



Review

Metal fate and effects in estuaries: A review and conceptual model for better understanding of toxicity



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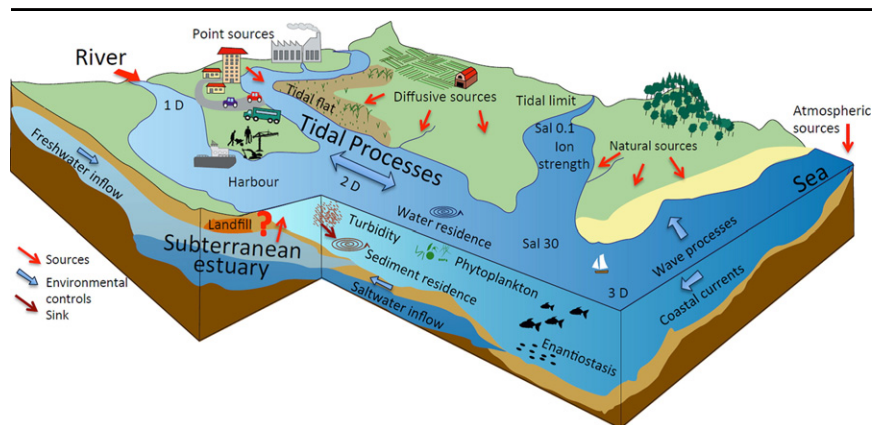
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HIGHLIGHTS

- A multidisciplinary overview of metal fate and toxicity in estuaries is provided.
- Physical and biogeochemical gradients cause non-conservative behaviour of metals.
- Water chemistry generally explains metal toxicity in freshwaters.
- Organism physiology generally explains metal toxicity in saltwater.
- Quantitative interdisciplinary models of metal fate and toxicity are yet required.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 30 July 2015

Received in revised form 9 September 2015

Accepted 9 September 2015

Available online 25 September 2015

Editor: D. Barcelo

Keywords:

Metal fate

Biogeochemistry

Estuarine processes

Organism physiology

Metal toxicity

ABSTRACT

Metal pollution is a global problem in estuaries due to the legacy of historic contamination and currently increasing metal emissions. However, the establishment of water and sediment standards or management actions in brackish systems has been difficult because of the inherent transdisciplinary nature of estuarine processes. According to the European Commission, integrative comprehension of fate and effects of contaminants in different compartments of these transitional environments (estuarine sediment, water, biota) is still required to better establish, assess and monitor the good ecological status targeted by the Water Framework Directive. Thus, the present study proposes a holistic overview and conceptual model for the environmental fate of metals and their toxicity effects on aquatic organisms in estuaries. This includes the analysis and integration of biogeochemical processes and parameters, metal chemistry and organism physiology. Sources of particulate and dissolved metal, hydrodynamics, water chemistry, and mechanisms of toxicity are discussed jointly in a multidisciplinary manner. It is also hypothesized how these different drivers of metal behaviour might interact and affect metal concentrations in diverse media, and the knowledge gaps and remaining research challenges are pointed. Ultimately, estuarine physicochemical gradients, biogeochemical processes, and organism physiology are jointly coordinating the fate and potential effects of metals in estuaries, and both realistic model approaches and attempts

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to postulate site-specific water criteria or water/sediment standards must consider such interdisciplinary interactions.

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1. Introduction

Estuaries are defined as water bodies that connect land and ocean and extend from fully marine conditions to the effective limit of tidal influence, and where seawater is diluted by freshwater inflow (Hobbie, 2000). These environments have traditionally been zones of intense human occupation. They provide a multitude of ecosystem services such as drinking freshwater supply, fisheries, climate regulation, sheltered access to coastal water, coastal protection, water purification and waste treatment (Millennium Ecosystem Assessment, 2005). Estuaries also serve as nursery areas for several species, provide habitat to a high diversity of organisms for the whole or part of their life cycle, and are characterized by a high biological productivity. However, estuaries have also been used for the dilution and disposal of waste worldwide (Kennish, 1991; Spencer et al., 2006b) which contributes to their deterioration.

In this context, trace metals are pollutants of concern (Kinne, 1984; Nemerow, 1991; Fairbrother et al., 2007). Although metals are naturally ubiquitous in aquatic systems (usually within $\mu\text{g}\cdot\text{L}^{-1}$ -range in surface waters), they are increasingly present as a result of anthropogenic activities. Förstner and Wittmann (1979) concluded that the world's six most heavily polluted aquatic environments by trace metals are estuaries. In countries with long historic industrialization, such as United Kingdom, Germany and Netherlands, thousands of tons of metals were systematically deposited in the estuarine and coastal areas (Förstner and Wittmann, 1979). This legacy of contamination is currently aggravated via freshwater input, increasing urbanization and discharges of domestic effluents, industry, fossil fuel burning, mining, groundwater use, surface runoff and soil erosion, and mobilization of historic contaminated sediment (Phillips, 1980; Heath, 1995; Deboudt et al., 2004; Paytan et al., 2009; Bai et al., 2015). Additionally, these contaminants are persistent

in the environment, and all metals are potentially bioavailable and toxic to aquatic biota at high concentrations (Kennish et al., 1991; Wood et al., 2012a,b; Machado et al., 2014b).

Integrative models of behaviour and threats of metals are required to better set environmental quality standards and goals. Currently, North American scientists put significant effort into better understanding metal transport, mobilization and toxicity and derive scientifically defensible site-specific water quality criteria for metals in salt water environments – as has already been successfully established in freshwater (Paquin et al., 2003). While in Europe, a lack of knowledge of the ecological status and function of transitional waters is hindering the setting of standard baselines with consequences for the implementation of the Water Framework Directive (European Commission, 2012b). In both cases, it is relevant to ensure that management is based on a better understanding of the main risks and pressures on these systems (Elliotta and McLusky, 2002; Millennium Ecosystem Assessment, 2005; European Commission, 2012b). At best of our knowledge, such an integrated model is still lacking within the literature. Therefore, it is fundamental to discuss metal fate and consequences for their toxicity in a holistic and systematic manner.

The aim of this work is to propose a conceptual model for the distribution, environmental fate, and toxicity of metals in estuaries, including environmental dynamics, metal chemistry and organism physiology. In a first step, the estuarine features important for the environmental dynamics of metal fate are described (Section 3). Secondly, the main biogeochemical processes by which these features affect metal transport, distribution and partitioning concentrations in the different estuarine sub-compartments are introduced (Sections 4, 5 and 6). Finally, the organism physiology and metal toxicity under estuarine conditions are discussed (Section 7), enabling a unique transdisciplinary overview of the threats of these chemical elements in estuarine environments.

2. Scope and terminology

The physiography of estuaries has been shown to vary widely impacting physical mixing processes and consequently biogeochemistry (Bianchi, 2007). The present study thus focuses on river-dominated or tidal-river estuaries. Details on processes that are characteristic for metal behaviour and effects in estuaries were identified from scientific literature and are discussed from an ecotoxicological point of view. A broad range of information sources, which include peer-reviewed scientific articles, specified books, technical articles, and on-line information provided by environmental protection agencies have been considered. Notwithstanding, estuarine functioning and metal literature is extensive, and readers interested in more specific details beyond the scope of this study are encouraged to refer to further reviews (Drexler et al., 2003; Paquin et al., 2003; Bianchi, 2007; Fairbrother et al., 2007; Monserrat et al., 2007; Wood et al., 2012a,b). Likewise, in the mosaic of gradients typical of estuaries, the relative importance of different variables and processes might vary several orders of magnitude in time and space. Quantifying the extent of such variation at whole estuary scales constitutes an open research question, and is not deeply discussed in this review.

2.1. Terminology

In aquatic systems metals are known to be simultaneously present as different chemical species (Fairbrother et al., 2007; Gonzalez et al., 2007). For the purpose of this work 'total metal' is defined as the concentration of metal measurable in water or in sediment after strong acid digestion, including metal precipitates, metals associated with the mineral lattice, adsorbed to sediment, adsorbed to and absorbed into particulate organic matter and dissolved metals (Förstner and Wittmann, 1979). Dissolved metal is defined as the fraction of total metal that passes through 0.45 µm filter, including metal-organic matter complexes, colloids, inorganic complexes, dissolved gases and free ion forms (Bianchi, 2007; Du laing et al., 2009a). 'Non-filterable metal' is the fraction of total metal that does not pass the 0.45 µm filter. In turn, 'particulate metal' is here referred to as the portion of non-filterable metal that is relatively mobile to the dissolved phase, measurable in water after acid strong extraction. It includes metal in inorganic precipitated or co-precipitated form (e.g. carbonates and hydrous Fe-Mn oxides), and adsorbed to sediment and organic matter particles.

'Bioaccumulated metal' describes the metal fraction internalized (from outside to inside through membrane or tissue) by organisms, while 'bioavailable metal' includes metal species that are bioaccessible and have the potential for distribution, metabolism, elimination, and bioaccumulation by a given organism (Drexler et al., 2003). The term 'effect of metal' on organisms denotes deleterious consequences of metal exposure, while the term 'responses' points to a more generic physiological alteration due to metal exposure (Machado et al., 2013).

