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Effect of organic complexation on the behaviour of dissolved Cd, Cu and Zn in the Scheldt estuary

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Abstract

Samples from the Scheldt estuary have been assayed for dissolved Cd, Cu and Zn using differential pulse anodic stripping voltammetry, either as total (after UV irradiation) or labile concentrations. Under these experimental conditions, labile concentrations ranged between 51–65% of total Cu, 16–66% of total Zn and 53–91% of total Cd. The metal–organic interactions were assessed by evaluating (a) the distribution coefficient K_d for the distribution of the metals between the liquid phase (complexation) and their binding to particulate matter, and (b) the competitive effect exerted by inorganic complexing ligands using a multi–element interaction model. The proportion of organically bound metals (strong and labile) was estimated, in this speciation scheme, to range from 86 to 99% for Cu, from 90 to 96% for Zn, and from 10 to 35% for Cd. From the dissolved organic carbon distribution in the Scheldt ($\leq$ 10 mg C l^{-1}) and taking into account competition from major cations Ca and Mg, free ligand concentrations available for heavy metal complexation were estimated to be $\leq$ 0.15 mg C l^{-1}. With these values, conditional stability constants for the chelation of Cu, Zn and Cd were calculated assuming either a single-step or a two-step complexation in the dissolved phase. Given the assumptions made in these models, stability constants in the range of $10^{7.8}$–$10^{10.6}$ for Cu, $10^{7.0}$–$10^{9.1}$ for Zn and $10^{6.9}$–$10^{8.9}$ for Cd were obtained. The relevance of these data to previous *in vitro* and *in situ* studies is discussed taking into consideration current concepts of metal binding affinity for organic ligands.

Introduction

The distribution of Cd, Cu and Zn between the dissolved and particulate matter phase has been discussed in a related paper (Baeyens et al., 1998). The behaviour of these trace metals in the Scheldt estuary is dominated by remobilisation processes from suspended matter (Wollast, 1976; Zwolsman & van Eck, 1991). During the summer period, both the dissolved Cu and Cd concentrations are found to decrease concomitantly with the dissolved oxygen content of the water in the area of maximum turbidity, suggesting that redox processes control their behaviour. Two redox processes have been identified: (a) metal sulphide formation in the water column or close to the water/sediment interface (Zwolsman & van Eck, 1991; Panutrakul & Baeyens, 1991), the sulphide being probably introduced in the water column through porewater diffusion, and (b) coprecipitation by dissolved iron and manganese compounds released from the sediments. The reduction of iron and manganese occurs in the Scheldt especially in the organic rich sediments found in the upper and mid-estuarine region (Elskens et al., 1991).

In the case of dissolved Zn, a linear decrease from the riverine end member towards the river mouth has been observed (Monteny et al., 1993). It was further suggested that scavenging of the dissolved Zn compounds was due to (a) the increasing amount of fine particles in the area of maximum turbidity offering an enhanced adsorption surface, and (b) freshly discharged or precipitated particulate organic matter containing large amounts of functional groups with high adsorption affinity (Baeyens et al., 1998).

Extensive mobilisation of dissolved metals was observed once the particles left the maximum turbidity area at 20 psu for copper, 15 psu for cadmium and zinc (Monteny et al., 1993). The mobilisation processes involved include degradation, oxidation and desorption. Exo-enzymes produced by microbial organisms can degrade particulate organic carbon and simultaneously release scavenged metals. Oxidation of authigenic metal monosulphides is a second pathway that can explain the increase of dissolved metals, in the mid to downstream estuary especially for Cd and Cu. Resuspension of fine-grained sediments can be a source of metal sulphides to the water column. Progressive shifting of the oxic borderline, hence of metal mobilisation due to oxidation further upstream, was observed from fall to winter (Monteny et al., 1993). Finally, the enhanced competition between alkaline and earth alkaline ions with the adsorbed metals and the gradual decrease of adsorption surfaces after the zone of high turbidity contribute both to the desorption flux from the particulate phase (Mantoura et al., 1978). However, to keep metal ions into solution, complexation by organic ligands might also be a leading factor (Houghton, 1979; Valenta et al., 1986, van den Berg et al., 1987). It is now well established that speciation measurements are required to investigate into the toxicity of metals for aquatic organisms, and to understand trace metal transport in rivers and estuaries (Florence, 1989).

In this report, dissolved metal-organic interactions are discussed with regard to the competitive effect exerted by complexing ligands. A limited speciation of the dissolved metal burden was carried out on the samples. It was assumed that the fraction which was determined by electrochemical analysis (DPASV) at pH = 1 consisted of free and labile bound metal, including inorganic colloids, while the fraction determined at pH = 1 after UV-destruction represented metal bound in inert organic complexes and to organic colloids, according to the usual ASV labile/non-labile discrimination (Florence, 1989). The speciation of Cd, Cu and Zn was subsequently assessed by determining ligand concentrations and conditional stability constants for these metals, using a multi-element interaction model. The competition between complexation of metals in the dissolved phase and their binding to particulate matter has also been considered.

