

Dispelling the myths: Is the existence of L1 and L2 ligands necessary to explain metal ion speciation in natural waters?

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Abstract

A critical analysis and interpretation of the data published for complexation (conditional stability constants and complexation capacities) of trace metals (copper, Cu(II); zinc, Zn(II); lead, Pb(II); and cadmium, Cd(II)) in whole seawater and freshwater samples over the past 34 yr is presented. We propose that it is not necessary to invoke the presence of any particular classes of ligands (e.g., the so-called L1 and L2 ligands) to explain the degree of metal complexation observed. Consideration of the presence of a range of many different types of ligands could explain the observed linear relationship between the stability of complexes ($\log \bar{K}$) and the effective metal ion loading (complexation capacity, \bar{C}_c), without the need to assume the presence of any stable specific complexants. Apparently stronger binding sites are utilized at lower metal ion loadings; progressively weaker sites contribute to complexation at higher metal ion concentrations. Taking into account the detection window of the technique employed greatly improves the internal consistency of the overall data. When comparable windows are used, no significant difference is observed between the $\log \bar{C}_c$ - $\log \bar{K}$ relationship for Cu(II) and that for Zn(II), Pb(II), or Cd(II). In natural waters, the complexing material present will be a combination of that derived from terrestrial sources and that formed in situ in the water column. An important observation arising from our analysis is that the binding curves for natural ligands in whole natural-water samples lie between the binding curves for compounds representative of each these sources, viz. isolated aquatic fulvic acids and biota.

Studies on metal ion complexation in seawater often propose the existence of two main classes of binding sites: (1) strong binding sites (L1), with $\log K$ values of 12–14 and concentrations in the range of 1–40 nmol dm⁻³ and (2) weaker binding sites (L2), with $\log K$ values of 8–10 and concentrations in the range of 6–150 nmol dm⁻³.

Many authors have proposed the existence of specific chelators that would account for the very strong binding sites observed in seawater (and also in freshwaters) (e.g., Hirose 1994; Gordon 1998). However, despite a concerted effort (e.g., Gordon et al. 1996; Midorikawa and Tanoue 1996, 1998), nobody has yet isolated or identified such ligands. Indeed, the term “mysterious” has recently been employed in their description (Gordon 1998). Typically, a biological source of L1 is proposed (Capodaglio et al. 1989; Moffett et al. 1990; Xue and Sigg 1993), with possible candidates being various intracellular components, such as metallothioneins (Zhang et al. 1990), phytochelatins (Gekeler et al. 1988; Ahner and Morel 1995; Ahner et al. 1995), porphyrins (Zhang et al. 1990; Rue and Bruland 1995; Qian et al. 1998), and phosphate granules (Jensen et al. 1982; Twiss and Nalewajko 1992; Fernandez-Piñas et al. 1995), all of which would be released into the aquatic medium after death of the organism. Although such components could form very stable metal complexes (Rue and Bruland 1995; Leal and van den Berg 1998), there is a paucity of information on the chemical stability (e.g., to oxidation or hydrolysis) of these biological materials in the open ocean (Coale and Bruland 1988). In addition to intracellular complexation (detoxification) mech-

anisms, biota are known to excrete extracellular compounds in response to metal ion exposure (Capelo et al. 1993; Geringa et al. 1995; Gonçalves et al. 1988). Many of these compounds are polysaccharide-type materials that are weakly complexing for metal ions, although there are reports of more strongly complexing protein-type exudates being produced (Lombardi and Vieira 1998). Measurements on whole natural waters are universally made on filtered samples (typically 0.45 μm); hence, the majority of whole biota (and the potential for intracellular sequestration or exudate production) is not present during analysis.

Recently, an onion model has been proposed by Zirino and coworkers as an alternative to the L1, L2 approach (Mackey and Zirino 1994; Zirino et al. 1998). This model considers trace metals in seawater to exist predominantly in colloidal forms in association with organic matter (OM). Our analysis is not inconsistent with this approach (see Discussion).

In this work, we present a critical analysis and interpretation of the data published for complexation of trace metals (copper, Cu(II); zinc, Zn(II); lead, Pb(II); and cadmium, Cd(II)) in whole seawater and freshwater samples over the past 34 yr. In natural waters, the complexing material present will be a combination of that from terrestrial sources (pedogenic, with properties comparable to soil-derived humic substances) and that generated in situ (aquagenic, derived from phytoplankton or their exudates); the relative contributions of each source are dependent on the particular sample involved. Therefore, any natural water is expected to contain a diverse range of potential metal-complexing components. The relative importance of refractory organic matter