3. The conceptual model of metal fate and effects in estuaries

In aquatic systems, potentially toxic metals are transported in dissolved or particulate form, where sediment and suspended particles play important roles in metal adsorption, desorption and dissolution, and sedimentation processes (Boyle et al., 1974; Gonzalez et al., 2007). Solubilisation, speciation, precipitation as well as diffusion and advection are also critical processes that determine metal spatial distribution (Benoit et al., 1994). All these processes are influenced by physical, chemical and biological parameters in estuaries. Thus, estuarine circulation, river and groundwater discharge, tidal flooding, sediment input and re-suspension, exchange with neighbouring environments, water properties (e.g. salinity, redox and pH) and the presence of organisms jointly determine the mobility of metals. The consequence of such interactions is often metal accumulation in estuaries (Förstner and

Wittmann, 1979), turning the estuarine fate of metals into a complex dynamics with interdisciplinary nature.

Moreover, metal concentrations and environmental conditions jointly determine organism physiology and toxicity (Monserrat et al., 2007). Indeed, several parameters act on metal behaviour and toxicity simultaneously. Salinity, for example, affects flocculation and hence sedimentation of fine particulate matter (Bianchi, 2007), metal speciation (Drexler et al., 2003), and animal physiology (Martins and Bianchini, 2009) at the same time. Although studies regarding metal fate and toxicity in estuarine conditions exist (Falconer and Lin, 1997; Baeyens et al., 1998; Mao et al., 2006; Trento and Alvarez, 2011; Mwanuzi and De Smedt, 1999; Liu et al., 2007; Di Toro et al., 2001), very few attempts have been made to consider the system in a broader context and to integrate physical, chemical and biological processes simultaneously (Paquin et al., 2003). Thus, the interacting aspects of metal fate in estuaries remain widely unexplored.

3.1. Physico-chemical estuarine features: environmental gradients and non-conservative metal behaviour

Estuarine waters are characterized by strong physicochemical gradients in e.g. salinity, density, flow velocity, and suspended matter composition (Elliotta and McLusky, 2002), which are important influences on the fate of metals (Fig. 1). The interaction of these environmental gradients and metal sources in estuaries normally yields a non-conservative behaviour for the majority of metal elements. Oceanic waters usually have higher salinity and lower trace metal concentrations than inland freshwaters. Thus, metals are interpreted to behave conservatively if concentrations decrease linearly with salinity increase (Boyle et al., 1974). A model from Boyle et al. (1974) predicted Fe as highly non-conservative in Merrimack Estuary due to mobilization processes discussed later. Indeed, environmental partitioning coefficients of Cd, Cu and Pb and other metals are observed to vary non-linearly under different estuarine conditions (e.g. salinity, suspended matter, chlorophyll), which confirms the non-conservative behaviour (Valenta et al., 1986; Benoit et al., 1994; Spencer and MacLeod, 2002; Wang et al., 2009).

Another important estuarine feature is river discharge, which affects mixing processes, the salinity gradient and the input of dissolved organic matter, suspended material, and phytoplankton (Liu et al., 2007; Couceiro et al., 2009; Falconer and Lin, 1997; Van den Berg et al., 2001). The input of freshwater (low density) and saltwater (high density) typically generate the estuarine circulation schematized in Fig. 1, with vertical salinity gradients and freshwater flushing out mainly at the surface (Kundu and Cohen, 2004). Deviations from this pattern are not unusual and well or partially mixed estuaries are commonly reported (Bianchi, 2007). Consequently, river input, tidal flows and coastal processes interact with estuarine physiography, to determine the salinity distribution within the estuary (Valle-Levinson, 2010).

The salinity gradient is one of the main estuarine characteristics responsible for non-conservative metal behaviour. Salinity results in several major changes in water chemistry and ionic strength (Benoit et al., 1994), accounting for metal mobilization (Sections 4–5). Salinity also significantly enhances the water pH buffering capacity due to high concentrations of carbonate and bicarbonate ions, such that pH often increases to slightly basic conditions (~8.08–8.33) along the estuary transect in a seawards direction. Therefore, pH is expected to have a greater influence on metal behaviour at lower salinities.

Moreover, differences in salinity induce a water density gradient within the estuary that is strong enough to affect water circulation. Thus, at very low salinities the flow is mainly barotropic, e.g. dictated by relative gradient in water column elevation. However at higher salinity ranges the flow is also baroclinic, e.g. influenced by pressure caused by distinct water densities. The misalignment of barotropic and baroclinic gradients also generates vorticity increasing turbulent mixing processes and potentially metal solubilisation (Kundu and Cohen, 2004).

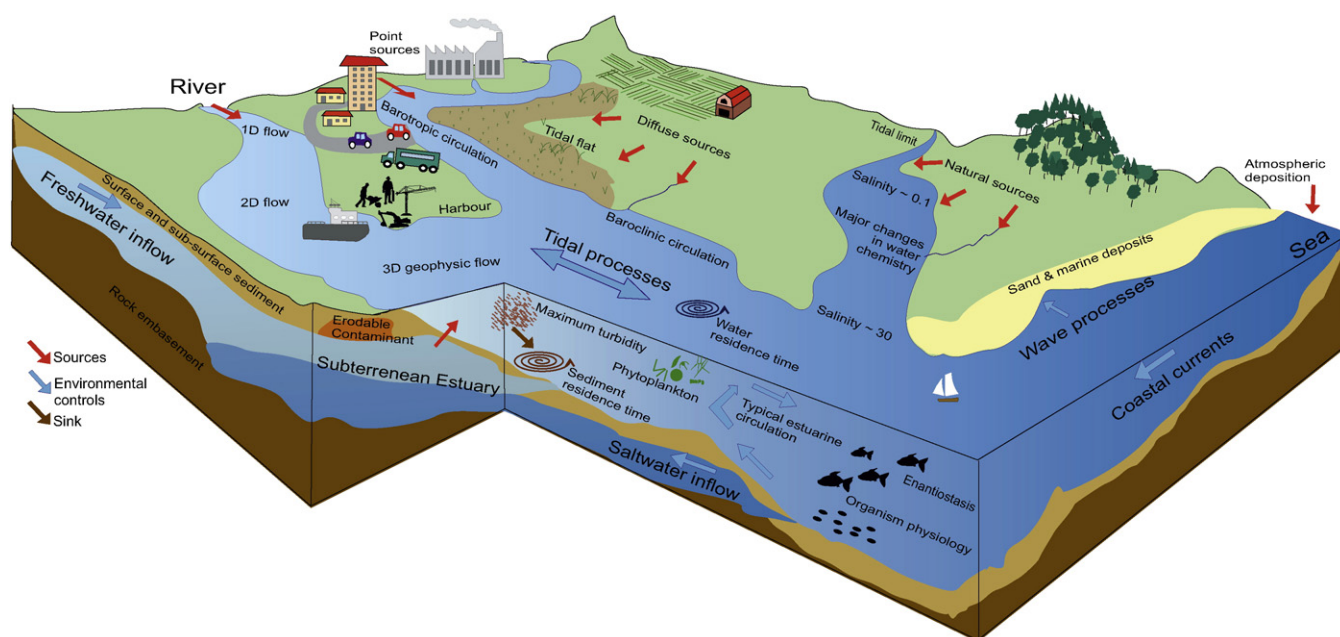


Fig. 1. Conceptual model of physical, chemical and biological variables and processes for behaviour and toxicity of metals. Adapted from Bianchi (2007).

An important estuarine feature resulting from above discussed riverine discharge and salinity gradient is the maximum turbidity zone (Fig. 1). Maximum turbidity zones are one or more estuarine regions with high concentrations of aggregates of particles and colloids from river and other multiple sources formed by physical dynamics of sediment supply and settling velocity, tidal mixing and estuarine stratification (Hobbie, 2000). Change in ionic strength of water is known to be one of the main processes responsible for the formation of an estuarine maximum turbidity zone (Valenta et al., 1986; Bianchi, 2007). In fact in many estuaries, the maximum turbidity is found at the salinity front, usually less than 5 psu (practical salinity units; Jassby et al., 1995; Lanceleur et al., 2013). Very high turbidity zones might also be consequence of dredging activities (Van den Berg et al., 2001; Spencer et al., 2006a). The strong gradient in suspended matter alters physical and chemical mobilization of metal, as discussed later for adsorption and precipitation. In addition, maximum turbidity is often a hotspot for microbial metabolic processes, and has major biogeochemical impacts on organic matter of estuaries.

In terms of metal contamination, special focus has also to be put on the subterranean estuary, in which gradients that affect metal mobilization also occur. In a typical unconfined subterranean estuarine system saltwater intrusion occurs from sea, while freshwater flows from continental side (Fig. 1). The location of the salinity front in these underground systems depends upon freshwater inflow and sea level, i.e. the hydraulic gradient between the aquifer and the sea (average sea level, tides, surges) (Moore, 1999; Fleury et al., 2007), and also upon the geological structure, sediment permeability and hydraulic conductivity (Bianchi, 2007).