Material and methods

Data collection and treatment

Data presented in this paper have been obtained during five cruises on the Scheldt estuary from spring 1982 and spring 1983 (12/05/1982; 25/05/1982; 24/01/1983; 08/03/1983 and 18/05/1983). Determination of trace metals was performed by differential pulse anodic stripping voltammetry (DPASV). The ASV labile metal fraction, measured after acidification of the sample at pH 1, included the free metal ion and metal dissociated from complexes and colloids in the diffusion layer, whereas total metal concentrations were determined after acidification and UV-irradiation. The difference between the two fractions was assumed to represent metal bound in inert organic complexes and to organic colloids (Florence, 1989). Full details of the analytical procedures are set out in publications by Baeyens et al. (1998).

Longitudinal profiles of total dissolved metals *versus* salinity were obtained for each cruise using polynomial regressions. Average metal concentrations at seven salinity intervals (1, 3.5, 7.5, 12.5, 17.5, 25 and 32.5 psu) were subsequently fitted and used in speciation models to determine organo-metal interactions (see Equation 3–4).

Inorganic metal complexation was calculated with thermodynamic equilibrium data of Woods & Garrels (1987). In this model, the activity coefficients of all species have been calculated for consistency by means of the Davies equation (Ramette, 1981). Although this approach may be not satisfactory at high ionic strengths, it is a reasonable approximation for a preliminary survey of speciation. Unless otherwise stated, the overall relationship between the conditional stability constant β, used in the model, and the thermodynamic equilibrium constant at infinite dilution in pure water K^{∞} is as follows:

$$\beta = K^{\infty} \cdot \frac{\gamma_{\text{metal}} \cdot \gamma_{\text{ligand}}}{\gamma_{\text{complex}}} \qquad (1)$$

For hydroxide complexes, it was more convenient to use mixed stability constants defined by:

$$K' = \frac{[\text{Complex}]}{[\text{Metal}].a_{\text{OH}}} \text{ and } K' = K^{\infty} \cdot \frac{\gamma_{\text{metal}}}{\gamma_{\text{complex}}} \qquad (2)$$

The resulting set of non-linear equations provided by the mass law and the mole balance equations (see Equation 5) was solved by use of the Newton-Raphson iteration procedure (Morel & Morgan, 1972). Free metal and free organic ligand concentrations were subsequently used to assess values for the conditional stability constants of the various organo-metal complexes (see Equations 6–7).