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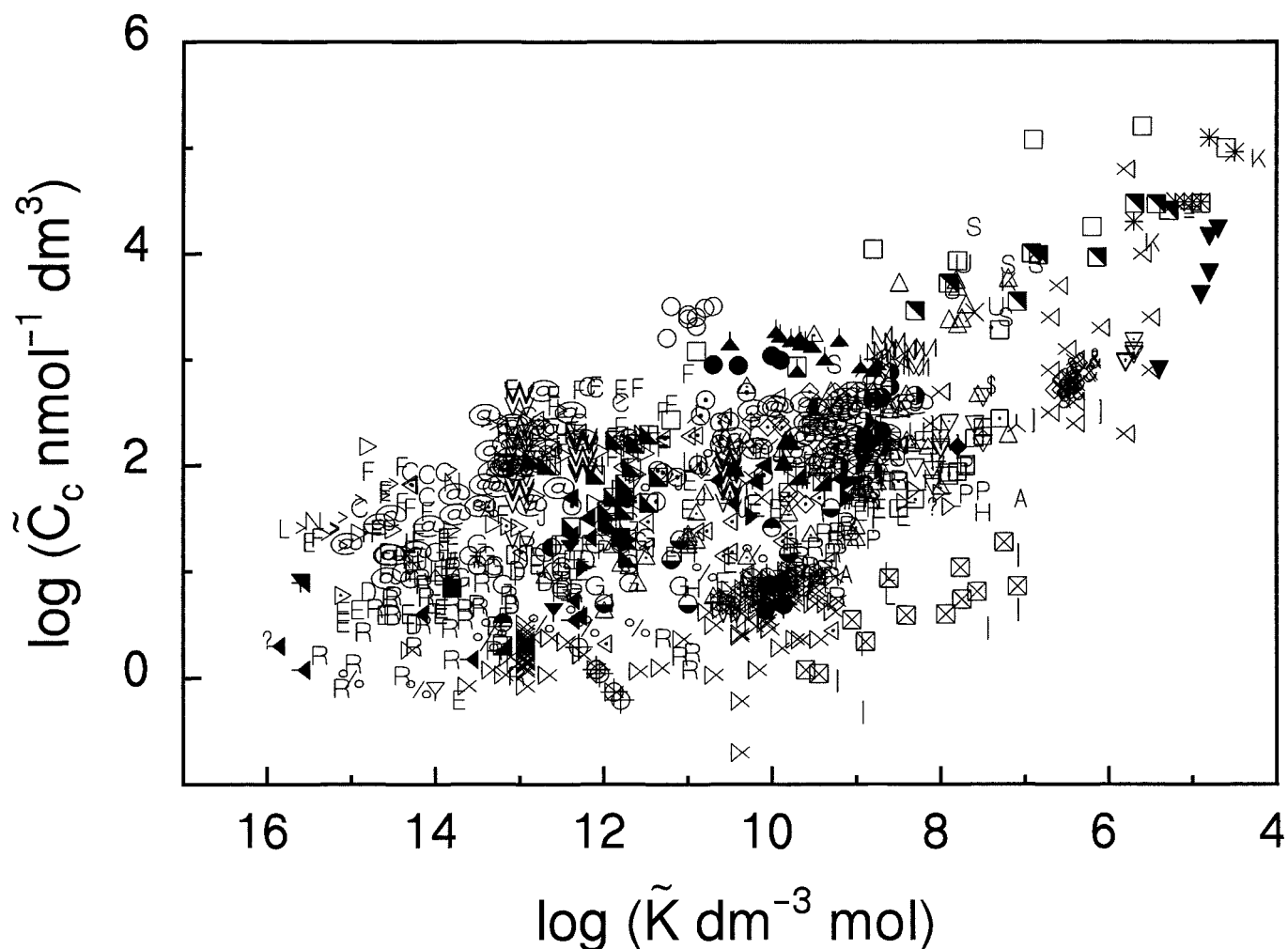


Fig. 1. Compilation of Cu(II) binding parameters ($\log \tilde{C}_c - \log \tilde{K}$) reported for whole natural waters. Each symbol represents a separate publication. + Ramamoorthy and Kushner 1975; * Shuman and Woodward 1977; ● Gächter et al. 1978; O Baccini and Suter 1979; x van den Berg et al. 1979; Δ van den Berg and Kramer 1979a,b; \square Sunda and Hanson 1979; \diamond Srna et al. 1980; ∇ Hart and Davies 1981; ● Hoffman et al. 1981; \blacktriangle van den Berg 1982; \blacksquare Hirose et al. 1982; \blacklozenge Plavšić et al. 1982; \blacktriangledown Ryan and Weber 1982; \bullet Sunda and Ferguson 1983; ! Waite and Morel 1983; \odot Anderson et al. 1984; γ van den Berg 1984a,b; \triangleright van den Berg 1984c; \wedge van den Berg et al. 1984; ∇ Hart and Jones 1984; \boxplus Kramer and Duinker 1984; [Sunda et al. 1984; \triangleleft Nelson 1985; \odot van den Berg and Rebello 1986; \diamond van den Berg et al. 1986; Δ Buckley and van den Berg 1986; \triangleright Kramer 1986; \triangleright van den Berg et al. 1987; \blacktriangleright Hering et al. 1987;] Kyle 1987; \triangleleft Moffet and Zika 1987; \blacktriangleleft Sunda and Hanson 1987; \oplus Coale and Bruland 1988; \bullet Hanson et al. 1988; \setminus Jones and Hart 1989; \bullet Morelli et al. 1989; \square Verweij et al. 1989; \square Apte et al. 1990a; \triangleleft Apte et al. 1990b; \triangleright van den Berg et al. 1990; \times Coale and Bruland 1990; \vee Gonzalez-Davila and Millero 1990; \times Holm and Curtiss 1990; \boxtimes Midorikawa et al. 1990; \blacksquare Moffett et al. 1990; W Gardner and Ravenscroft 1991; \bullet Sunda and Huntsman 1991; = Ventry et al. 1991; \blacktriangleleft van den Berg and Donat 1992; \blacktriangledown Donat and van den Berg 1992; ∇ Donat and Bruland 1992; Δ Donat and Bruland unpublished; \blacktriangle Kawamoto et al. 1992; A Midorikawa et al. 1992; B Scarano et al. 1992; C Xue and Sigg 1993; D Campos and van den Berg 1994; # Donat et al. 1994; \$ Midorikawa and Tanoue 1994; % Miller and Bruland 1994; & Pardo et al. 1994; ? Capodaglio et al. 1995; < Gonzalez-Davila et al. 1995; > Xue et al. 1995; @ Gerringa et al. 1996; | Midorikawa and Tanoue 1996; E Muller 1996; F Xue et al. 1996; G Achterberg et al. 1997; H Donat et al. 1997; J Moffett et al. 1997; K Verweij and Ružić 1997; L Xue and Sunda 1997; M Antelo et al. 1998; N Knauer et al. 1998; P Kozelka and Bruland 1998; R Muller 1998; S Midorikawa and Tanoue 1998; U Presa et al. 1998.

