relatively lower than for humic acids extracted from various sources (Table 1; Sonke and Salters (2006)).

The conditional stability constants for Hg^{2+}-DOM complexation are 4.34–5.20 for the peak C at Ex/Em = 340–355/430–455 nm of humic substances (possibly fulvic acid) at pH 4.37–7.01 (Wu et al. 2004a). The conditional stability constants for Hg^{2+}-FA complexation are 5.01–5.55 ($\log_{10} K_1$) for peak C detected at Ex/Em = 330–335/426–434 nm and 5.62–5.72 ($\log_{10} K_2$) for peak A detected at Ex/Em = 245/432–438 nm (Fu et al. 2007). Complexation of river DOM with Hg^{2+} is 3.54–4.93 and 3.64–4.85, determined using linear and non-linear model fitting of the fluorescence maxima at Ex/Em = 304, 306/426, 430 nm (Bai et al. 2008). The fluorophores or functional groups bound for peak C at longer wavelength are presumably considered to be the fast binding sites and the fluorophores bound for peak A, correspondingly, the slow binding sites in fulvic acid. Generally, $\log_{10} K_1$ should be higher than in $\log_{10} K_2$ but the result is, in fact, the opposite. The reason behind this phenomenon is that during the complexation of the fast binding site at peak C, the decrease in fluorescence intensity occurs simultaneously at peak A of fulvic acid. Note that the fluorophores bound at a particular peak (e.g., peak C, peak A, peak T or T_{UV}) of a fluorescent molecule are the result of all fluorophores existing in the molecule. Therefore, any changes in the molecule by photoinduced or microbial degradation can alter its fluorescence properties, inducing new peak position and fluorescence intensity (Mostofa et al. 2009a, 2011; Senesi 1990). For the fast-reacting binding site of fulvic acid, the kinetic rate constant (k_1) ranges from 0.18 to 0.55 s^{-1}, whilst the k_2 values for the slow reacting binding site are similar for all metals (Cu^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, and Ca^{2+}) at 0.01–0.02 s^{-1} (Wu et al. 2004c).

The conditional stability constants for the complexation of humic acid (HA, two binding sites) with metal ions are 3.99–5.60 ($\log_{10} K_1$) and 8.55 ($\log_{10} K_2$) for Cu^{2+}-HA; 0.40 ($\log_{10} K_1$) for Mn^{2+}-HA; 0.50 ($\log_{10} K_1$) for Co^{2+}-HA; 0.50–4.43 ($\log_{10} K_1$) for Zn^{2+}-HA; 6.79–10.0 ($\log_{10} K_1$) for Fe^{3+}-HA; 6.00 ($\log_{10} K_1$) for Cr^{3+}-HA; 15.0 ($\log_{10} K_1$) for Sc^{3+}-HA; 2.1 ($\log_{10} K_1$) for $VO_2{}^{+}$-HA; 5.50 ($\log_{10} K_1$) for Hg^{2+}-HA; 4.5–5.3 ($\log_{10} K_1$) and 5.8–7.2 ($\log_{10} K_2$) for As^{3+}-HA; 2.80–4.94 ($\log_{10} K_1$) and 7.15–9.80 ($\log_{10} K_2$) for H^{+}-HA; 4.0 ($\log_{10} K_1$) for Be^{2+}-HA; 1.0 ($\log_{10} K_1$) for Ca^{2+}-HA; 0.5 ($\log_{10} K_1$) for Sr^{2+}-HA; 0.5 ($\log_{10} K_1$) for Ba^{2+}-HA; 10.0 ($\log_{10} K_1$) for Ga^{3+}-HA; 4.95–5.81 ($\log_{10} K_1$) for Pb^{2+}-HA;

4.24–4.63 ($\log_{10} K_1$) for Cd^{3+}-HA; 4.27 ($\log_{10} K_1$) for $UO_2{}^{2+}$-HA; 3.3–3.7 ($\log_{10} K_1$) for Np^{5+}-HA; and 6.22 ($\log_{10} K_1$) for Cm^{3+}-HA in aqueous solution except lanthanides (Table 1) (Liu and Cai 2010; Cao et al. 2004; Antunes et al. 2007; Tipping 1994; Buckau et al. 1992; Kim et al. 1991). Lanthanides (Ln) are strong complexing agents for humic acid, showing relatively high conditional stability constants that range from 10.34 to 16.50 for Ln-LHA (Leonardite coal humic acid standard) and from 12.17 to 16.22 for Ln-EHA (Elliot soil humic acid standard) at pH 7–9 in aqueous media (Table 1) (Sonke and Salters 2006). The stability constants of the standard Aldrich humic acid show significantly low values (2.65–2.75 at 5 mg L^{-1}) for lanthanides. These values are greatly increased with increasing the humic acid concentration (e.g. La ranges from 2.65 to 3.85 for humic acid concentrations of 5 and 20 mg L^{-1}, respectively) for each lanthanide (Pourret and Martinez 2009). For the concentration of 20 mg L^{-1} of the standard Aldrich humic acid, the stability constants increase from La (3.85) to Eu (4.15) and then decrease from Gd (4.06) to Lu (3.95).

The stability constants of the M-HA complexes reached high values ($\log_{10} K_{Pb} = 5.81$, $\log_{10} K_{Cu} = 5.55$, $\log_{10} K_{Cd} = 4.63$ and $\log_{10} K_{Zn} = 4.43$) for the peak C (Ex/Em = 440/510 nm) of humic acid extracted from the corresponding soil (nonamended and control) compared to those of sewage sludge (4.95, 4.65, 4.24 and 4.08, respectively) and to soil amended with sewage sludge at a rate of 40 tons ha^{-1} (5.53, 5.36, 4.47 and 4.31, respectively), for metal ions such as Pb^{2+}, Cu^{2+}, Cd^{2+}, and Zn^{2+} in aqueous solution at pH 8.1 (Table 1) (Plaza et al. 2006). The overall stability constant for M-HA complexes can follow the order Pb(II) > Cu(II) > Cd(II) > Zn(II) (Plaza et al. 2006). These results suggest that the fluorophores or functional groups of humic acid of different origin are highly variable and may affect the metal-DOM complexation and transport in soil and natural water ecosystems.

The conditional stability constants for complexation between humic acid and alkaline earth metal ions follow the order Be > Ca > Sr, Ba humate. It can be hypothesized that the humates of alkaline earth metal ions with smaller ionic radii are more stable, and that humic acid is a 'hard' ligand (Takahashi et al. 1997). Correspondingly, Sc and heavy lanthanide elements (Yb and Lu) can form more stable complexes than light lanthanide elements (Ce and Eu), depending on the ionic radii (Takahashi et al. 1997). In addition, humic acid complexes of trivalent Fe and Ga are more stable than those of rare earth elements (REEs) except for Sc, while the Cr(III)-humic acid complex is less stable than REE-humic acid complexes (Takahashi et al. 1997). It has also been shown that the conditional stability constant ($\log_{10} K$) between lanthanides and humic substances (fulvic and humic acids) of varied sources significantly increases from La to Lu and increases with increasing pH and decreasing ionic strength (I) of the solution (Fig. 5a–c) (Sonke and Salters 2006). The log K values also significantly increase with decreasing ionic radius (Fig. 6) (Sonke and Salters 2006). The strength of M-DOM complexation between lanthanides and humic substances (fulvic and humic acids) thus follows the order Lu > Yb > Tm > Er > Ho > Dy > Tb > Gd > Eu > Sm > Pm > Nd > Pr > Ce > La).

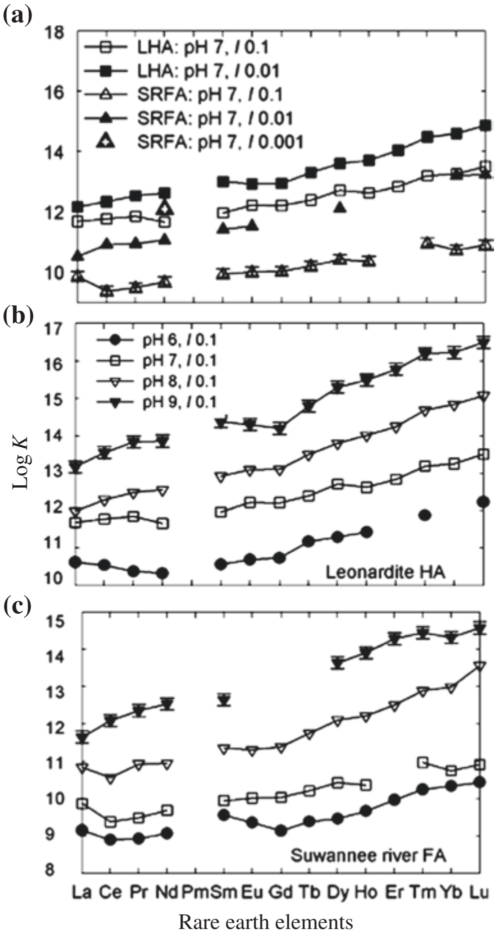

Fig. 5 Conditional binding constants (log *K*) for rare earth elements (REE)–Leonardite coal humic acid standard (LHA) and REE–SRFA complex formation at pH 7 with various ionic strength (**a**) and the effect pH on log *K* for complexation of LHA (**b**) and SRFA (**c**) with REE. Error bars (0.16 log units) are only shown for pH 7 (**a**), but apply to all log *K*c values. Nominal total concentrations are 100 nM REE; 20 mg L^{-1} SRFA, 130 nM EDTA for SRFA experiments (**b** and **c**). *Data source* Sonke and Salters (2006)

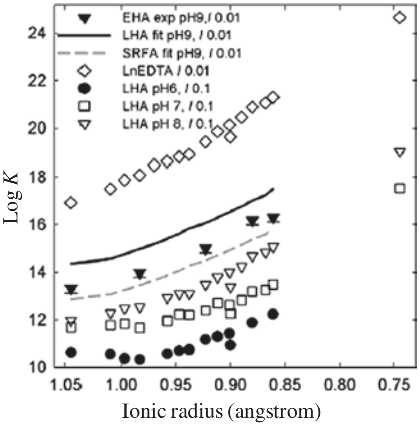

Fig. 6 Log *K* for lanthanides and organic ligands of various sources at pH 6, 7, and 8 as a function of ionic radius (sixfold hydrated), including Sc and Y transition metals. Log *K* for Y and Leonardite coal humic acid standard (LHA) values are represented by singular data points, floating just below the main rare earth elements (REE) data trends at an ionic radius of 0.900 Å. Log *K* for lanthanides and Elliot soil humic acid standard (EHA) values observes at pH 9, and 0.01 mol L^{-1} *I* and extrapolated log *K* values for SRFA and LHA under those conditions. EDTA conditional binding constants for 0.01 mol L^{-1} *I* are included for comparison. *Data source* Sonke and Salters (2006)

The conditional stability constants for the complexation of hydrophobic or hydrophilic fractions of DOM or autochthonous DOM with metal ions are 7.8–9.6 ($\log_{10} K_1$) for Zn^{2+}-DOM; 8.92–16.3 ($\log_{10} K_1$) and 6.87–12.9 ($\log_{10} K_2$) for Cu^{2+}-DOM; 11.6–31.0 ($\log_{10} K_1$) and 10.5–11.2 ($\log_{10} K_2$) for Hg^{2+}-DOM in lakes, estuaries and oceans (Table 1) (Haitzer et al. 2002; Xue and Sigg 1993; Xue et al. 1995; van Den Berg et al. 1987; Sunda and Huntsman 1991; Sunda and Hanson 1987; Sunda and Ferguson 1983; Moffett et al. 1990; Coale and Bruland 1988, 1990; van Den Berg 1984; Midorikawa and Tanoue 1996; Benoit et al. 2001; Drexel et al. 2002; Gasper et al. 2007; Hsu and Sedlak 2003; Sander et al. 2005; Shank et al. 2006). It has been shown that the conditional stability constants between autochthonous DOM of phytoplankton or algal origin and Cu^{2+} are much higher ($\log_{10} K_1 = 13.9–15.6$ and $\log_{10} K_2 = 11.8–13.4$) in lakes, and they are similar to those of organic ligands of phytoplankton or biological sources (11.1–13.2 and 9.2–10.2, respectively) in estuaries and seawater compared to those of allochthonous fulvic and humic acids (Table 1) (Xue and Sigg 1993; Xue et al. 1995; van Den Berg et al. 1987; Moffett et al. 1990; Coale and Bruland 1988; Coale and Bruland 1990; Sunda 1988). Surface water from the Irish Sea and the Atlantic Ocean contains ligand concentrations of 1.7×10^{-7} and 1.1×10^{-7} M, with conditional stability constants ($\log_{10} K$) of 9.84 ± 0.13 and 9.86 ± 0.23, respectively, at pH 8.0 (van Den Berg 1982).

The conditional stability constants of the Hg^{2+}-DOM complexation are significantly higher for the hydrophobic fraction of DOM or DOM in natural surface waters (Table 1) (Haitzer et al. 2002, 2003; Benoit et al. 2001; Drexel et al. 2002; Gasper et al. 2007; Hsu and Sedlak 2003; Ravichandran et al. 1998; 1999; Lamborg et al. 2003, 2004; Waples et al. 2005). The high affinity of the Hg^{2+}-DOM complexation is responsible for the reduced S-containing binding sites (thiol and disulfide/disulfane functional groups) bound in DOM, presumably autochthonously produced in natural waters (Haitzer et al. 2002; Benoit et al. 2001; Gasper et al. 2007; Dyrssen and Wedborg 1986; Schuster 1991; Guentzel et al. 1996; Wallschlager et al. 1996; Xia et al. 1999). The possible complexation reaction can be written as [$Hg^{2+} + R\text{-}SH^{n-} = HgR\text{-}S^{(n-1)-} + H^+$] (Benoit et al. 2001; Dyrssen and Wedborg 1991).

The conditional stability constants for the complexation of the two major binding sites of tryptophan with metal ions are 4.88–4.90 ($\log_{10} K_1$) for Cu^{2+}-tryptophan; 7.82–9.56 for Cu^{2+}-tryptophan; and 4.99–5.33 for Hg^{2+}-tryptophan for peak T at Ex/Em = 275–285/330–360 nm in aqueous solution (Table 1) (Fu et al. 2007; Hays et al. 2004; Wu and Tanoue 2001b).