3.2. Sources and emission pathways of metals to estuaries

The main sources of metal to estuarine systems are usually riverine particulate and dissolved metal, point (harbour activities, urban centre and runoff, and sewage and industrial effluents) and diffuse natural and anthropogenic sources (runoff from road surface, and agricultural landscapes), atmospheric deposition, and groundwater enrichment (Förstner and Wittmann, 1979; Nemerow, 1991) (Fig. 1). According to Zwolsman et al. (1993) riverine input of Cd, Cu, Pb and Zn to tidal flats of Scheldt estuary was significantly larger

(97.5% to 99.5%) than atmospheric deposition (2.5% to 0.5%). Notwithstanding, Deboudt et al. (2004) added that atmospheric-derived metals might be dominant on the coast, thus potentially affecting metal in the lower estuary. Indeed, atmospheric wet deposition might account for 20–70% of total metal input to coastal areas in Western Europe (Deboudt et al., 2004).

Since groundwater inflow is usually orders of magnitude lower than surface discharge, subterranean metal input was often considered to be negligible. In fact, some models have represented trace metals in estuaries by only considering riverine sources (Boyle et al., 1974; Falconer and Lin, 1997), assuming groundwater accounting for less than 1% of metals discharged (Förstner and Wittmann, 1979). However, more recently the term subterranean estuary has gained relevance for estuarine and coastal biogeochemistry (Moore, 1999). Bone et al. (2007) demonstrated the role of groundwater as the major source of Hg in the Waquoit Bay-Massachusetts ($0.47\text{--}1.9\text{ nmol Hg m}^{-2}\text{ day}^{-1}$). Although the volume of groundwater advection to the surface water might be low compared to the river-derived flow, percolation through sediments that can normally store metals up to 100,000 more than surface waters increases proportionally metal concentrations in groundwater (Förstner and Wittmann, 1979), and results in significant mass transfer (Bone et al., 2007). Also, the flow in subterranean estuaries changes at different temporal scales than their river-fed counterparts and coastal circulation. Such subterranean variation in freshwater-saltwater interface changes the vertical location of the pycnocline and, subsequently the flow paths of solutes (Mao et al., 2006). This implies that the relative importance of surface and groundwater metal sources may alternate.

An additional potential source of metal to both surface and subterranean estuaries is the subsurface of contaminated sediments and landfills, which are common in industrialized countries. The leachate of coastal landfills can carry metals to the neighbouring groundwater and eventually surface waters. Leachate contamination is an emerging threat in historic deposits challenged by sea level rise and consequent coastal erosion and coastal hydrodynamics shifts (Spencer and O'Shea, 2014), groundwater salinization (Bone et al., 2007), presence of colloids and organic matter, and low pH (Jensen et al., 1999; Sañudo-Wilhelmy et al., 2002), all common stressors in estuarine landfills.

4. Physical processes: metal transport in estuaries

4.1. Changes in flow affecting metal distribution

At the riverine end, water flow and sediment transport are primarily determined by barotropic gradients, i.e. flow velocities are proportional to gradients of water surface elevation. Thus, the resulting metal transport is mainly one-dimensional along the river course on a large (several km) scale (Fig. 2). As water flows into the part of the estuary that is more affected by tidal flow, the mass transport tends to occur in two directions (Geyer and MacCready, 2014), where either vertical or horizontal stratification occurs (Kundu and Cohen, 2004). This bi-directional stratified flow is usually associated with gradients of salinity and acceleration of particles transported in the water column, which affect sedimentation, partitioning and metal mobilization, and ultimately the transport of metals in the water column (Wu et al., 2005). Moreover, the density stratification produced by salinity gradients affects turbulence. Salt wedge intrusion and vertical stratification may reduce turbulent mixing by increasing water column stability, i.e. higher Brunt-Väisälä frequency (Kundu and Cohen, 2004). Differently, horizontal salinity stratification might increase mixing due to misalignment of baroclinic and barotropic forces. Both processes potentially affect redistribution of metals within the water column because stronger turbulence causes disaggregation of large cohesive flocs by means of more frequent collisions, therefore enhancing metal transport and dissolution.

In the lower estuary, circulation might be reasonably described by considering three-dimensional hydrodynamics only (Fig. 2). Estuaries are typically funnel-shaped, so the horizontal scales can increase several orders of magnitude moving seaward with respect to the upstream river, causing a reduction in the Rossby number (quotient between velocity and the product of the horizontal scale of movement and Earth's rotational velocity) (Azevedo et al., 2008). As a consequence, a higher influence of Earth's rotation in the circulation of low velocity and large horizontal scale flows is observed at high latitudes. Thus, in the most coastal estuary the flow is geophysical, and the circulation interacts with Coriolis acceleration influencing the dispersion of estuarine contaminant plumes (Falconer and Lin, 1997). In large, open estuaries this effect determines the location of preferential sediment-associated metal accumulation both within and outside the estuarine system (Bianchi, 2007). The resultant transport, also known as Ekman transport, deviates to the right or the left (north or south hemisphere, respectively) of the predominant flow direction (Kundu and Cohen, 2004), and can produce regions of upwelling and downwelling along the coast and within the estuary.

Due to longitudinal salinity gradients other transport mechanisms can be identified in estuaries in addition to gravitational mixing. Among these tidal pumping can be observed which is associated with residual circulation that results from the presence of tidal loops with ebb- and flood-dominated channels. Also tidal trapping is a transport mechanism whereby the asymmetric flow over lateral shallower areas and the effect of halophytic vegetation determine a residual particle

transport (Savenije, 2012). These processes are usually summarized in the one-dimensional concept of tidal dispersion, a process describing the increased longitudinal dispersion of tracers along the estuary (Geyer and MacCready, 2014).

Ultimately, the interaction of river flow and tidal cycles generates a rotational circulation that increases water and sediment residence times within the estuary (Valenta et al., 1986; Kundu and Cohen, 2004). The respective extent varies strongly with tidal amplitude and frequency, estuary geomorphology and riverine discharge, and usually results in the accumulation of particulate metal within the estuary and buffering metal input to marine coastal systems.

4.2. Changes in flow that affect metal concentration and partitioning

Not considering marine processes outside the estuary, low river discharge is usually associated with low suspended particulate matter input into the estuary, resulting in lower levels of particulate metals (Falconer and Lin, 1997; Wang et al., 2009). Under low fluvial discharge salinity increases due to higher relative contribution of seawater within the estuary. This causes dissolution and desorption of metals from particulate matter (Fig. 3, see Section 4.3 for sorption) (Mwanuzi and De Smedt, 1999). Increasing river discharge, in contrast, implies higher suspended material input (Couceiro et al., 2009), potential for re-suspension of settled particles (Savenije, 2012), and decreased salinity. This can intensify adsorption and precipitation processes, resulting in a maximum of particulate and a minimum of dissolved metal in the water column (Fig. 3). However, with higher riverine discharge the riverine influence is larger throughout the estuary and the flow pattern is modified such that the relative importance of tidal processes is decreased (Cai et al., 2014). This can trigger a reduction in water and particle residence times including flushing of contaminants out of the estuary. In such a situation, smaller fractions of adsorbed metal might also occur due to the increment of coarse material with lower sorptive capacity, while increasing river turbulence might promote the solubility of some metal precipitates (Förstner and Wittmann, 1979), i.e. these are processes contributing to an increase in the relative importance of the dissolved fraction (Benoit et al., 1994). Also at very high concentrations of suspended material the dissolved metal correlates with colloid concentrations, which is ultimately dependent on total suspended matter concentration (Benoit et al., 1994). Although major differences in dissolved and particulate metal concentrations have been reported as varying with discharge, the general effect is often only a slight reduction in total water metal concentration (Förstner and Wittmann, 1979; Wang et al., 2009). The extent of the influence of these variables and processes at whole estuary scales remains yet to be investigated.

4.3. Sorption and desorption: the physico-chemical interactions of sorbents and salinity

The absolute distribution of metals between the particulate and the dissolved phase predominantly depends on the availability of sorbents,

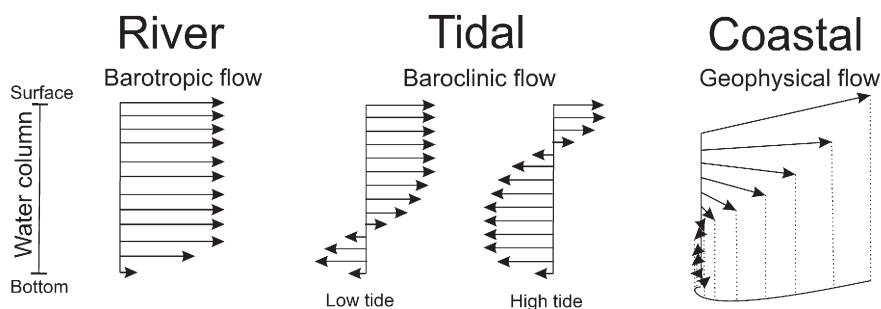


Fig. 2. Comparison of river, tidal and coastal processes determining estuarine circulation, and mass transport of dissolved and particulate metal. Note: bi-directional tidal flow might occur either vertically, horizontally, or both.