versus biotic components has been tentatively assessed by comparison of the binding curves for whole-water samples with those for isolated aquatic fulvic acids and biota.

Data analysis

Trace-metal complexation properties have often been represented by means of average parameters; typically, one or

more site concentrations (C_c) and stability constant (K) couples are reported. Most often, 1:1 stoichiometry ratios and a Langmuir model with one to two sites have been assumed. Different linearization methods have been applied (Klotz 1982, 1983; Buffle 1988). These binding parameters derived from Langmuir isotherms are curve-fitting parameters, and they are valid for the given experimental conditions. It has

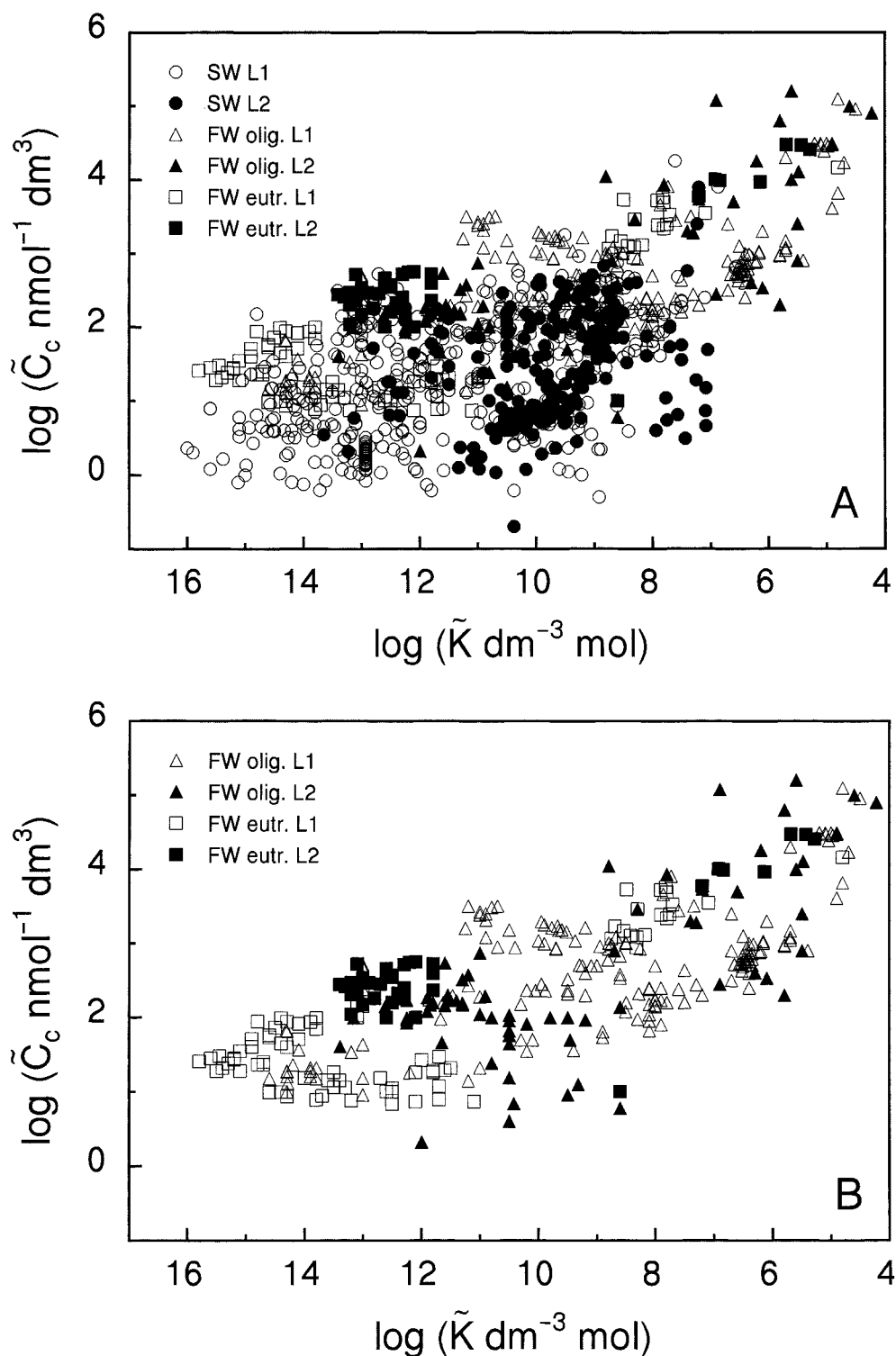


Fig. 2. Cu(II) binding parameters shown in Fig. 1 grouped by proposed type of ligand (L1, L2) and by type of natural water: (A) seawater and freshwater, or (B) only freshwater.

