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Trace metal behavior in surface waters: emphasis on dynamic speciation, sorption processes and bioavailability

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Abstract

Trace metals (TMs) play a central role in the functioning of aquatic systems. Both essential and toxic trace metals are involved in different chemical, biological and physical processes that determine their concentration and chemical forms. The various TM chemical form include free hydrated ions; metal complexes with various small inorganic and organic ligands as well as with ill-defined dissolved organic matter; metals adsorbed on the surface of natural or anthropogenic colloids and particles. TMs reactivity, fate and impact on the organisms depend on the proportion of these different forms which in turn is influenced by the biophysicochemical conditions of the media. In this overview, dedicated to the father of the modern limnology François-Alphonse Forel, we present current concepts of chemical speciation, sorption processes and biological availability that play a key role in metal cycling and regulation occurring in the aquatic systems. These concepts are illustrated with examples of field case studies performed in the Riou-Mort stream in France and the plume of the Po River in the Northern Adriatic Sea. The necessity of more environmental realism as well as the exciting gaps and challenges in TM cycling in the aquatic systems are also discussed.

Keywords: *trace metals, speciation, sorption, colloids, bioavailability, phytoplankton, in situ measurements*

Résumé

Comportement des métaux trace dans les eaux de surface: aspects dynamiques de la spéciation, des processus de sorption et de la biodisponibilité. – *Les métaux traces jouent un rôle majeur dans les systèmes aquatiques. Ils sont persistants et impliqués dans de nombreuses réactions chimiques, biologiques et processus physiques qui au final déterminent leurs concentrations et leur répartitions sous une grande variété de formes chimiques. Ces différentes formes incluent: les ions métalliques hydratés; les métaux complexés à des ligands organiques et inorganiques simples, ainsi qu'à de la matière organique d'origine très diverse; les métaux adsorbés à la surface de colloïdes et particules. La réactivité, le devenir et l'impact écotoxicologique des métaux traces dans les milieux aquatiques dépendent de la proportion de ces différentes formes chimiques, qui elles-mêmes dépendent des conditions bio-physicochimiques du milieu. Dans cet article de synthèse, en hommage à François-Alphonse Forel, Père fondateur de la limnologie moderne, nous présentons les concepts les plus récents dans le domaine, en soulignant l'importance de la spéciation chimique, des processus d'adsorption et de la disponibilité biologique qui jouent un rôle clef et déterminant dans le cycle des métaux, leurs comportements et régulation dans les systèmes aquatiques. Ces concepts sont également illustrés à l'aide d'exemples d'études de terrain effectuées en eau douce (rivière Riou-Mort – France), et milieu marin côtier (panache du fleuve Po dans le nord de la mer Adriatique). La nécessité d'accentuer le réalisme environnemental ainsi que les différentes pistes et futurs challenges dans le domaine sont également abordés.*

Mots-clés: *métaux traces, spéciation, processus de sorption, colloïdes, biodisponibilité, phytoplancton, mesures in situ*

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In the aquatic systems, trace metals (TMs) interact with a broad spectrum of biotic and abiotic components via dynamic interrelated processes. As a result they exist in different forms such as free uncomplexed ions, bound to small inorganic and organic ligands, bound to particles, and inorganic and organic colloids, with different reactivity, biological availability and ultimate impact. In this overview in tribute to the father of the modern limnology François-Alphonse Forel, we present the important concepts on the TM behavior in surface waters that have emerged over the last forty years from both laboratory and field studies and discuss some persistent gaps and challenges in this field. Given the rather large subject to be covered, we focus on the chemical speciation, sorption processes of cationic forms of TMs and their biological availability to the planktonic microalgae representing the base of food chain in aquatic systems.

1. Trace metals in aquatic systems

1.1. A new old environmental problem

The biogeochemistry of TMs in fresh and marine waters has received increasing consideration since seventies of the 20th century as present in different reviews (Gaillardet et al. 2003; Batley et al. 2004; Sigg and Behra 2005; Slaveykova and Wilkinson 2005; Tercier-Waeber and Taillefert 2008; Hamilton-Taylor et al. 2011). This attention has been triggered by the technical advances allowing determining very low concentrations of TMs together with the development of the ecotoxicology and increasing public awareness of the possible detrimental effect of some TMs on the human and ecosystem health (Neumann and McIntish 1991; Dallinger and Rainbow 1993; Tessier and Turner 1995; Nordberg et al. 2007). Indeed, TMs can exert biological effects that can be beneficial or harmful for the life. Some trace metals such as Cu(II), Zn(II), Fe(III), Mn(II), Co(II), Ni(II), Mo(IV) and V(V) are essential for the life development and allow a normal metabolic functioning under optimal concentration range. Below or above this optimal range, toxicity can occur due to their deficiency or excess. By contrast, TMs such as Cd(II), Hg(II), Pb(II) can be toxic for organisms even at very low concentration.

TMs are ubiquitous and diverse natural components of the various environmental compartments. Their distributions among these compartments are regulated by different physical, chemical and biological processes (Fig. 1). TMs are exploited from natural accumulation sites and used over thousands of years (Hong et al. 1996; Nriagu 1996; Davis et al. 2000). However, the early 19th century industrial revolution, coupled with the explosion of the population growth that followed, brought about unprecedented demand for metals and an exponential increase in the intensity of anthropogenic metal emissions, both in absolute masses and in the number and type of metal compounds release. TMs are therefore highly sensitive indexes of human impact from local to global scale, as highlighted by global budgets of trace metal pollution and sources (Nriagu 1990; Pacyna et al. 1995); which clearly showed that anthropogenic activities have become the most important catalyzer of the global biogeochemical cycling of TMs. These inventories have also shown that aquatic ecosystems are the ultimate sinks of most of metals emitted in the atmosphere and soils, in addition to those from direct release. Consequently, TM concentrations can exceed the background levels normally found in surface and coastal waters. Since TMs are among the conservative pollutants that are not subject to degradation or other breakdown processes with infinite persistence, bioaccumulative capacity and toxicity, they could represent a potential risk to the environment and public health.

Nowadays, the anthropogenic perturbation of the TM biogeochemical cycles embraces new dimensions given the increased demand for metals to satisfy the rapid development of new technologies, such as electronics, fuel cell, semiconductors, car exhaust technology etc., and associated "new waste" problems. In addition, some of these problems, *e.g.* e-waste, are further exacerbated by the export of electronic waste from developed to developing countries (Nnorom and Osibanjo 2008). Moreover, exponentially growing nanotechnology can also lead to un-foreseen environmental and (eco)toxicological problems caused by consumer products and combustion of material based on engineered nanoparticles (ENPs) (Dunphy Guzman et al. 2006). The development of technologies for efficient remediation of metal contaminated systems is still challenged by the complex behavior of metals in aquatic systems. Metals can exist in a variety of forms in water, not all of which are equally toxic, mobile, or bioavailable. Moreover, metal associations are dynamic and reversible, reflecting changing in physicochemical conditions in surface waters. Therefore, even with reduced loading scenarios, potential effects from metals released from sedimentary compartments can be envisioned for some time to come. Such processes may be driven indirectly by anthropogenic activities leading to significant change in the natural equilibrium of the media, *e.g.* acidification, seasonal anoxia, climate variability. The implementation of appropriate legislation and successful remediation strategies is therefore intimately related to a fundamental understanding of the key processes and dominant variables controlling metal dynamics in surface waters.

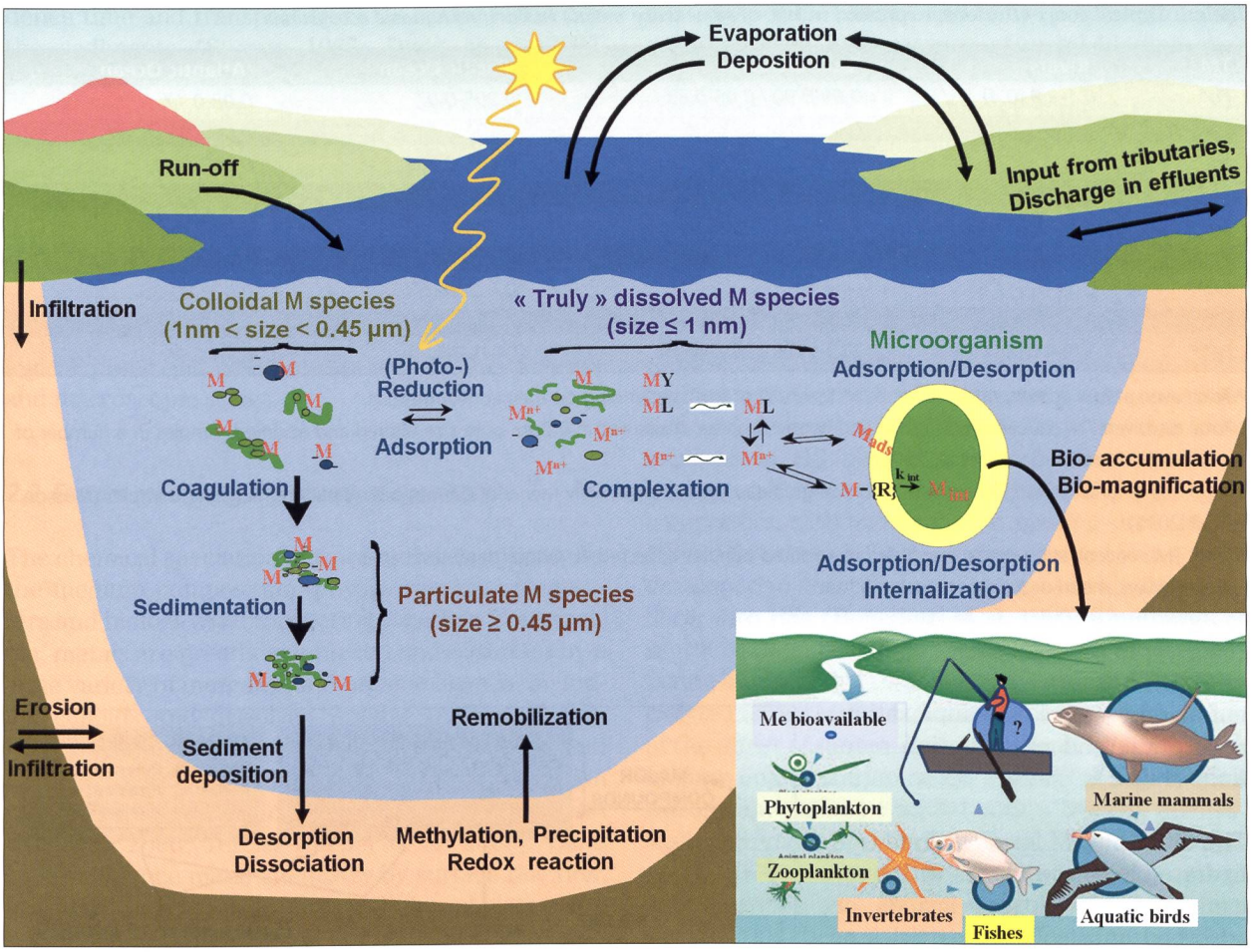

Fig. 1. Conceptual representation of the different fluxes of trace metals in aquatic ecosystems, with emphasis on the physical, chemical and biological processes regulating metal cycling in the water column. TMs reach aquatic systems via: direct release, input from tributaries, atmospheric deposition, surface runoff of surrounding lands, exchange with groundwater, and release from sediments. They are involved in different processes and distributed under different chemical forms: free metal ions (M^{n+}), complexes with various inorganic and organic ligands forming small labile and mobile complexes (ML), or inert or non-mobile complexes (MY); adsorbed into a variety of colloids and particles. M-Rs represents the metal bound to the receptor sites of the biological membrane; M_{ads} is the metal adsorbed to the microorganism surface. TMs can be eliminated through: discharge in effluents, evaporation, and uptake by surrounding vegetation and organisms and subsequent bioaccumulation along the food chain, infiltration into subterranean waters, reactions in the upper sediment layers and sedimentation.