The conditional stability constants ($\log_{10} K_1$ and $\log_{10} K_2$) for the complexation of two major binding sites of EPS with metal ions are 3.98–4.12 ($\log_{10} K_1$) and 4.28–4.48 ($\log_{10} K_2$) for Hg^{2+}-EPS; 0.45–4.9 ($\log_{10} K_1$) and 3.2–4.6 ($\log_{10} K_2$) for Pb^{2+}-EPS; 1.54–3.5 ($\log_{10} K_1$) and 2.7–3.8 ($\log_{10} K_2$) for Cd^{2+}-EPS; 3.0–4.4 ($\log_{10} K_1$) for Cu^{2+}-EPS; and 2.6–3.0 ($\log_{10} K_1$) for Ni^{2+}-EPS in aqueous solution (Table 1) (Zhang et al. 2010; Guibaud et al. 2004, 2006; Comte et al. 2006). The conditional stability constants of Hg^{2+}-EPS complexes are relatively low (3.98–4.12: $\log_{10} K_1$) at peak T (Ex/Em = 275–280/328–334 nm) compared to those (4.28–4.48: $\log_{10} K_2$) at peak T_{UV} (Ex/

Em = 220–230/322–336 nm) in aqueous solution (Zhang et al. 2010). Note that the fluorescence properties of EPS are similar to those of tryptophan, with higher fluorescence intensity at peak T_{UV}-region than at peak T-region and similar Ex/Em maxima.

The conditional stability constants ($\log_{10} K$) for the complexation of some standard organic and inorganic ligands with metal ions are identified in aqueous solution as 3–6 for Cu^{2+}-carboxylic or HO group; 2.8 for Cu^{2+}-amine (NH_2); 4.4 for Cu^{2+}-ethanol amine; 6.6 for Cu^{2+}-glycine (NH_2–CH_2–COOH or NH_3^+–CH_2–COO^-); 8.5 for Cu^{2+}-ethylene diamine (NH_2–CH_2–CH_2–NH_2); 7.2 for Cu^{2+}- aspartic acid [HOOC–CH(NH_2)–CH_2–COOH]; 9.4 for Cu^{2+}- iminodiaacetic acid (HOOC–CH_2–NH_2–CH_2–COOH); 8.3 for Cu^{2+}- diaminopropanol; 11.4 for Cu^{2+}- ethylenediamine-N-acetic acid; 13.0 for Cu^{2+}-diethylenetriamine; 6.27 and 11.76 for Eu^{3+}-5-sulfosalicylic acid; 6.4 and 11.99 for Cm^{3+}-5-sulfosalicylic acid; 8.06 and 15.34 for Am^{3+}-5-sulfosalicylic acid; 8.56 and 17.51 for Pu^{3+}-5-sulfosalicylic acid; 59.65 and 14.06 for Th^{4+}- catechol; 48.51 and 64.86 for Th^{4+}-hydroquinone; and 16.98, 46.46, and 59.65 for Th^{4+}- resorcinol (Table 1) (Nair and Chander 1983; Martin and Prados 1974; Thakur et al. 2006; Smith 1974). The stability constants of various standard organic substances are similar to those of allochthonous and autochthonous DOM in natural waters, indicating that carboxylic, phenolic and amino group-containing carboxylic acid bound in allochthonous and autochthonous DOM may form complexes with metal ions in aqueous solution.

The binary complexes of Cu^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+} with resorcinol and some biologically important aliphatic dicarboxylic acids (adipic, succinic, malic, malonic, maleic, tartaric and oxalic) in aqueous solution at 25 °C and $I = 0.1$ mol dm^{-3} $NaNO_3$ suggest the following hypotheses (Radalla 2010): (i) Stability of the 1:1 binary complexes for all investigated ligands is higher than that of the corresponding 1:2 ones. (ii) Normal 1:1 and 1:2 complexes of resorcinol are formed with all of the metal ions studied. (iii) The order of stability constants of the 1:1 complexes of all investigated ligands with respect to the divalent transition metal ions follows the Irving-Williams series (Zn $<$ Cu $>$ Ni $>$ Co) (Irving and Williams 1948). (iv) Stability of the 1:1 metal-complexes of aliphatic dicarboxylic acids follows their basicity ($pK_{a1} + pK_{a2}$), where K_{a1} and K_{a2} are the first and second dissociation constants, respectively.

Experimental studies show that photoinduced degradation can decrease the conditional stability constants of M-DOM complexes in stream waters ($\log K_1 = 4.9$ to 4.1) and in wetland waters ($\log K_1 = 8.02$ and $\log K_2 = 6.1$ to 7.61 and 5.85, respectively), whilst their values are increased in river DOM ($\log K_1 = 7.59$ and $\log K_2 = 5.83$ to 7.63 and 5.88, respectively) and in lake DOM ($\log K_1 = 16.3$ to 16.8). The values remain the same ($\log K_1 = 13.5$) before and after irradiation of estuarine waters (Wu et al. 2004a; Brooks et al. 2007; Sander et al. 2005; Shank et al. 2006). The decomposition of the functional groups in DOM is susceptible to decrease the stability constants of the M-DOM complexes in natural waters. In contrast, the increase in stability constants of the M-DOM complexes is likely caused by the formation of new photoproducts with stronger functional groups for M-DOM complexation. Irradiation of waters with high contents of DOM is not able to substantially modify the degree of M-DOM complexation, which is the most likely explanation for the cases where the same stability constants have been observed before and after irradiation. The effect of photoinduced degradation on M-DOM complexation will be explained in details later.

3.6.1 Conditional Stability Constants for Ternary Complexes in Waters

Ternary complexes are operationally defined as complexes involving two newly successive bonds between a metal ion and two different types of DOM components or organic ligands (e.g. allochthonous fulvic acid and tryptophan). It is assumed that fulvic acid (FA) acts as a primary ligand to form bonds with a metal ion (M). Therefore, one initially observes binary complex formation such as $\mathrm{M-FA}$ $(\mathrm{M+FA} \leftrightarrows \mathrm{M-FA})$. Then another molecule, e.g. tryptophan (T) acts as a secondary ligand and forms ternary complexes that can be represented as such as $\mathrm{T-M-FA}$ $(\mathrm{M-FA+T} \leftrightarrows \mathrm{T-M-FA})$.

Formation of ternary complexes is observed in aqueous solution (Martin and Prados 1974; Khalil and Radalla 1998; Khalil and Attia 1999; Khalil 2000a, b; Khalil and Taha 2004; Khalil and Fazary 2004; Radalla 2010; Rosas et al. 2010).

For the formation of ternary complexes of the selected bivalent metal ions (M) in the presence of resorcinol = R and aliphatic dicarboxylic acid = A, the following equilibria may be considered (Eqs. 3.11 and 3.12) (Radalla 2010):

$$\mathrm{M} + \mathrm{A} \rightleftharpoons \mathrm{MA}, \quad K_{\mathrm{MA}}^{\mathrm{M}} = \frac{[\mathrm{MA}]}{[\mathrm{M}][\mathrm{A}]} \tag{3.11}$$

$$\mathrm{MA} + \mathrm{R} \rightleftharpoons \mathrm{MAR}, \quad K_{\mathrm{MAR}}^{\mathrm{MA}} = \frac{[\mathrm{MAR}]}{[\mathrm{M}][\mathrm{A}]} \tag{3.12}$$

In the presence of both ligands, A is presumably considered to interact first with M forming a 1:1 MA binary complex. It follows interaction of R in a stepwise manner. The overall stability constant $\beta_{\mathrm{MAR}}^{\mathrm{M}}$ can be described as below (Eq. 3.13):

$$\mathrm{M} + \mathrm{A} + \mathrm{R} \rightleftharpoons \mathrm{MAR}, \quad \beta_{\mathrm{MAR}}^{\mathrm{M}} = \frac{[\mathrm{MAR}]}{[\mathrm{M}][\mathrm{A}][\mathrm{R}]} = K_{\mathrm{MAR}}^{\mathrm{MA}} \times K_{\mathrm{MA}}^{\mathrm{M}} \tag{3.13}$$

The $\beta_{\mathrm{MAR}}^{\mathrm{M}}$ constant expresses the stability of the mixed-ligand species and it does not represent the binding strength between R and M^{2+} ions directly in the presence of A. This effect is much better reflected by the equilibrium constant, $K_{\mathrm{MAR}}^{\mathrm{MA}}$ calculated according to Eq. 3.14:

$$\log_{10} K_{\mathrm{MAR}}^{\mathrm{M}} \rightleftharpoons \log_{10} \beta_{\mathrm{MAR}}^{\mathrm{M}} - \log\, K_{\mathrm{MA}}^{\mathrm{M}} \tag{3.14}$$

The equilibrium constant expressed in Eq. 3.14 indicates how tightly R is bound to the simple MA binary complex in aqueous solution (Radalla 2010).

The ternary complexes of Cu^{2+}, Ni^{2+}, $Co^{2+}$and Zn^{2+} with resorcinol as primary ligand and with some biologically important aliphatic dicarboxylic acids (adipic, succinic, malic, malonic, maleic, tartaric and oxalic) as secondary ligands in aqueous solution at 25 °C and $I = 0.1$ mol dm^{-3} $NaNO_3$ show very high conditional stability constants, such as $\log_{10} \beta^{M}_{MAR} = 13.05 - 16.48$ for Cu^{2+}, 11.72–13.32 for Ni^{2+}, 10.01–11.29 for Zn^{2+}, and 8.78–10.01 for Co^{2+}, compared to those of the respective binary complexes (2.29–7.30, 2.29–6.10, 2.33–5.80 and 2.19–5.80, respectively, ranges including both $\log_{10} K_1$ and $\log_{10} K_2$) (Table 2) (Radalla 2010). The ternary complexes between vanadium(III)-picolinic acid (or dipicolinic acid) and the amino acids cysteine, histidine, aspartic acid and glutamic acid have been examined in aqueous solution at 25 °C and 3.0 mol·dm^{-3} KCl as ionic medium (Table 2) (Rosas et al. 2010; Shiozawa et al. 2011). The results show that the stability constants range from −1.96-4.8 for three ternary complexes of aspartic acid, 0.74–2.26 for three ternary complexes of glutamic acid, 1.71–8.69 for four ternary complexes of cysteine, and −0.35-10.22 for histidine (Rosas et al. 2010)· For dipicolinic acid as a primary ligand, the stability constants range from −5.98 to 8.17 for five ternary complexes of aspartic acid, −6.2 to 10.59 for five ternary complexes of glutamic acid, −1.54 to 13.91 for four ternary complexes of cysteine, and −9.7 to 14.1 for five ternary complexes of histidine (Shiozawa et al. 2011). The significantly high values of the stability constants of ternary complexes are similar to those of DOM in natural surface waters or hydrophobic acids extracted from surface waters ($K_1 = 8$–15; Table 1) (Midorikawa and Tanoue 1998; Sunda and Hanson 1987, 1991; Sunda and Ferguson 1983; Moffett et al. 1990; Coale and Bruland 1988, 1990; van Den Berg 1984; Midorikawa and Tanoue 1996). Therefore, formation of ternary complexes could be vital to understand the M-DOM complexation in natural waters. The results of a species distribution obtained for the Cu^{2+} + malonic acid + resorcinol system shows that the formation of MA starts at pH < 3, reaches a maximum concentration (70 % total Cu^{2+}) at pH 4.2 and decreases to a minimum when MAR becomes predominant. The maximum concentrations of MA_2 and MR are less than 5 % of total Cu^{2+} in solution (Radalla 2010).

These results suggest the following hypotheses (Radalla 2010): (i) Stabilities of ternary complexes with respect to the aliphatic dicarboxylic acids follow the order: adipic > succinic > malic > malonic > maleic > tartaric > oxalic; this behavior can be explained in terms of the decrease in basicity of the aliphatic dicarboxylic acids in the same direction. (ii) The complex stability of the ternary complexes with respect to the metal ion present follows the Irving-Williams series (Irving and Williams 1948). (iii) The stabilities of the ternary complexes are higher than for the 1:1 binary complexes of the corresponding aliphatic dicarboxylic acid or resorcinol for all systems studied; this behavior can be attributed to some cooperative interaction in the ternary complex between the carboxylic acid and resorcinol, such as H-bond formation. Finally, it is vital to examine the formation of ternary complexes of metal ions with fulvic acids and tryptophan in aqueous media, under laboratory conditions. This should be the focus for future studies.

Table 2 Conditional stability constants for Cu^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+} binary and ternary complexes in aqueous media at 25 °C and $I = 0.10$ mol.dm^{-3}

Elements or groups	Cu^{2+}				References
	$\mathrm{Log}_{10}K_1$	$\mathrm{Log}_{10}K_2$	$\log_{10}K^{MA}_{MAR}$	$\log_{10}\beta^{M}_{MAR}$	
Resorcinol (1,3-dihydroxybenzene)	7.30	5.50			Radalla (2010)
Adipic acid (HOOC–CH_2–CH_2–CH_2–CH_2–COOH)	3.84	2.29	9.17	13.81	Radalla (2010)
Succinic acid (HOOC–CH_2–CH_2–COOH)	3.2	–	9.83	13.05	Radalla (2010)
Malic acid [HOOC–CH_2–CH(OH)–COOH]	6.8	–	7.3	16.48	Radalla (2010)
Malonic acid (HOOC–CH_2–COOH)	4.82	3.37	7.3	14.31	Radalla (2010)
Maleic acid (HOOC–CH=CH–COOH)	4.08	2.82	7.3	13.5	Radalla (2010)
Tartaric acid [HOOC–CH(OH)–CH(OH)–COOH]	6.69	5.7	7.3	16.31	Radalla (2010)
Oxalic acid (HOOC–COOH)	4.66	4.06	7.3	13.75	Radalla (2010)
	Ni^{2+}				
Resorcinol (1,3-dihydroxybenzene)	6.10	5.30			Radalla (2010)
Adipic acid (HOOC–CH_2–CH_2–CH_2–CH_2–COOH)	3.54	2.29	9.17	12.71	Radalla (2010)
Succinic acid (HOOC–CH_2–CH_2–COOH)	3.17		8.84	12.01	Radalla (2010)
Malic acid [HOOC–CH_2–CH(OH)–COOH]	4.63		8.69	13.32	Radalla (2010)
Malonic acid (HOOC–CH_2–COOH)	3.93	2.92	8.58	12.51	Radalla (2010)
Maleic acid (HOOC–CH=CH–COOH)	3.68	2.82	8.42	12.10	Radalla (2010)
Tartaric acid [HOOC–CH(OH)–CH(OH)–COOH]	4.67	3.38	8.36	13.03	Radalla (2010)
Oxalic acid (HOOC–COOH)	3.46	2.96	8.26	11.72	Radalla (2010)
	Zn^{2+}				
Resorcinol (1,3-dihydroxybenzene)	5.15	3.90			Radalla (2010)
Adipic acid (HOOC–CH_2–CH_2–CH_2–CH_2–COOH)	3.41	2.35	7.88	11.29	Radalla (2010)
Succinic acid (HOOC–CH_2–CH_2–COOH)	3.06		7.79	10.83	Radalla (2010)
Malic acid [HOOC–CH_2–CH(OH)–COOH]	3.38		7.54	10.72	Radalla (2010)
Malonic acid (HOOC–CH_2–COOH)	2.98	2.33	7.48	10.82	Radalla (2010)
Maleic acid (HOOC–CH=CH–COOH)	3.40	2.45	7.37	10.77	Radalla (2010)

(continued)

Table 2 (continued)