been common, particularly in the field of marine chemistry, to postulate the existence of real ligand entities corresponding to each site. Verification of this hypothesis requires isolation and identification of such compounds, and many

workers have pursued this analytically challenging approach (e.g., Hirose 1994; Gordon et al. 1996; Midorikawa and Tanoue 1996, 1998).

More than 20 yr ago, it became obvious that comparison

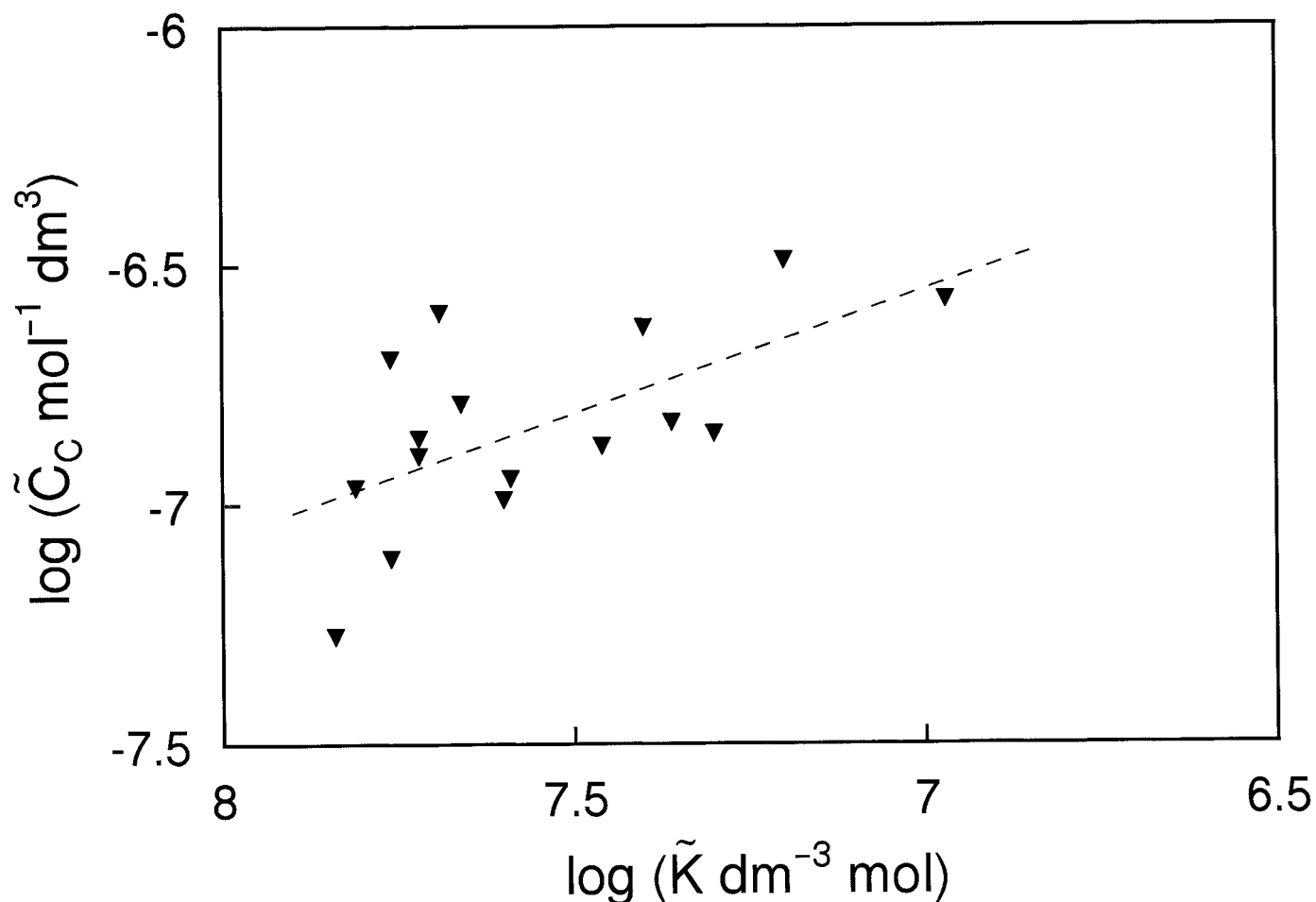


Fig. 3. Binding parameters calculated from data reported by Robinson and Brown (1991) for a bloom of dinoflagellate *Gymnodinium sanguineum* in a lagoon (Vancouver Island, Canada).

of complexation constants reported in the literature for humic-type substances was difficult because of the variety of experimental conditions used (such as pH, temperature, ionic strength, ligand concentration, reaction time, and metal:ligand ratio). The importance of the last factor was underlined by several authors (Gamble and Schnitzer 1973; Gamble et al. 1980) and led to the postulation of the existence of a continuum of ligand sites with varying metal affinity in the humic molecules. Different strategies were then established for the determination of continuous binding-site distribution functions (Buffle et al. 1990a; de Wit et al. 1990; Nederlof et al. 1990), however these strategies have been infrequently applied to real systems. Interesting implications related to the ecological role played by humic substances in the buffering of free-metal ion concentrations in natural systems have been inferred from the observed gradual change of $\log K$ with metal loading (Buffle et al. 1990b).