1.2. Main sources and concentration levels

Trace metals are released by various natural and anthropogenic sources (Nriagu 1990; Pacyna et al. 1995) and reach aquatic systems via different pathways, including: direct inputs, atmospheric deposition, run-off from land/soil and rock erosion (Fig. 1).

The concentration ranges for both essential and toxic TMs in the various aquatic ecosystems differ from one metal to another and are highly variables (Table 1). They range typically from pM to few nM in open ocean and slightly impacted freshwater bodies, and may reach locally values of 2 to 3 order of magnitude higher in heavily polluted areas (Davis et al. 2000; Pauwels et al. 2002, Audry et al. 2004).

2. Trace metal speciation

2.1. Chemical speciation concept

The chemical speciation is defined as the distribution of an element amongst defined chemical species in a system (Templeton et al. 2000). Trace metals are inherently persistent, i.e. they are neither created nor degraded by natural and anthropogenic processes. Once they have entered aquatic ecosystems, trace metals are involved in biogeochemical processes and distributed under many different chemical forms (Fig. 1). Their reactivity is determined by the interaction with a large variety of inorganic and organic compounds and controlled by interdepend-

Table 1. Typical concentrations, expressed in nM, of some trace metals in various aquatic ecosystems.

TM	[1]Rivers	[2]Lake Geneva	[3]Pacific Ocean	[3]Atlantic Ocean
*Pb**	*0.1-3.9 (0.4)*	*0.09-0.90 / 0.08-0.83*	*0.005-0.07*	*0.08-0.17*
*Cd**	*0.009-1.6 (0.7)*	*0.04-0.18 / 0.03-0.16*	*0.002-0.10*	*0.007-0.25*
*Hg**	—	—	*0.001-0.002*	*0.002-0.01*
Cu**	3.6-40.9 (23.3)	14.9 / 25.3 / 14.4-22.1	0.5-5.3	1.1-2.0
Zn**	3.1-44.7 (9.18)	3.2-77.4 / 2.9-68.0	0.08-9.0	0.04-2.0
Ni**	3.4-85.2 (13.6)	—	2.5-10.9	2.0-3.0
Co**	0.34-7.3 (2.51	—	0.013-0.054	0.022-0.056
Mn**	8.38-923.4 (619)	16.2-85.0 / 0.3-60.4	0.1-0.77	0.15-0.96
Fe**	179-11925 (1182)	225-1116 / 24.3-253.7	01-0.77	0.15-0.96

* *Toxic even at low concentration.* ** Essential or toxic depending on their concentrations.

[1] Total dissolved TM concentration range and (world average) measured in 0.2µm-pore size filtered and acidified samples in a number of worldwide rivers (Gaillardet et al. 2003).

[2] Total and total dissolved TM concentration range measured in respectively raw and 0.2µm-pore size filtered acidified samples (Masson et al. 2012).

[3] Total TM concentration range monitored in acidified samples collected at various depth and area (http://www. mbari.org/chemsensor/pteo.htm).

ent acid/base, redox, complexation, adsorption, and precipitation reactions (Fig. 2).

The different TM forms include free hydrated ions, complexes with inorganic species, natural ligands or adsorbed onto colloids, particles or microorganisms. The chemical speciation of metal ions plays an important role in geochemistry and in life cycle of TMs and determines to a large extent their mobility, toxicity and bioavailability (Campbell 1995; Wilkinson and Buffle 2004). As it may vary continuously in space and time, it is thus obvious that measurements of total metal concentrations alone do not yield sufficient information on the fate, biological effects and ultimate environmental impact of TMs. The measurement of relevant specific metal species or groups of homologous metal species, *i.e.* speciation analysis, and their variation as a function of time is essential. Important groups of homologous species, defined based on their size, include (Fig. 1): particulate (> 0.45 µm), colloidal (1 nm - 0.45 µm) and "truly" dissolved metal species (≤ 1 nm). The latter includes free (hydrate)-metal ions which are known to be related to metal bioavailability as well as labile and mobile inorganic and organic complexes with anthropogenic or natural organic ligands which are potentially bioavailable (Campbell et al. 2002; Wilkinson and Buffle 2004). Colloidal and particulate materials play a key role in sorption, coagulation, and sedimentation processes (Fig. 1) which influence trace metal resi-

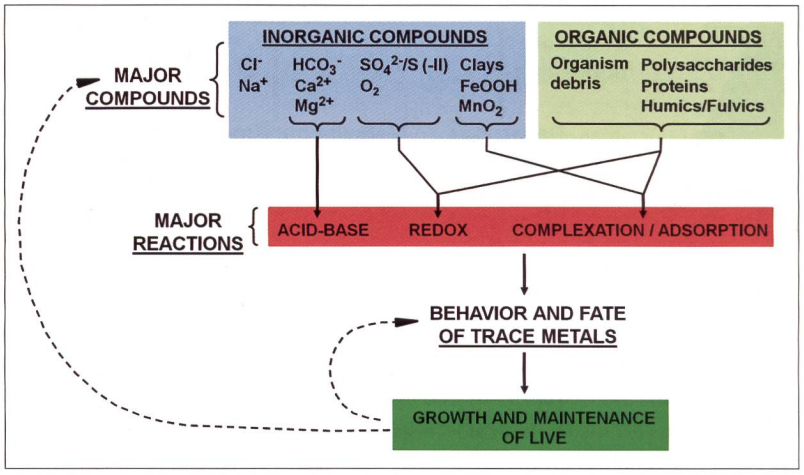

Fig. 2. Classification of aquatic major compounds and trace metals as a function of their environmental role. The major compounds involve dissolved inorganic cations and anions found in concentrations typically >10⁻⁵ M; dissolved organic compounds such as humic and fulvic acids (FA / HA), polysaccharides, proteins; colloids such as Fe and Mn (oxy-hydr)oxides; aluminosilicates (clays); colloidal and particulate silicates, and debris of plants and animals. These major compounds are formed by only 15 elements of the periodic table, i.e: H, C, N, O, P, S, Ca, Na, Mg, K, Cl, Mn, Fe, Al, Si. They control the chemical conditions of the natural waters by means of in particular four basic chemical reactions: acid/base, redox, complexation, adsorption, and precipitation. The behavior of the trace metals is constrained by these chemical conditions. Many of the TMs are essential, and their bioavailability is a limiting factor in the development and maintenance of life. Conversely, life exerts a strong feedback on major chemical conditions (acid-base, redox, etc.) through processes such as photosynthesis and respiration (Buffle and De Vitre 1994).

dence time and transport from the water column to the sediments (Stumm 1985; Dong et al. 2007; Wilkinson and Lead 2007). Metals transported to the sediments can be either buried or remobilized via various diagenetic processes including reduction of manganese and iron minerals during mineralization of natural organic matter or reaction with sulfides produced during sulfate reduction (Berner 1980; Canfield et al. 1993; Audry et al. 2006; Burdige 2006). TM speciation in aqueous environment is thus controlled by complex linkages of geochemical and biological processes which occur at both microscopic and macroscopic scales.

2.2. Factors modifying the chemical speciation

The chemical speciation of trace metals is affected by the medium composition, physicochemical parameters and biological factors acting together. For example, metals are greatly complexed and adsorbed by a large variety of inorganic and organic ligands, including colloids and dissolved organic matter (DOM). Physicochemical parameters, such as salinity and water hardness, pH and biota affect metal speciation (Buffle 1988; Wilkinson and Buffle 2004; Lead and Wilkinson 2006; Tercier-Waeber et al. 2009). Biota could influence metal chemistry by surface reaction, biouptake, synthesis of intracellular chelator and secretion of low molar mass exudates, as well as production of extracellular polymeric substances (EPS) with complexing properties (Dedieu et al. 2006; Worms et al. 2006; Tercier-Waeber et al. 2009; Slaveykova et al. 2010). Transformations among the various metal species may occur continuously on time scales that vary depending on the element, and the bio-physicochemical conditions of the medium in which it is found (Buffle 1988; Buffle et al. 2009). As a consequence, a correct interpretation of the behavior, fate and environmental impact of trace metals must consider the reactivity and fluxes of the specific metal species (or group of homologous metal species), their exchange between compartments and biota via interface processes, and the relative time scale of the various processes.

2.3. Trace metal binding to dissolved organic matter

Among the factors affecting metal speciation in natural waters, DOM plays a key role by buffering the free metal ion concentration in a wide concentration range. With respect to metal binding, DOM exhibits three general characteristics (Buffle 1988; Dudal and Gerard 2004; Benedetti 2006): (i) polydispersity, incorporating a range of molecular size and structures; (ii) polyelectrolyte character, resulting from presence of a large density of charge due to numerous dissociable sites; and (iii) polyfunctionality, resulting from the presence of metal binding sites with different chemical nature. Among the different components of DOM, humic substances (HSs) are most often studied, in particular humic and fulvic acid fractions (HA and FA). Two major types of sites, carboxylic and phenolic, are considered to bind metal ions in HSs. Different factors including salt concentration, type of ions in the medium and pH can influence the inter- and intramolecular interactions of HSs and thus change their metal binding properties (Buffle 1988; Benedetti et al. 1996). In general, metal binding will decrease with increasing salt concentration and decreasing pH. These effects are due to an increase of HS charge screening when the ionic strength increase, and to an increase of proton (H^+) competition with metals for the binding sites (Buffle 1988; Christl et al. 2005). Various models have been developed to describe metal and proton binding equilibria with HSs (Benedetti et al. 1995; Kinniburgh et al. 1999; Benedetti 2006; Van Riemsdijk et al. 2006) taking into account their heterogeneity and polyelectrolyte character. Such models enable the prediction of the effect of different physicochemical parameters in large concentration range. Non-Ideal Competitive Adsorption (NICA) coupled with a Donnan electrostatic sub-model (Benedetti et al. 1995; Kinniburgh et al. 1999; Benedetti 2006; Van Riemsdijk et al. 2006), and model VI (Tipping 1998; Dudal and Gerard 2004) are among the most widely used.