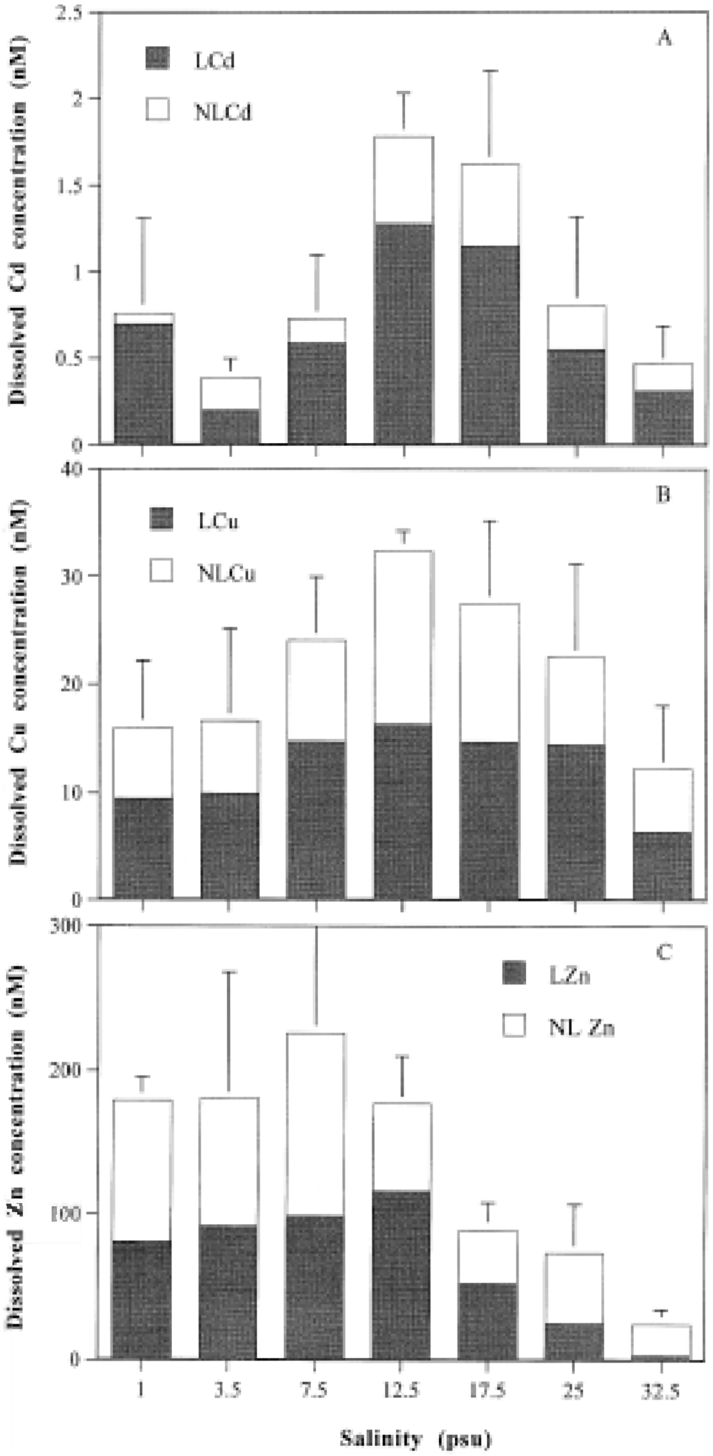

Figure 1. Dependance of total, labile and non-labile metal concentrations on salinity. LMe and NLMe represent the labile and non-labile metal fraction, respectively.

Results and discussion

Labile and non-labile metal fractions

Average longitudinal profiles of dissolved metal concentrations *vs.* salinity are shown in Figure 1 with standard deviation error bars representing the interannual variability. Total dissolved Cd concentrations (Cd_d) ranged from 0.38 to 1.8 nM with mid-estuarine maxima at 12.5–17.5 psu. The percentage of labile bound Cd (LCd_d) was higher in the upstream estuarine area with values up to 90% and slightly decreased down to 60% in the plume of the estuary (Figure 1a). It seems likely that the production of dissolved Cd contributed initially to the labile bound fraction, while afterwards, a transfer to the non-labile species occurred. As a matter of fact, the non-labile Cd concentrations ($NLCd_d$) reached a maximum, when Cd_d already decreased. Average $NLCd_d$ amounted to 28%. This is in agreement with previous anodic stripping voltammetry (ASV) speciation analyses reporting ASV non-labile Cd $< 30\%$ in both seawater and freshwaters (Florence, 1989). In a study of the Belgian coastal zone, we found that the average organically bound Cd fraction was higher in the vicinity of the Scheldt plume than in the coastal waters and in the offshore area (Baeyens et al., 1982). It is thus possible that the dissolved organic matter produced in the muddy organic rich sediments of the coastal-estuarine zone has a high complexing capacity and contributes also to keep Cd in solution in the downstream estuary.

Total dissolved Cu concentrations (Cu_d) ranged from 12.3 to 32.4 nM and exhibited a maximum at 12.5 psu (Figure 1b). The percentages of labile bound dissolved Cu (LCu_d), as observed in the Scheldt during May 1982 to 1983, varied from 14 to 80% with a mean of 57%, while van den Berg et al. (1987) reported values between 5 and 58%. Cu appeared to be more strongly bound to complexing ligands than Cd, with an average non-labile fraction ($NLCu_d$) of 43%, a value falling within the 40 to 60% range usually quoted for coastal surface seawater (Florence, 1989). According to van den Berg et al. (1987), dissolved organic Cu (the sum of labile and non-labile Cu complexes) represented more than 99.99% of the total dissolved Cu content in their samples, suggesting that organic complexation alone almost controls the behaviour of Cu in the Scheldt estuary. The total Cu-complexing ligand concentrations were generally higher than the dissolved Cu concentrations, but assuming a two-site model, van den Berg and co-workers (1987) estimated that the concentrations of the strong Cu-complexing ligands should be similar to that of the dissolved Cu. Hence, it can be assumed that the dissolved to particulate Cu ratio results from competition between complexation by organic ligands in the dissolved phase and scavenging by suspended particles. It was also observed that $NLCu_d$ was highest (about 50%) when Cu_d reached its maximum value.

Total dissolved Zn concentrations (Zn_d) ranged from 25 to 226 nM (Figure 1c). The percentages of labile dissolved Zn (LD_{Zn}) found during May 1982 to 1983 in the Scheldt varied from 26 to 68% with a mean of 45%, while van den Berg et al. (1987) provided values ranging from 34 to 69%. It was also reported that only about 50% of total Zn in seawater and river water is ASV labile (Florence, 1989). van den Berg et al. (1987) found that the concentration of dissolved Zn co-varied with the Zn-complexing ligand throughout the Scheldt estuary, and it was argued that the Zn concentration was mediated by interactions with dissolved organic complexing ligands. On average the non-labile Zn fraction ($NLZn_d$) amounted to 55% indicating that, in the estuary, half of the dissolved Zn was strongly bound to dissolved organic ligands. Therefore, the distribution between the dissolved and particulate Zn is, as for Cu, probably the result of a competition between complexation by organic ligands in the dissolved phase and scavenging by suspended particles. However, at high salinity NLD_{Zn} represented the main fraction of this metal in the dissolved phase.