Extensive review of Cu(II) complexation by aquatic fulvic acids (Buffle 1984; Buffle et al. 1984; Buffle 1988) showed that (1) published $\log K$ values increased gradually with decreasing $\log C_c$ (or corresponding ligand concentration) values and (2) the relationship between $\log C_c$ and $\log K$ mimicked the actual underlying continuous binding-site

distribution. The existence of such continuous binding distributions results from the fact that humic substances are made up of a collection of highly complex and poorly characterized molecular structures that are able to form a large number of metal cation complexes that have different molecular scale bonding characteristics. Indeed, $\log K$ and $\log C_c$ parameters alone have no physicochemical meaning since (1) they are not constant over the entire titration, as the free-metal ion is successively controlled by sites of different nature, and (2) the measured values depend on the analytical window of the method used (see below). In fact, these parameters represent only average equilibrium quotients or overall site concentrations for a range of site types having different metal affinities. Thus, so-called equilibrium constants will hereafter be called equilibrium quotients, $\log \tilde{K}$. Published ($\log \tilde{C}_c$ and $\log \tilde{K}$) values for humic substances have proven to be extremely useful, since they allow for estimation of the underlying continuous binding function, provided that \tilde{C}_c and \tilde{K} are computed for the same window using the same interpretative mode.

In this work we have applied the same data analysis approach used for humics to an extensive compilation of published complexation parameters for whole-water systems