Elements or groups	Cu^{2+}				References
	$\text{Log}_{10}K_1$	$\text{Log}_{10}K_2$	$\log_{10}K^{MA}_{MAR}$	$\log_{10}\beta^{M}_{MAR}$	
Tartaric acid [HOOC–CH(OH)–CH(OH)–COOH]	3.81	2.75	7.21	11.02	Radalla (2010)
Oxalic acid (HOOC–COOH)	3.03	2.47	6.98	10.01	Radalla (2010)
	Co^{2+}				
Resorcinol (1,3-dihydroxybenzene)	5.80	4.90			Radalla (2010)
Adipic acid (HOOC–CH_2–CH_2–CH_2–CH_2–COOH)	3.31	2.19	6.70	10.01	Radalla (2010)
Succinic acid (HOOC–CH_2–CH_2–COOH)	2.97		6.58	9.55	Radalla (2010)
Malic acid [HOOC–CH_2–CH(OH)–COOH]	3.12		6.39	9.51	Radalla (2010)
Malonic acid (HOOC–CH_2–COOH)	2.94	2.28	6.35	9.29	Radalla (2010)
Maleic acid (HOOC–CH=CH–COOH)	3.08	2.24	6.09	9.17	Radalla (2010)
Tartaric acid [HOOC–CH(OH)–CH(OH)–COOH]	3.15	2.37	6.00	9.15	Radalla (2010)
Oxalic acid (HOOC–COOH)	2.88	2.38	5.90	8.78	Radalla (2010)
	$\mathbf{V^{3+}}$				
Picolinic acid (pyridine-2-carboxylic acid)					
Picolinic acid + aspartic acid [HOOC–CH(NH_2)–CH_2–COOH]	–	–	–	2.12–4.8	Rosas et al. (2010)*
Dipicolinic acid + glutamic acid [HOOC–CH_2–CH_2–CH(NH_2)–COOH]	–	–	–	0.74–2.26	Rosas et al. (2010)*
Dipicolinic acid + cycteine [HOOC–CH(NH_2)–CH_2–SH]	–	–	–	1.7–8.69	Rosas et al. (2010)*
Dipicolinic acid + histidine [2-amino-3-(1H-imidazol-4-yl)propanoic acid]	–	–	–	5.48–10.22	Rosas et al. (2010)*
Dipicolinic acid (pyridine-2,6-dicarboxylic acid)					
Dipicolinic acid + aspartic acid [HOOC–CH(NH_2)–CH_2–COOH]	–	–	–	3.15–8.17	Shiozawa et al. (2011)*
Dipicolinic acid + glutamic acid [HOOC–CH_2–CH_2–CH(NH_2)–COOH]	–	–	–	5.57–10.59	Shiozawa et al. (2011)*
Dipicolinic acid + cycteine [HOOC–CH(NH_2)–CH_2–SH]	–	–	–	5.30–13.91	Shiozawa et al. (2011)*
Dipicolinic acid + histidine [2-amino-3-(1H-imidazol-4-yl)propanoic acid]	–	–	–	5.7–14.1	Shiozawa et al. (2011)*

* indicates the positive stability constant values of several ternary complexes formed in the aqueous solution

3.7 *Homogeneous and Heterogeneous Complexation of Fulvic and Humic Acids to Metals*

Homogeneous complexation is operationally defined as a chemical binding equilibrium behavior (approximately in the ratio of 0.8–1:1) between metal ion and a ligand, when they are mixed up under specific conditions in aqueous media. Heterogeneous complexation is operationally defined as a chemical binding equilibrium behavior with a <0.8:1 ratio between metal ion and a ligand.

It has been shown that fulvic and humic acids (humic substances) can behave as homogeneous and heterogeneous complexants to trace metals in waters (Filella 2008; Chakraborty et al. 2007; Pinheiro et al. 1994; Town and Filella 2000; Murimboh 2002). A comparison of the heterogeneity parameter (Γ, termed Gamma: Greek Capital Letter) for Zn(II), Cd(II), Pb(II) and Cu(II) complexes in different model solutions of SRFA shows that ΓCd > ΓZn > ΓPb > ΓCu (Chakraborty et al. 2007). The results show that the value of Γ remains about the same for a given metal: the value of Γ for Cu(II) is ~0.50 in all metal to SRFA solutions with $C_{\mathrm{M}}/C_{\mathrm{SRFA}} = 0.01$, 0.001, and 0.0005, suggesting that SRFA behaves as an heterogeneous complexant for Cu(II) (Chakraborty et al. 2007). The value of Γ for Pb(II) is ~0.70 in comparable solutions, suggesting that SRFA behaved as a less heterogeneous complexant for Pb(II) compared to Cu(II). The Γ value obtained for Cd is ~0.94, suggesting that SRFA almost behaved as a homogeneous complexant for Cd, and a bit lower for Zn ($\Gamma \sim 0.86$) (Chakraborty et al. 2007). These results in combination with other studies suggest that SRFA behaves as a relatively homogeneous complexant for Zn(II) and Cd(II), as a relatively heterogeneous complexant for Pb(II) and as an even more heterogeneous complexant for Cu(II) (Chakraborty et al. 2007; Town and Filella 2000; Murimboh 2002). Coherently, it has also been shown that humic acid derived from peat performs more heterogeneous complexation of Pb(II) compared to Cd(II) (Pinheiro et al. 1994).

4 Binding Sites (or Functional Groups) in Fulvic Acid, Humic Acid and Other Ligands

Humic substances (fulvic and humic acids), autochthonous fulvic acids of phytoplankton origin (see chapters "Dissolved Organic Matter in Natural Waters" and "Fluorescent Dissolved Organic Matter in Natural Waters" for detailed discussion), polysaccharides, proteins, peptides, nucleic acids, extracellular polymeric substances and amino acids, which have properties of polyfunctionality, polyelectrolyticy, size polydispersity, physical heterogeneity and structural lability, are natural organic ligands with binding sites (or functional groups) that can form complexes with trace metals (Malcolm 1985; Mostofa et al. 2009a, b; Zhang et al. 2009, 2010; Merroun and Selenska-Pobell 2008; Filella 2008; Mandal et al. 1999; Xue et al. 1995; Hatch et al. 2009). The molecular structure of allochthonous fulvic and humic acids is composed of a variety of functional groups (or fluorophores) such as benzene-containing carboxyl, methoxylate and phenolic groups (catechol-type), carboxylic and di-carboxylic groups, alcoholic OH, carbohydrate OH, –C=C–, hydroxycoumarin-like structures, fluorophores containing Schiff-base derivatives, chromone, xanthone, quinoline, O, N, S, and P-atom-containing functional groups including aromatic carbon (17–30 %) and aliphatic carbon (47–63 %) (Malcolm 1985; Senesi 1990; Morel and Hering 1993; Leenheer et al. 1998; Steelink 2002; Leenheer and Croue 2003; Stenson et al. 2003; Fimmen et al. 2007). In humic substances, 60–90 % of the acidic groups are carboxylic and the remainder are phenolic (Morel and Hering 1993). S-XANES measures have shown that sulphur is present in humic substances in many different oxidation states: thiol, thiophene or disulfide, sulfoxide, sulfone, sulfonate and sulfate esters (Morra et al. 1997; Xia et al. 1998; Fimmen et al. 2007). A typical humic acid containing 0.2 % reduced sulphur has only 63 μmol g^{-1} of thiol sites (Bloom et al. 2001).

Fulvic and humic acids can be roughly classified into two categories: minor (approximately 1–10 %), strong sites, and major (approximately 90–99 %), weak sites (Mandal et al. 1999; Chakraborty et al. 2007; Buffle and Filella 1995).Wu and Tanoue 2001c The strong binding sites are first occupied entirely, after which the weak binding sites are occupied (Mandal et al. 1999; Chakraborty et al. 2007; Buffle and Filella 1995; Wu and Tanoue 2001c). The major sites are less diverse in type, but in number they represent the majority of the complexation sites (e.g., carboxylate and phenolate groups in humic substances). The minor sites, which are fewer in number, include all those which exhibit a wide range of binding energies, including strongly complexing nitrogen- and sulphur-containing sites (Filella 2008). Major sites represent the largest proportion of dissociable groups. They play an important role in the polyelectrolytic properties of the complexant (Filella 2008). The distinction between major and minor sites also reflects a chemical selectivity: since the major sites have oxygen-containing groups, they will preferably react with hard metals such as calcium and magnesium (Filella 2008).

EEM spectroscopy (EEMS) during the complexation between extracted fulvic acid and several metals [Cu(II), Ni(II), Co(II), Cd(II) and Ca(II)] also showed two major kinetically distinguishable binding sites on fulvic acid, ‘fast’ and ‘slow’, having reaction half-lives of 1.3–3.9 and 34.7–69.3 s, respectively (Wu et al. 2004c). The ‘fast’ sites in fulvic and humic acids are susceptible to be the fluorophores (or functional groups) bound at the longer wavelength peak C-region, which are rapidly complexed with metal ions. In contrast, the ‘slow’ sites are considered to be the fluorophores at the shorter wavelength peak A-region.

Another time-resolved fluorescence study demonstrates that fulvic acid during its compexation with metal ions has three binding sites, which can be assigned lifetimes and wavelength maxima as follows: ~50 ps at 392 nm, ~430 ps at 465 nm and 4.2 ns at 512 nm (Cook and Langford 1995). The functional groups in humic acid exhibit different proton binding properties as their concentration increases from 20 to 100 mg L^{-1}. The acidic functional group content values suggest that 40 % of the total acidity is accounted for by carboxylic-type sites in humic acids extracted from peat and soil (Vidali et al. 2009). Proton affinity constants to humic acids extracted from various sources suggest that carboxylic and phenolic-type groups in humic acids can bind to H^+, whereas carboxylic-type binding sites exhibit a smaller apparent heterogeneity than phenolic-type sites in humic acids (Vidali et al. 2009; Benedetti et al. 1996; Kinniburgh et al. 1996; Pinheiro et al. 1999; Plaza et al. 2005). Copper(II) complexation with DOM suggests that sites characterized as phenolic based on alkalimetric titration, and not carboxylic sites, account for the majority of Cu complexation under natural water conditions. Cu–DOM complexation mainly takes place through replacement of H^+ by Cu^{2+} at the phenolic binding sites (Lu and Allen 2002). Ca/Mg–Cu exchange experiments with DOM suggest that Ca and Mg are preferably bound by carboxylic sites, particularly at relatively high concentrations, which results in a weakened apparent competition effect (Lu and Allen 2002).

Conditional distribution coefficients (K'_{DOM}) for the binding of Hg(II) to dissolved organic matter (extracted hydrophobic acids) shows that very strong interactions ($K'_{\mathrm{DOM}} = 10^{23.2\pm1.0}$ L kg^{-1} at pH = 7.0), at Hg/DOM ratios below approximately 1 μg of Hg/mg of DOM, are indicative of mercury − thiol bonds. In contrast, much weaker interactions ($K'_{\mathrm{DOM}} = 10^{10.7\pm1.0}$ L kg^{-1} at pH = 4.9 − 5.6), at Hg/DOM ratios above approximately 10 μg of Hg/mg of DOM, are consistent with Hg binding to oxygen functional groups (Haitzer et al. 2002). Similar results have been found in another study where conditional distribution coefficients (log K'_{DOM}) for Hg(II) binding to extracted humic acids, fulvic acids and hydrophobic acids from diverse aquatic environments indicate very strong interaction, suggesting the involvement of thiol groups (Haitzer et al. 2003). It has also been shown that K'_{DOM} values decrease at low pH (4) compared to pH 7, suggesting proton competition for the strong Hg(II) binding sites that is consistent with bidentate binding of Hg(II) by one thiol group ($p^{Ka} = 10.3$) and by another group ($p^{Ka} = 6.3$) in the DOM (Haitzer et al. 2003). In addition, the hydrophobic fraction of DOM is composed of thiol functional groups with high conditional stability constant of the Hg^{2+}-DOM complexes (Benoit et al. 2001; Dyrssen and Wedborg 1986, 1991; Schuster 1991; Xia et al. 1999).

It has been shown that each SRFA molecule has approximately 3 carboxyl sites available for coordination in the pH 6–9 range (Sonke and Salters 2006). However, coordination depends on other environmental factors such as metal concentration, ligand concentration, pH and so on (Sonke and Salters 2006; Thomason et al. 1996). Lanthanide ion probe spectroscopy (LIPS) suggested that an increase in the metal/DOM ratio can result in progressively less chelated complexes, with a gradual succession from 4 to 3 to 2 to 1 carboxyl groups bound to Eu^{3+} (Thomason et al. 1996)· The LIPS study indicated that Eu^{3+} complexation by Suwannee river DOM (mixture of HA and FA) at pH 3.5 is dominated by tetra-dentate complexes at low metal to ligands ratios (100 nM Eu^{3+}, 30 mg L^{-1} DOM) (Thomason et al. 1996). The complex formation of quinonoid-enriched humic derivatives with actinides (ca. Np^{5+}) demonstrated that hydroquinone-enriched derivatives have higher stability constants than the catechol ones, and that enriched humic derivatives are more effective than the parent humic acid (Shcherbina et al. 2007). Moreover, interaction between HA and Np^{5+} in the neutral pH range is dominated by carboxylate groups in aqueous media (Sachs et al. 2005). Conditional stability constants between Cm^{3+} and 5-sulfosalicylic acid ($\log_{10} K = 6.44$), fulvic acid ($\log_{10} K = 5.90$), and humic acid ($\log_{10} K = 6.22$) are all very similar, suggesting the salicylic acid-like functional groups may be present in the molecular structure of humic substances (fulvic and humic acids) (Panak et al. 1995). This can be further highlighted by the observation of enhanced fluorescence intensity in the complexes of Cm^{3+} with 5-sulfosalicylic acid, fulvic acid, and humic acid (Panak et al. 1995). The U^{4+} complexation with humic acids with different sulfur contents (1.9, 3.9, 6.9 wt %) shows that increasing sulfur (>2 wt %) leads to an increase of the number of humic acid binding sites. This is also reflected in increased U^{4+} loading capacities and increased total humic acid ligand concentrations for U^{4+} (Sachs et al. 2010). This increase of the fraction of humic acid binding sites for U^{4+} indicates an involvement of reduced sulfur functionalities, such as thiol groups, in the complexation between U^{4+} and humic acid (Sachs et al. 2010). However, for environmentally relevant sulfur contents of humic acids (<2 wt %), compared to the oxygen functionalities and in particular to carboxylic groups, reduced sulfur functionalities play only a subordinate role in U^{4+} complexation in the acidic pH range. Notes that reduced sulfur species such as thiols, dialkylsulfides and/or disulfides are the dominating sulfur functionalities in extracted humic acids with different sulfur contents (Sachs et al. 2010). Therefore, the functional groups in fulvic and humic acids that form complexes with trace metals are phenolic OH and acidic OH groups, among which are hydroquinone-like moieties and non-quinoid phenols, O-, N- and S-containing functional groups or thiol groups (Lu and Allen 2002; Schmeide and Bernhard 2009; Haitzer et al. 2003; Zhang et al. 2004; Smith et al. 2002).