Partition between dissolved and particulate matter phase

The potential for organic complexation was assessed from the distribution coefficient (K_d), since K_d depends on the composition of both the liquid and solid phases. It was calculated according to Valenta et al. (1986): i.e. K_d = particulate metal [μg kg^{-1} of suspended matter]/dissolved metal [μg kg^{-1} of water]. As previously mentioned (Baeyens et al., 1998), the K_d-values varied from 0.2 to 2.7 10^5 for Cu, from 0.1 to 6.1 10^5 for Cd and from 0.3 to 0.7 10^5 for Zn (Figure 2). These values are in agreement with those reported by others in estuarine waters (Valenta et al., 1986; Balls, 1989). They are, however, substantially lower, except for Cd, than those observed in areas with low suspended matter (ocean) with 1–4 10^6 for Cu, 0.3–1 10^5 for Cd and 0.4–1 10^6 for Zn. Since estuarine water is generally richer in dissolved organic matter than oceans, the lower K_d values observed here were assumed to be the result

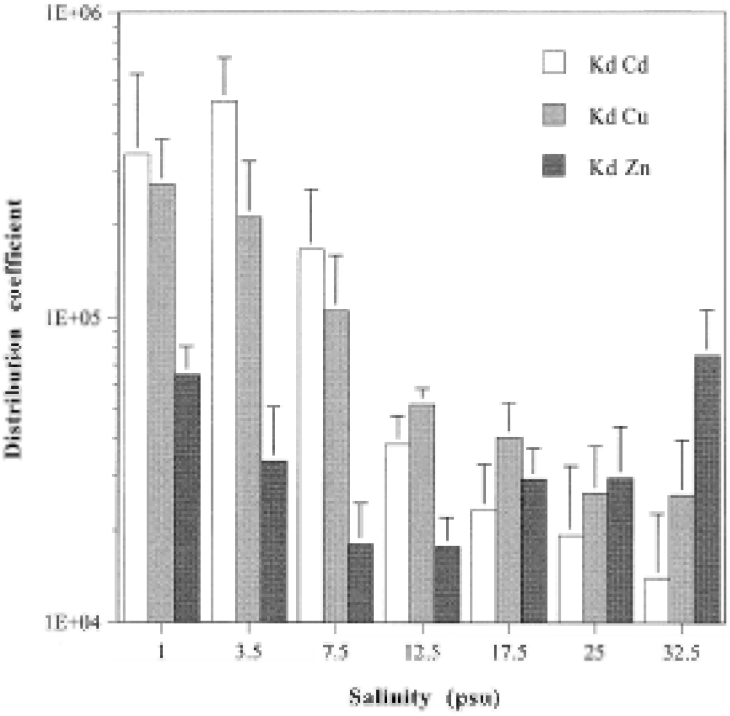

Figure 2. Dependance of the distribution coefficient K_d for Cd, Cu and Zn on salinity. K_d = the ratio of particulate metal (μg kg^{-1}) of suspended matter) over dissolved metal (μg kg^{-1} of water).

of organic complexation (Valenta et al., 1986). Hence the fraction of dissolved organo-metal complexes was assessed according to:

$$K_d = \frac{Me_p}{Me_d} = \frac{Me_p}{MeL_{in} + MeL_{or}} \tag{3}$$

where K_d is the actual distribution coefficient measured in the Scheldt (Figure 2), Me_p the particulate metal concentration (Baeyens et al., 1998), Me_d the dissolved metal concentration (Figure 1), MeL_{in} the inorganic metal species and MeL_{or} the organic metal complexes. Let K'_d be the distribution coefficient observed in the absence of dissolved organic species = Me_p/MeL_{in} with values close to those reported in the surface water of the oceans, i.e. 2.10^6 for Cu and $0.7.10^6$ for Zn (Valenta et al., 1986; Balls, 1989). Then from (3):

$$MeL_{or} = Me_p \cdot (1/K_d - 1/K'_d) \tag{4}$$

For both Cu (12–31 nM) and Zn (23–215 nM), MeL_{or}-values are significantly higher than the corresponding $NLMe_d$ ones (Table 1). This might be expected since speciation schemes based on physicochemical separations and ASV measurements have demonstrated the electrochemical lability of several Cu and Zn organic complexes (Batley & Florence, 1976). Moreover, it is known that with DPASV, the labile metal fraction increases dramatically as pH is lowered (Duinker & Kramer, 1977; van den Berg & Dharmvanij, 1984). Therefore, at natural pH values, a greater potential for organic complexation than the one resulting from our DPASV measurement at pH 1 might be expected. Accordingly, it is suggested to subdivide the organic complexing ligands of Cu and Zn into two groups, mono and bidentate, corresponding to weak (labile) and strong (non-labile) complexing sites (Figure 3). The same reasoning can not hold for Cd. The stability constants for organic complexation of Cd are substantially smaller than those of analogous Cu and Zn complexes (Mantoura et al., 1978; Valenta et al., 1986). This suggests that only $NLCd_d$ has a chance to act as a competitive ligand in keeping Cd into solution.