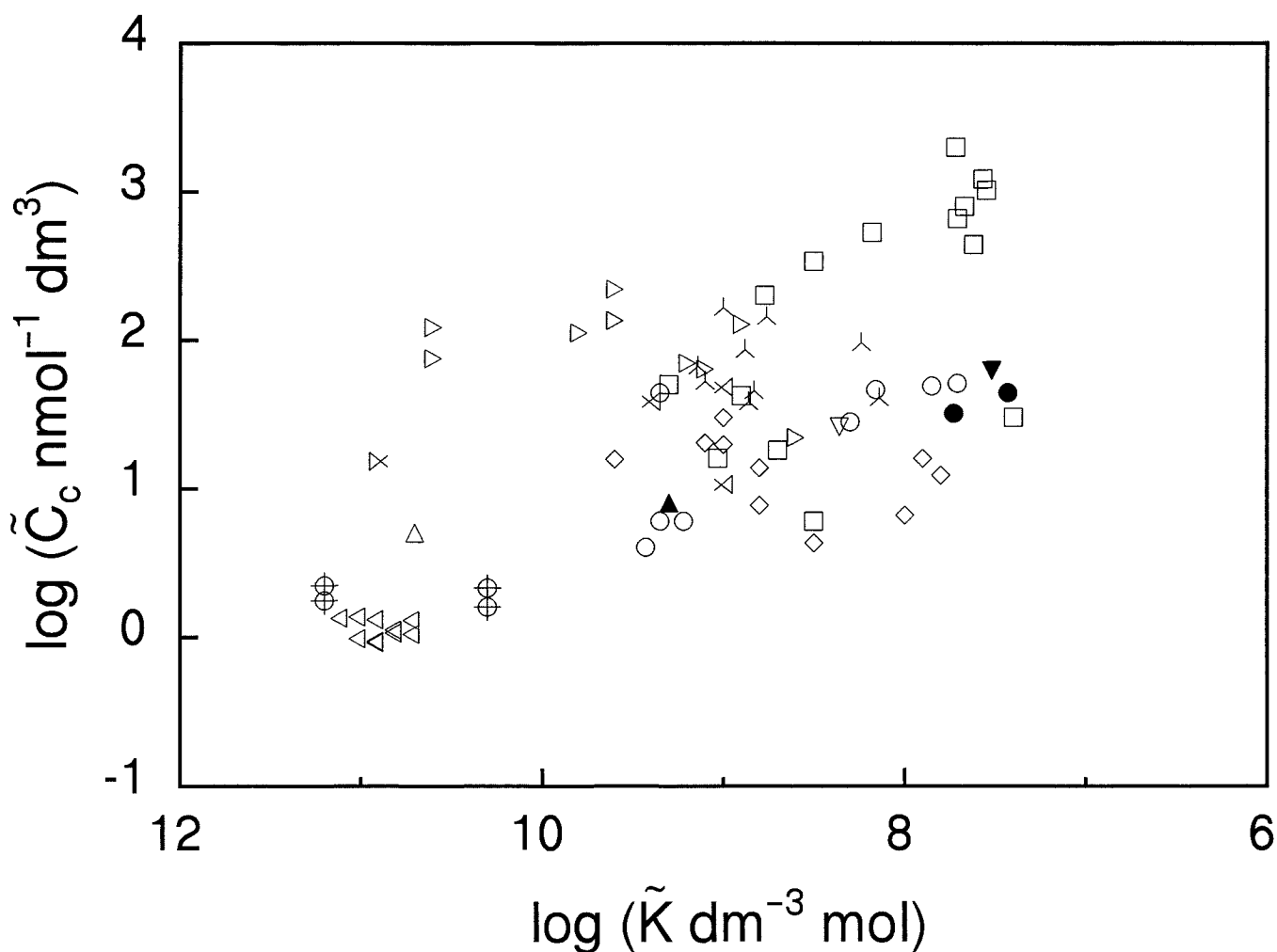


Fig. 4. Compilation of Zn(II) binding parameters ($\log \tilde{C}_c - \log \tilde{K}$) reported for whole natural waters Δ L1, \blacktriangle L2 Hirose et al. 1982; \square van den Berg and Dharmvanij 1984, van den Berg et al. 1984; ∇ L1, \blacktriangledown L2 van den Berg 1985; γ van den Berg et al. 1986; \triangleright van den Berg et al. 1987; \triangleleft Bruland 1989; \oplus Donat and Bruland 1990; \circ L1, \bullet L2 Muller and Kester 1991; \times Lewis et al. 1995; \diamond Xue et al. 1995; \bowtie Kozelka and Bruland 1998.

(i.e., data from measurements performed on natural-water samples that have been subjected only to filtration). For whole-water samples, ligand concentrations (i.e., effective complexation capacities) are typically reported in the following unit of measure: $\text{nmol metal dm}^{-3}$. Although these values are often quoted as being complexation capacities (e.g., Srna et al. 1980; Kyle 1987; Verweij and Ružić 1997), in fact they are not; complexation capacity is the number of moles of metal per gram of complexant (Buffle 1988). Published data can be converted into "real" \tilde{C}_c values by assuming a certain dissolved organic carbon (DOC) concentration. However, since the majority of publications do not report DOC values (and a wide range is possible, especially for freshwaters), we have not converted the original published units and thus present the effective ligand concentrations as being \tilde{C}_c values. The validity of this approach is further discussed below.

Results and discussion

Relationship between \tilde{K} and \tilde{C}_c —Inspection of the collected literature data reveals some important features (Fig. 1). Notably, the $\log \tilde{K}$ values span a remarkably wide range (4–16). A similar result was observed for isolated humic substances (Buffle 1988). It can be argued that some of this dispersion results from differences in the pH and the ionic strength at such time as the measurements were made (different functional groups on the OM will become available for complexation at different pH values). However, for the whole-natural-water samples, differences in their physicochemical properties, such as pH and ionic strength, are not sufficient to explain such a high degree of dispersion.

As noted above, $\log \tilde{K}$ values alone have no physicochemical meaning and should not be considered in isolation. Compilation of $\log \tilde{C}_c - \log \tilde{K}$ couples provides a more valid