Strong organic ligands for copper (II) in seawater are likely to derive from biological sources, rather than being refractory organic materials (Wu and Tanoue 2001a, b; Midorikawa and Tanoue 1998; Moffett et al. 1990). The exudates from certain phytoplankton and bacteria, which are important sources of protein-like fluorescence, are strong Cu chelators (Zhang et al. 2009; Mcknight and Morel 1980; Determann et al. 1998). Autochthonous DOM from phytoplankton or algal biomass may contain amino and sulfidic functional groups in its molecular structure, which may form complexes with trace metals in water (Xue and Sigg 1993; Xue et al. 1995).

EEMS of tryptophan amino acid shows two fluorescence peaks: peak T for the amino carboxylic acid functional group [–CH–(NH_2)–COOH] and peak T_{UV} for the –NH group in the aromatic functionality [C_8H_5(NH)–] (Mostofa et al. 2009a, 2011). Interestingly, proteins and oligopeptides are important constituents of high molecular mass-DOM that contains primary amines in seawater (Lee and Bada 1975; Tanoue et al. 1996).

The EPS is primarily composed of polysaccharides, proteins, uronic acid, fatty acids, nucleic acids and lipids containing ionizable functional groups such as carboxyl, phosphoric, amine, acidic amino acids, hydroxyl, phenolic, sulfates, and organic phosphates (Beech and Sunner 2004; Quiroz et al. 2006; Merroun and Selenska-Pobell 2008; Zhang et al. 2008; Wingender et al. 1999; Liu and Fang 2002, 2003; Guibaud et al. 2003; Merroun et al. 2003; Sponza 2003; Guibaud et al. 2005). Such groups can form complexes with trace metals depending on the different environmental conditions. The complexation between EPS and metal ions (ca. Pu^{4+}) suggests that the carboxylic groups in EPS are the primary binding sites at pH 4 and with a ionic strength of 0.1 M NaCl (Harper et al. 2008). The amide functional groups of proteins in the EPS are susceptible to form complexes with trace metals, which have been detected using spectroscopic analysis (Guibaud et al. 2005a; b; Zhang et al. 2006). Polysaccharides can form complexes with trace metal ions, and the hydroxyl groups of neutral polysaccharides and the carboxyl groups of anionic polysaccharides are the probable binding sites for such complexes (Guibaud et al. 2005; Zhang et al. 2006; Brown and Lester 1982). The organic phosphate groups of EPS of *A. ferrooxidans* or phosphate groups in nucleic acids play a major role in the binding of U from aqueous solutions, although this bacterium contains small amounts of phosphates (Merroun and Selenska-Pobell 2008). Bacteria, algae and their exudates also consist of a mosaic of functional groups (i.e., amino, phosphoryl, sulfhydryl and carboxylic groups) and the net charge on the cell wall depends on the pH of the medium (Filella 2008). In addition, trace metals such as Th^{4+} and U form complexes with organic ligand in particulate matter that might be a nonmetal-specific chelator originating from the cell surface of microorganisms (Hirose 2004).

4.1 The Mechanism for Complex Formation Between Trace Metals and DOM in Waters

A relationship between the lability of metal−DOM complexes involving 3*d* transition metals in freshwater and their *d*-electron configuration is discussed by Sekaly and his colleagues (2003). The order of the lability of the metal complexes, Co(II) d^7 > Ni(II) d^8 > Cu(II) d^9 < Zn(II) d^{10}, follows the reverse order of the ligand field stabilization energy (LFSE). There is an exception for Cu(II), the behavior of which is due to the Jahn–Teller effect that shortens the equatorial bonds and lengthens the axial bonds of a tetragonally distorted Cu(II)-L_6 complex (Sekaly et al. 2003). Another mechanism is commonly provided by several studies (Chen et al. 1986; Dixon and Larive 1999; Sharpless and McGown 1999; Simpson et al. 2002; Wrobel et al. 2003), which hypothesize that macromolecules (humic substances) can form aggregations that can be induced by complexation with metal cations. These mechanisms do not explain how the functional groups in DOM or the organic ligands can bind to trace metal ions. Recently, Mostofa and his colleagues (2009a, 2011) firstly provided a comprehensive mechanism for M-DOM complexation using tryptophan as a model compound. A strong π-electron bond between the functional group (F:) of tryptophan and the empty *d*-orbitals of the transition metal (M^{n+}) is formed by donation of electrons (Fig. 7).

Based on the available literature data that concern M-DOM complexation, it is suggested that the functional groups in DOM (or the organic ligands) can form complexes with trace metal ions by two ways. First, donation of electrons occurs

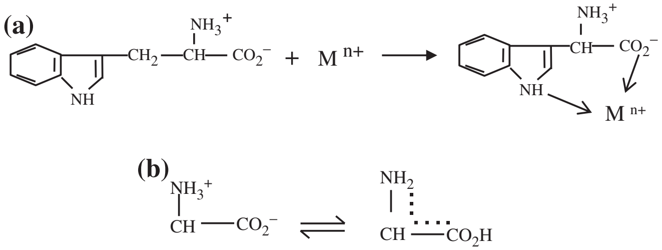

Fig. 7 The mechanism of the chemical bonding of two functional groups (amino-carboxylic and amide) in tryptophan with metal ion (M^{n+}) (**a**) and the resonance configuration of the amino-carboxylic group [–CH(NH_2)–COOH] (**b**). *Data source* with modifications Mostofa et al. (2009a; 2011)

from O-containing functional groups (carboxylic, amino-carboxylic, phenolic, alcoholic and so on) of DOM or organic ligands to metal ions. Second, donation to metal ions of non-bonding π-electrons occurs from S-, N-, and P-containing functional groups in DOM or highly unsaturated π-electrons either in aromatic or aliphatic systems. In both cases, donation of electrons takes place from the functional groups (F:) of DOM to the empty or partially filled *d*-orbitals of transition metals/lanthanides/actinides. Complex formation can also involve *s*- and *p*-orbitals in alkali/alkaline earth metals (M^{n+}) that form a strong covalent or π-electron bonding system with DOM functional groups ($F{:}M^{n+}$). This can simply be expressed as follows (Eq. 4.1):

$$F{:} + M^{n+} \rightarrow F{:}\, M^{n+} \qquad (4.1)$$

The formation of such a bond in M-DOM complexes can greatly reduce the electron density of the functional groups (F:) in DOM. Donation of electrons from functional groups (F:) then causes the *s*-, *p*- or *d*-orbitals in metal ions (M) to be either stabilized or destabilized. Therefore, the M-DOM complexation causes the fluorescence properties of DOM to be either decreased or increased (Mostofa et al. 2009a, 2011). A stabilizing effect from a functional group in DOM lowers the energy of the interacting *s*-, *p*-, *d*-orbitals, which can considerably decrease the electron transfer probability and decrease as a consequence the fluorescence intensity of the functional groups (or fluorophores) in DOM. It is generally known that the metal ions are excellent Lewis acids and accept electron density from many molecules or ions that act as Lewis bases. On the other hand, M-DOM formation may enhance the probability of an electron transfer if a destabilizing effect from the functional groups in DOM raises the energy of the *s*-, *p*-, *d*-orbitals. This effect subsequently leads to an increase in the fluorescence intensity of the functional group (or fluorophores) in DOM.

The two types of mechanism for M-DOM complexation can be clarified by considering tryptophan amino acid as an example, because its molecular structure $(C_8H_5\,(NH) - CH_2\left(NH_3^+\right)CHCOO^-)$ is composed of two functional groups such as $[-CH_2\left(NH_3^+\right)CHCOO^-]$ and [C_8H_5(NH)–] (Mostofa et al. 2009a, 2011). The two functional groups of tryptophan can electronically bind metal ions, creating strong covalent and π-electron bonds, respectively, with the metal-ion *d*-orbitals (Fig. 7). In fact, the $[-\mathrm{CH_2}\left(\mathrm{NH_3^+}\right)\mathrm{CHCOO^-}]$ functional group has a strong affinity toward a resonance configuration, and the $[C_8H_5(NH)–]$ moiety has the nonbonding electrons of the NH- group in the aromatic ring (Fig. 7) that can form complexes with metal ions. The formation of the M-DOM bonding system can greatly reduce the electron density of the functional groups, i.e. the fluorophores in tryptophan, which results in a lower probability of electron transition of those fluorophores and decreases as a consequence the fluorescence intensity of tryptophan after complexation with the metal ions. On the other hand, an increase in fluorescence intensity of the DOM in M-DOM complexation may arise from enhanced probability of electron transition of the fluorophores due to M-DOM complexation that depends on the occurrences of the trace metal ions. The fluorescence peak T in the longer wavelength region is caused by the functional group $[-\mathrm{CH_2}\left(\mathrm{NH_3^+}\right)\mathrm{CHCOO^-}]$ and the peak T_{UV} at shorter wavelengths is linked with the group $[C_8H_5(NH)–]$ (Mostofa et al. 2011). The conditional stability constant ($\log_{10} K_1$) for tryptophan-like or protein-like material of biological origin is 7.82–9.56 for peak T that has stronger bonding capacity than the humic-like component ($\log K_1 = 7.05–8.78$) in lake water (Wu and Tanoue 2001b). Moreover, the order of complex formation of the transition metals and other metals to DOM in natural waters depends on several associated effects (Mostofa et al. 2011). Therefore: (i) Fluorophores with high electron density will merely compete for the metal ions with stabilizing effects of *d*-orbitals.

(ii) Among the transition metals, the size or atomic radius generally decreases with increasing nuclear charge, because the electrons that experience a greater nuclear charge are pulled more strongly towards the nucleus. However, the last few elements (Cu, Zn, Ag, Cd, Pt, Au, Hg, etc.) in each row of the *d*-block are slightly larger than those preceding them because in these cases the electron–electron repulsions caused by the filling of the *d*-orbitals outweigh the increasing nuclear charge. Therefore, the two competing effects of nuclear charge and electron–electron repulsion affect the chemical binding of the *d*-orbital metals with the fluorophores. As one moves across a period, the increasing nuclear charge is usually more significant than the electron–electron repulsion. These combined effects make Cu and other metals of the same row more susceptible to complexation with DOM than the transition metals of the second and third rows. Moreover, the ground states of Sc, Ti, Fe, Co and Ni are ferromagnetic because of the presence of one unpaired electron, while V, Cr, and Mn are antiferromagnetic (Tung and Guo 2007; Iota et al. 2007). It can be proposed that the unpaired electron of ferromagnetic transition metals would easily form a bond with an electron donated by functional groups in DOM, which is not possible for antiferromagnetic metals. This hypothesis can account for the rapid complexation of the ferromagnetic transition metals compared to the antiferromagnetic elements. For example, Fe^{3+} has one electron each in its outer *d*-orbitals and no electron in its outer shell *s*-orbital (Fe^{3+}: $1s^2 2s^2 2p^6 3d^5 3s^0$). Therefore, it can accept electrons in its outer unpaired *d*-orbitals from functional groups in DOM to form M-DOM complexes. Cr^{3+} has

3 electrons in its outer d-orbitals and an empty s-orbital in its outer shell (Cr^{3+}: $1s^2 2s^2 2p^6 3d^3 3s^0$). It can accept electrons in its two empty and three unpaired *d*-orbitals from functional groups in DOM to form complexes. Cu^{2+} has one unpaired *d*-orbital and one empty *s*-orbital in its outer shell (Cu^{2+}: $1s^2 2s^2 2p^6 3d^9 3s^0$), to which the functional groups in DOM can donate electrons when forming complexes. It is assumed that donation of electrons from functional groups in DOM to unpaired *d*-orbitals can enhance the stability of the M-DOM complexes. This gives a strong bonding capacity of Cu^{2+} ions toward functional groups in DOM or organic ligands. Finally, Sc^{3+} has empty *d*- and *s*-orbitals (Sc^{3+}: $1s^2 2s^2 2p^6 3s^2 3p^6 3d^0 4s^0$) that can accept electrons when forming M-DOM complexes. Entirely empty *d*-orbitals of Sc^{3+} ions gives exceptionally strong bonding properties toward fulvic and humic acids (ca. $\log_{10} K = 17.57$ for fulvic acid and 17.54–20.47 for humic acid) among all the metal ions ($\log_{10} K = 3.26$–14.58 and 0.5–16.50, respectively) studied in aqueous solutions (Table 1).

(iii) For alkali/alkaline earth metals and metalloids, the functional groups in DOM can donate electrons to the outer empty *s*- and/or *p*-orbitals. For example, H^+ has no electrons in its outer empty *s*-orbital (H^+:1s) and can accept electrons. Ca^{2+} has one empty *s*-and one *p*-orbital in its outer shell (Ca^{2+}: $1s^2 2s^2 2p^6 3s^0 3p_x^0 3p_y 3p_z$) that can accept electrons. Donation of electrons to *s*- and *p*-orbitals possibly explains the weak bonding properties of Ca^{2+} toward functional groups in DOM. Al^{3+} has an empty *s*- and two *p*-orbitals in its outer shell (Al^{3+}: $1s^2 2s^2 2p^6 3d^3 3s^0 3p_x^0 3p_y^0 3p_z$) that can accept electrons. However, the involvement of these orbitals in complexation with functional groups in DOM would ultimately destabilize the Al^{3+}-complexes. This effect can enhance the fluorescence intensity of Al-DOM complexes in aqueous media. Sb^{3+} has empty *p*-orbitals in its outer shell (Sb^{3+}: $1s^2 2s^2 p^6 3s^2 p^6 d^{10} 4s^2 p^6 d^{10} 5s^2 p_x^0 p_y^0 p_z^0$) that can receive electrons from functional groups in DOM. Therefore, alkali and metals/metalloids can form complexes with DOM or organic ligands in aqueous media.

5 Factors Affecting the Metal-DOM Complexation in Natural Waters

The formation of M-DOM complexes is greatly affected by several factors in natural waters, which can be distinguished as (i) Quantity, nature and molecular size of DOM; (ii) Occurrence and affinity of trace metals; (iii) Effect of pH; (iv) Effects of ions (cations and anions) and their ionic strength (*I*); (v) Effects of photoinduced processes; (vi) Effects of microbial processes; and (vii) Effects of freshwater and sea waters.

5.1 Quantity, Nature and Molecular Size of DOM

The most common organic ligands in DOM are allochthonous fulvic acid, allochthonous humic acid, tryptophan and extracellular polymeric substances (EPS).

The complexation between DOM and trace elements depends on the nature and quantity of DOM in natural waters (Sonke and Salters 2006; Iskrenova-Tchoukova et al. 2010; Sanchez-Marin et al. 2010; Cao et al. 2004; Kaiser 1998; Reszat and Hendry 2007; Jansen et al. 2003; Naka et al. 2006).