Similarly to HSs, EPS components contain functional groups able to bind metals (*e.g.* carboxylic, phosphoric, amino and hydroxylic groups) and thus to affect TM speciation. EPS, make up a quantitatively important fraction of DOM in natural waters. They represent a large proportion of flocs and biofilms (Flemming and Wingender 2001), up to 25% of DOM in freshwaters, especially during algal blooms (Wilkinson et al. 1997) and up to 80% of organic carbon in marine systems (Verdugo et al. 2004). Nonetheless much less is known about their metal binding properties. Complexation of Cu(II) by EPS has been observed for a number of algae, including *Dunaliella tertiolecta* (Gonzalez-Davila et al. 1995), *Chlorella spp.* (Kaplan et al. 1987), *C. reinhardii* (Xue et al. 1988), *Phaeodactylum tricornutum*, and *Thalassiosira weissflogii* (Gonzalez-Davila et al. 2000). Mixture of carbohydrates, proteins and siderophores produced by *Sinorhizobium meliloti* also complexed Cd(II) (Dedieu et al. 2006; Slaveykova et al. 2010). Some progress in modeling the binding of TMs by the EPS based on the approaches developed for HSs was also achieved. For example, NICA-Donnan model has been successfully applied to interpret proton and Cd(II) binding by exopolysaccharides (Lamelas et al. 2006) and alginates (Lamelas et al. 2005a). Furthermore, new pow-

erful hyphenated techniques such as asymmetrical flow field-flow fractionation coupled to differential refractive index and UV detection, multiangle laser light scattering and the ICP-MS (AFlFFF-ICP-MS), allowed demonstrating the size dependence of the metal binding to the EPS. For example Cd(II), Zn(II), Ca(II) bound preferentially to lower molar mass fractions of the EPS of different origin (Alasonati et al. 2010; Worms et al. 2010; Hassler et al. 2011) and alginate (Alasonati et al. 2006; Alasonati et al. 2007), while Pb(II), Al(III) and Fe(III) were found to predominate in the high molar mass fraction. Nonetheless, to understand the role of various fractions of DOM, it is necessary to improve our knowledge on metal binding by EPS, as well as to explore their significance in the presence of humic substances and inorganic colloids.

3. Interaction of TMs with aquatic colloids

3.1. Concept of TMs binding with colloids

The interaction of the TMs with colloids strongly modifies their transport and elimination in surface waters (Doucet et al. 2006; Lead and Wilkinson 2006). The primary justification comes from the importance of the aquatic colloidal phase in binding large fractions of TMs. Based upon colloid number, and surface area, TMs are associated to colloids through TM-surface binding processes. TM-surface binding involves adsorption of TMs at oxy-hydroxide surface sites (Lyven et al. 2003), ions exchange within clay minerals (Plaschke et al. 2002), complexation by organic colloidal material or organically coated particulate matter, or adsorption of a metal-ligand complex (Tipping et al. 1988; Weber et al. 2006). For example it has been shown that the mobility of As is primarily controlled by adsorption onto colloid clay minerals and metal oxide surfaces (Dixit and Hering 2003; Ilwon et al. 2004; Sharma et al. 2012). TM sorption reactions show both metal ion and solid surface specificity. Several speciation codes containing equilibrium models and surface complexation constants are available to predict the speciation of TMs in the presence of colloids and evaluate the role of pH, ionic strength and chemistry of the colloidal phase (Tipping 2002). Some important stability constants for metals (Al(III), Cu(II), Fe(III), Mn(II), Ni(II), Pb(II), Zn(II)) and different binding phases have been summarized in (Doucet et al. 2006). In all cases, results indicate a high affinity of TMs to aquatic colloids. As a result mobile colloids facilitate but also strongly control the transport and speciation of TMs. However the mobility of colloids and associated TMs also strongly depends on colloid dispersion and aggregation processes (Buffle et al. 1998). Dispersion leads to a release of stable colloids, thereby generating mobile colloids. Thus associated TMs can be transported over long distances. If the colloids are unstable, meaning that they aggregate, colloid aggregation will facilitate TM elimination from the water column through aggregate sedimentation processes. However in such heterogeneous and complex systems, prediction of colloid aggregation or stabilisation, and thus TM transport and elimination cycles, remains difficult due to colloid heterogeneity, dynamic and reversible association processes and distribution of reactive sites at the colloid surfaces. Consequently when making predictions on the circulation, speciation or bioavailability of TMs in the aquatic systems, it is important to determine not only their binding to each type of colloids but also the interactions of the major groups of colloids between themselves. The above interactions are largely influenced by the water chemistry such as pH, ionic strength, temperature, ion composition, as well as the type and concentrations of the DOM leading to colloid surface coating and hence modification of their chemical reactivity. DOM will then also strongly affect the dynamic properties of inorganic colloids, such as aggregation versus dispersion, immobilization versus diffusion, and consequently the bioavailability of the associated TMs.

3.2. Major TM sorbing colloids in aquatic systems

Colloidal size range in aquatic systems is defined as the range from 1nm to 0.45 µm. Colloids are inorganic or organic entities large enough to have supra-molecular structures and properties but small enough not to sediment quickly over reasonable timescales (hours-days) in the absence of aggregation (Gustafsson and Gschwend 1997). Three major components of the colloidal pool can be considered; inorganic colloids, HSs, and non-humic components (Buffle et al. 1998).

The most abundant *inorganic colloids* found in oxic waters include aluminosilicates (clays), calcium carbonates, silica, iron and manganese oxy-hydroxides. Inorganic colloids are usually considered as "compact" charged and spherical entities. Excepted iron oxy-hydroxides positively charged in the circumneutral pH range, most of the inorganic colloids are negatively charged in water. Inorganic colloids are characterized by a net negative surface charge and diffuse layer charge which is mainly controlled by the solution pH, co- and counter- ions distribution around them, adsorption of multivalent ions, binding of charged TMs, and adsorption of organic material. Using AFIFFF-ICP-MS and mass balance calculations (Lyven et al. 2003), it has been found that the smallest

colloid size fraction (<30nm) contains the most trace elements. *Non-humic components* such as fibrillar polysaccharides, EPS, cellular debris, peptides, peptidoglycans, proteins, also referred here to as biopolymers (BPs), constitute a significant proportion of colloidal pool. Their total contour length can be larger than 1 µm, whereas their thickness is often only a few nanometers. They can behave as rigid, semi-flexible or rigid linear polyelectrolyte and undergo important conformational transitions (Fig. 3) and changes in TM binding properties as a function of the temperature, pH, and ionic strength (Balnois et al. 2000; Avaltroni et al. 2007). Their particular properties arise from the long-range nature of the Coulomb interactions between their charged monomers. In the vicinity of biopolymers, small charged mobile counterions and TMs can interact strongly with the chain backbone, leading to a rich conformational behaviour (from extended conformations to coils) by reducing Coulomb repulsions and introducing ion-ion mediated attractions (Carnal and Stoll 2011).

Humic substances generally represent an important and active TM binding fraction of DOM (Redwood et al. 2005). They can be considered macromolecules with a relatively low molar mass with high charge negative densities at neutral or alkaline pH values. Due to the significant degree of branching of HSs, their high charge density and degree of hydration, they are less flexible than biopolymers, less subject to conformational changes and behave as small rigid globular macromolecules with diameters in the range 1-5 nm (Rizzi et al. 2004). In addition to their TMs strong binding properties, HSs also adsorb on inorganic surfaces and interact with non-humic substances, hence facilitating the adsorption of TMs on inorganic particles, aggregate formation and TM elimination (Seijo et al. 2009).

3.3. Major colloid interactions and aggregation processes

Colloidal interaction and aggregation processes are determined to a large extent by *electrostatic interactions*, which in turn are conditioned by the colloids charge density, and the ionic strength of the medium typically embodied in the Debye-Hückel parameter (Ulrich et al. 2006a). Depending of the solution chemistry which is expected to control surface charge net values and charge screening effects (ionic strength), colloids of a given type may aggregate together resulting in *homoaggregation* processes, or with colloids of other types resulting in *heteroaggregation* processes. Theory exists only for the prediction of the homoaggregation kinetics of compact colloids (Stoll and Diez 2008) and considers both electrostatic and van der Waals forces. There is no such general theory for heteroaggregation, especially for aggregation involving large biopolymers, despite an important literature on the modelling of strong charged (and/or hydrophobic) polyelectrolyte chains, and their complexation with nanometric colloids such as nanoparticles and metal cations (Ulrich et al. 2005; Ulrich et al. 2006b). *Homoaggregation of colloids.* The interaction energy between two compact, spherical colloids and associated TMs results essentially from (i) long range electrostatic interactions, and (ii) short range attractive van der Waals forces between them. Electrostatic interactions are always repulsive in homoaggregation, whereas they are attractive or repulsive in heteroaggregation, depending on the nature of colloids. Homoaggregation models that assume that all particles have similar surface properties have been found reasonably successful (O'Melia 1980; Filella and Buffle 1993) This is partly due to the fact that most inorganic colloids in freshwaters are covered by a similar layer of fulvic compounds and can be treated as a single class of colloidal compounds, irrespective of their nature. *Heteroaggregation of inorganic colloids with comparatively small biopolymers and HSs.* For

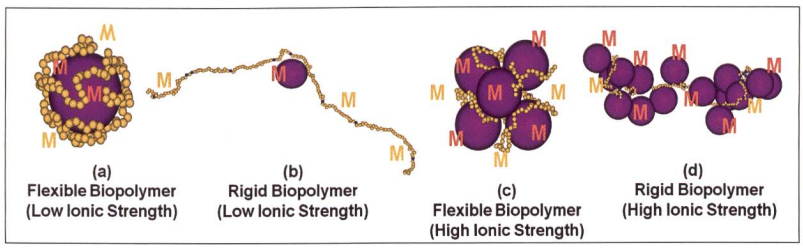

Fig. 3. Computer modelling of biopolymer-inorganic colloid (purple spheres) aggregate formation processes (Ulrich et al. 2006a). According to the pH (controlling the biopolymer ionization degree but also surface charge density of the inorganic colloid and TM binding affinity), conformation of ionisable biopolymers (yellow chain) may change drastically with charge density. Ionic strength is also expected to control the importance of the electrostatic interactions (biopolymer monomer-monomer, inorganic colloids-biopolymers, TM-biopolymer (M) and TM-inorganic (M) adsorption processes). Biopolymer flexibility (intrinsic and electrostatic) is also expected to control the final structures of the TM-inorganic and biopolymer aggregates hence resulting in the formation of very heterogeneous structures from dense and collapsed conformations to more extended structures (important effect on the settling velocities). At low ionic strength the flexible biopolymer is found here to stabilize the inorganic colloid mainly by steric and electrostatic effects whereas the rigid biopolymer to promote bridging processes between inorganic colloids. At high ionic strength the biopolymer is expected to promote the aggregate formation in the two cases.