Inorganic and organic metal speciation

Considering dissolved metal speciation in estuarine waters, it should be borne in mind that the extent of complexation markedly fluctuates with the nature and the concentration of the inorganic and organic compounds, as well as with the values of the stability con-

Table 1. Speciation of Cu, Zn and Cd (Equation 4–5) and conditional stability constants (M^{-1}) from complexes formed with humic (Equation 6) and amino acids (Equation 7). Data were calculated as described in the text

Salinity (psu)	Experimental Me_d (nM)	$NLMe_d$ (nM)	Equation (4) MeL_{or} (nM)	Equation (5) Me_f (nM)	MeL_{or}/Me_f	Equation (6) L_f ($\mu g\ C\ l^{-1}$)	Equation (7) L_f ($\mu g\ C\ l^{-1}$)
Copper						$\beta_{Hum} = 10^{10.6}$	$\beta_1 = \beta_2 = 10^{7.8}$
1	16.0 ± 6.0	6.6 ± 2.6	14.3 ± 1.0	0.06 ± 0.03	251 ± 149	21 ± 12	25 ± 9
3.5	16.7 ± 8.3	6.7 ± 4.8	14.0 ± 1.3	0.33 ± 0.16	42 ± 21	5 ± 3	15 ± 4
7.5	24.1 ± 11.8	9.3 ± 5.3	21.6 ± 2.0	0.42 ± 0.34	52 ± 42	7 ± 6	19 ± 10
12.5	32.4 ± 1.7	16.0 ± 5.2	31.3 ± 0.1	0.16 ± 0.01	194 ± 18	32 ± 3	45 ± 2
17.5	27.5 ± 7.6	12.7 ± 3.5	27.1 ± 0.5	0.04 ± 0.01	721 ± 52	121 ± 9	91 ± 3
25	22.5 ± 8.7	7.9 ± 4.4	22.2 ± 0.1	0.02 ± 0.01	891 ± 421	146 ± 69	98 ± 25
32.5	12.3 ± 5.7	5.8 ± 3.4	11.9 ± 0.4	0.04 ± 0.03	272 ± 186	43 ± 29	49 ± 20
Zinc						$\beta_{Hum} = 10^{9.1}$	$\beta_1 = \beta_2 = 10^{7.0}$
1	179 ± 15	98 ± 9	167 ± 8	7.9 ± 5.3	21.2 ± 14.2	50 ± 33	38 ± 16
3.5	180 ± 87	89 ± 28	172 ± 3	6.4 ± 2.3	26.9 ± 9.5	94 ± 33	67 ± 14
7.5	226 ± 73	127 ± 16	215 ± 5	8.5 ± 3.7	25.4 ± 11.2	103 ± 45	76 ± 20
12.5	177 ± 32	61 ± 34	170 ± 2	5.2 ± 1.4	32.5 ± 8.8	149 ± 40	99 ± 14
17.5	90 ± 17	37 ± 11	86 ± 1	2.7 ± 0.6	31.6 ± 7.5	149 ± 36	101 ± 13
25	74 ± 32	48 ± 16	69 ± 3	2.8 ± 1.5	24.7 ± 13.1	114 ± 61	85 ± 26
32.5	25 ± 9	21 ± 2	23 ± 1	1.5 ± 0.5	15.1 ± 5.1	67 ± 23	62 ± 12
Cadmium						$\beta_{Hum} = 10^{8.9}$	$\beta_1 = \beta_2 = 10^{6.9}$
1	0.77 ± 0.53	0.06 ± 0.02	–	0.31 ± 0.01	0.2 ± 0.1	1 ± 0.3	2 ± 1
3.5	0.38 ± 0.11	0.19 ± 0.06	–	0.05 ± 0.02	3.4 ± 1.5	22 ± 10	29 ± 8
7.5	0.73 ± 0.36	0.14 ± 0.10	–	0.09 ± 0.02	1.5 ± 1.1	11 ± 9	18 ± 11
12.5	1.78 ± 0.24	0.50 ± 0.16	–	0.13 ± 0.02	3.9 ± 1.4	33 ± 12	41 ± 9
17.5	1.62 ± 0.53	0.47 ± 0.15	–	0.08 ± 0.01	6.2 ± 2.1	54 ± 19	56 ± 12
25	0.81 ± 0.50	0.26 ± 0.12	–	0.02 ± 0.01	10.5 ± 5.3	90 ± 45	74 ± 23
32.5	0.46 ± 0.22	0.16 ± 0.05	–	0.01 ± 0.00	18.4 ± 6.2	150 ± 51	99 ± 19