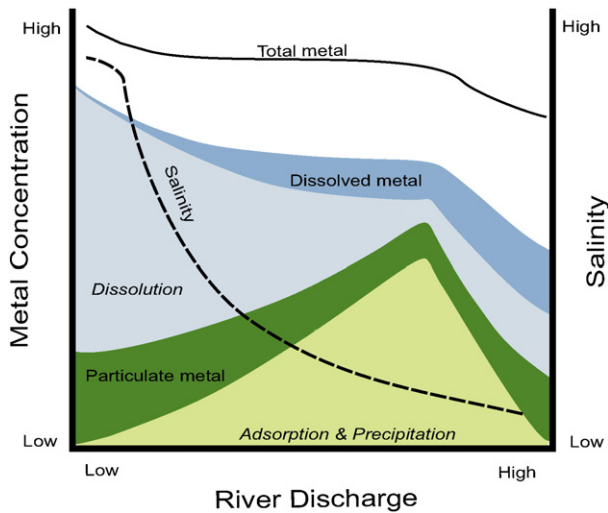


Fig. 3. Conceptual river discharge effects on particulate (dark green area), dissolved (dark blue area) and total metal (continuous line) concentrations, as well as on dissolution (light blue area), adsorption and precipitation (light green area) processes. The sketch is based on Wilson model (1976) for river discharge and metal concentration, and modified to address metal interaction with salinity (dashed line) under estuarine conditions.

and results from the operation of surface forces, i.e. usually the attraction of negatively charged metal ions to positively charged surfaces (Förstner and Wittmann, 1979). Consequently, highest concentrations of suspended particles and total and particulate metal concentrations are found within the maximum turbidity zone, where dissolved metal concentrations are low (Gonzalez et al., 2007), as observed in environmental samples of maximum turbidity for Ag (Lanceleur et al., 2013), Cu, Cd, Pb and Zn (Van den Berg et al., 2001; Müller and Förstner, 1975). Higher sorption, and consequently higher metal inventories and potential to water purification services are also expected in tidal flats or estuarine areas of deposition of finer sediments (Zwolsman et al., 1993).

The partitioning of dissolved metal phases is also affected by salinity (Förstner and Wittmann, 1979). Metal desorption from sediments and suspended matter increases with increasing salinity (Mwanuzi and De Smedt, 1999) due to complexation of metals such as Cd with chloride and sulphate forming soluble inorganic complexes (Greger et al., 1995). At the same time, cations, notably Ca and Na, compete with metals for adsorption sites displacing both weakly and moderately sorbed metals such as Cr, Cu, Zn and Pb (Fairbrother et al., 2007). For example, waterborne Cd has been reported up to 4-fold higher if salinity changes from 0 to 10 psu (Greger et al., 1995). Thus, gradients of suspended matter concentration, sediment types, and salinity are decisive for metal mobility within the estuary.

4.4. Sedimentation and metal removal from surface waters

Metal adsorbed to particulate material can settle out of suspension, providing one of the main sinks of metals in the estuarine system. Gradients that are responsible for water column stratification also affect sedimentation, precipitation and flocculation of particles and colloids (Förstner and Wittmann, 1979). In turn, high turbidity areas contribute to enlarge sub-surface contaminated deposits. Thus, residence time and mobilization of particulate metal in estuaries are strongly influenced by the turbidity maximum. Sorption of metals to depositing sediments is a major removal mechanism for these contaminants from the overlying waters (Mwanuzi and De Smedt, 1999), providing important ecosystem service in terms of water quality improvement (Millennium Ecosystem Assessment, 2005). This transfer of metals from the aqueous to the

sediment compartment may be a temporary removal from the water phase but not from the whole estuarine system.

5. Metal remobilization processes

Metals sorbed to bottom sediments do not necessarily stay in that condition, but may be released due to resuspension or desorption and act as a diffuse pollution source (Zwolsman et al., 1993; Mwanuzi and De Smedt, 1999). The sediment texture, hydrological regime, salinity and organic content are responsible for the mobility and availability of metals in the superficial sediment layer especially in inter-tidal environments (Du Laing et al., 2009b). Especially in tidal flats, autochthonous (in situ precipitated) metals are constantly remobilized by interaction with organic matter, water level, redox conditions and water chemistry changes, and diagenesis (Förstner and Wittmann, 1979) (Fig. 4).

5.1. Chemical metal remobilization

In freshwater saturated sediments, the mobility of metals is strongly determined by metal–sulphide interaction and Mn or Fe oxide co-precipitation (Paquin et al., 2003), or phosphate and organic matter interactions (Förstner and Wittmann, 1979) since under specific redox conditions such compounds are able to sequester metal ions from solution by reducing their reactivity. Under saltwater saturated conditions, ionic interactions with carbonate oxides, oxide-hydroxides, silicates, and chloride gain additional relevance for metal mobilization (Fairbrother et al., 2007). In this case, water filtration into sedimentary layers introduces ions (such as Ca, Cl, K, Mg, Na, SO₄) which leads to the mobilization of sediment bound metal to pore water and promotes the reactive transport of these elements (Fig. 4).

In sediment layers also redox conditions determine metal remobilization (Zwolsman et al., 1993). Under oxidizing conditions the co-precipitation and adsorption of metals with Fe-Mn oxides acts as sink of dissolved metal (Fairbrother et al., 2007; Lu et al., 2014). At more reductive conditions, first Mn and then Fe are reduced, both releasing adsorbed and co-precipitated metals including Co, Ni, Cr, Cd, Cu, Pb and Zn (Zwolsman et al., 1993). This release is amplified by sulphate–metal interaction, increasing pore or surface waterborne metal (Du Laing et al., 2009b). At even stronger reductive conditions sulphate is reduced to sulphide, which can complex metals and reduces mobility and toxicity (Du Laing et al., 2009c; Lu et al., 2014).

5.2. Chemical and biologically mediated metal remobilization

For those metals that form stable methyl species (e.g. Hg, As, Cr), salinity is also expected to promote bacterial methylation by providing additional sulphate, which is the substrate for sulphate reducing bacteria (Zhang et al., 2014). In turn, methylation increases both metal toxicity and mobility by shifting metal affinity to organic matter. Notwithstanding, reduction on metal bioavailability and further production of sulphide might play a role at decreasing methylation in fully saline conditions (Fairbrother et al., 2007) altho.

Particulate metals are also released as a result of chemical changes caused by aquatic plants (Greger et al., 1995) and benthic organisms (French and Turner, 2008). The organic matter provided by biota detritus and roots exudates plays an important role as food source for micro-organisms, which catalyse a series of redox reactions in the presence of electron acceptors. Together with sulphate, Fe and Mn, organic matter in dissolved, colloidal and particulate forms provide redox-buffering capacity to sediments (Wright, 1995; Paquin et al., 2003). Organic matter also chelates metals and increases mobility (Du Laing et al., 2009c), e.g. increases of 25% in organic matter content might raise metal concentrations up to 200 times (Du laing et al., 2009a). Frequently flooded and higher salinity areas, in general, present higher organic matter due to lower optimal redox conditions for its degradation and higher chemical stability (Du Laing et al., 2009b). Moreover, the respiration and

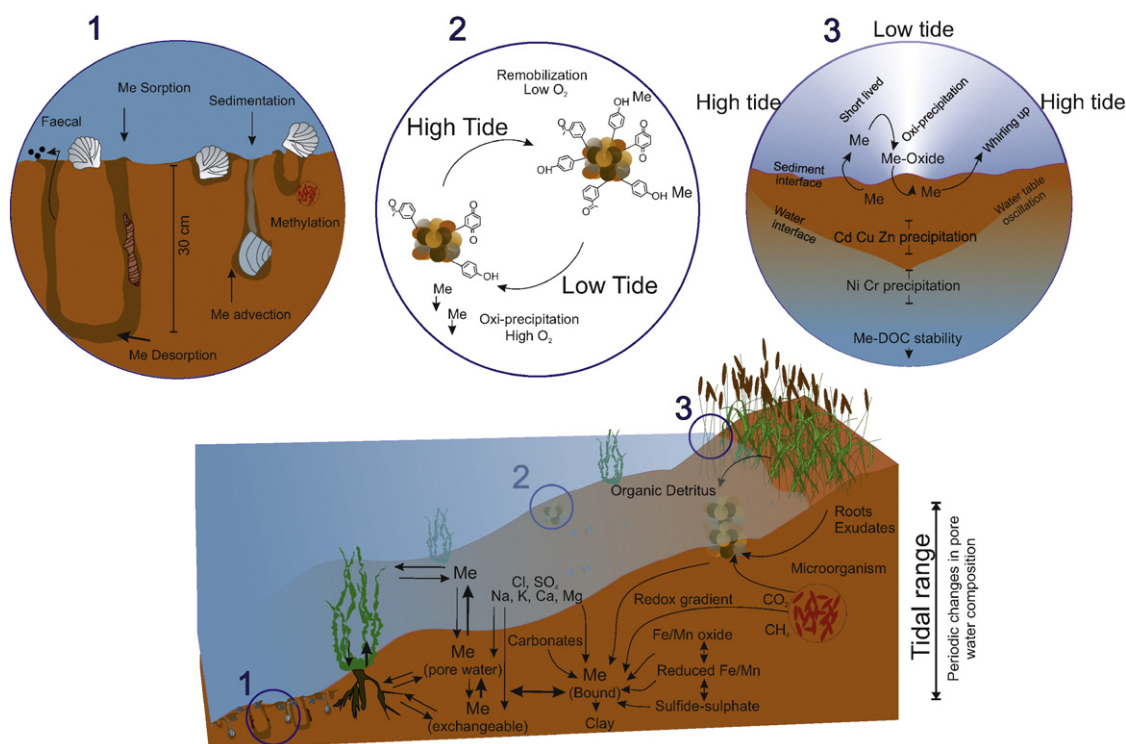


Fig. 4. Physical, chemical and biological variables and processes affecting the behaviour of trace metals in tidal flats. Subplots represent processes associated to benthic invertebrates in sub-tidal zone (1), organic matter and redox cycles in inter-tidal zone (2) and water table and metal precipitation in inter-tidal zone (3). Inspired by Greger et al. (1995); Du Laing et al. (2009c), and Klüpfel et al. (2014).

degradation products of organic matter causes reductive conditions and changes pH altering ionic composition of pore water and overall increasing mobility (Lu et al., 2014). Du Laing et al. (2009b) found that when organic matter is being decomposed Ca, Mn, Ni and Fe are released upon CO_2 accumulation and Fe/Mn oxide reduction if no sulphide is present.