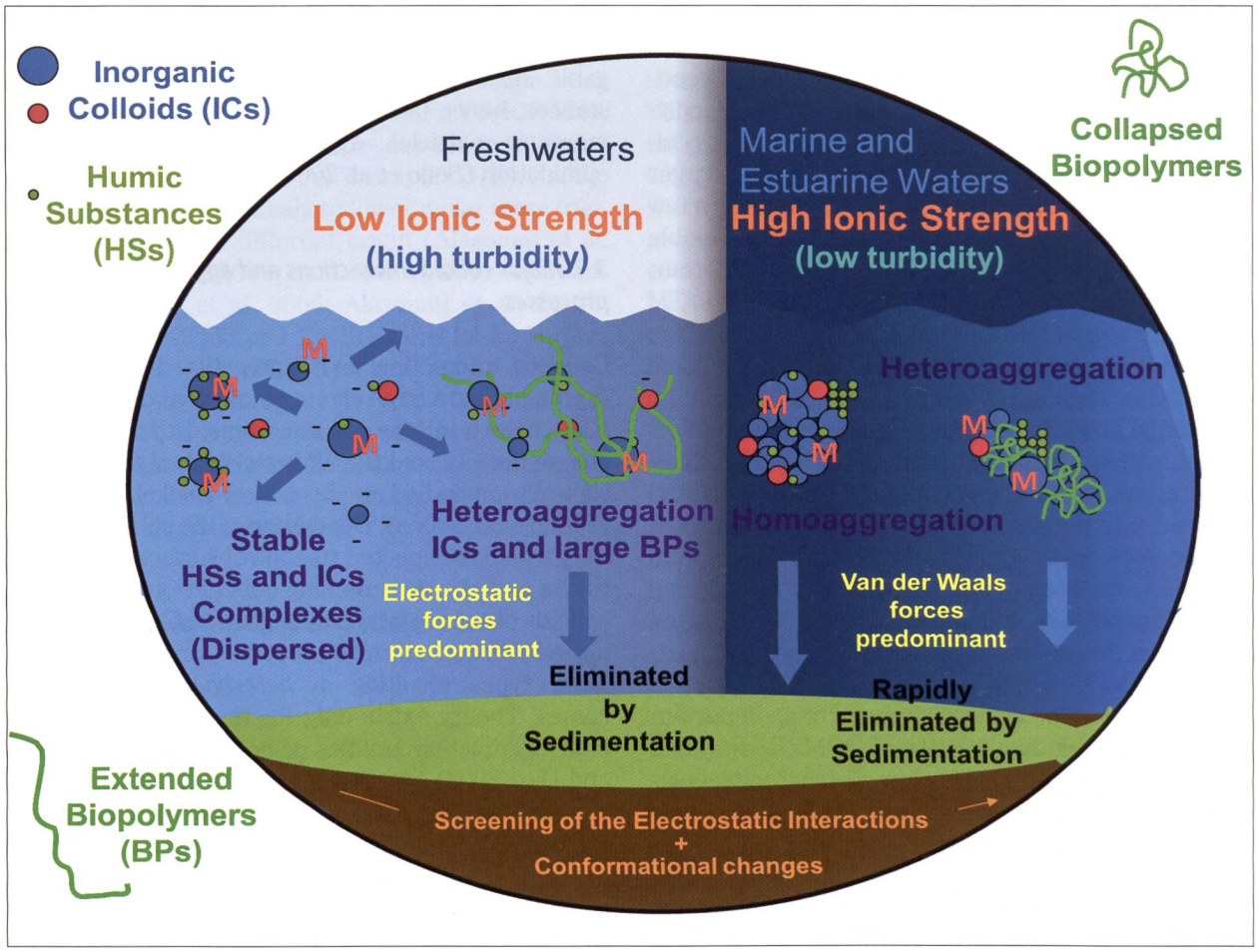

Fig. 4. Major colloidal interactions and aggregation processes relevant in natural waters. At low ionic strength (freshwaters), the electrostatic is expected to play a key role and form stable complexes (high turbidity). Bridging of inorganic colloids by large biopolymers represents the major route for aggregate formation and TM elimination by sedimentation processes. In marine and estuarine waters, due to the increase of the ionic strength and resulting charge screening effects, van der Waals forces are predominant. Homoaggregation as well as heterocoagulation are rapidly promoted resulting in the rapid destabilization, and sedimentation (low turbidity) of the aquatic colloids and thus transport of TMs. Biopolymers are expected to undergo large conformational changes from coils (compact structures) in marine waters to rod-like (extended structures) conformations in freshwaters.

inorganic colloids larger than 100 nm the interaction between HSs and inorganic colloids will correspond to their adsorption on the colloid surface resulting in a modification of the surface properties (Seijo et al. 2009). Similar effects are expected to occur by considering small biopolymers. The net effect (aggregation and TM elimination) will then depend on the amount of adsorbed DOM and the corresponding degree of charge neutralization or charge inversion. For model compounds, it has been shown that adsorption of negatively charged fulvic compounds on positively charged inorganic colloids (e.g., hematite) will result in destabilization only for surface coverage very close to charge neutralization (Ferretti et al. 2003). *Heteroaggregation of large rigid biopolymers with comparatively small compact inorganic colloids.* In such cases, the small, compact colloids interact with a small part of a large rigid biopolymer. Since the rigid biopolymer is much longer than the diameter of the inorganic colloids, biopolymers will serve as rigid (or semi-rigid) long distance bridges between the inorganic colloids. Several biopolymers may be associated to form a network by attaching to the same compact colloids. The net result will be the formation of a network of biopolymers connected together either by isolated or aggregated compact colloids, which will facilitate in the elimination of TM-colloid complexes.

3.4 Salinity effects on colloid aggregation and TMs - elimination

In freshwaters (*low salinity, low ionic strength*), a suspension containing only one type of compound in the colloidal size range is expected, from a kinetic point of view, to be stable for long periods of time

owing to the electrostatic repulsions, and weeks or months will be necessary for inorganic colloids and associated TMs to reach aggregate sizes large enough to allow sedimentation. As a result, inorganic colloids and HS mixtures as well as inorganic colloids and small biopolymer mixtures, due to electrostatic and steric effects, will not lead to rapid destabilization, whereas large rigid biopolymers will promote aggregation (Chen et al. 2005). Bridging mechanisms will lead to the formation of large entities which will constitute a major pathway leading to sedimentation and thus TMs elimination. This is in agreement with colloid aggregation and sedimentation rates which have been shown in many cases to be largely dependent on the concentration of organic material released by biota, specifically the large polysaccharides (Buffle and Leppard 1995a, b; Pizarro et al. 1995). According to these general considerations, in freshwaters, the aggregation of inorganic colloids can therefore be described as the result of two major but opposite effects: their stabilization by HSs and small biopolymers and their destabilization by large biopolymers (Fig. 4).

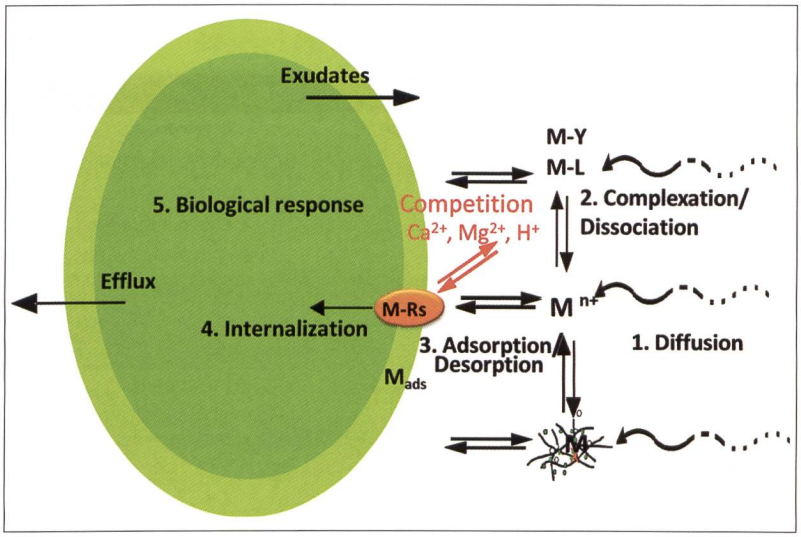

Fig. 5. Conceptual diagram of the key processes at the vicinity of the freshwater microalgae determining metal bioavailability. In this case, M^{n+} represents a trace metal ion, ML represents small labile and mobile complexes, MY - inert or non-mobile complexes; ✱ is the metal bound to dissolved organic matter, colloids, particles and their aggregates, M-Rs represents the metal bound to the receptor sites of biological membrane; M_{ads} is the metal adsorbed to the microorganism surface. The biological effects are related to the concentration of any of the metal species in equilibrium, e.g. *free metal ion concentrations in the solution M^{n+} (base of free ion activity model, FIAM) or metal bound to sensitive sites at the membrane surface (base of biotic ligand model, BLM). Generally, the metal complexation would be expected to reduce metal biouptake by reducing the steady-state concentration of M^{n+}. Water hardness ions Ca^{2+} and Mg^{2+}, as well as H^+ could compete with metal for the sensitive sites at the membrane surface and thus could decrease the uptake. Metal efflux or exudates production will influence the metal speciation.*

In estuarine and marine waters (*high salinity, high ionic strength*) the aggregation behaviour of aquatic systems and TMs mobility is expected to significantly change – indeed high ionic strength will enhance in almost all cases aggregation since the attractive van der Waals forces will dominate. This will destabilize both inorganic colloids and dissolved organic matter resulting in the rapid *heteroaggregation* and *homocoagulation* of all the colloids and associated TMs (Fig. 4).

4. Interaction of trace metals with aquatic phytoplankton

4.1. Bioavailability concept

The biological availability (or bioavailability) is defined as "the extent of absorption of a substance by a living organism compared to a standard system" (Nordberg et al. 2010). Bioavailability of various metals is significantly dependent on the medium speciation and chemodynamics (Buffle et al. 2009). Bioavailability is thus considered as a key concept allowing to quantitatively relating the changes in the TM concentrations and speciation with the intensity of the biological effects induced to biota.

Based on the extensive research over the last 30 years, there is currently a consensus that to be bioavailable different metal species should be first transported from the medium to the vicinity of the microorganisms (Fig. 5) (*e.g. by diffusion,* ***1***). Metal complexes are not necessarily inert during transport; dynamic complexes can *dissociate / associate* (***2***) in the time that it takes to diffuse to the surface of the organism. To invoke a biological effect, the trace metal must first react with receptor sites on the biological membrane (***3***, considered as the site of toxic action), often (but not necessarily) followed by transport across the membrane (*internalization,* ***4***). Once inside the cell, metals interact with different intracellular components and thus affect the cellular processes. The biological endpoints considered as indication of metal bioavailability includes uptake (*e.g.* internalization fluxes, metal cellular content *per se*) as well as effects on the growth rates, respiration, reproduction or photosynthesis.

Table 2. Examples illustrating the effect of the water hardness ions and pH on the TM bioavailability to different freshwater algae. J_{int} is the uptake flux.