Extracted humic acid shows a 65-fold higher affinity for lanthanide metals than SRFA, which suggests that affinity depends on the sources and nature of fulvic and humic acids (Sonke and Salters 2006). Allocthonous fulvic and humic acids have poly-carboxylic, phenolic (catechol), iminodiacetic and aminocarboxylic functionalities in their molecular structures; furthermore, the quantity of the functional groups and the aromaticity are significantly varied between them (Malcolm 1985; Senesi 1990; Morel and Hering 1993; Leenheer et al. 1998; Steelink 2002; Sonke and Salters 2006; Fimmen et al. 2007). Each SRFA molecule has ~3 carboxylic sites available for coordination that can be judged from the carboxyl site density in SRFA (Sonke and Salters 2006; Ritchie and Perdue 2003). The study of SRFA complexation with the lanthanide series hypothesizes that poly-dentate carboxylic, phenolic, and N-containing carboxylic binding sites may be involved under environmental conditions (Sonke and Salters 2006). Correspondingly, autochthonous DOM of phytoplankton or algal origin is composed of amino and sulfidic functional groups, although its exact structure remains unknown (Xue and Sigg 1993; Xue et al. 1995). The conditional stability constants of the M-DOM complexation are thus significantly variable for different DOM sources, even for the same metal ion (Table 1).

DOM in sediment elutriates and sewage-influenced water (i) was enriched by 1.4–1.7 times in DOC; (ii) absorbed and reemitted more light; and (iii) presented higher Cu complexation capacities than the natural seawater (Sanchez-Marin et al. 2010)· This suggests that differences in DOC and DOM may control the metal toxicity in natural waters (Sanchez-Marin et al. 2010). The total DOC concentrations decrease slightly when the C/metal ratio is less than 10, particularly for Al^{3+} and Fe^{3+}. Hydrophilic DOC increases and hydrophobic DOC decreases with increasing concentrations of polyvalent metal cations in the order $Ca^{2+} < Al^{3+} < Fe^{3+}$, whilst Na^{+} remains unaltered (Fig. 8) (Kaiser 1998). Such an effect is more pronounced at low DOC concentrations and high pH values (Fig. 8). This result suggests that the formation of insoluble M-DOM complexes is susceptible to reduce DOC concentrations, whilst soluble metal-DOM complexes may induce an alteration of the distribution between hydrophilic and hydrophobic DOC in natural waters (Kaiser 1998). Therefore, the polyvalent cations and their concentrations can considerably affect the distributions of the DOM fractions and their contents, determined using XAD-8 resins, particularly at low DOC and high pH (Kaiser 1998).

The interaction of several elements (Cu, Mn, Mo, Ni, Sr, U, and Zn) with high dissolved organic carbon (DOC) concentrations (21–143 mg C L^{-1}) showed that only U and Zn can form complexes with all DOC samples. The in situ association constant (K'_d) for U decreases with depth in pore waters (Reszat and Hendry 2007). It is also suggested that minor amounts of U and Zn (less than or equal to 4 % of total) can form complexes with this DOC under in situ pH conditions. Competitive complexation by other ligands may limit the importance of

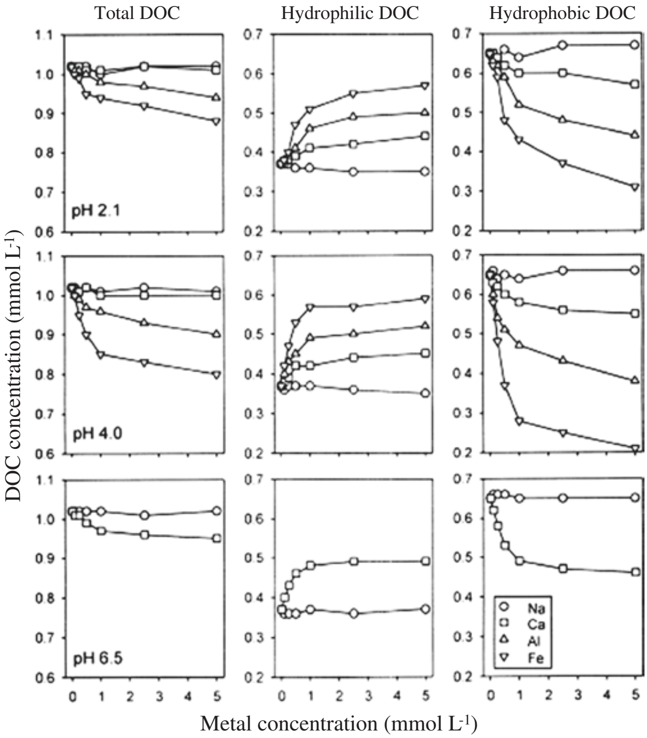

Fig. 8 Effects of increasing concentrations of Na^+, Ca^{2+}, Al^{3+} and Fe^{3+} on the total, hydrophilic and hydrophobic DOC at three pH levels (2.1, 4.0 and 6.5) and an initial total DOC concentration of 1.0 mmol L^{-1}. *Data source* Kaiser (1998)

DOC-facilitated transport of elements (Reszat and Hendry 2007). The ability of actinide ions such as uranyl to form complexes with DOC is very variable depending on the occurrence of humic fractions (fulvic and humic acids) in DOM, which undergo in turn large variations (5–80 %) in the aquatic environments (Mostofa et al. 2009a; Kim and Czerwinski 1996; Giesy et al. 1986; Kim et al. 1994; Artinger et al. 2002; Crancon and van der Lee 2003; Jackson et al. 2005). Interestingly, the complexation of U to DOM is kinetically controlled (Artinger et al. 2002).

Studies on M-DOM complexation suggest that trace metals with high binding strength are primarily distributed in the larger molecular size fractions, and metals with low binding strength are mostly distributed in the smaller molecular size fractions (Wu et al. 2004b; Shin et al. 2001; Lakshman et al. 1993; Midorikawa and Tanoue 1996, 1998; Lin et al. 1995; Rao and Choppin 1995; Sekaly et al. 1998; Sposito et al. 1982). The larger molecular size fractions have a higher affinity for metals such as Al, Cu, UO_2(II), and Np(V) than smaller molecular size fractions (Shin et al. 2001; Lakshman et al. 1993; Midorikawa and Tanoue 1998; Lin et al. 1995; Midorikawa and Tanoue 1996; Rao and Choppin 1995; Sekaly et al. 1998; Sposito et al. 1982). Kinetic studies show that strong affinity metals can form complexes with DOM more rapidly than the weak metals, and that their DOM complexes undergo a slower dissociation process (Wu et al. 2004c; Lin et al. 1995). It is also shown that the distribution of transition metals in DOM is shifted towards the larger molecular size fractions as the binding strength increases. Fe along with V and Ce is distributed mainly in the larger size fractions, but heavy metals such as U, Th and Mo are distributed mainly in the smaller molecular size fractions (Wu et al. 2004b). These studies hypothesize that the strong affinity metal ions can form complexes with the functional groups or replace the weak affinity metal ions in the larger molecular size fractions in the first binding site. Weaker metals then occupy other available sites in the small molecular size fractions.

Molecular size (or mass) distribution and levels of organic ligands suggest that new organic ligands with high molecular masses are produced during periods of high biological productivity in natural waters (Midorikawa and Tanoue 1996, 1998; Mopper et al. 1996). Such new organic ligands are considered to be the autocthonous fulvic acids (C-like) that have recently been found to originate under photoinduced or microbial assimilation of algal or phytoplankton biomass (Fig. 1c, d) (Mostofa and Sakugawa 2009; Zhang et al. 2009). The fluorescence excitation-emission maxima of autocthonous fulvic acid (C-like) are similar to those of the allochthonous fulvic acid and humic acid (C-like), showing two fluorescence peaks in the C- and A-regions (Fig. 1a–d). In the molecular mass distribution of organic ligands, the relative contribution of the fraction with <5 kDa molecular masses is dominant (67–79 %), while 17–30 % of the total organic ligands are in the 5 kDa-0.1 μm fraction, leaving 3–6 % in the 0.1 μm-GF/F fraction in lake water (Wu and Tanoue 2001a). The contribution of organic ligands in the <1 kDa fraction is 41 % of the total in estuarine water (Gordon et al. 1996). The contribution of total organic ligands in DOM accounts for 10–62 % in the case of molecular masses of >1 kDa and 50–90 % for the <1 kDa fraction in sea waters (Midorikawa and Tanoue 1998, 1996; Maurer 1976; Zsolnay 1979; Carlson et al. 1985; Benner et al. 1992; Guo et al. 1994, 1995, 1996; Buesseler et al. 1996; Guo and Santschi 1996). Contributions of total organic ligands are 0.63–4.68 % of the bulk DOM in the water of rivers, lakes and oceans (Wu and Tanoue 2001a, c; Midorikawa and Tanoue 1998; Wu et al. 2001). It has also been observed that the quantities of the weak ligands are relatively high, approximately 0.54–1.21 % of the total DOM whilst the quantities of strong ligands are low, approximately 0.06–0.21 % in stream waters (Wu and Tanoue 2001a). High-affinity nitrogenous moieties account for only 2–4 % of DOM in water (McKnight et al. 1997; Croue et al. 2003). Finally, the M-DOM complexation significantly depends on the quantities, nature and molecular size of DOM in natural waters.

5.2 *Occurrences and Affinity of Trace Metals in M-DOM Complexation*

The M-DOM complexation significantly depends on the occurrence of the trace metal ions and on their affinity toward organic ligands. Complexation shows high variation in the conditional stability constants for alkali, alkaline, heavy metals, transition metals, lanthanides and actinides (Table 1) (Nair and Chander 1983; Kim et al. 1990, 1991; Buckau et al. 1992; Xue et al. 1995; Takahashi et al. 1997; Mandal et al. 1999; Sekaly et al. 2003; van Loon et al. 2004; Wu et al. 2004b; Sonke and Salters 2006; Thakur et al. 2006; Shcherbina et al. 2007). The trace metals have different affinities toward the functional groups and the donor atoms in DOM. Affinity depends on the occurrence of trace metals and on their outer-shell electronic configuration in aqueous media (Wu et al. 2004b, c; Sonke and Salters 2006; Konstantinou et al. 2009; Fu et al. 2007; Mostofa et al. 2011; Duffus 2002; Konstantinou et al. 2007; Marang et al. 2008; Kolokassidou and Pashalidis 2006). The complexation of various trace transitional metals with different molecular size fractions of DOM in natural waters indicates that the number-averaged molecular weight follows the order: Cu > Ni > (Co, Zn, Cr) > Pb > Cd for the DOM-bound complexes. This is consistent with the Irving-Williams series. For DOM-bound complexes with other metals, the order is followed (Fe, V, Ce) > Th > U > Mo (Wu et al. 2004b). The complexation between fulvic acid and several metals (Cu^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+} and Ca^{2+}) at pH 7 demonstrates that for the fast-reacting binding site, the rate constant and the site relative contribution shows an order of $Cu^{2+} > Ni^{2+} > Co^{2+} > Cd^{2+} > Ca^{2+}$ that agrees with the Irving-Williams series. This indicates that the complexation kinetics is affinity-dependent (Wu et al. 2004c). The competitive binding of Cu(II), Co(II) and Ni(II) ions by fulvic acid shows that in the absence of Cu(II) and Co(II), the strong binding sites of the fulvic acid are occupied by Ni(II), forming strong complexes that are inert. In contrast, in the presence of Cu(II) and Co(II), the strong binding sites of the fulvic acid are occupied by Cu(II). Co(II), Ni(II) and the remaining Cu(II) occupy the weak binding sites of the fulvic acid, forming weak complexes that are labile (Mandal et al. 1999). The enhanced lability of the Ni-FA complexes in the presence of Cu(II) and Co(II) suggests that Cu(II) and Co(II) successfully compete with Ni(II) for the strong binding sites of the fulvic acid (Mandal et al. 1999). The order of the lability of the metal complexes, Co(II) > Ni(II) > Cu(II) < Zn(II), follows the reverse order of the ligand field stabilization energy with the exception of Cu(II). The behavior of Cu(II) is also due to the Jahn–Teller effect, which shortens the equatorial bonds and lengthens the axial bonds of a tetragonally distorted Cu(II)-L6 complex (Sekaly et al. 2003).

It is known that the rate of octahedral ligand substitution reactions of 3*d* transition metal complexes is influenced by several factors such as effective nuclear charge, ionic radius (Douglas et al. 1994; Margerum et al. 1978) and ligand field stabilization energy (LFSE) (Morel and Hering 1993; Butler and Harrod 1989). The dissociation kinetics of the 3*d* transition metal complexes agrees with the trend predicted by the LFSE (weak field): Co(II) d^7 > Ni(II) d^8 > Cu(II) d^9 < Zn(II) d^{10} whereas the behavior of Cu(II) is also due to the Jahn–Teller effect cited above (Sekaly et al. 2003). A gradual increase in complexation strength is observed with decreasing ionic radius, an expression of the lanthanide contraction during the complexation between lanthanides and humic substances (fulvic and humic acids) in waters (Sonke and Salters 2006).

Trace metal ions have a specific interaction affinity with organic ligands or DOM that generally depends on the size and outer electronic configuration level of the metal ions. Replacement of a metal ion by a higher affinity metal ion in M-DOM complexation occurs because the number of complexing sites remains constant for a specific amount of DOM. A decrease in fluorescence intensity is often detected after further addition of Cu^{2+} to Hg–DOM complexes, which suggests that Cu^{2+} can act as a stronger quencher for DOM than Hg(II) in aqueous media (Fu et al. 2007). The complexation of humic acid, olive cake and its hydrophilic extracts with Cu^{2+} and Eu^{3+} shows that Cu^{2+} is replaced by Eu^{3+} in the aqueous solution (Konstantinou et al. 2007, 2009; Konstantinou and Pashalidis 2010). Eu^{3+} has only one electron in its outer *d*-orbital shell, which gives it strong affinity to bind to DOM compared with Cu^{2+}. Comparison between Ca–DOM and Cu–DOM complexation demonstrates that (i) Ca–DOM complexation increases of much less than an order of magnitude per pH unit and decreases at higher Ca concentration, differently from Cu–DOM complexation; and (ii) Cu–DOM complexation is highly non-linear, in contrast to the very reduced extent of non-linearity of Ca–DOM complexation (Lu and Allen 2002).

The effective distribution of affinities (Conditional Affinity Spectrum, CAS) of a metal ion binding to a humic substance under natural water conditions suggests three groups of cations (Rey-Castro et al. 2009): (a) Al, H, Pb, Hg, and Cr, which are preferentially bound to the phenolic sites of the fulvic ligand; (b) Ca, Mg, Cd, Fe(II), and Mn, which display a higher affinity for carboxylic sites, in contrast to expectations based on the individual complexation parameters; and (c) Fe(III), Cu, Zn, and Ni, for which phenolic and carboxylic distributions are overlapped.