stants. To provide a better insight into the speciation of the dissolved metal burden, the potential for inorganic complexation was first examined with the following relation:

$$Me_d = Me_f \cdot \left(1 + \sum_i K_i \cdot L_i^{\alpha}\right) + MeL_{or} \qquad (5)$$

where Me_f = the free metal ion concentration, L_i the concentration of free inorganic complexing ligands ,K_i the stability constant for the i^{th} complex, valid at the ionic strength of the sample, α the stoichiometric coefficient of the i^{th} reactant and MeL_{or} the organic metal complexes given by Equation (4) for Cu and Zn, and set equal to $NLCd_d$ for Cd.

As discussed later, an estimation of Me_f was required to asses the stability constants involved in the organic complexation. Calcium, magnesium, sulphate and chloride were considered to behave conservatively during mixing of river water and sea water (Mantoura et al., 1978 and references cited), while the concentrations of carbonate, bicarbonate and hydroxide were obtained from longitudinal profiles measured in the Scheldt estuary by Frankignoulle et al. (1996). The stability constants of the inorganic metal complexes were taken from Woods & Garrels (1987).

The results of the computation are given in Table 1 and Figure 4. According to previous estimates (Mantoura et al., 1978; Zuehlke & Kester, 1983; Florence, 1989), the major inorganic Cu complexes are the hydroxide and carbonate species. They vary between 1 and 10% of Cu_d throughout the estuary (not illustrated), while the free Cu ion still exceeds 2% at a salinity of 3.5 psu (Figure 4a). On the other hand, Cu exists to a significant extent (> 90%) as organic complex even at a salinity of 30 psu (Table 1). Zn and Cd exhibit a different behaviour. The major inorganic Zn species in

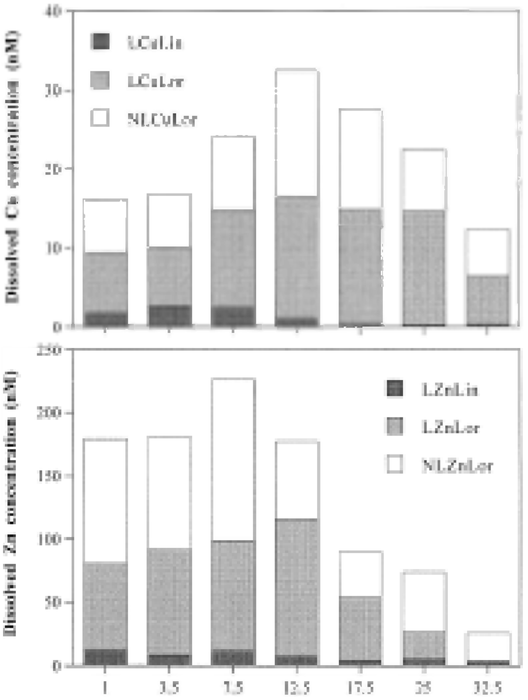

Figure 3. Speciation of Cu and Zn according to results of Equation (3–4). $LMeL_{in}$, $LMeL_{or}$ and $NLMeL_{or}$ represent the labile (inorganic and organic) and the non-labile organic metal fractions.

the estuary is the free ion with values between 3 to 6% (Figure 4b). Only in the downstream area do the sulphate, mono and dichloride Zn species exceed the 3% limit (not illustrated). As for Cu, the organic complexes are predominant with values > 90% (Table 1). In the upstream area, the free Cd-ion was the major species with 40%, and this percentage decreased gradually to the mouth where only 2% of the free ion was left (Figure 4c). The monochloride complex is important in the whole estuary. At 1 psu it represents 26% of Cd_d, increasing to 60% in the middle estuarine area where Cd_d is highest, and decreases slightly at the mouth to 24%. The dichloride complex shows an reverse trend: in the upstream estuary where the chloride concentration is very low, it represents only 2% of Cd_d, whilst it increases up to 31% at the mouth of the Scheldt (not illustrated). As previously mentioned, the organically bound fraction, equal to $NLCd_d$, represents on average less than 30% of Cd_d (Table 1).

A sensitivity analysis of the model (Equation 5) shows that even small fluctuations in the concentration of MeL_{or} results in large variability of Cu^{2+} (C.V: 10–72%) and Zn^{2+} (CV: 23–67%). Obviously, the uncertainties on the inorganic metal fraction increase dramatically as the concentration of MeL_{or} approaches that of Me_d. Yet, when MeL_{or} was set equal to zero in Equation (5), a drastic increase was observed in the free Cu and Zn ions, presumably the most toxic forms for aquatic organisms, while the distribution of Cd^{2+} remained almost unaffected (Figure 4). This suggests that chloride acts as a significant competitor for organic ligands with Cd.