Parameter	Metal	Microorganism	Effect	Reference
Water hardness ions Mg^{2+}, Ca^{2+}	Ni(II)	*C. reinhardtii*	$J_{int,Ni}$ decrease (Mg), competitive inhibition $J_{int,Ni}$ decrease (Ca), competitive interaction doesn't explain the observation	(Worms et al. 2007)
Ca^{2+}	Pb(II) Zn(II)	*C. kesslerii*	$J_{int,Pb}$ decrease, competitive inhibition $J_{int,Zn}$ decrease	(Slaveykova and Wilkinson 2002, Hassler et al. 2004)
Mg^{2+}, Ca^{2+}	Cd(II)	*C. reinhardtii*	$J_{int,Cd}$ inhibition (Ca), No inhibition for similar [Mg^{2+}]	(Kola and Wilkinson 2005)
K^+, Na^+, Mg^{2+} or Ca^{2+}	Tl(I)	*Chlorella sp*	Reduction of Tl uptake and toxicity by K^+ No effect of Na^+, Mg^{2+} or Ca^{2+}	(Hassler et al. 2007)
Mg^{2+}	Ni(II)	*P. subcapitata*	Increasing [Mg] decrease Ni toxicity	(Deleebeeck et al. 2009)
Ca^{2+}	Cu(II)	*P. subcapitata* *C. vulgaris*	No significant effect on the toxicity	(De Schamphelaere and Janssen 2006)
Mg^{2+}, Ca^{2+}	U(IV)	*C. reinhardtii*	Inhibition of uranyl-uptake	(Fortin et al. 2007)
pH	Cu(II), Zn(II)	*Chlorella sp*	Reduction of the cellular metal and growth inhibition Competition effect prevails over effect of speciation; pH 5.5-8.0	(Wilde et al. 2006)
pH	Cd(II), Mn(II)	*C. reinhardtii*	$J_{int,max}$ increase with pH Non-competitive inhibition of metal uptake	(Francois et al. 2007)
pH	Cd(II)	*C. reinhardtii*	$J_{int,Cd}$ decrease at low pH Competition of H^+ with Cd uptake sites	(Kola and Wilkinson 2005)
pH	Pb(II)	*C. kesslerii*	J_{int} increase with pH from 4.0-5.0, and pH 7.0 - 8.0., no effect for pH 5.5-6.5	(Slaveykova and Wilkinson 2003)
pH	Cu(II)	*P. subcapitata* *C. vulgaris*	Higher pH resulted in an increased toxicity	(De Schamphelaere and Janssen 2006)
pH	Hg(II)	*C. reinhardtii*	Uptake increased by a factor of 1.6 to 2 when the pH was lowered from 6.5 to 5.5, Hg(II) as lipophilic complex $HgCl_2$	(Le Faucheur et al. 2011)
pH	U(IV)	*C. reinhardtii*	Complex effect including speciation alteration, competitive and non-competitive inhibitions	(Fortin et al. 2007)

4.2. Limiting cases of diffusion and biological membrane transport

Depending on the nature of metal, biota and physicochemical conditions of the medium any of the above processes can be rate limiting. Two cases will be further considered: (i) If the mass transport in the external medium (*e.g.* the diffusion, Fig. 5) is rate limiting step, metal complexes are expected to contribute to the metal bioavailability depending of their chemodynamics (including their mobility and lability). Thus measurement of the dynamic species (*i.e.* free metal ions plus sufficiently labile and mobile complexes) concentrations should be better suited as predictors of metal bioavailability. Diffusion limitation was experimentally observed for rapidly accumulating metals, such as bioavailability of Ag(I) to *C. reinhardtii* (Fortin and Campbell 2000) and Zn(II) uptake by *Chlorella kesslerii* under starvation conditions (Hassler and Wilkinson 2003). It can be also expected for colloidal or particulate metal species or under conditions of the restrained diffusion (*e.g.* in biofilms). (ii) If the transport of metal across the biological membrane is rate limiting step, the biological

response (*e.g.* internalization flux, Fig. 5) can be directly related to any metal species in equilibrium including metal bound to the receptor sites on the organisms, base of the biotic ligand model, BLM (Campbell et al. 2002; Slaveykova and Wilkinson 2005) or free metal ion in the solution, base of the free ion activity model, FIAM (Sunda and Guillard 1976; Morel and Hering 1983; Campbell 1995; Worms et al. 2006) . Examples of biological transport limitation includes Pb(II), Cd(II) and Cu(II) uptake by *C. kesslerii* (Slaveykova and Wilkinson 2002; Lamelas and Slaveykova 2007), Cd(II) (Kola and Wilkinson 2005) and Ni(II) (Worms et al. 2007) uptake by *C. reinhardtii*. Under such conditions, measurements of the free metal ion concentration in the medium could be better surrogate of metal bioavailability. However, the BLM and FIAM are based on a number of simplifying assumptions that are not always confirmed in natural waters and exceptions were documented (Campbell et al. 2002; Slaveykova and Wilkinson 2005; Worms et al. 2006).

4.3. Factors modifying TM bioavailability

The entire bioavailability process can be influenced by (Wilkinson and Buffle 2004): (i) the characteristics of the biological membrane; (ii) the reactivity of the metals towards the biological membrane; (iii) the water quality parameters, such as pH, water hardness and alkalinity; (iv) the presence and concentrations of other TMs, both toxic or essential; (v) the presence of different ligands from natural (*e.g.* DOM) or anthropogenic (*e.g.* EDTA, NTA) origin in medium that influence chemical speciation. In the presentation below we will focus on the effect of water hardness, pH, and dissolved organic matter content, on the TM bioavailability to phytoplankton.

Effect of water hardness on metal bioavailability. There are quite a lot experimental evidences from laboratory studies and field validation showing that metal bioavailability to different organisms including algae varies largely with the water hardness. In general, hardness cations Ca^{2+} and Mg^{2+} play protective role with respect to organism by competing with metal ions for the binding sites on water - organism interface (Table 2). Most often the total hardness or Ca^{2+} are taken into consideration, but recently it was shown that the variable ratios between the Ca^{2+} and Mg^{2+} affect differently the toxicity of Cu(II) (Naddy et al. 2002); important finding given that the $Ca^{2+}$and Mg^{2+} ratio in standard laboratory-reconstituted waters often differs from the ratio in natural surface waters.

Effect of pH on metal bioavailability. More complex seems to be the effect of pH on metal bioavailability (Table 2). In general metal uptake, *e.g.* internalization flux, decreases when lowering pH. Protons affected Pb availability to the *C. kesslerii* surface by competing directly for surface sites, by modifying the overall algal surface charge and by modifying the chemical speciation of Pb(II) in solution (Slaveykova and Wilkinson 2003). However, modulation of Mn(II) and Cd(II) transport rates in the green alga *C. reinhardtii* by H^+ through non-competitive interactions was also reported (Francois et al. 2007). In addition, the interdependence of the effect of water hardness ions and pH was also shown to play important role in the chronic toxicity responses. For example, the alga *P. kirchenella* was more sensitive to chronic exposure to Mn(II) than invertebrates and fish at high pH when Ca^{2+} concentrations were low (Peters et al. 2011).

Effect of DOM on bioavailability. DOM and colloids could affect metal bioavailability by (i) complexing trace metals, (ii) affecting the diffusion of the metal species to the biological surface, (iii) altering the lability of the metal bound to the colloids or (iv) interacting directly with microorganisms. According to the currently accepted paradigms, DOM is expected to reduce metal bioavailability by binding metals in the medium and thus decreasing both the free metal concentration and the metal bound to the biotic ligand. Indeed, good agreement between prediction by BLM and FIAM and experimental observations were obtained for Cd(II) uptake by green alga *P. subcapitata* (Vigneault and Campbell 2005), *C. kesslerii* (Bayen et al. 2006), *C. reinhardtii* (Kola and Wilkinson 2005; Vigneault and Campbell 2005) and *P. subcapitata* (Koukal et al. 2003; Vigneault and Campbell 2005). Similarly, in two Connecticut rivers, Cu and Cd uptake by diatom *Stephanodiscus hantzschili* and chlorophyte *C. vulgaris* was in agreement with their speciation measurement (Mylon et al. 2003). By contrast, some experimental evidences exist that models, such as FIAM and BLM, do not reliably predict metal uptake in natural waters. For example, FIAM and BLM predictions underestimated the protective role of fulvic acid to Pb(II) uptake by *C. kesslerii* (Slaveykova and Wilkinson 2003; Lamelas et al. 2005b) and of river DOM to the green alga *Chlorella* and the diatom *S. hantzschili* (Mylon et al. 2003). On the other hand, although Pb(II) is significantly complexed by humic acid fraction, its toxicity to *Vibrio fischeri* increased; while for Zn(II) it remained almost constant, resulting from a weak complexation of Zn(II) with HA (Kungolos et al. 2006). Similarly, DOM from terrestrial and river origin increased Pb(II) toxicity for sea urchin embryos, mussel *Mytilus sp.* and the microalga *Isocrysis galbana*, as observed in previous studies (Sanchez-Marin et al. 2007; Sanchez-Marin et al. 2010a; Sanchez-Marin et al. 2011; Sanchez-Marin and Beiras 2012), however decreased Cu(II) and

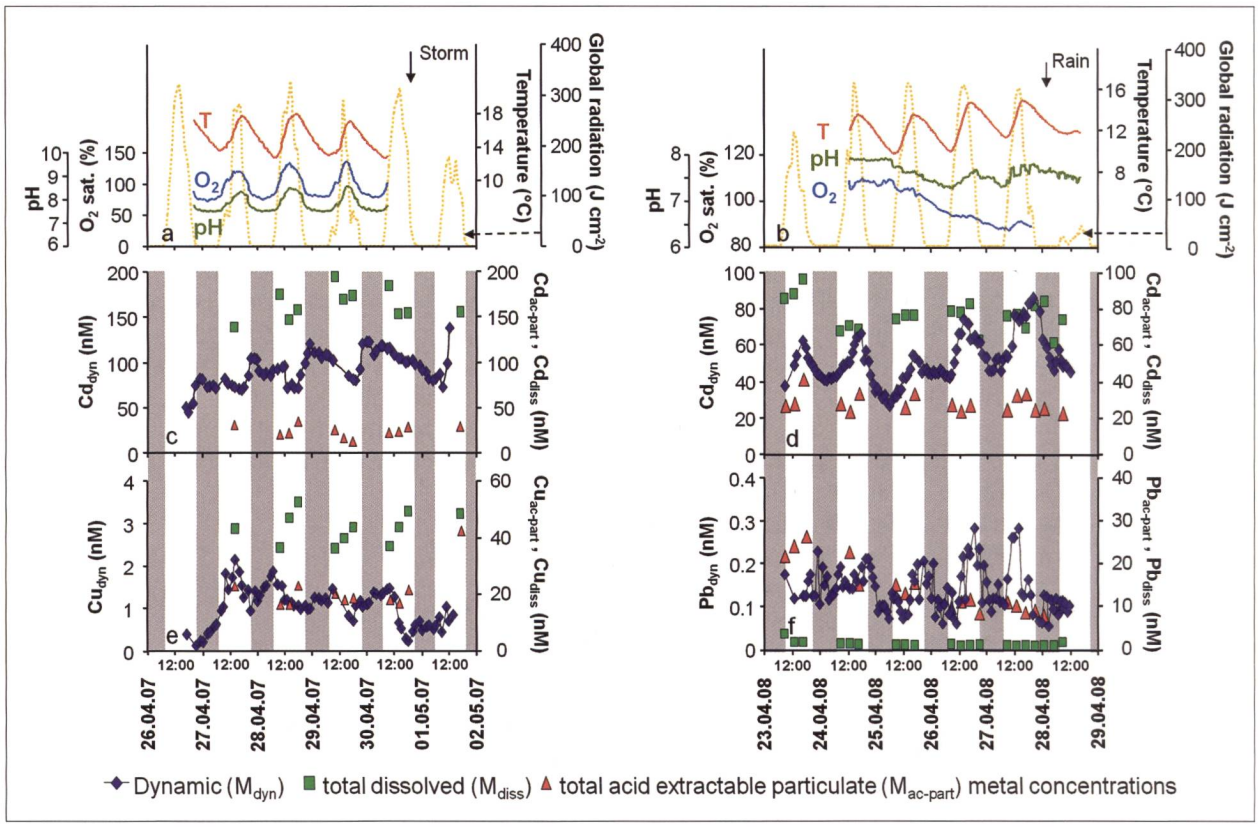

Fig. 6. Summary of continuous ancillary in situ measurements (temperature, pH, oxygen saturation, global solar radiation) and trace metal (Cd, Cu and Pb) concentrations for dynamic, total dissolved and total acid extractable particulate fractions monitored during two field campaigns in the Riou-Mort River (Joanis site). Shaded bars correspond to nighttime. Modified from (Tercier-Waeber et al. 2009). Left column: First campaign. Right column: second campaign.