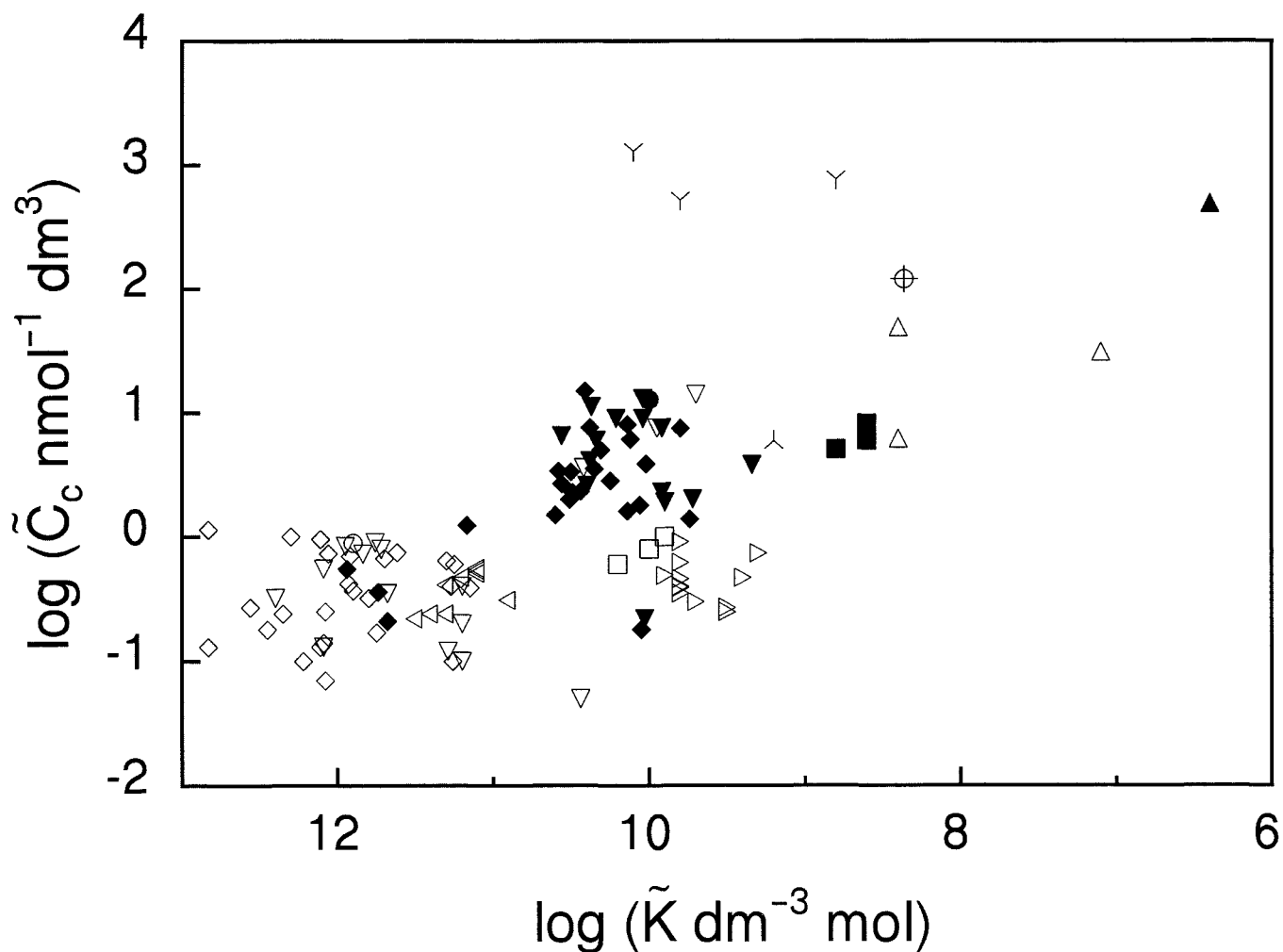


Fig. 5. Compilation of Pb(II) binding parameters ($\log \tilde{C}_c - \log \tilde{K}$) reported for whole natural waters Δ L1, \blacktriangle L2 van den Berg et al. 1984; \triangleright Capodaglio et al. 1989; \triangleleft Capodaglio et al. 1990; γ Bothelo et al. 1994; \wedge Capodaglio et al. 1995; \oplus Santana-Casiano et al. 1995; ∇ L1, \blacktriangledown L2 Muller 1996; \circ L1, \bullet L2 Kozelka et al. 1997; \square L1, \blacksquare L2 Kozelka and Bruland 1998; \diamond L1, \blacklozenge L2 Muller 1998.

basis for data interpretation. Plots of $\log \tilde{C}_c$ versus $\log \tilde{K}$ for isolated humic (Buffle 1988) and biotic (Filella and Town unpubl. data) systems show a linear relationship, with the apparent binding strength increasing as the metal ion loading is decreased. This is in agreement with the fact that stronger sites will be saturated first. Analogous plots for the data from whole-water samples are shown for Cu(II) in Fig. 1. This figure summarizes all the $\log \tilde{C}_c$ - $\log \tilde{K}$ data reported for Cu(II), with each symbol representing a separate publication. The same general trend that was observed for the isolated humic and biotic systems is indicated here, thus indicating the presence of many different binding sites, which form a range of complexes with different stabilities. However, a significant degree of dispersion is obvious in this natural-water data.

As discussed above, each study typically reports one or two $\log \tilde{C}_c$ - $\log \tilde{K}$ couples, and these are ascribed to the presence of L1 and L2 sites. In Fig. 2, the data presented in Fig. 1 have been grouped according to their original assign-

ment as L1 or L2 and have been grouped as well by water type (marine, oligotrophic freshwater, or eutrophic freshwater). Figure 2 clearly shows that there is no significant difference observable between reported L1 and L2 values; hence, there does not seem to be any basis for ascribing these measured ligand concentrations to any real entities.

Moreover, it is also apparent from Fig. 2 that there is no significant difference between the $\log \tilde{C}_c$ - $\log \tilde{K}$ plots for whole seawaters and freshwaters. Even if the organic material is inherently different in detailed composition, it exhibits apparently similar global metal binding behavior. However, this observation should be viewed with caution; these results could be so methodology dependent that any underlying differences are masked.

In totality, the data appears to be highly dispersed (Fig. 1); however, few cases exist in which data have been collected for the same water system under conditions that allow the postulated relationship between metal loadings and equilibrium constants to be tested. Robinson and Brown (1991)