A recent study suggested that organic matter might be prone to sequential oxidation and reduction cycles in the environment (Klüpfel et al., 2014). These results have implications on metal behaviour because oxidized and reduced organic matter present lower and higher metal binding capacities, respectively. Thus, during organic matter oxidation metal would be released and susceptible to Fe-Mn-oxi-precipitation or co-precipitation (Fig. 4.2). At reductive conditions, in contrast, organic matter could act as catalytic factor for metal mobilization through metal chelation.

5.3. Chemical and physically mediated metal remobilization

In the inter-tidal flats reductive and oxidative conditions are cyclic with tides what implies an additional type of organic matter-mediated metal remobilization and precipitation. Similarly, the consecutive high and low tide cycles also affect inorganic precipitation of metals in surface and sub-surface sediments (Fig. 4.3). Even for supratidal areas, fluctuation in groundwater table level causes the migration of organic matter among layers, changes ion compositions and affects the preferential region of metal deposition (Du Laing et al., 2009c). Experimental results of the interaction of water table level, organic matter and pore water composition across sedimentary layers showed that Cd, Cu and Zn are deposited right above the water level, while Ni is deposited right below (Du Laing et al., 2009b). Ultimately, the physical component of water level oscillation and tidal current shear implies sediment re-suspension (Hawkins et al., 1996) and is an important

factor in the release of metals from particles and from pore solutions (Lindberg et al., 1975).

6. Interactions with biological processes

6.1. Physical, chemical and biological interactions for metal remobilization

Physical water intrusion favours advection of exchangeable and pore water metal to the water column or bioaccumulation by roots of aquatic macrophytes (Fig. 4). In turn, roots transfer metal to above-ground biomass which constitutes a route of particulate organic metal to the water column (Greger et al., 1995). The interaction of roots and bacteria is also able to modify the microenvironment (up to 1 cm around roots) increasing the metal amount in the exchangeable weakly bound fraction (Du Laing et al., 2009c). This might have a great impact on metal remobilization if considered the remarkable portion of surface sediments covered by their dense root system.

In the tidal flat, benthic fauna also alters metal biogeochemistry in sediments because some of the metabolites produced (as CO_2 , CH_4 , NH_2 , PO_4) are chemical species that increase metal mobility (Du Laing et al., 2009b). Organic residues and abandoned spaces within the sediment might be hotspots for microbial activity affecting redox conditions as well as methylation processes (Fairbrother et al., 2007). Maybe even more importantly, in bioturbated sediments the thickness of the layer of exchange and advection of water and sediment might be increased from <1 cm to ~30 cm (Fairbrother et al., 2007) (Fig. 4.1). The burrowing organisms form preferential channels for groundwater flow, which, depending on sediment permeability, can reach up to $7.0 \cdot 10^{-6} \text{ m s}^{-1}$ (Brand et al., 2013) and thus promote metal advection. Brand et al. (2013) pointed that transport of oxygen-containing surface water into the deeper zones of the sediment by burrowing organisms could trigger a sequence of redox reactions with potential impacts on Fe behaviour and solute transport. Indeed, French and Turner (2008) found that

platinum metals introduced via water show increased sorption behaviour when sediments are colonized by the deposit feeder *Arenicola marina*. On the other hand, the same species increased pore water metal concentrations up to 2 orders of magnitude by remobilizing contaminated sediment (French and Turner, 2008). Relocation of metals as faecal pellets might also affect their distribution. Assimilation rates about 10% of Pt from water and sediment imply that 90% of ingested metal is transferred to faecal pellets (French and Turner, 2008). The high filtration capabilities of some molluscs also have a potential to significantly affect sedimentation of particles at whole estuary scale. Hawkins et al. (1996) found that *Mytilus edulis* from Marennes-Oléron bay (France) showed a clearance rate (water cleared of suspended matter per mussel) of up to $\sim 5.28 \text{ L h}^{-1}$, from which about 93% of filtered material was deposited as pseudofaeces, e.g. filtered from water column but not ingested. Thus while deposit-feeders polychaeta might have effects in both deposition and remobilization of metal, bivalves would affect mainly deposition. The extension of such interactions in sedimentation and metal behaviour remains to be studied however.

Finally, estuarine sediments' remobilization and their changes in pore water release might be enhanced by physical advection events, e.g. wave action, storm surge, and dredging activities. This can affect dissolved and (mainly) particulate metal concentrations within the estuary if contaminated sediments are present (Van den Berg et al., 2001). Spencer et al. (2006b) found that historically contaminated sediments from Thames and Medway estuaries (UK) represented a risk of mobilization of Cr, Cu, Pb and Zn. These authors suggested that in case of natural or anthropogenic reworking of these historic sediments, associated metal would become readily available to cause toxicity at Thames and Medway estuaries, in which bioavailability of metals vary up to 70% of the total content.

6.2. Phytoplankton affecting partitioning and advection of metals

One of the most important biotic factors influencing the behaviour of metals in the water column is phytoplankton (Fig. 5). This group of organisms shows high metal bioconcentration (Phillips, 1980), with uptake occurring predominantly from dissolved fractions (Paquin et al., 2003). Fisher et al. (1981) reported Cu concentrations on *Asterionella japonica* of approximately 10,000 times as high as culture media. Similarly, Fisher et al. (1984) comparing *Thalassiosira pseudonana*, *Dunaliella tertiolecta*, *Emiliana huxleyi*, *Oscillatoria woronichinii* found bioconcentration factors (BCF, volume based) about 10^4 for Ag, 10^3 for Cd, 10^4 to 10^5 Hg and 10^2 to 10^3 for Zn. Also data from Yap et al. (2004) suggests BCF (volume based) about 10^4 to 10^5 for *Isochrysis galbana* for Cd, Cu,

Pb and Zn, ratifying the role of phytoplankton on the metal distribution between aqueous, solid and organism phase in an estuarine system.

Two ways of metal–phytoplankton interaction can be distinguished: external adsorption of the metal to the cell wall and uptake through the cell wall into the cell. The surface of phytoplankton cells provides a significant area for metal adsorption, while the increase of up to 2 units of pH around photosynthesizing microalgae might favour metal oxiprecipitation on the cell wall (Brøek et al., 1980). With dividing cells producing new cells and thus new reactive surface material, the particulate metal content increases linearly (Fisher et al., 1984).

Phytoplankton also passively and actively absorbs (internalizes) metals. Biological membranes are considerably impermeable to electrically charged and highly polar neutral and organic metal species. However, passive absorption happens when metals cross these biological membranes diffusing into the organisms, which might occur with neutrally charged metals (e.g. Hg^0 , HgCl_2 , AgCl , Cu(I)Cl) or organic–metal complexes (Sunda and Huntsman, 1998). The resulting diffusion process of metals in this case is forced by external/internal gradient concentrations according to Fick's law. On the other hand, active absorption is more common for most of metal ions and polar metal–organic complexes. This uptake is performed by specific membrane transporters like channels and ion exchange pumps. It might thus occur more independently of gradient concentrations as a saturation-like curve response ruled by kinetics and thermodynamic metal/transporter interactions constants (Sunda and Huntsman, 1998; Drexler et al., 2003) and transporter density in cell membrane (Fisher et al., 1984).

Under steady-state conditions, for both processes (adsorption and absorption of metals by phytoplankton) the cellular metal concentration equals the cellular uptake divided by the specific growth rate (Sunda and Huntsman, 1998). Considering that phytoplankton is usually included on suspended matter measurements and its metal cellular content remains relatively constant after metal exposure under steady-state (Fisher et al., 1984), both adsorption and absorption increase particulate metal and alter partitioning of metal between water and suspended matter. This is significant at environmental level, for instance the distribution coefficient (L kg^{-1}) for particulate and dissolved Cd concentration was reported to vary from $17 \cdot 10^3$ up to $100 \cdot 10^3$, with highest anomalies of particulate Cd attributed to seasonal phytoplankton bloom in Scheldt Estuary (Valenta et al., 1986). Wang et al. (2009) similarly observed inverse relationship of dissolved copper and chlorophyll in the Changjiang Estuary during phytoplankton bloom.