The complexation of trace metals with the functional groups in EPS varies depending on the environmental conditions. The order is Pb $>$ Cd $>$ Zn for exopolysaccharides of bacterial origin (Loaec et al. 1997); Cu $>$ Cd $>$ Ni $>$ Cr(III) $>$ Cr(VI) for contaminated effluents (Bux et al. 1994); Cu $>$ Cr $>$ Zn $>$ Pb for activated sludge microorganisms (Chua et al. 1999); Zn $>$ Cu $>$ Co $>$ Cd $>$ Cr(III) $>$ CrO_4^{2-} $>$ Ni for activated sludge (Liu et al. 2001); Pb $>$ Cd $>$ Cu $>$ Zn for polluted waters of sewage sludge and paper mill waste (Lister and Line 2001); Cu $>$ Cd $>$ Ni for pure cultures of bacteria originated from activated sludge (Kim et al. 2002); Pb $>$ Cu $>$ Zn $>$ Ni for the first site and Pb $>$ Cu $>$ Ni $>$ Zn for the second site of an acidogenic thermophilic anaerobic reactor (Leighton and Forster 1997); Cd $>$ Pb $\approx$ Cu for activated sludge originated from wastewater treatment plants (Guibaud et al. 2003); and Cu $>$ Ni $>>$ Zn for activated sludge (Guibaud et al. 2003).

Trace metals have highly variable affinity towards various functional groups in DOM and they show strong differences in the conditional stability constants of M-DOM complexation (Table 1) (Sonke and Salters 2006). The complexation between lanthanides and humic substances (fulvic and humic acids) suggests that a gradual increase by 2–3 orders of magnitude in the conditional stability constants from La to Lu follows the decreasing ionic radius and is an expression of the lanthanide contraction (Sonke and Salters 2006).

Overall, the M-DOM complexation greatly depends on the outer-shell electronic configuration of the metal ions in aqueous media.

5.3 Effects of pH

Complexation between DOM and trace elements is highly dependent on pH (Fig. 5) (Zhang et al. 2009, 2010; da Silva et al. 1998a, b, 2002; Sonke and Salters 2006; Shcherbina et al. 2007; Christoforidis et al. 2010; Iskrenova-Tchoukova et al. 2010; Liu and Cai 2010; Cao et al. 2004; Takahashi et al. 1997; Naka et al. 2006; Ghassemi and Christman 1968; Glaus et al. 2000). Fe can strongly form complexes with colored humic substances at low pH (Ghassemi and Christman 1968) and the iron-holding capacity of color increases with pH up to a value of 10, after which it decreases rather abruptly (Shapiro 1964). The complexation of Aldrich humic acid with As^{3+} shows that the stability constants for the first binding site are maximum under acidic conditions ($\log_{10} K_1 = 6.9$–7.2 at pH 5.2), gradually decrease under neutral conditions (6.2–7.1 at pH 7.0) and become lowest at basic pH (5.8–6.2 at pH 9.3) (Table 1) (Liu and Cai 2010). In contrast, in the case of the second binding sites the constants remain similar or increase a little, from $\log_{10} K_1 = 4.5$–5.0 (mean = 4.6) at pH 5.2 to $\log_{10} K_1 = 4.7$–5.3 (mean = 4.9) at pH 9.3 (Table 1) (Liu and Cai 2010). As^{3+} complexation to Aldrich humic acid increases with pH (particularly at and above pH 9.3), whereas the conditional stability constants decrease for the strong binding sites and remain approximately constant for weak binding sites (Liu and Cai 2010). The interaction of Cu(II), Ni(II), and Fe(III) with extracted soil fulvic acids results into quite stable soluble complexes in the acidic pH range from 3 to 6 (da Silva et al. 1998b, 2002). The pH effect can change the complexation capacity toward metals. In fact, the metal order at pH 6 is $Pb > Cu > Cd$ and at pH 7 and 8 it is $Cu > Pb >> Cd$ (Comte et al. 2008). The conditional stability constants (K) between lanthanide series (14 elements) and humic substances (standard fulvic and humic acids) increases with increasing pH in waters (Sonke and Salters 2006).

The fluorescence intensity of EPS at both peak T- and T_{UV}-regions is strongly dependent on the solution pH in the absence and presence of Hg(II), with the maximal fluorescence intensity at neutral pH (Fig. 2) (Zhang et al. 2010). EPS shows higher fluorescence intensity at the peak T_{UV}-region than at peak T, and the trend resembles that of a tryptophan standard (Mostofa et al. 2009a; Zhang et al. 2010). The effects of pH on M-DOM complexation in water imply two things. First, the pH variation (low-pH or high-pH) generates protonation-deprotonation phenomena in functional groups of DOM (or of organic ligands) that subsequently alters the complexation capacity between DOM and trace metals. An increase in pH generally increases the binding capacity between the trace metals and DOM in aqueous media. Second, the presence of cations (ca. Mg^{2+}) can significantly influence the generation of the high-pH forms of functional groups in DOM, even under circumneutral conditions, which increases M-DOM complexation (Fig. 9) (Christoforidis et al. 2010). For example, at neutral pH the resonance configuration of the amino-carboxylic group [$-CH(NH_2)-COOH$] in tryptophan can exist in the highest form that can require the lowest energy for the excitation of electrons (Fig. 2). This effect can result in high fluorescence intensity at

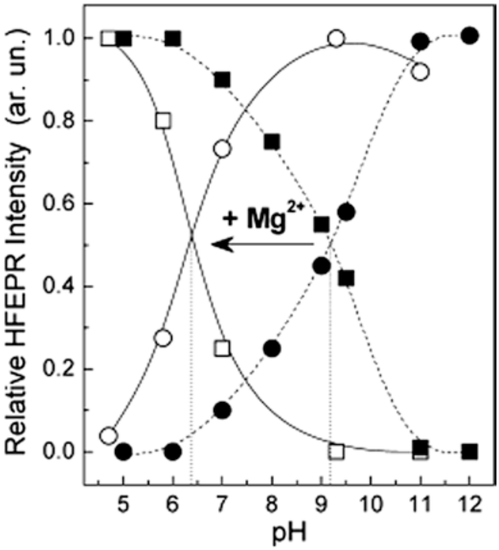

Fig. 9 Relative intensities of the HFEPR spectra of [HA-Mg^{2+}] sample (*solid lines*, *open symbols*) and metal-free HA (*dashed lines*, *solid symbols*) at different pH. The intensities are measured at $g = 2.0041$ {for [HA-Mg^{2+}] (*square*) and the metal-free HA (*filled square*)}, at $g = 2.0054$ for [HA-Mg^{2+}] (*circle*) and at $g = 2.0057$ for metal-free HA (●). *Squares* correspond to the low-pH radical forms and circles correspond to the high-pH radical forms. Data source: Christoforidis et al. (2010)

pH 7 and in donation of electrons from carboxylic functional groups to metal ions. Changes of pH (either acidic or basic) can result in protonation and deprotonation that ultimately increase the excitation energy of the functional groups, thereby decreasing the fluorescence intensity and the electron donation from the functional group to the metal ions. The changes of the fluorescence intensity of EPS and its complexation with Hg(II) as a function of pH are depicted in Fig. 2. Similarly, donation of unpaired electrons from the –NH group in the aromatic ring [C_8H_5(NH)–] of the tryptophan structure is highest under neutral condition (pH 7). Donation of electrons is significantly reduced with changes of pH (either acidic or basic), following either protonation of the unpaired electrons by H^+ in acidic conditions or deprotonation by OH^- in basic solution.

Overall, the pH effect therefore plays a very significant role in metal-DOM complexation in natural waters.

5.4 *Effects of Ions (Cations and Anions) and of Ionic Strength (I)*

The M-DOM complexation is significantly affected by the occurrence of ions (cations ca. $Na+$, $Ca^{2+}$and Mg^{2+} and anions ca. Cl^-, $CO_3{}^{2-}$, OH^-) and by the ionic strength (I) (Cabaniss 1992; Sonke and Salters 2006; Christoforidis et al. 2010; Iskrenova-Tchoukova et al. 2010; Smith and Martell 1987; Cabaniss and Shuman 1988; Fu et al. 2007; Cao et al. 2004; Takahashi et al. 1997; van Loon et al. 2004; Lu and Jaffe 2001; Glaus et al. 2000; Pinheiro et al. 2000). A computational molecular dynamics (MD) study of the interactions of Na^+, Mg^{2+}, and Ca^{2+} with the carboxylic groups of a model DOM suggests that aggregation of the DOM molecules occurs in the presence of Ca^{2+} but not of Na^+ or Mg^{2+} (Iskrenova-Tchoukova et al. 2010). These results suggest that Ca^{2+} ion bridging between NOM molecules can decrease repulsion due to the reduced net charge of the NOM−metal complexes (Iskrenova-Tchoukova et al. 2010).

An increase of Ca^{2+} can enhance the fluorescence intensity of fulvic acid involved in Hg-DOM complexation (Fu et al. 2007). In contrast, the Mg^{2+} ion has no effect on fluorescence and, therefore, on Hg-DOM complexation under natural conditions at pH 7.5 in urban river waters (Fu et al. 2007). On the other hand, the Cl^- ion is an inorganic ligand that can complex Hg(II) to form $HgCl_n^{2-n}$ depending on the concentration of Cl^- in natural waters (Lu and Jaffe 2001). It has been shown that the addition of Cl^- to Hg–DOM complexes increases the fluorescence emission intensity at both pH 7.50 and 9.50 due to the competition between DOM and Cl^- for Hg(II) (Fu et al. 2007). For divalent transition metal ions (Mn^{2+}, Co^{2+} and Zn^{2+}), carbonate complexes may be as important species as humate complexes (Takahashi et al. 1997). Hydroxide species are important for Fe^{3+}, VO^{2+} and Ga^{3+}, and significantly affect the complexation between humic acids and trace metal ions (Takahashi et al. 1997).

Mg^{2+} generates only electrostatic interaction with humic acid during complexation with heavy metals such as Cd^{2+}, Pb^{2+} and Sr^{2+} (Christoforidis et al. 2010). Furthermore, the two types of indigenous radicals that exist in all humic acids are influenced by the metal cations in a similar way, because of the presence of a unique phenolic moiety in humic acid (Christoforidis et al. 2010). Mg^{2+} ions can change the pH profile of the two radical types of humic acid, downshifting their interconversion pK_a by ca. 3 pH units (Fig. 9) (Christoforidis et al. 2010). The competition of Al^{3+} with divalent metal ions such as Pb(II) occurs primarily at carboxylic-type groups. Aluminum can reduce by a factor of 2 to 3 the amount of lead bound to humic acid (Pinheiro et al. 2000). In the case of Cd(II), the aluminum competition mainly affects the bound Cd, with only smaller changes in $[Cd^{2+}]$. Therefore, Al^{3+} competition is likely to increase Pb(II) toxicity and bioavailability and Cd(II) transport in aqueous solution (Pinheiro et al. 2000).

The conditional stability constants of the M-DOM complexation are decreased with increasing supporting electrolyte concentration in aqueous media (Sonke and Salters 2006; Cao et al. 2004; Benoit et al. 2001; Pinheiro et al. 2000; Stevenson et al. 1993; Bryan et al. 2002). It has been shown that conditional stability constants (K) values increase with decreasing ionic strength (I) (from 0.1 to 0.001 mol L^{-1} $NaNO_3$) for complexation of lanthanides with SRFA and extracted humic acids (Fig. 5a) (Sonke and Salters 2006). Similarly, the conditional stability constants of the Hg^{2+}-DOM complexes were increased by 7–12 % at $I = 0$ compared to those at $I = 0.06$ for the hydrophobic fraction of DOM extracted from the Florida Everglades surface waters (Benoit et al. 2001). An increase of ionic strength can lead to a decrease of Pb and Cd bound to humic acid in aqueous solution (Pinheiro et al. 1999, 2000). With a ionic strength of 0.1 M NaCl, the EPS from *P.*

fluorescens exhibited a much stronger affinity for the Na^+ ion compared to other EPS that originated from *S. putrefaciens* and *Clostridium* sp. This finding allows the hypothesis that the deprotonated carboxylic sites of EPS from *P. fluorescens* are mostly bound to Na^+ in solution at pH 4 (Harper et al. 2008). The mechanism behind the lower binding capacity at high ionic strength is probably that complexation at one site will decrease the tendency of a neighboring functional group to form an electrostatic complex with another metal ion. The increasing counter ion condensation in the diffuse double layer of the macromolecule will also weaken the affinity of the binding site (Stevenson et al. 1993; Bryan et al. 2002).

5.5 Effect of Photoinduced Processes

Photoinduced processes have a significant role in metal-DOM complexation in natural waters (Wu et al. 2004a; Bergquist and Blum 2007; Brooks et al. 2007; Yang and Sturgeon 2009; Sanchez-Marin et al. 2010; Vidali et al. 2010; Yin et al. 2010; Zheng and Hintelmann 2009). The protective effect of DOM on Cu and Pb toxicity greatly disappears when the samples are irradiated with high intensity UV-light (Sanchez-Marin et al. 2010). After UV irradiation, the bulk DOC has been found to decrease by between 60 and 75 %, whereas the decreases in fluorescence and absorbance of CDOM range from 85 to 99 % (Sanchez-Marin et al. 2010). The capacity of humic acid to bind copper appears significantly reduced for irradiated humic acid solutions in the pH range from 3 to 6 (Vidali et al. 2010). The observed apparent convergence of the percentage of copper bound to humic acid for photolytically unaltered and irradiated humic acid in the pH range from 6 to 7 is due to the precipitation of copper-soluble species and to the binding on available ionized binding sites (Vidali et al. 2010).

The photoinduced reduction of ionic Hg in natural water can result in the production of elemental Hg in presence of DOM, which is strongly affected by the Hg–DOM interaction. The subsequent reoxidation of elemental mercury to ionic mercury can occur in the presence of DOM in natural waters (Bergquist and Blum 2007; Zheng and Hintelmann 2009; Xiao et al. 1995; Costa and Liss 1999; Zhang and Lindberg 2001; Ravichandran 2004). Photochemistry can thus affect loss by volatilization and bioavailability of mercury to organisms (Ravichandran 2004).

Photoinduced degradation processes can significantly decrease the absorbance (approximately 8–100 %) and fluorescence intensity of humic substances (fulvic and humic acids) (up to 84 %), tryptophan (up to 88 %) and tyrosine (0–100 %) upon irradiation for hours to months in natural waters (Brooks et al. 2007; Mostofa et al. 2011; Stedmon et al. 2007; Moran et al. 2000; Mostofa et al. 2007, 2010; Skoog et al. 1996; Winter et al. 2007; Abboudi et al. 2008; Osburn et al. 2009; Zhang et al. 2009);Norman et al. 2011 Photoinduced degradation is able to sequentially decompose the functional groups of DOM, particularly in macromolecular fulvic and humic acids. It is induced the formation of low molecular weight photo-products, with simultaneous mineralization of dissolved organic carbon (DOC) in natural waters (Brooks et al. 2007; Corin et al. 1996; Amador et al. 1989; Allard et al. 1994; Moran et al. 2000; Mostofa et al. 2007; Backlund 1992; Kulovaara 1996; Bertilsson and Tranvik 1998; del Vecchio and Blough 2002; Vahatalo and Wetzel 2004; Lou and Xie 2006; Vione et al. 2009).