Pb(II) intracellular content in diatom *T. weissflogii* (Sanchez-Marin et al. 2010b). The above examples illustrated the existing controversy in our current knowledge, as well as showed that the effect of DOM and in particular the HSs on the metal bioavailability and toxicity can vary as a function of the type of metal, the nature of organism and the concentration of DOM.

Opposite to the recent advances concerning the role of HS fractions on the metal bioavailability, the role of the non-humic component, such as EPS on metal bioavailability is not fully elucidated. EPS released from the organisms, are expected to reduce metal bioavailability by reducing free metal concentrations in the medium. For example, extracellular ligands released by the alga *Oocystis nephrocytioides* decreased Cu bioavailability at low Cu concentrations (Soldo et al. 2005). Moreover, a gram-negative bacterium *S. meliloti* produced some proteins, carbohydrates and siderophores that were able to complex Cd and reduce both its free ion concentration and its cellular metal content (Dedieu et al. 2006). The bioaccumulation of Cd(II) by another gram-negative bacterium *Rhodospirillum rubrum* was greatly decreased by the production of exudates but, in that case, the type of extracellular ligand was not identified (Smiejan et al. 2003). The protective effect of exudates against Cd(II), Cu(II), Pb(II) and Zn(II) toxicity on *P. subcapitata* has also been observed (Koukal et al. 2007). In spite of these relatively recent advances our fundamental understanding of the role of EPS in metal bioavailability remains largely unexplored and it is still unclear whether and under what conditions EPS can play significant role in metal bioavailability, in particularly when present as mixtures with humic substances.

5. Field case studies

Recent progress in the development of different speciation techniques (Buffle and Horvai 2000; Buffle and Tercier-Waeber 2005; Tercier-Waeber et al. 2005; Van Leeuven et al. 2005; Sigg et al. 2006), as well as metal specific biomarkers (Ohsawa 1997; Elder and Colins 2001), that can be applied *in situ* or *on field*, gave great promise for improvement of the basic understanding of the major processes governing the behavior of the TMs in surface waters. Below, we will

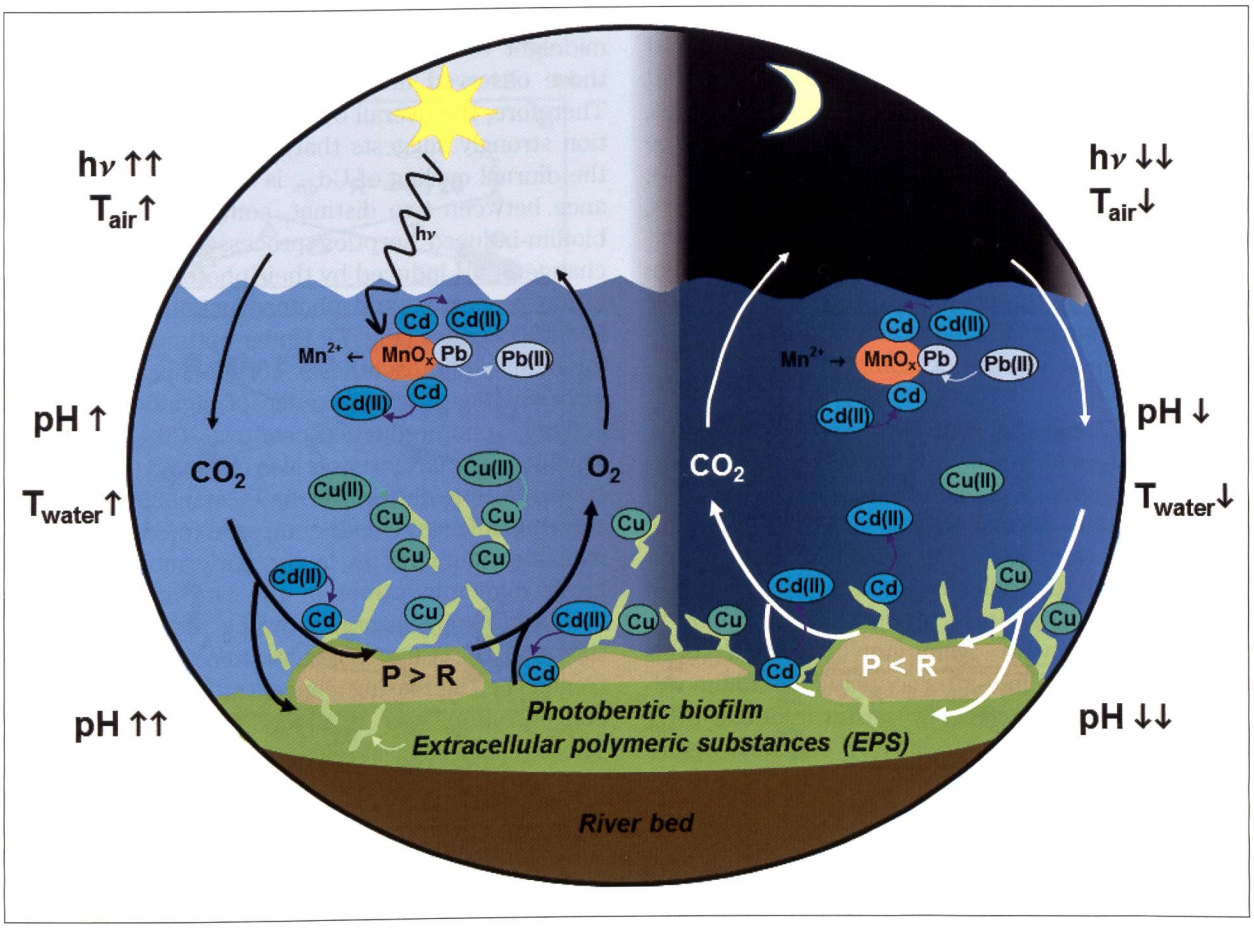

Fig. 7. Schematic representation of the main processes which control the dynamic metal speciation of Cu, Pb and Cd in the Riou-Mort River (France). Diurnal Cu_{dyn} cycle appears to be related to complexation with Cu-binding EPS released by photobenthic biofilm during the day. The diurnal Pb_{dyn} cycle was attributed mainly to photoreduction of colloidal Mn oxides, whereas the diurnal Cd_{dyn} cycle reflected the balance between both sorption onto biofilms and photoreduction of colloidal Mn oxides.

illustrate some of the basic concepts discussed in the sections 2 to 4 with examples of results obtained from field studies performed in the Riou-Mort stream in France (Tercier-Waeber et al. 2009; Simon et al. 2011) and the plume of the Po river in the Northern Adriatic Sea (Tercier-Waeber et al. 2005; Tercier-Waeber and Taillefert 2008).

5.1. Influence of sorption processes on potentially bioavailable metal fractions

The variation of the so-called dynamic fractions (M_{dyn}) of Cd(II), Pb(II) and Cu(II), defined as the sum of free metal ions and small labile and mobile complexes with size typically of few nm (Buffle and Tercier-Waeber 2000; Buffle and Tercier-Waeber 2005), was monitored *in situ*, at hourly time scale (Tercier-Waeber et al. 2009) near the outlet of the Riou-Mort watershed (France) which is impacted from former open-cast coal mining and Zn ore treatment (Audry et al. 2004). This was achieved by using a field voltammetric analyzer (Tercier-Waeber et al. 2009), based on a gel integrated microsensor (GIME) (Belmont-Hébert et al. 1998; Tercier-Waeber et al. 2008), and square wave anodic stripping voltammetry (SWASV). In parallel, dissolved O_2, pH, temperature and conductivity were monitored continuously using a submersible multiparameter probe. Water samples were collected three times per day for ancillary measurements of: acid extractable particulate metal ($M_{ac\text{-}part}$) and total dissolved metal (M_{diss}) concentrations as well as water composition (Tercier-Waeber et al. 2009). These measurements were performed under contrasting photobenthic biofilm activity and hydrological conditions (Tercier-Waeber et al. 2009). Namely low water flow and discharge, and presence of important photobenthic biofilm communities during a first campaign, and high water flow and discharge, and absence of photobenthic biofilm community during a second campaign. Ancillary measurements showed that, except for discharge

peaks during major flooding events, the water composition (major ions, organic and redox species) at the monitored site was not fundamentally different for contrasting hydrological conditions. Diel T cycles, induced by diurnal cycle of solar radiation, were observed during the two field campaigns (Fig. 6 a,b). Well defined diurnal pH and dissolved oxygen cycles, reflecting biological photosynthesis/respiration activity, were also observed during the first field campaign characterized by the presence of important benthic biofilm community (Fig. 6a), but not during the second campaign in absence of biofilm (Fig. 6b).

The *in situ*, near real-time GIME-SWASV measurements of Cd_{dyn} showed significant temporal variation (ratio: Cd_{dyn} max – Cd_{dyn} min/ Cd_{dyn} min up to 100%) with persistent diurnal trends, albeit different for the two field campaigns (Fig. 6 c,d). During the first field campaign, the daily evolution of Cd_{dyn} matched inversely the variation in pH from around noon to midnight, showing minimum concentrations between noon and mid-afternoon and maximum values around midnight (Fig. 6c). After midnight Cd_{dyn} decreased, then stayed relatively constant before slightly increasing between sunrise and noon (Fig. 6c). $Cd_{ac\text{-}part}$, measured by classical techniques in the collected samples (9 am, 1 pm, 5 pm), was relatively low and constant while Cd_{diss} concentrations varied similarly to those of the Cd_{dyn} (Fig. 6c). During the second campaign, Cd_{dyn} was at minimum in the early morning; then increased by 70-100% from sunrise to afternoon, and decreased during the night, while the Cd_{diss} and $Cd_{ac\text{-}part}$ were found to be relatively constant (Fig. 6d). Despite the low Pb concentrations, a similar significant and persistent behaviour was monitored for Pb_{dyn} (Fig. 6f). The increase during daytime and decrease during the night of the Cd_{dyn} and $Pb_{dyn,}$ with constant total dissolved concentrations of both metals observed during the second campaign (Fig. 6 d,f) suggest source/sink of Cd(II) and Pb(II) induced by photoreduction/reoxidation of colloidal Mn or Fe (hydrous)oxides. Results obtained for ancillary measurements of Fe and Mn speciation (Tercier-Waeber et al. 2009), showed that 78% of Mn_{tot} was present as colloids with size < 0.020µm, while 90% of Fe_{tot} was under acid extractable particulate form (size > 0.45µm). Accordingly, photoreduction of Mn oxides (daytime) followed by the re-oxidation of the Mn(II) formed (night time) appears to be the most plausible explanation for the concomitant release/fixation of Cd(II) and Pb(II) (Fig. 7). Although the effect of light on dissolved Mn reactivity and transport is still not well understood, some previous studies reported that synthetic and natural MnO_x are photo-reduced in sea and freshwater, and that this process is catalyzed by FA-HA (Sunda and Huntsmann 1994; Scott et al. 2002).