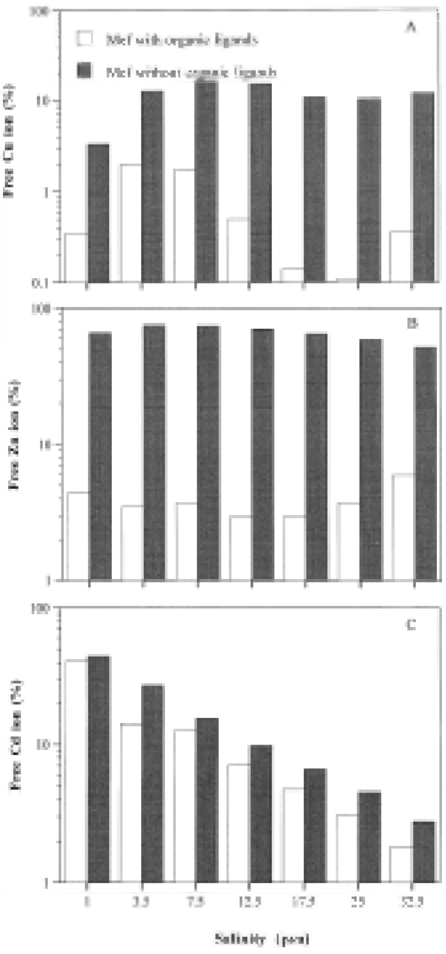

Figure 4. Predicted fraction of free Cu, Cd and Zn ions *versus* salinity in the presence of dissolved organic ligands and in their absence, i.e. MeL_{or} was set equal to zero in Equation (5).

Conditional stability constants and ligand concentrations for organic complexation

Data from Table 1 allow calculations of the product between the free organic ligand concentration and the conditional stability constant assuming either a single site complexing model, as proposed by Mantoura et al. (1978) for the complexation of metals with humic materials;

$$\beta_{\mathrm{Hum}} \cdot \mathrm{L_f} = \frac{\mathrm{MeL_{or}}}{\mathrm{Me_f}} \tag{6}$$

or a two-site complexing model as reported by Valenta et al. (1986) for the complexation of metals with amino-acids;

$$\beta_1 \cdot \mathrm{L_f} + \beta_1 \cdot \beta_2 \cdot \mathrm{L_f^2} = \frac{\mathrm{MeL_{or}}}{\mathrm{Me_f}} \tag{7}$$

where β is the conditional stability constant and $\mathrm{L_f}$ the free organic ligand concentration. Note that for cadmium $\mathrm{MeL_{or}}$ was set equal to $\mathrm{NLCd_d}$ and that β_1 and β_2 in Equation (7) were assumed to be of the same order of magnitude.

The results are shown in Table 1. In Equation (6) humic substances were assumed to be of average composition $C_{10}H_{11}O_6N_{0.3}$ and average molecular mass of 3.5 10^3 (Schwarzenbach et al., 1993) yielding the following relationship: 1 mM of humic material = 1817 mg of C $\mathrm{l^{-1}}$. Values for the conditional stability constant were then derived from Equation (6) with the constraint that $\mathrm{L_f}$ must be $\leq$ 0.15 mg C $\mathrm{l^{-1}}$. This results from the fact that (i) the dissolved organic matter levels in the Scheldt estuary usually do not exceed 10 mg C $\mathrm{l^{-1}}$ (Wollast, 1997; unpublished results), (ii) humic acids may represent up to 30% of the total organic matter in seawater (Stumm & Morgan, 1981), and (iii) the competition from Ca and Mg is sufficiently high to utilise > 95% of the complexing capacity of the humic ligands (Mantoura et al., 1978). A same procedure was applied to assess interactions between heavy metals and amino acids. For the two-site model, however, an average composition of $C_{10}H_{18}O_5N_3$ and an average molecular mass of 134 were considered yielding the following relationship: 1 mM of amino acids = 62 mg of C $\mathrm{l^{-1}}$. Furthermore, since amino acids rarely exceed 20% of the dissolved organic matter levels in seawater (Stumm & Morgan, 1981), the constraint applied to Equation (7) was 0.1 mg C $\mathrm{l^{-1}}$.

Throughout the various calculations, the ratio of the activity coefficients $\gamma_{\mathrm{ligand}}/\gamma_{\mathrm{complex}}$ has been assumed to be unity, therefore, $\beta = \mathrm{K}^{\infty} \cdot \gamma_{\mathrm{Me}}$ where K^{∞} is the thermodynamic equilibrium constant at infinite dilution and γ_{Me} was assessed with the Davies equation as aforementioned. The longitudinal profiles of organic ligands obtained for the various metals with the corresponding minimum values of β at infinite dilution is given in Table 1.