The most important functional groups or fluorophores in humic substances (fulvic and humic acids), which are responsible for complex formation with trace metals, are carboxylic and phenolic-type groups containing hydroquinone-like moieties and non-quinoid phenols, as well as non-bonding O-, N- and S-atoms contained in functional groups of DOM, such as thiols (Vidali et al. 2009; Mostofa et al. 2011; Lu and Allen 2002; Schmeide and Bernhard 2009; Haitzer et al. 2003; Benedetti et al. 1996; Kinniburgh et al. 1996; Pinheiro et al. 1999; Plaza et al. 2005; Zhang et al. 2004; Smith et al. 2002). High-affinity nitrogenous moieties that make up 2–4 % of DOM can dominate complexation at low Cu concentrations (McKnight et al. 1997; Croue et al. 2003), but model and natural organic nitrogen are readily decomposed by photoinduced processes (Langford 1973; Bushaw et al. 1996). Photoinduced losses of amide carboxyls (15 %) are responsible for a decrease of complexation between Cu and DOM in river water (Brooks et al. 2007). It is suggested that the photoinduced changes in the strong binding sites of parent organic compounds are responsible for the decrease of the stability constants of M-DOM complexes in natural waters.

On the other hand, photoirradiation can increase the conditional stability constants between Cu and DOM in river water whilst it decreases the corresponding values in wetland water (Table 1) (Brooks et al. 2007). Coherently, the ligand concentrations in DOM after photoinduced degradation are decreased by 41–45 % in rivers whilst they are increased by 65–161 % in wetland waters (Brooks et al. 2007). Cu complexation is increased by approximately 150 % in wetland DOM after photoinduced degradation, differently from riverine DOM at the same dissolved organic carbon concentrations (Brooks et al. 2007). It has similarly been found that the conditional stability constants remained the same ($\log_{10} K = 13.5$) after photoinduced degradation of estuarine DOM, but they increased a little in lake DOM after hours to days of photoirradiation. In contrast, in both cases the ligand concentrations were decreased photolytically by up to 95 and 24 %, respectively (Table 1) (Sander et al. 2005; Shank et al. 2006). DOM in wetland, lake and estuarine water is mostly composed of algal-derived material that contains comparatively high proportions of lipids, autochthonous fulvic acids and allochthonous DOM (Parlanti et al. 2000; Mostofa and Sakugawa 2009; Zhang et al. 2009; Brooks et al. 2007; Mostofa et al. 2009b; McCarthy et al. 1998; McCallister et al. 2006). Photolysis can decrease the binding affinity via scission of the allochthonous fraction of lignin-derived polyphenols in wetland DOM (Opsahl and Benner 1998; Sun et al. 1998). Simultaneously, it is hypothesized that photoirradiation can double the binding-site density of wetland DOM by polymerizing and photooxidizing the polyunsaturated lipids produced by microbial communities. This assumption is supported by the experimental observation that photooxidation of polyunsaturated fatty acids and triacylglycerides can increase their content of oxygen functional groups and produce aliphatic aldehydes (Kieber et al. 1997).

Therefore, lipid photooxidation causes a 165 % increase in ketone and aldehyde carbonyls (C-II region), which also increases the ratio of carbonyl groups to aromatic ones (Brooks et al. 2007). Correspondingly, the number of metal-binding substituents per aromatic moiety can increase, producing binding sites with weaker conditional stability constants in the residual (or photobleached) wetland DOM (Brooks et al. 2007).

Photoinduced irradiation is unable to cause complete degradation in waters having high levels of DOM, such as 2.3–32.2 mg L^{-1} in rivers, 43.3 mg L^{-1} in wetland, and 22.6–24 mg L^{-1} in estuaries. In contrast, photochemistry can degrade most of the DOM in waters having low levels of DOM, such as $\leqq 1$ mg L^{-1} in upstream rivers (Brooks et al. 2007; Moran et al. 2000; Mostofa et al. 2007). Therefore, 16–23 % DOM losses can occur in waters with high levels of DOM, whilst higher losses (32–36 %) occur in waters with low levels of DOM. As a consequence, the effects of the photoinduced degradation on M-DOM complexes are expected to be lower in high-DOM waters than in low-DOM ones.

The functional groups in protein or tryptophan are amino carboxylic acid [–CH–(NH_2)–COOH] and –NH group in an aromatic system [C_8H_5(NH)–] (Mostofa et al. 2009a, 2011). Similarly, EPS is primarily composed of several ionisable functional groups such as carboxyl, phosphoric, amine, acidic amino acids, hydroxyl, phenolic, sulfates and organic phosphates. These groups can form complexes with trace metals depending on the environmental conditions (Beech and Sunner 2004; Quiroz et al. 2006; Merroun and Selenska-Pobell 2008; Zhang et al. 2008; Merroun et al. 2003; Guibaud et al. 2005)

It has recently been shown that the functional groups of humic substances (fulvic and humic acids) and tryptophan undergo preferential photoinduced decomposition in natural waters (Xie et al. 2004; Minakata et al. 2009). Decomposition and mineralization of the functional groups of DOM by solar irradiation are responsible for the disappearance of complexation between DOM and metal ions (Sachs et al. 2010; Kulovaara 1996; Kulovaara et al. 1996; Bertilsson and Tranvik 2000). Therefore, photoinduced processes can produce a marked increase of metal toxicity in natural waters.

5.6 Effect of Microbial Processes

Microbial processes such as bacterial reductive precipitation, immobilization of soluble metals and M-DOM complexation can significantly affect the mobility (or transport) and toxicity of the trace metals and radionuclides in the aquatic environments (Bergquist and Blum 2007; Yin et al. 2010; Tabak et al. 2005; Francis and Dodge 2008; Fletcher et al. 2010). Key microbial processes such as biotransformation, biosorption and bioaccumulation, as well as degradation or synthesis of DOM can alter the solubility of metals and radionuclides (Fig. 10) (Bergquist and Blum 2007; Yin et al. 2010; Tabak et al. 2005; Francis and Dodge 2008; Fletcher et al. 2010). Metal-reducing or sulfate-reducing microorganisms can directly or indirectly reduce the soluble and mobile trace metals (Cr^{6+}, U^{6+}, Tc^{7+}) to metals (Cr^{3+}, U^{4+},

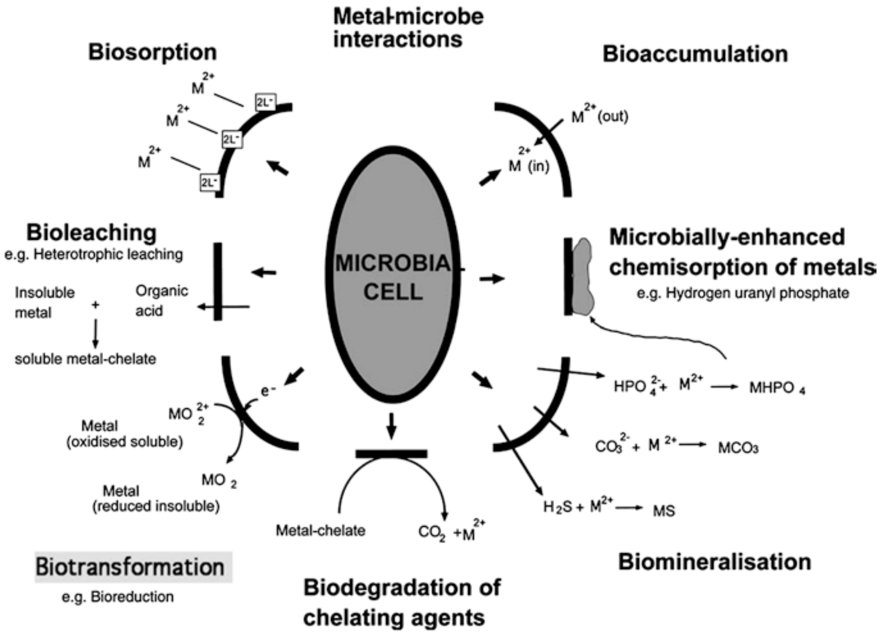

Fig. 10 Metal-microbe interactions impacting bioremediation. *Data source* Tabak et al. (2005)

Tc^{4+}) that are insoluble and less mobile in water, also affecting the M-DOM complexation (Tabak et al. 2005). Another study has shown that the anaerobic spore-forming bacteria *Clostridia*, ubiquitous in soils, sediments, and wastes, are able to reduce Fe^{3+} to Fe^{2+}, Mn^{4+} to Mn^{2+}, U^{6+} to U^{4+}, Pu^{4+} to Pu^{3+}, and Tc^{6+} to Tc^{4+}. They also reduce U^{6+} associated with citric acid in a dinuclear 2:2 U^{6+}:citric acid complex to a biligand mononuclear 1:2 U^{4+}:citric acid complex that remains in solution, in contrast to the reduction and precipitation of uranium (Francis and Dodge 2008). The bioreduction of U^{6+} to U^{4+} also occurs by environmentally relevant bacteria (Gram-positive and Gram-negative), yielding a phase or mineral composed of mononuclear U^{4+} atoms that can form inner-sphere bonds with C/N/O- or P/S-containing ligands (Fletcher et al. 2010; Bernier-Latmani et al. 2010).

Bioaccumulation and biosorption occurs in three ways (Tabak et al. 2005): (i) Sorption on surface sites: sorption of metals and actinides can take place with cell surface active sites. (ii) Surface precipitation: following initial surface sorption, additional surface complexation of metals and actinides can happen by precipitation. (iii) Precipitation with bacterial cell lysate: it occurs by both complexation and precipitation. In addition, microbes can degrade the functional groups of DOM (e.g. fulvic acid, humic acid and tryptophan amino acid) and of synthetic organic ligands (ca. EDTA), or complexes between DOM and trace metals (Tabak et al. 2005). Microbial processes can alter the functional groups (or chromophores or fluorophores) of DOM, causing significant changes (either increase or decrease) in their absorption and fluorescence properties (Moran et al. 2000; Mostofa et al. 2007; Winter et al. 2007; Helms et al. 2008). Microbial changes in the functional groups of DOM that are responsible for complex formation with trace metals are thus susceptible to alter M-DOM complexation in natural waters.

In addition, accumulation of metals by plants (phytoremediation) can reduce the toxicity and content of metals (Tabak et al. 2005; Salt et al. 1995, 1998; Raskin et al. 1997; Pulford and Watson 2003; Schwitzguébel et al. 2002) and, as a consequence, the metal complexation with DOM in the aquatic environments. The key processes are: (i) phytoextraction; the use of metal-accumulating plants to remove toxic metals from soil; (ii) rhizofiltration; the use of plant roots to remove toxic metals from polluted waters; and (iii) phytostabilization or phytoremediation; the use of plants to eliminate the bioavailability of toxic metals in soil.

5.7 *Effects of Freshwater and Marine Water (Salinity)*

The ionic strength and the occurrence of trace elements can affect the complexation between DOM and trace metals and can induce structural modifications of DOM in waters (Wu et al. 2004a, c; Fu et al. 2007; Harper et al. 2008). Sea water is mostly a solution of NaCl where Na and Cl contribute for more than 86 % of the salt content by mass. The order of the other cations is $Mg^{2+} > Ca^{2+} > K^{+} > Sr^{2+}$ and the other main anions are $SO_4{}^{2-}$, $HCO_3{}^{-}$, Br^{-}, and F^{-} (Livingstone 1963; Carpente and Manella 1973; Hem 1985). Comparison of river and sea water shows that Na^{+}, Ca^{2+}, Mg^{2+}, K^{+}, $HCO_3{}^{-}$, Cl^{-} and $SO_4{}^{2-}$ in the sea are typically 1670 times, 27 times, 330 times, 170 times, 2.4 times, 2,400 times and 245 times, respectively, higher than in rivers (Livingstone 1963; Hem 1985). Recently it has been shown that the affinity of EPS for complexing Pu^{4+} decreases in the order of *Clostridium* sp. > *S. putrefaciens* > *P. fluorescens*, although the concentrations of carboxylic groups in EPS isolated in laboratory cultures decrease in the order of *P. fluorescens* > *S. putrefaciens* > *Clostridium* sp. (Harper et al. 2008). The deprotonated carboxylic sites of EPS from *P. fluorescens* are mostly bound by Na^{+} at a ionic strength of 0.1 M NaCl, which might be caused by a much stronger affinity of the Na^{+} ion for the EPS from *P. fluorescens* compared to other EPS (Harper et al. 2008). The high quantities of cations in sea water can induce more rapid complex formation in DOM, even with metals that are relatively less effective in fresh waters. Therefore, DOM is expected to form more complexes with cations in sea waters than in fresh waters.

6 Shifts in Fluorescence Spectral Patterns Due to Metal-DOM Complexation

The complexation of trace elements with fulvic and humic acid (extracted from sewerage sludge) can shift the excitation-emission wavelengths to longer wavelength regions during the initial complexation process (Wu et al. 2004a, c; Plaza et al. 2006). During the Hg–DOM complexation, both excitation and emission wavelengths are increased by up to Ex/Em = 40/25 nm in the first 20 s, after which they remain stable over a period of 100 s (Wu et al. 2004a). The mixing of standard fluorescent organic substances with Milli-Q water and seawater shows that the excitation-emission wavelength maxima of SRFA, DAS1, tyrosine, benzoic acid, *p*-hydroxy benzoic acid, *p*-hydroxy benzaldehyde and *p*-hydroxy acetophenone in seawater are significantly shifted from shorter to longer wavelength regions compared to Milli-Q water (Nakajima 2006). For example, the fluorescence peak C of SRFA dissolved in seawater is detected Ex/Em = 345/452 nm whilst the same peak in Milli-Q water is detected at Ex/Em = 325/442 nm. The peak A remains almost the same in both aqueous media (Nakajima 2006). The fluorescence peak C of autochthonous fulvic acid (C-like) of algal origin is detected at Ex/Em = 340/442–448 nm in Milli-Q water, and at Ex/Em = 340/454–455 nm in river water during the photoinduced and microbial assimilations of algae (Mostofa et al. 2009b). In another study, the same autochthonous fulvic acid (C-like) of algal origin has been detected at Ex/Em = 365/453 nm and 270/453 nm in an isotonic solution during the microbial assimilation of lake phytoplankton (0.5 ‰ salinity) (Zhang et al. 2009). The autochthonous fulvic acid or marine humic-like material of algal origin (peak M) at the peak C-region is found to be shifted from shorter excitation wavelengths (290/400–410 nm in pure Milli-Q water) to a longer wavelength region (300–310/400–410 nm in seawater) (Parlanti et al. 2000).