If not consumed, phytoplankton-bound metal will be regulated by residence times and fate of particles and organic matter in the estuary, and similarly be subject to sedimentation or advection to coastal

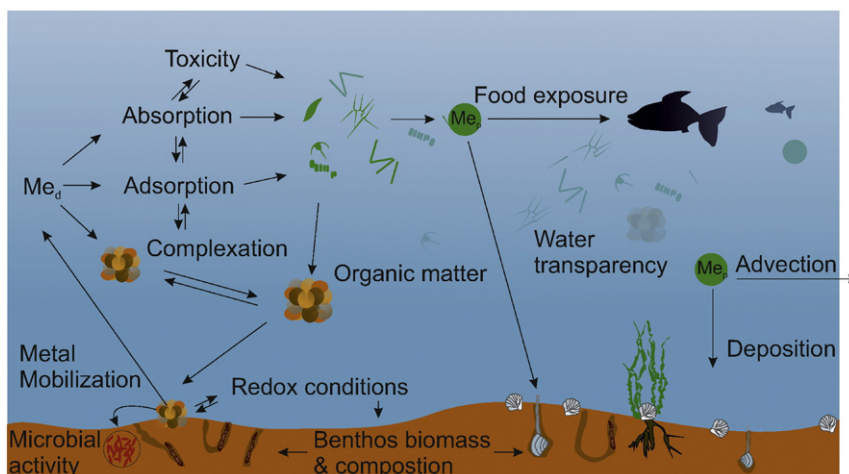


Fig. 5. Conceptual model of phytoplankton consequences on dissolved (Me_d) and particulate (Me_p) metal and organisms exposure route on estuaries.

areas. In this sense, the sedimentation of dead phytoplankton might contribute to additional particulate metal input to surface sediments since dead cells might accumulate even more metal than living ones (Fisher et al., 1984). In fact, Valenta et al. (1986) noticed an efficient removal of Cd, Cu, and Pb from water column taking place during the months of sedimentation of plankton detritus.

7. Organism physiology and effects of metals

7.1. Phytoplankton and environmental toxicity of metals

The important role of phytoplankton in transferring metal from the dissolved to the particulate fraction, i.e. decreasing dissolved concentrations, does not represent necessarily a decrease in metal bioavailability (Fig. 5). In fact, it implies potential increment in food transfer of metals to higher trophic levels (Sunda and Huntsman, 1998). Thus, phytoplankton alters metal chemistry and exposure routes to other organisms simultaneously. Phillips (1980) noticed that metal concentrations in the blue mussel *M. edulis* (Swedish coast), pike (Stockholm archipelago), and the scallops *Pecten maximus* and *Chlamys opercularis* (English Channel) were related to metal levels rather in phytoplankton than in water. It is evident that dietary accumulation of metals is at least as important as metal uptake from the aqueous phase and in many cases dominates metal accumulation, e.g. in bivalves from marine environments (Ahlf et al., 2009). King et al. (2005) compared metal bioaccumulation by the deposit-feeding amphipod *Melita plumulosa* from water, sediment and microalgae sources under laboratory conditions. They report that feeding rates of 0.19 g algae g⁻¹ organism day⁻¹ implied about 95 and 75% of total Cd and Cu uptake, respectively. The same authors found that the rate of Cd and Cu accumulation from sediments was actually increased when microalgae were present due to higher feeding rates.

Although mechanisms and consequences of diet-borne exposure are widely unknown, the consensus is that it has the potential to cause toxicity (Fairbrother et al., 2007). Comparing contaminated waterborne and diet-borne effects of Cu exposure to the copepod *Acartia tonsa*, Pinho et al. (2007) found that feeding over Cu contaminated microalga *Thalassiosira weissflogii* overall improved nourishment conditions, and only highly contaminated food caused more adverse than benefiting effects. In contrast, Moreno-Garrido et al. (1999) reports that reproduction of the planktonic rotifer *Brachionus plicatilis*, which under seawater control conditions occurred within three to four days, was delayed by one to two days when this rotifer was fed with four different Cu-contaminated microalgal species.

It has been questioned even if the same underlying toxicity mechanism is occurring for metal absorbed from water (dissolved) and for metal absorbed from suspended material (particulate). Simpson and King (2005) found that Cu uptake by *M. plumulosa* and the bivalve *Tellina deltoidalis* was higher from dissolved metal compared to suspended matter metal, while toxicity was higher for particulate-exposed individuals. According to them, the uncoupling of bioaccumulated metal and toxicity regarding the different exposure routes suggests distinct toxicity mechanisms for food and waterborne related metals.

Phytoplankton growth also has indirect important effects on metal behaviour and toxicity by affecting organic matter. Phytoplankton exudates and decomposition increase significantly the dissolved organic matter in water column (Cloern, 2001), potentially increasing organic-metal complexation and solubilisation (Fig. 5). Additionally, there is evidence for higher excretion of organic matter by metal-treated phytoplankton populations (Bentley-Mowat and Reid, 1977). In terms of implication for toxicity, freshwater organic matter has a shielding effect by chelating metals and reduced bioavailability and toxicity (Di Toro et al., 2001). The protective effect of humic and fulvic acids on toxicity of copper in freshwater, for instance, is well acknowledged (Paquin et al., 2003). However, in terms of saltwater, this issue is still unsolved. Studies available so far point to a more specific response of organic matter, organism, metal toxicity and salinity than overall trends. Sánchez-

Marín et al. (2007) found that humic acids actually increased absorption of Pb by gills of *M. edulis* and toxicity to the purple sea urchin *Paracentrotus lividus* larvae, even when 25–75% of this metal was bound to organic forms.

Phytoplankton-derived organic matter can further deposit as particulate or colloidal matter in sediments, where mineralization processes take place and favour reductive conditions, which affect microbial activity and fauna in the sediment (Du Laing et al., 2009c) and lead to a remobilization of metals. In this case, shifts in oxygen availability are known to cause changes in the benthic community composition, i.e. to reduce biodiversity and increase biomass and to change the community towards the direction of polychaeta species (Cloern, 2001). Polychaetes, in turn, might affect metal desorption from sediments (Fig. 4.1) (French and Turner, 2008). Ultimately, phytoplankton growth and organic enrichment of water column decrease water transparency, and consequently the light availability of metals to submerged plants, limiting plant growth and transfer of particulate metal as discussed above (Fig. 5).

7.2. Metal impact on estuarine biota

In contrast to freshwater systems, where nutrients are the main factor limiting primary production, the growth of phytoplankton in coastal zones is primarily limited by the interplay of nutrient loading, filter capacity of the system, contaminants (Fig. 5), light availability, and ecological interactions (Cloern, 2001). For instance, growth reductions of up to 50% related to exposure to less than 90 µg·L⁻¹ of Cu or Zn have been demonstrated for cosmopolitan phytoplankton species (Fisher et al., 1981; Machado et al., 2014a). In fact, laboratory metal exposure inducing phytoplankton reduced cytokinesis, photosynthesis disturbance and increasing cell size has been reported for several metal and species (Shrift, 1959; Erickson, 1972; Davies, 1974; Blankenship and Wilbur, 1975; Bentley-Mowat and Reid, 1977; Rivkin, 1979). It is clear that in contaminated estuaries cellular concentrations of metals may reach toxic levels, controlling growth and potentially resulting in alterations of phytoplankton productivity (Phillips, 1980) and species composition (Fisher et al., 1984). Its quantitative relevance under environmental conditions remains to be clarified, however (Cloern, 2001).

Beyond phytoplankton, metals might also cause toxicity to other organisms according to the physiological adaptations of organisms to the estuarine environment as discussed below (Monserrat et al., 2007).

7.3. Physiology interfering the mechanisms of metal toxicity in estuaries

Most of the knowledge regarding metal toxicity mechanisms to aquatic organisms has been established in freshwaters. From these studies, especially for the metals Ag, Cd, Cu, Pb, and Zn the concept has emerged that metals react with organism's ligands at organism/environment interface similarly to metal interaction with abiotic chemical ligands (Paquin et al., 2003). Once absorbed, metals could either be located in a non-toxic pool or bound to the organ or molecule target of toxicity, often referred to as site of action (Drexler et al., 2003). The current most accepted idea is that toxicity is proportional to the metal accumulated at this toxicity target. A classic example is the correlation between Cu in the gills (as toxicant), Na uptake (as toxicity mechanism), NaK-ATPase inhibition (as site of action), and mortality (as toxicity endpoint) in rainbow trout after Cu waterborne exposure (Grosell et al., 2002). Indeed, effects on ion homeostasis have been demonstrated for several metals and freshwater organisms in both laboratory and field exposures (Heath, 1995; Wright, 1995; Grosell et al., 2002; Wood et al., 2012a,b; Machado et al., 2014b). Thus, it has been suggested that the common mechanism of metal toxicity is related to ion and osmotic disturbances driven by ion gradients between the organism's plasma and environmental water (Alsop and Wood, 2011). These concepts have been successfully demonstrated for Ag, Cd, Cu

and Zn in several freshwater species, and broadly propagated as Biotic Ligand Models (Paquin et al., 2003).

However, metal toxicity to estuarine organisms might be more complex because the physiology of the organisms changes with increasing salinity. For instance, freshwater fishes are hypertonic compared to their environment (Alsop and Wood, 2011). These hyper-osmoregulating fish avoid drinking water, produce high amounts of diluted urine and uptake ions from food and especially through gills, which are the main tissues of ion regulation (Wood et al., 2012a). On the contrary, saltwater teleost fishes are hypotonic (Grosell et al., 2007), drink saltwater, produce small amounts of concentrated urine and excrete ions through gills (Wood et al., 2012a). In saltwater fishes, water is absorbed by actively producing an osmotic gradient in the intestine, which is a further important organ to ionic regulation. Thus, in an estuarine system, the importance of exposure routes as well as toxicity targets, and consequently organism sensitivity might change.