With copper, β_{Hum} amounts to $10^{10.6}$ which is in close agreement with values reported for humic interactions $10^{8.9}$–$10^{11.4}$ (Mantoura et al., 1978; Zuehlke & Kester, 1983), but is significantly lower than values reported by van den Berg et al. (1987) for the Scheldt, assuming a single-step complexation: $10^{11.8}$–10^{14}. When the data are fitted to a two-site model, β_1 and β_2 amounts to $10^{7.8}$ according to values reported for complexes with isoleucine and methionine, i.e. $10^{8.1}$–$10^{8.6}$ and $10^{6.7}$–10^{7} (Valenta et al., 1986), while the constants reported by van den Berg et al. (1987) still remain higher, ranging from 10^{12} to 10^{13} for a weaker complexing site and 10^{13} to 10^{15} for a strong complexing site. From these results, it can be stated that free humic acids with concentrations between 0.01 to 0.15 mg C $\mathrm{l^{-1}}$ and free amino acids with concentrations between 0.019 and 0.100 mg C $\mathrm{l^{-1}}$, as may occur in the Scheldt estuary, can be ligand species for copper, competing with suspended particulate matter for the binding of $\mathrm{Cu^{2+}}$ (Table 1).

It was calculated that between 90 and 96% of zinc should be complexed by organic material to reproduce the pattern of $\mathrm{K_d}$ *versus* salinity. Therefore, given the assumptions made in the models (Equation 6–7), $\mathrm{Zn^{2+}}$ required β_{Hum} of $10^{9.1}$ and β_1, β_2 of $10^{7.0}$ (Table 1). These values are two to three orders of magnitude higher than the corresponding stability constant of Zn complexes with humic compounds, i.e. up to $10^{5.9}$ (Mantoura et al., 1978) and amino-acids, i.e. up to 10^{5} (Stumm & Morgan, 1981), but fall within the range reported by van den Berg et al. (1987) in the Scheldt, i.e. $10^{8.6}$–$10^{10.6}$. Since the organic Zn fraction ($\mathrm{MeL_{or}}$) reported in Table 1 is in agreement with the one reported by others in estuarine waters (van den Berg & Dharmvanij, 1984; van den Berg et al., 1987), it seems that models considering only humic and/or amino acid interactions, excluding the formation of mixed ligand complexes and polynuclear complexes, can underestimate the potential of Zn for organic complexation. The position of the metal ions within the Iving-Williams series does not alone determine metal binding affinity. Other factors, including hard-soft acid-base and ligand-field stabilisation energy effects can be important determinants as well. Fulvic acids, for instance, exhibit stronger interactions with Zn than with Cu and Cd (Piotrowicz et al., 1983). This was also demonstrated in studies investigating the metal binding specificity of Zn-finger peptides (Lippard & Berg, 1994).

Tabulated stability constants of the Cd complexes with humic and amino acids are about 10^{5} and 10^{4}, respectively (Mantoura et al., 1978; Valenta et al., 1986) and are thus substantially smaller than the values reported in Table 1: $\beta_{\mathrm{Hum}} = 10^{8.9}$ and $\beta_1, \beta_2 = 10^{6.9}$. Investigations of the required ligand concentrations for complexation of Cd^{2+} by amino acids in seawater have shown that to achieve a complexation of about 20%, the organic ligand concentrations should reach the mM level (Valenta et al., 1984; Sugawara et al., 1984). This is much higher than the ligand concentrations provided in Table 1 and those reported in the Scheldt estuary for the complexation of Zn and Cu (van den Berg et al., 1987). Yet, a significant fraction of the inert Cd can be inorganic, probably adsorbed on colloidal particles of Fe_2O_3, Al_2O_3 and MnO_2 coated with humic acid (Florence, 1989). Hence, only 28% of the total dissolved Cd concentration should be associated with colloidal particles coated by humates to fit the $NLCd_d$ fraction reported in Table 1. Alternatively, as pointed out for Zn, higher values for stability constants might be expected with respect to the hard-soft acid-base effect. One example are the complexes with metallothioneins where Cd^{2+} exhibits higher affinity for cysteinyl residues than Zn^{2+} and Cu^{2+}.

To conclude, we have related our model calculations to electrochemical measurements of metals by grouping the metal species into two categories: electrochemical labile and non-labile. For Cd, the ratio of particulate metal over labile dissolved metal provide K_d-values close to those reported in oceans, while for Cu and Zn this ratio was much lower. This discrepancy can be explained by a high affinity of Cu and Zn for complexation with components of natural dissolved organic matter. In this context, the speciation model provides estimates for conditional stability constants and ligand concentrations in the Scheldt. It supports the data of van den Berg et al. (1987) suggesting that in the Scheldt, interactions of Cu and Zn with dissolved organic complexing ligands determine almost completely the biogeochemical pathway (toxicity and transport) of these metals. On the contrary, for Cd, competition from chloride ion is the leading factor since not only are the stability constants of its chlorocomplexes very high, but also because the concentration of the nth complex is dependent on the nth power of the chloride concentration.

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