The overlay of the trends observed for Cd_{dyn} from midnight to noon during the second campaign fit those observed during the first one (Fig. 6 c,d). Therefore, the overall observation for Cd(II) speciation strongly suggests that, in the Riou-Mort River, the diurnal cycling of Cd_{dyn} is controlled by the balance between two distinct, competitive processes: biofilm-induced sorption processes, related to the change in pH induced by their photosynthesis/respiration processes, and photoreduction of small colloidal Mn oxides (Fig. 7). Under optimum conditions for biofilm photosynthesis, sorption processes prevail, whereas in the absence of significant biofilm activity, photoreduction control Cd_{dyn} behavior. Finally, a diurnal cycle was also observed for the Cu_{dyn} concentration during the first campaign (Fig. 6e), showing different trends compared to Cd_{dyn} (Fig. 6c), with maximum values in the early morning, 2-fold lower minimum values in the late afternoon, and increasing concentrations during the night. During the day, the Cu_{diss} concentration showed inverse trends, i.e. increasing concentration, while $Cu_{ac\text{-}part}$ concentration was relatively constant (Fig. 6e). These results suggest release of a non-labile Cu-complex into the water column during the day from a phase other than suspended particulate matter (SPM). Excretion of Cu-binding EPS from the biofilm could explain these observations (Fig. 7), suggesting that free ligand sites on EPS may outcompete the weak ligands of the dynamic Cu complexes, and thus decrease the proportion of these latter species. Of course, a more detailed study is required to better understand the diurnal cycling of the Cu_{dyn} and Cu_{diss} species, but the relative constant concentrations of the three Cu fractions observed during the whole monitoring period of the second campaign (i.e. in absence of significant benthic biofilm community) (Tercier-Waeber et al. 2009), and the similar behavior observed for Cu_{dyn} and Cu_{diss} from GIME-VIP profiling in a lake during summer productivity (Tercier-Waeber and Buffle 2000; Tercier-Waeber et al. 2008), support this hypothesis.

Several original results of the present work may be important to the definition of monitoring procedures for water quality control, and in particular for metal ecotoxicity assessment. They demonstrate that the phases and amplitudes of diurnal cycles of the dynamic, i.e. potentially bioavailable, fraction of trace metals can change significantly as a function of the bio-chemical conditions of the media. Diurnal Cu cycles with opposite trends may occur for the total dissolved and the dynamic fractions. Finally, photoreduction of small photoreactive (hydrous)oxide colloids may control diurnal cycles of bioavailable metal species. Impact of such processes may not be identified by classical dissolved metal measurements in <0.45µm or even 0.02µm filtered samples if small col-

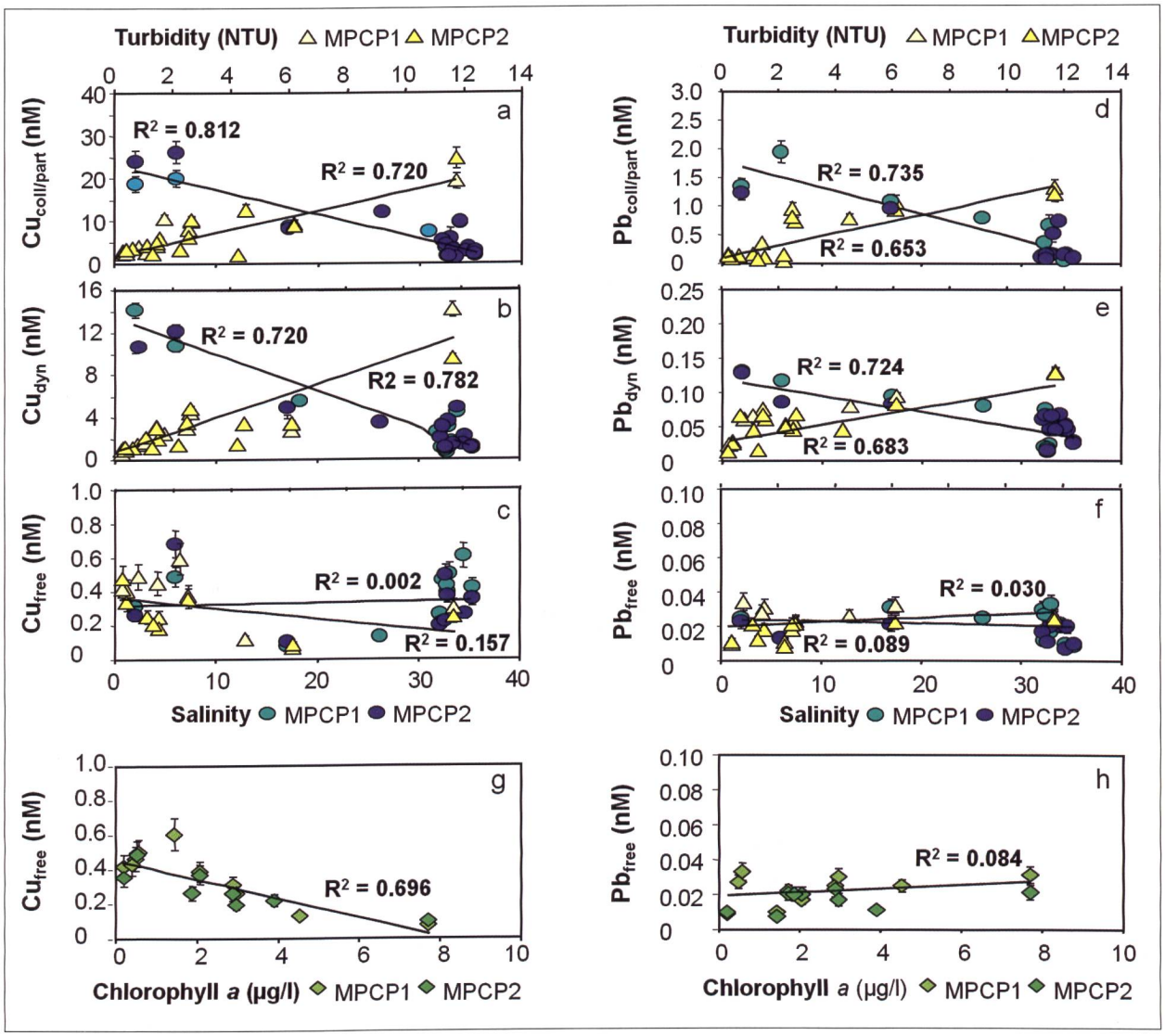

Fig. 8. Concentrations of Cu and Pb specific fractions as a function of salinity and turbidity (a,b,c for Cu and d,e,f for Pb) as well as chlorophyll a *(g, h). Trace metal speciation and master variable data were obtained from in situ, simultaneous measurements performed using two MPCPs deployed at various stations and depths in the Po plume in the Northern Adriatic Sea. Modified from (Tercier-Waeber and Taillefert 2008).*

loids (<0.02µm) are involved as observed in the Riou-Mort River. Note that similar results may be expected for dissolution of other natural or engineered nanoparticles. These results suggested that TMs released by nanosized material may significantly contribute to TM bioavailability to microorganisms, hypothesis further developed in the section 5.2.

5.2. In situ *sensitivity of cadmium biomarkers to metal speciation*

Laboratory-determined biomarkers of Cd(II) stress that were previously identified for the green alga *C. reinhardtii* (Simon et al. 2008) were also tested during the second field campaign in the Riou-Mort watershed (Simon et al. 2011). *C. reinhardtii*, cultured in standard laboratory conditions and resuspended in 0.45µm pore size filtered river water samples, was exposed to the natural media using three Stirred Underwater Biouptake Systems (Davis et al. 2009). Analysis of relative transcript abundance normalized to cellular viability (Livak and Schmittgen 2001, Simon et al. 2008) demonstrated overexpression of four biomarkers out of ten identified from laboratory tests. mRNA levels were best correlated to [Cd^{2+}], determined by ICP-MS after in-situ extraction and pre-concentration using hollow fiber liquid permeation membrane (HF-PLM) technique (Buffle et al. 2000), rather intracellular Cd content as initially hypothesized. One explanation could be that Cd(II) concentrations in the Riou-Mort were generally much higher than potential interfering ions such as Cu(II) and Pb(II) (Fig. 6). In the absence of competing met-

als, [Cd^{2+}] is thought to be strongly correlated with the flux of metal crossing the biological membrane (Slaveykova and Wilkinson 2005; Lavoie et al. 2009). Finally, the four genes that appeared to be the most sensitive to [Cd^{2+}], appeared to be those involved in detoxification abiotic stress tolerance (Simon et al. 2011). These results demonstrated that release of metals under "truly dissolved forms" from *e.g.* nano-sized colloids (i.e. Mn oxides in the case of the Riou-Mort) or particles, occurring even at short time scale (Fig. 6d), may have a significant impact on biota. Moreover, these findings highlight that single measurements of total dissolved metal concentrations in collected samples are inappropriate for reliable assessment of trace metal impact in highly dynamic aquatic systems.