Freshwater fishes are expected to absorb waterborne metal mainly by gills, while in saltwater drinking fishes gills and gut might be important (Wood et al., 2012a). Moreover, by drinking water, organisms ingest higher amounts of particulate metal that could later be mobilized under the acidic conditions of the digestive tract. Indeed, metal sensibility and toxicity mechanisms that have been traditionally attributed to and modelled based on dissolved fractions in freshwater (Ahlf et al., 2009) might require reconsideration for saltwater organisms. As mentioned earlier, dissolved and particulate metal appear to have distinct accumulation rates and, eventually, toxicity mechanisms. Simpson and King (2005) observed faster Cu uptake by gills, but higher toxicity (lower accumulation at LC50–concentration lethal for 50% of organisms) when accumulated by intestine.

Additionally, some groups of organisms can change from osmoregulatory to osmo-conforming behaviour as a function of salinity. This is the case for several crustaceans like the blue crab *Callinectes sapidus*. In lower salinities (0–20 psu) the organism is hyper-osmoregulating, i.e. its haemolymph is denser than the surrounding water. At higher salinities (>20 psu) the crabs are osmoconforming, i.e. their haemolymph has approximately the same osmolality of the surrounding water. In fact, changes in salinity typically cause metabolic changes in crustaceans and estuarine animals (Monserrat et al., 2007). Thus, if ion disruption is the toxicity mechanism of metals, physiology may become more important than water chemistry in predicting metal toxicity under varying salinities (Grosell et al., 2007; Zimmer et al., 2012).

7.4. A conceptual model for metal toxicity in estuaries

There is an overall negative relationship between salinity and dissolved metal accumulation or toxicity (Wright, 1995), i.e. salinity reduces the availability of metals and thus diminishes their toxicity (Fig. 6.1). This protective effect against waterborne metal exposure predicted by biotic ligand models is due to the introduction of cationic competition for sites of toxicity on the organism, and due to increasing metal-complex forms (Pinho et al., 2007; Pinho and Bianchini, 2010). The mechanisms of toxicity in varying salinity are also affected by several factors. Salinity directly affects waterborne ions, increasing ion diffusive gain (from water to the organism) and decreasing ion diffusive loss (from the organism to the environment). Consequently, at higher salinities less active ion uptake is performed. On the other hand, high salinity requires higher ion excretion rates to cope with the excess of ion gained.

Both ion uptake and excretion are regulated by several enzymes and channels, which constitute direct targets for metals (Wood et al., 2012a, b) (Fig. 6.1). Thus, by impairing biologically controlled pathways, metal toxicity ultimately reflects the disturbance of the ion-osmo-regulation and the physiologically required gradient of ions inside and outside the organism (Fig. 6.2). In freshwater organisms that are not exposed to metals, the internal concentration of a certain ion over time is relatively constant (Grosell et al., 2002), and concentrations of osmolytes inside the organism are much bigger than in the surrounding water.

Under such conditions, all organisms hyper-osmoregulate, and active uptake is high in order to cope with ion diffusive loss while diffusive gain into the organism and ion excretion are minimal. Thus, osmolyte loss is the possible ion-osmoregulatory effect at freshwater conditions. Indeed, ion loss is often reported in freshwater organisms after metal exposure. If ion loss is higher than a critical level, the organism dies. For instance, empiric data suggest that death for 50% of some invertebrates occurs after Ag, Cd, and Cu exposure when about 30% of Na is lost (Vitale et al., 1999; Grosell et al., 2002).

At higher salinities the situation is different since the gradients of ions inside and outside the organisms are smaller. Thus, with increasing salinity various ion-osmoregulation and ion-osmoconformation strategies coexist. The higher salinity per se has implications usually increasing ion diffusive gain, such that ion excretion gains physiological relevance. In this case, diverse metal effects can be expected (Fig. 6.2). Notice that lethal toxicity caused by metals might be observed even in osmoconforming organisms, suggesting that either ionic disruption is the cause of death or other toxicity mechanisms are to be discovered.

The interaction of possible organism physiologies and metal effects has generated some apparent disagreement on factors accounting for metal toxicity in the scientific literature. For instance, some authors claimed that Cu toxicity in different salinities could be explained simply by changes in water chemistry and free ion activity instead organism physiology (Martins et al., 2011). Other authors stated that physiology is important to cover effects of Cu not explained by water chemistry (Wright, 1995; Grosell et al., 2007). Thus it is appropriate to mention that for estuarine organisms enantiostasis might be important to explain metal toxicity and these conflicting results. In order to keep homeostasis, organisms change the properties of interface membranes (transporters, channels, and lipid composition) (Wright, 1995) and adapt cytosolic enzymes activities among other changes, referred as enantiostasis processes (Monserrat et al., 2007). Thus, for several estuarine organisms enantiostasis drives significant changes in membrane properties or internal availability of toxic metal (Drexler et al., 2003) that affect effective metal exposure. Such changes in membrane transporters and channels shift the affinity of biotic ligands to metals, while changes in lipid composition additionally affect the membrane permeability to distinct metal species (Sunda and Huntsman, 1998). Variability of internal metal availability can also be caused by salinity-driven metallothionein adaptation (Martins and Bianchini, 2009). Indeed, Oguma and Klerks (2013) recently demonstrated in field populations of the shrimp *Palaemonetes pugio* the importance of native salinity acclimation to Cd sensibility, which might be attributed to enantiostatic related processes affecting metal exposure, and consequently, toxicity.

Major modifications in water chemistry that affect metal speciation are expected to occur primarily under low salinity conditions, and secondary at intermediate salinities. At higher salinities relatively minor changes in metal speciation take place (Grosell et al., 2007). Concomitantly, in freshwater organisms (mandatory hyper ion-osmoregulatory physiology) only body size is a relevant component of the physiology, because it is known to affect ion turnover rates (Grosell et al., 2002). Differently, at higher salinities the physiology is more variable. Thus, although water chemistry might play a role, we conceptualize that physiological processes gain importance as salinity rises (Fig. 7). For practical purposes, physiology is here distinguished from enantiostasis derived from the memory of native water chemistry condition and related interface membrane responses. Note that such enantiostasis processes are expected to be highest in brackish water, where salinity variations are higher and more instable.

Some considerations are necessary regarding metal toxicity mechanisms in saltwater. Firstly, although organisms acclimated to higher salinity are usually less sensitive to metals, the lowest sensibility often occurs at intermediate salinities when organisms are isotonic to the surrounding environment (Blanchard and Grosell, 2006; Grosell et al., 2007).

Secondly, marine species usually demonstrate lighter or non-detectable ion and osmotic disruptions when exposed to metals