The shift in excitation and emission wavelength maxima with salinity is presumably caused by the anions and cations present in sea water. Such a shift in excitation-emission from shorter to longer wavelengths is termed the red shift of fulvic acid-like fluorescence. The mechanism behind this red shift in sea water is attributed to the complex formation of the functional groups (or flurophores at peak C-region) in fulvic acid with trace elements or ions markedly present in sea water. The complexation of trace elements with the SRFA functional group (or fluorophore) can significantly enhance the electron transfer of that functional group bound at peak C from the ground state to the excited state upon absorption of longer wavelength radiation. The effect is a shift of the excitation-emission maxima of the peak C to longer wavelengths. This is evidenced by the photoinduced formation of aqueous electrons (e_{aq}^{-}) from organic substances, which is higher in the presence of NaCl than with organic substances alone in aqueous solution (Gopinath et al. 1972; Zepp et al. 1987; Assel et al. 1998; Richard and Canonica 2005; Fujiwara et al. 1993). Rapid excitation of electrons in ionic (saline) FDOM solution is susceptible to shift both excitation and emission maxima of fluorophores (or functional groups) associated to the peak C of allochthonous fulvic and humic acids or autochthonous fulvic acids or other autochthonous DOM. This effect is presumably responsible for the high production of hydrogen peroxide in natural waters (Mostofa and Sakugawa 2009; Fujiwara et al. 1993). Indeed, photogeneration of H_2O_2 from ultrafiltered river DOM is substantially increased with salinity, from 15 to 368 nM h^{-1} at circumneutral pH (Osburn et al. 2009). The mechanism behind this phenomenon apparently can be factors: first, irradiated CDOM can induce photoinduced production of hydrogen peroxide (H_2O_2) that is a hydroxyl radical ($HO^{\bullet}$) source via photolysis or the Fenton reaction, and the photoinduced generation of H_2O_2 is enhanced by salinity. Trace metal ions (M) in salinity or sea waters can complex with DOM (M-DOM) forming a strong π–electron bonding system between metal ions and the functional groups in DOM. This π–electron in M-DOM complex is rapidly excited photolytically, which is responsible for high production of aqueous electrons (e_{aq}^{-}) and subsequently the high production of superoxide ion ($O_2^{\bullet -}$), H_2O_2 and $HO^{\bullet}$, respectively.

On the other hand, the mixing of some standard FDOM (e.g. DSBP, phenol, and tryptophan) with seawater show that the fluorescence excitation-emission wavelength maxima (peak C- region and peak T- region) are shifted from longer to shorter wavelength regions compared to Milli-Q water (Nakajima 2006). Such changes in fluorescence excitation-emission maxima are termed as blue-shift of the flurophores in FDOM. The blue-shift of the FDOM fluorescence peaks can be hypothesized to occur upon loss of high molecular weight fluorescent components by physico-chemical modifications such as flocculation, aggregation or precipitation when the ionic strength is increased (Sholkovitz 1976; Carlson and Mayer 1983; Sierra et al. 1997; van Heemst et al. 2000; Benner and Opsahl 2001; McCarthy et al. 1996). The mechanism behind the blue-shift phenomenon in metal-DOM complexation is presumably the fact that anions or cations can form complexes with the fluorophores (or functional groups) of few fluorescent organic components and may increase the excitation energy of the fluorophores associated to the peak C or peak A-region. The result can be a change in the excitation-emission from longer to shorter wavelengths.

7 Importance of the Metal-DOM Complexation

The complexation of DOM with trace metals is connected to several major biogeochemical phenomena that can be distinguished as: (i) Chemical speciation of the trace metals is of key importance for their biological effects and biogeochemical cycling in natural water, sediment and soil environments (Sekaly et al. 2003; Huber et al. 2002; Hughes et al. 1995; Markich 2002). (ii) DOM including humic substances can control the occurrence of free toxic metals through formation of M-DOM complexes that can significantly reduce the bioavailability and toxicity to organisms in natural waters (Shcherbina et al. 2007; Mostofa et al. 2011; Filella et al. 2007; Hörnström et al. 1984; Markich 2002; Managaki and Takada 2005; Yadav and Trivedi 2006). The bioavailability of toxic metals, their ability to bind to or traverse the cell surface of an organism, is generally dependent on the metal speciation or physicochemical form in the aquatic environments. For example, U^{4+} complexes with humic substances (fulvic and humic acids) and inorganic ligands (e.g., carbonate or phosphate) apparently reduce the bioavailability of U by reducing the activity of UO_2^{2+} and UO_2OH^{+}, which are the major forms of U^{4+} available to organisms, rather than U in strong complexes (e.g. uranyl fulvate) or adsorbed to colloidal and/or particulate matter (Markich 2002). (iii) Formation of M-DOM complexes can influence the transport or migration of metals, the acid–base balance and the solubility in water, sediment and soil environments (Shcherbina et al. 2007; Sekaly et al. 2003; Lippold and Lippmann-Pipke 2009). The complexation of trace metal ions with specific binding sites in fulvic acid can result in conformational folding, thereby changing the outer appearance of the molecule and affecting its mobility (Chakraborty et al. 2007). (iv) Toxic metals and radionuclides can form complexes with humic substances (fulvic and humic acids) in natural aquatic ecosystems (Tabak et al. 2005; Mantoura et al. 1978; Choppin 1988; Higgo et al. 1993; Christensen et al. 1996; Lenhart et al. 2000; Freyer et al. 2009). These trace metals can subsequently undergo facilitated transport with natural DOM via groundwater into the biosphere. As an alternative, they can remain in waters under both oxic and anoxic conditions by forming various soluble complexes with DOM (Francis and Dodge 2008; Fletcher et al. 2010; Kim and Czerwinski 1996; Kim et al. 1994; McCarthy et al. 1998; Bernier-Latmani et al. 2010; Freyer et al. 2009; Artinger et al. 1998; Schussler et al. 2001; Ranville et al. 2007). These results suggest that the complexation of DOM with toxic metals, actinides and radionuclides may affect mobility, toxicity and fate of these elements in the contaminated subsurface environments. (v) Due to the steady-state existence of DOM as M-DOM complexes in natural waters, DOM itself may substantially enhance the production of H_2O_2 by rapid charge transfer, excited-state electron transfer or intramolecular energy transfer in natural waters (Mostofa and Sakugawa 2009; Fujiwara et al. 1993; Dalrymple et al. 2010). The H_2O_2 upon irradiation of river DOM is substantially increased (from 15 to 368 nM h^{-1}) with increasing salinity at circumneutral pH values (Osburn et al. 2009). Therefore, M-DOM complexation plays a significant role in the initiation of photoinduced processes in natural waters. (vi) The M-DOM complexes are highly susceptible to inducing the photo-Fenton reaction, in the presence of the H_2O_2 produced photolytically in surface waters. Such a process is a potential pathway to produce the hydroxyl radical ($HO^{\bullet}$) (Mostofa and Sakugawa 2009; Fujiwara et al. 1993; Zepp et al. 1992; Vermilyea and Voelker 2009). The photo-Fenton reaction for iron can be depicted as follows (Zepp et al. 1992):

$$\mathrm{Fe}^{2+} + \mathrm{H_2O_2} \xrightarrow{h\nu} \mathrm{Fe}^{3+} + \mathrm{HO}^{\bullet} + \mathrm{OH}^{-} \tag{6.1}$$

$$\mathrm{Fe}^{3+} + h\nu/\mathrm{H_2O}^{\bullet}/\mathrm{O_2^{-\bullet}} \rightarrow \mathrm{Fe}^{2+} + \mathrm{O_2} + \mathrm{H}^{+} \tag{6.2}$$

$$\downarrow$$

$$\boxed{\text{Recycling of reaction 6. 1}}$$

Therefore, the M-DOM complexation can regulate the photoinduced degradation processes of DOM and other solutes in natural waters. (vii) Production of pure chemicals in petrochemical industry for extraction, separation and recycling of metals from aqueous and organic phases, where the M-DOM complexes are selectively dissolved (Mostofa et al. 2011; Bhattacharyya et al. 2010). (viii) Metal–organic ligand complexes are less bioavailable than metals and thus are less toxic to a variety of aquatic species (Koukal et al. 2003; Knezovich et al. 1981; van Leeuwen et al. 2005). These complexes can undergo partitioning between liquid–solid and solid–solid phases, thereby affecting the environmental fate and transport of metals (Hays et al. 2004). (ix) The polyvalent cations and their concentrations are considerably affected by the distribution of DOM fractions and by their quantities (which can for instance be determined using XAD-8 resins), particularly at low DOC concentrations and high pH (Kaiser 1998). It is also shown that hydrophilic DOC increases and hydrophobic DOC decreases with increasing concentrations of metal cations, in the order Ca < Al < Fe, due to formation of metal-DOM complexes (Kaiser 1998). (x) Complexation of Fe^{2+} by catechol and thiol ligands leads to the formation of aqueous species that are capable of reducing substituted nitroaromatic compounds to the corresponding anilines, and thus play an important role in the reductive transformation of persistent organic contaminants (Naka et al. 2006). (xi) Competition of Cu(II), Co(II) and Ni(II) for the few (~1–10 % of the total) strong binding sites of humic substances suggest that Ni(II) is bound to the much more numerous (~99–90 % of the total) weak binding sites, forming labile nickel complexes. The consequence is that Ni(II) is largely present as free nickel ion (Ni(II)-aquo complex), which is reported to be toxic (Mandal et al. 1999, 2000; Lavigne et al. 1987). (xii) Metal binding properties of DOM and the interaction of iron-DOM complexes with phosphate can decrease the concentration of dissolved nutrients and subsequently act as nutrient reservoirs during periods of low availability (Francko and Heath 1983; Jones 1998; Vahatalo et al. 2003). (xiii) Complexes between As and humic acids are much stronger than As-protein or As-fulvic acids ones. Therefore, intake of black soil (or dark brown soil) with high amounts of humic acid could reduce As levels in the human body. Note that while eating soil is quite unusual for humans, in some rural Bangladesh villages it is customary for pregnant women to eat *shikal* (it sounds like "chain" in English), which consists of small (2 in. × 1 in. × 1/2 in.) pellets made out of flooded soil (information source is personal experience of one of the authors). While the origin of this tradition is quite uncertain, it is noteworthy that it was observed in one of the world's regions where human exposure to As is the highest.

8 Scope of the Future Research

It is reported that autochthonous fulvic acid of algal or phytoplankton origin, typically shows fluorescence properties at peak C- and A-regions, which is a similar behavior as allochthonous fulvic and humic acids (Coble 1996, 2007; Parlanti et al. 2000; Mostofa and Sakugawa 2009; Zhang et al. 2009; Yamashita and Jaffe 2008; Stedmon et al. 2007; Mostofa et al. 2009b; Fulton et al. 2004; Yamashita and Tanoue 2004; Fu et al. 2010). Currently, PARAFAC modeling has been applied to the identification of various fluorescent components and of their characteristic changes (optical and chemical) when autochthonous fluorescent DOM originates from algae or phytoplankton and undergoes photoinduced and microbial degradation in seawater (Mostofa and Sakugawa 2009; Zhang et al. 2009; Stedmon et al. 2007). The current works do not distinguish between the photoinduced, microbial and metal-complexation processing of the autochthonous DOM and do not differentiate between e.g. autochthonous fulvic acids and terrestrial fulvic and humic acids. Such a differentiation and the elucidation of the behavior of the single components should be a key focus for future research.

An increase in water temperature due to global warming may significantly alter the biogeochemical functions in natural aquatic ecosystems. However, there is currently no study that examines the temperature effect on the metal-complexation features of fluorescent DOM in waters, which might be another key issue for future study. It may lead to understand the impacts of global warming on M-DOM complexation in the aquatic environments.

Fluorescent whitening agents (FWAs) and household detergents show fluorescence properties and are widely distributed in the waters and sediments of rivers, lakes and oceans (Mostofa and Sakugawa 2009; Mostofa et al. 2005, 2010; Managaki and Takada 2005; Stoll and Giger 1998; Poiger et al. 1999; Baker 2002; Yamaji et al. 2010). The complexation of trace elements with FWAs such as diaminostilbene-type (DAS1) and distyryl biphenyl (DSBP) and household detergents might be an interesting concern for future research.

The stability constants for the formation of ternary complexes are similar to those of DOM in surface waters (Tables 1 and 2) (Khalil and Taha 2004; Khalil and Fazary 2004; Radalla 2010; Rosas et al. 2010; Shiozawa et al. 2011). Considering the similarity of the stability constants, ternary complex formation should be taken into account in a speciation description of M-DOM complexation. It could be vital to understand the high variability of stability constants in natural waters and should be a focus for future research.

9 Nomenclature

CDOM	Chromophoric dissolved organic matter
DOM	Dissolved organic matter
EDTA	Ethylenediaminetetraacetic acid
EHA	Elliot soil humic acid standard
EPS	Extracellular polymeric substances
FA	Fulvic acid
FDOM	Fluorescent dissolved organic matter
HA	Humic acid
I	Ionic strength
$\mathrm{Log}_{10}\,K$	Conditional stability constant (logarithm)
LFSE	Ligand field stabilization energy
LHA	Leonardite coal humic acid standard
SRFA	Suwannee River fulvic acid
SRHA	Suwannee River humic acid

Problems

(1) Mention the ten key organic ligands of DOM that can complex trace metal ions.
(2) Mention the key DOM components that show fluorescence properties. Explain the application of fluorescence properties to identify the complexation between metal ions (M) and DOM in aqueous media.
(3) Mention the DOM components of natural origin that can complex metal ions in aqueous solution.
(4) Mention the key possible fluorophores in the molecular structures of fulvic acid, humic acid, and tryptophan.
(5) Explain the theory of multisite Stern–Volmer equation for the determination of M-DOM complexation.
(6) Explain the kinetics of the M-fulvic acid complexation regarding the binding sites of fulvic acid.
(7) Derive the equation for the conditional stability constant of M-DOM complexes in aqueous solution.
(8) How does SRFA behave as homogeneous and heterogeneous complexant to trace metals?
(9) Explain the binding sites in fulvic acid and humic acid and how they differ from autochthonous DOM and tryptophan.
(10) Explain the mechanism for the complexation of metal ions with DOM and explain how the functional groups in tryptophan amino acid form complexes with trace metal ions.
(11) Describe the effects of the M-DOM complexation that depend on the order of complex formation of transition metals and other metals to DOM in natural waters.
(12) Explain the electronic configuration of transition metals (Cu^{2+} and Cr^{3+}), alkaline earths (Ca^{2+}), and heavy metals (Sb^{3+}) and how does it influence the interaction with functional DOM groups in M-DOM complexation.
(13) Explain why transition metals generally have higher bonding properties with DOM functional groups than alkali or alkaline earth metals.
(14) What are the controlling factors for the M-DOM complexation in aqueous solution? Describe four important factors that can regulate the M-DOM complexation in aqueous solution.
(15) Why are the fluorescence excitation-emission maxima shifted in metal-DOM complexes? Explain the mechanisms.
(16) Explain the importance of M-DOM complexation in the aquatic environment.

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