5.3. Influence of salinity gradient on metal speciation

In-situ measurements of the spatial speciation of Cu(II) and Pb(II) in the plume of the Po River in the Northern Adriatic Sea have been performed at various seasons using two Multi Physical Chemical Probes (MPCP) deployed at various depths of six stations located from the Po mouth toward open sea (Tercier-Waeber et al. 2005; Tercier-Waeber and Taillefert 2008). The MPCP is a unique completely submersible mini-laboratory, i.e. lab-on-cable system (Tercier-Waeber et al. 2005). It allows the simultaneous *in situ* monitoring and profiling of three major fractions of Cu(II), Pb(II), and Cd(II) species coupled to the master biogeochemical variables (pressure, temperature, pH, oxygen, conductivity, salinity, redox E, turbidity and chlorophyll *a*). The three metal fractions measured include (Tercier-Waeber et al. 2005): (i) the free metal ion concentration [M^{n+}] (Noël et al. 2006); (ii) the concentration of the dynamic metal species (M_{dyn}); and (iii) the total extractable metal concentration ($M_{tot\ ext}$). Subtracting (ii) from (iii) provides the concentration of metal bound to particles and colloids ($M_{coll/part}$).
Similar trends in the change of Cu and Pb speciation were observed for all the monitoring cruises (Tercier-Waeber et al. 2005; Tercier-Waeber and Taillefert 2008). They are summarized below and in the Fig. 8. The first observations that could be made from the monitored data were that: (i) the concentrations of the free ion and dynamic species of both metals are a small percentage of the total extractable metal concentrations; and (ii) the ratios of these species to total extractable concentrations vary, in various proportions for the two metals, as a function of depth and distance from the river mouth and period of the year (data not shown). Another observation was that while a decrease of the total extractable and dynamic concentrations of Cu(II) and Pb(II) was observed as a function of both distance from the Po mouth and depth (in particular between the 0.2 m surface water and the deepest layers), the variations observed for [Cu^{2+}] and [Pb^{2+}] were apparently independent of these parameters. Comparison of the Cu(II) and Pb(II) speciation data with those of the master variables measured simultaneously reveals that the decrease in concentration of the $Cu_{coll/part}$ and $Pb_{coll/part}$ fractions (i.e.: $Me_{tot\ ext} - Me_{dyn}$) with distance from the Po mouth is mainly related to the change in salinity and turbidity (Fig. 8 a,d). These results show that a significant proportion of these metal species is rapidly eliminated in coastal areas due to fast coagulation and sedimentation processes associated with the increase in salinity, i.e. the ionic strength of the media, as predicted in Fig. 4. The similar behavior observed for the Cu_{dyn} and Pb_{dyn} species (Fig. 8 b,e) suggests that a significant proportion of these species adsorbs on freshly formed particulate species and is thus eliminated by the same process. The [Cu^{2+}] and [Pb^{2+}] concentrations show different trends (Cu_{free} and Pb_{free} Fig. 8c,f). The [Cu^{2+}] was found to be strongly correlated to chlorophyll *a*, i.e to the primary productivity (Cu_{free}, Fig. 8g), whereas [Pb^{2+}] was very low (typically between 0.01 and 0.02 nM) and relatively constant (Pb_{free}, Fig. 8h). These results suggested that a significant proportion of Cu(II) is either assimilated by the phytoplankton or complexed by their exudates (*e.g.* EPS as observed in the Riou-Mort), while Pb(II) seems unaffected by biota. Similar behavior was observed for [Cu^{2+}] and [Pb^{2+}] in both surface lake water and open sea; while, in absence of significant variation of the ionic strength in these later cases, Cu_{dyn} and Pb_{dyn} were found to be respectively strongly related to the variation in chlorophyll *a* and relatively constant (Tercier-Waeber et al. 2008; Tercier-Waeber and Taillefert 2008).

The findings of the above field studies demonstrate the importance of continuous (hourly time scales) *in situ* measurements of relevant, in term of bioavailability and thus (eco)toxicity, metal species for different environmental conditions and at contrasting sites to deeper understand their behavior and fate, and thus ultimately better assess their potential health detrimental effect for the aquatic ecosystems and human (Fig. 1). Such information could not be obtained using traditional procedures based on laboratory measurements of total dissolved metal concentrations in discrete samples.

6. Conclusions and outlook

Important advances, mainly based on laboratory work, were achieved in the past four decades with respect to the fundamental understanding of the trace metal behavior in the aquatic systems and different modifying factors. They allowed the develop-

ment of key concepts such as TM speciation and bioavailability and revealed the utmost role of the interrelated dynamic biological, chemical and physical processes in TM cycling and regulation. Nonetheless, such knowledge is still lacking, especially for the conditions that are the most relevant to the natural (i.e. presence of multiple stressors and metal mixtures) and impacted environment (i.e. new emerging contaminants such as engineered nanoparticles, urban water cycle), as well as to the natural spatial and temporal variability (i.e. as illustrated by the reported examples of *in-situ* determination of metal speciation and bioavailability). Some of the persisting gaps and current challenges are briefly discussed below.

Multiple stressors. Combined effects of the environmental stressors (*e.g.* temperature or/and light extremes) and TMs on the phytoplankton have received more attention in the recent years. The interplay between variations and extremes in solar radiation, DOM and toxic TMs could affect the phytoplankton in the surface waters by: (i) altering the structure and reactivity of DOM (Gonsior et al. 2009; Porcal et al. 2009; Sulzberger and Durisch-Kaiser 2009), thus decreasing of the metal binding capacity and increasing metal bioavailability (Spierings et al. 2011); (ii) modifying chemical speciation of the redox sensitive metals, such as Fe(III) and Cu(II); and (iii) affecting the vital cellular functions of primary producers (Haeder et al. 2007). The above mentioned effects can be expected to be exacerbated by climate variability (Hegglin and Shepherd 2009) and other anthropogenic modification of aquatic ecosystems (van den Belt et al. 2006). Furthermore, the concentrations of metals in the water column vary over time and are highly responsive to biophysicochemical conditions and hydrological changes. Therefore any diurnal (see sections 5.1 and 5.2) seasonal dynamics as well as climate variability induced extremes such as floods or drought events may affect the metal concentration, speciation and thus TM bioavailabity and effects. In such a context the capability to perform *in-situ* high frequency measurements over the relevant spatial and temporal scales is highly sought.

Metal mixtures. The importance to consider metal-metal interactions in bioavailability is recognized since TMs in surface waters are present as mixtures of essential and toxic metals. Three major type of metal - metal interactions with respect to bioavailability are considered: (i) antagonistic, with a potential reduction of bioavailability when both metals are present; (ii) synergistic, resulting in enhanced bioavailability of one metal due to the presence of another, *e.g.* Cu(II) has been shown to increase Pb(II) and Zn(II) uptake fluxes to *C. kesslerii* (Hassler et al. 2004); or (iii) additive, where the observed effect is equivalent to the sum of the individual effects associated to the individual components. Cu(II) uptake was enhanced when Ni(II) was added to solutions resulting in a concurrent reduction of the respiratory rate and chlorophyll *a* contents of *Scenedesmus quadricauda* (Fargasova 1998). BLM was recently extended to predict the mixture effect on the base of the single metal toxicity data (Jho et al. 2011). Despite the importance of considering the effects of metal mixtures to aquatic organisms, this is still a challenging issue that needs future studies. The laboratory experiments allowed development of the concepts, such as concentration-addition model, assuming addition *within* modes of action and independence *between* different modes of action in mixture assessment. However the adequacy in natural waters is still to be proven.

Importance and role of the colloidal fraction. As discussed colloids may control to a large extend the transport of TMs. In some cases colloid-facilitated transport may be important in increasing distances travelled by TMs in comparison to the distances predicted for non-colloidally adsorbed elements. In other cases, colloid aggregate formation will facilitate the TM elimination through the formation of large sedimenting aggregates. Within the exception of as ligands that may bind TMs, colloid properties and aggregate formation are generally not explicitly considered in the main equilibrium-based and biotic ligand models. While TM uptake is often reduced in presence of colloids, the chemical heterogeneity and structure of the colloid-TM complexes may influence bioavailability (slow diffusion, reduced chemical lability, change in the biochemical route, etc). The validity of such assumptions is still to explore and verify and, given the recent findings, it is necessary to examine in details metal uptake from the colloidal phase and for the refinement of bioavailability models. Challenges also still remain in the understanding and importance of the various aggregation processes under commonly encountered environmental conditions which will collectively determine the persistence of the TMs as well as ENPs in the aquatic systems and their potential toxicity to exposed organisms.

Behavior and impact of engineered nanoparticles. ENPs can be considered as a new class of contaminants with a potential to persist, bioaccumulate and exercise toxic action in the environment (Behra and Krug 2008; Auffan et al. 2009). Due to their small size and high surface reactivity, the ENPs have a potential to interact with biota in a way different from that of the bulk substances. The properties of the ENPs that make them useful in manufacturing also make them *potentially* biologically disruptive and the potential health risks associated with exposure to ENPs. An

urgent need exists for the identification not only of the potential hazards posed by nanomaterials to the environment, but also if and under which conditions they can come in contact with living organisms. To achieve these goals a deeper understanding of the stability, transport, redistribution and ultimate fate of nanoscale materials under environmental conditions is strongly needed. Furthermore, it has been suggested that the interactions of ENPs with other environmental contaminants and their role as contaminant nanovector, may be as important as their intrinsic toxicity (Nowack and Bucheli 2007), issue that very recently was addressed for the aquatic phytoplankton interaction with quantum dot nanoparticles and Cu (II) and Pb(II) (Worms et al. 2012).

Development of new tools for in-situ measurements of TM speciation and bioavailability.

The findings of the examples of the field case studies presented in the section 5 demonstrate the importance of continuous *in situ* measurements of relevant (in term of ecotoxicity) metal species at appropriate time scale, for different environmental conditions, and at contrasting sites to better understand the behavior of in particular potentially bioavailable trace metal species, which may present high ecotoxicity risk for aquatic ecosystems. Thus it is essential to: (i) quantify specific metal species or groups of homologous metal species with appropriate temporal resolutions; (ii) study the influence of contrasting bio-physicochemical conditions on the proportion of these different metal species or groups of homologous metal species; and (iii) evaluate the impact of specific metals species on biota. Optimization of existing sensor techniques and development of new sensors to extend respectively the *in situ* long-term application of these tools without maintenance and the number of metals and bio-physicochemical parameters that can be monitored simultaneous is thus of prime interest. Deeper characterization of the metal species which are detected *in situ* with novel analytical tools for trace metal speciation is also highly needed. Due to the varying role of the natural environment on metal bioavailabity, site-specific approaches are becoming more appropriate. Indigenous organisms or those taken from culture collections have been used to relate the "natural" state of the water to specific toxicological or biological end points. Examples includes *in-situ* determination of Cd(II) availability to algae and Fe(III) to cyanobacteria using porous underwater chamber (Hassler et al. 2008), metal bioavailability to *C. reinhardtii* in SUBS (Davis et al. 2009) and evaluation of the specific transcriptomic (Simon et al. 2011) or metabolic biomarkers (e.g. thiol compounds, phytochelatins) (Le Faucheur et al. 2005a; Le Faucheur et al. 2005b; Lavoie et al. 2009). The opportunities of advancing the understanding about the TM dynamics in surface waters through the application of new "-omic" were specifically emphasized, when revisiting the 'big questions' in TM speciation and bioavailability (Hering 2009).

Overall, trace metal dynamics in freshwater environments are likely to be controlled by a suite of linked physical, chemical and biological processes. The understanding of these linkages will provide key insights into the prevailing controls on metal behavior in surface waters. In particular, the fields of biogeochemistry and ecotoxicology are implicit in any relevant paradigms. In a comprehensive review 11 years ago, Warren and Haack (2001) pointed out the multi- and inter-disciplinary nature of trace metal dynamics in the aquatic systems and the necessity of the quantification of the interactions and linkages amongst the traditional disciplines of chemistry, physics, biology and geology to advance the knowledge in this area. This is the objective of the current research in the Institute F.-A. Forel, and in particular, of the authors and within the spirit of François-Alphonse Forel ideas and legacy.

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