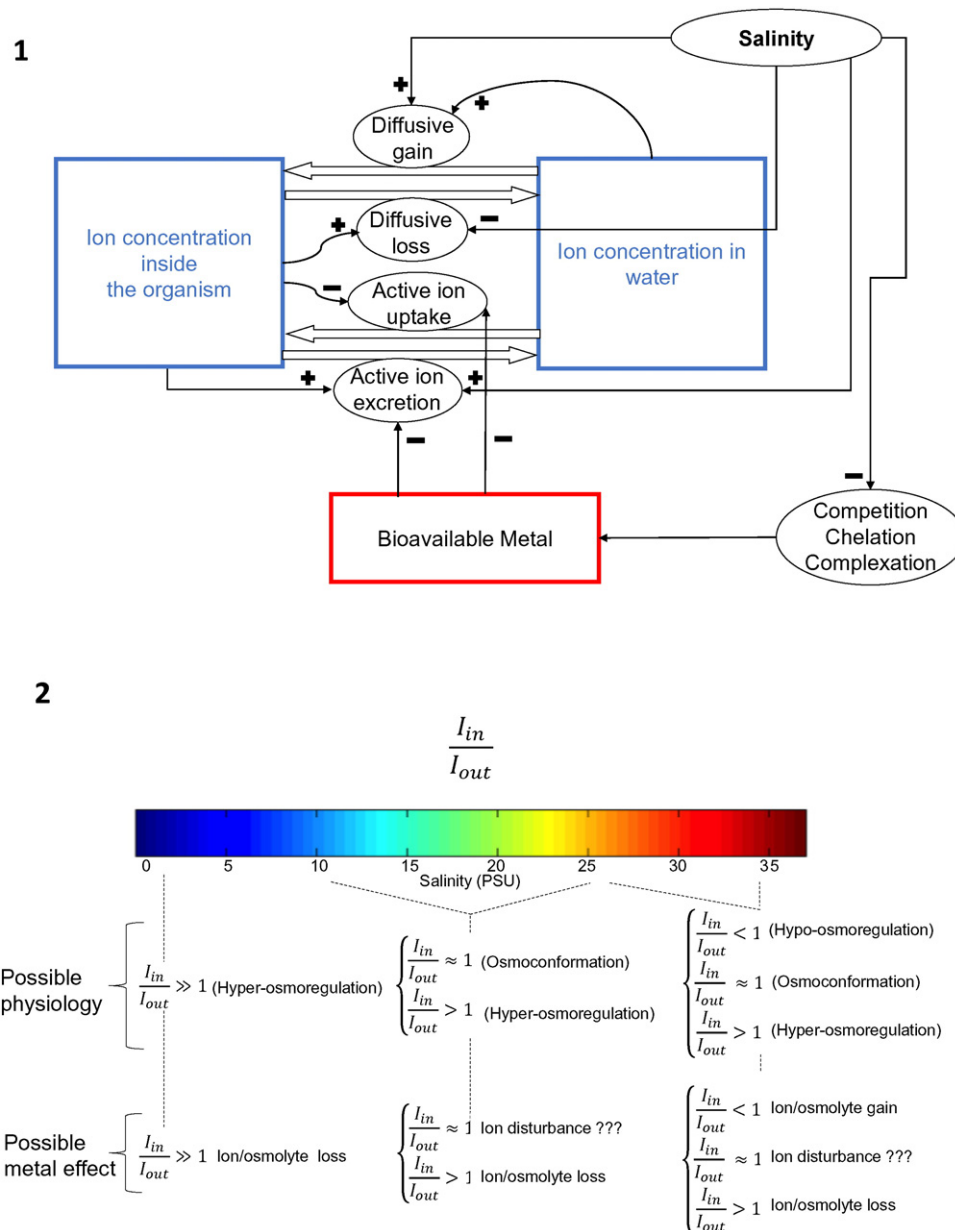


Fig. 6. Conceptual model of (1) metal effects on osmoregulation with three state variables (ions inside the organism, ions in water, bioavailable metals), large arrows represent flows, light arrows indicate interrelations and impacts of variables on processes; +: reinforcing interaction (e.g. “the higher the ion concentration inside the organism the higher the active ion excretion”), -: alleviative interaction (e.g. “the higher the ion concentration inside the organism the weaker the active ion uptake”); and (2) ion-osmoregulatory behaviour and potential metal effects in salinity gradients.

compared to freshwater organisms (Vitale et al., 1999; Zimmer et al., 2012). This holds even when the free ion activity is considered (Wright, 1995; Grosell et al., 2007). Blanchard and Grosell (2006) tested the hypothesis of ion-osmotic disruption in the killifish *Fundulus heteroclitus* by Cu across salinities. These authors found no evidence of such toxicity mechanism being homogeneous as salinity changes, and only freshwater acclimated killifish toxicity indicated consistently ionic stress. Vitale et al. (1999) observed similar results for Cd in the intertidal crab *Chasmagnatus granulata*. Thus, even if ionic and osmotic disruption is an important mechanism for metal toxicity at low salinities, other mechanisms remain to be discovered at higher salt concentrations (Fig. 7).

Some candidates for that are respiratory impairment, oxidative stress, ammonia excretion and acid–base unbalance. Although such effects have been consistent with metal exposure (Wood et al., 2012a,b; Loro et al., 2012; Zimmer et al., 2012; Machado et al., 2013; Giacomini

et al., 2014; Machado et al., 2014b), their ability in providing explanation to lethal toxicity across taxa still remains to be demonstrated, and other unknown mechanisms cannot be discarded.

8. Final considerations and future directions on modelling metal fate and effects on estuaries

While this conceptual model focused mostly on metal effects under estuarine gradients, the combined environmental pressure and interactions of metal pollution with other (anthropogenic) stressors, e.g. nutrient loads and pathogens, to affect environmental health remain a complex issue to be clarified. Along this, toxic exposure in the environment occurs as mixtures, and regulations as the Water Framework Directive consider not only individual toxics but also their combined effects (European Commission, 2012a,b). In this sense, Cravo et al. (2012) found complex patterns of spatial and temporal trends for co-

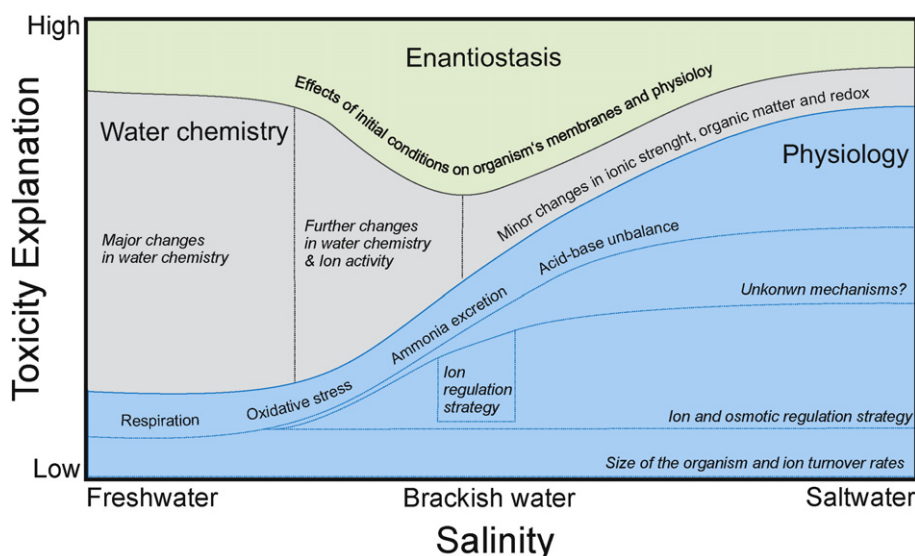


Fig. 7. Conceptual idea of potential explanatory variables for metal toxicity in salinity gradients. The blue areas refer to processes related to toxicity mechanisms depending upon organisms' physiology. In grey areas some of the main water chemistry processes controlling metal in the environment are shown. In the green area is hypothesized the interactions of native water chemistry and physiology on enantiostasis processes.

varying metals, hydrocarbons and tributyltin, with strong interaction of contaminants and water physicochemistry (temperature, salinity, dissolved oxygen) on driving physiological responses of the bivalve mollusc *Ruditapes decussatus*. Sures (2006, 2008) further demonstrates the influence of parasitism in either ameliorating or intensifying the effects of metal exposure. Additionally, Long et al. (1995) found that toxic sensitivity in modelling and bioassay methods differ considerably from those of field studies due to different effects of individual 28 compounds and mixtures in estuarine and marine sediments. It is yet required to identify where synergistic, additive or antagonistic effects exist (Cravo et al., 2012). This means that, especially for ecological effects, the exposure to mixtures of dissimilarly acting substances at low, but potentially relevant concentrations is a current concern, even if all substances are below the individual "predicted no observed effect concentrations" (European Commission, 2012a). The first concepts of combination effects of sequential exposure to both chemical and non-chemical stressors are still continuously derived from laboratory, models and field experiments (Altenburger and Greco, 2009; Altenburger et al., 2015). Therefore, the following future main research challenges are identified regarding effects of mixtures of metals and other stressors under estuarine conditions: (1) more information on mode of action of diverse toxicants and their mixtures (Cravo et al., 2012), (2) information on effects of sequential exposure with different concentration pulses and duration (Altenburger and Greco, 2009), and (3) more robust conceptual and quantitative models for where, how often and to what extent estuarine organisms are exposed to certain mixtures over time (European Commission, 2012a).

Mathematical and conceptual models are available to address some of the specific estuarine dynamics discussed in the present work (Boyle et al., 1974; Betty et al., 1996; Baeyens et al., 1998; Di Toro et al., 2001; Paquin et al., 2003; Simpson and King, 2005; Brand et al., 2013). However, it is evident that the coupling of different models is mandatory to represent the estuarine environment as conceptualized here. Therefore, future models integrating quantitatively physical, biogeochemical and physiological processes occurring in estuaries are required for a realistic approach.

Temporal and spatial scales are key factors to the discussed metal deposition, transport and toxicity processes. The relative importance of most variables and processes varies across orders of magnitude depending on estuary dimensions, riverine discharge, coastal hydrodynamics, as well as on organism size and physiology. Water and suspended matter residence times at the surface are often in the range of weeks and months,

respectively. Differently, subterranean estuary and sediments reside for much longer (months to years and decades). Metal precipitation, adsorption and dissolution processes are fast (mostly less than hours) and assumed to be instantaneous in most of the existing models, which is reasonable for the majority but not for all of the metals. Pd for instance might not be in equilibrium in estuarine systems, since time for partitioning reactions are longer than water residence time in several estuaries (French and Turner, 2008). Moreover, phytoplankton growth and consequent changes in dissolved to particulate metal affect the equilibrium partitioning in the range of days and in dependence on seasonal fluctuations. Thus, on the same time scale, fluctuations in the meaning of food-related metal exposure are expected. In this sense, the establishment of ecotoxicological and ecohydrological relevant scales constitute an additional open research topic to be addressed.

Finally, physical and chemical estuarine gradients affect biogeochemistry and organism physiology, impacting organism exposure and toxicity. The relative importance of each gradient on metal behaviour and toxicity might vary greatly and differ seasonally. The influence of salinity on partitioning coefficients can be significantly masked by oscillation in river flow, particulate and organic matter input and phytoplankton growth for instance (Valenta et al., 1986). Thus, behaviour and effects of metals in estuaries are dynamically depending upon both, environmental and biological causes, which have to be considered when establishing site-specific water quality criteria and environmental quality targets.

Acknowledgements

We thank Dr. Marianna Basso Jorge for the insightful discussions on metal toxicology. The present work was carried out within the Erasmus Mundus Joint Doctorate Program SMART (Science for the Management of Rivers and their Tidal Systems) funded with the support of the EACEA of the European Union.

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