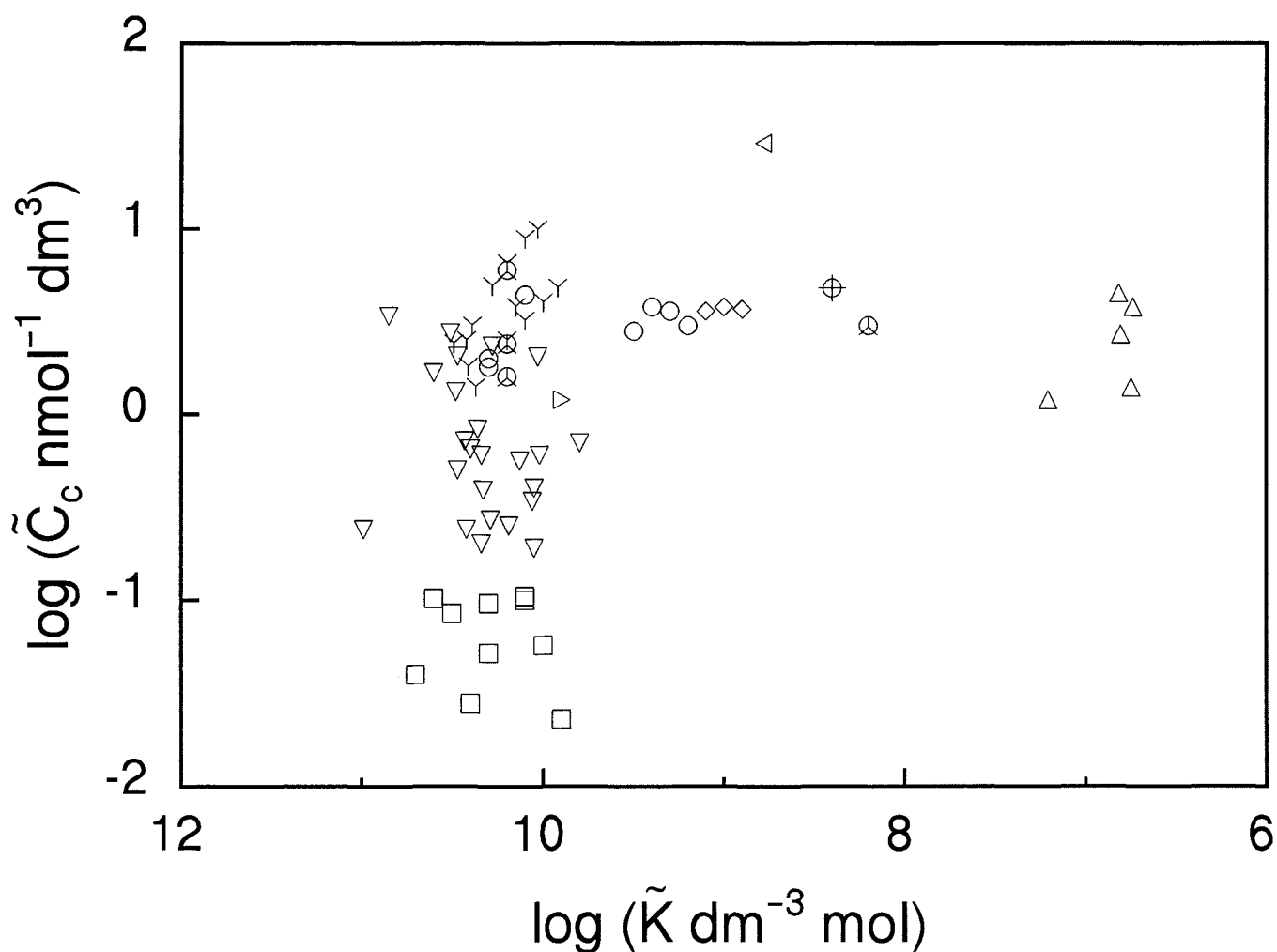


Fig. 6. Compilation of Cd(II) binding parameters ($\log \tilde{C}_c - \log \tilde{K}$) reported for whole natural waters; Δ Midorikawa et al. 1990; \square Bruland 1992; \triangleright Capodaglio et al. 1995; \triangleleft Santana-Casiano et al. 1995; Υ Muller 1996; \wedge Knauer et al. 1998; \oplus Kozelka and Bruland unpublished; \diamond Kozelka and Bruland 1998; ∇ Muller 1998; \circ Xue and Sigg 1998.

monitored Cu-complexation parameters over the course of a dinoflagellate bloom in a lagoon. Figure 3 presents the $\log \tilde{C}_c - \log \tilde{K}$ plot calculated from their data. The data show that there is a general tendency for $\log \tilde{K}$ to gradually increase as $\log \tilde{C}_c$ decreases, a trend that is consistent with an underlying continuous distribution of functional groups of different complexation strengths.

There is much less data published for other trace metals [Zn(II), Pb(II), and Cd(II)], but presentation analysis analogous to that given above for Cu(II) shows similar trends. Figures 4 through 6 summarize all the $\log \tilde{C}_c - \log \tilde{K}$ data published for Zn(II), Pb(II), and Cd(II), respectively. A linear relationship is also observed for Zn(II) and Pb(II). Cd(II), however, shows slightly different behavior. The vertical section of the plot at ca. $\log \tilde{K}$ 10–11 (Fig. 6) could indicate that the strongest binding sites for this metal have been detected within the available analytical window range (Benjamin and Leckie 1981).

Role of the analytical detection window—A more rigorous interpretation of reported binding parameters must also consider the analytical detection window of the techniques employed in their determination. A wide range of different techniques, with a corresponding range of detection windows, have been used in studies on complexation in whole-water samples.

The analytical detection window determines the concentration of ligands and the relative stability of their metal complexes that can be detected in a sample with a given technique. One limit of this window (corresponding to the weakest detectable complexation sites) is determined by the ability of a particular technique to detect a decrease in the labile metal signal resulting from the complexation of the metal by a natural ligand, and the other limit (strongest detectable sites) is determined by the method's detection limit. The position of this window (and hence, the accessible binding parameters) may be varied by using the same method

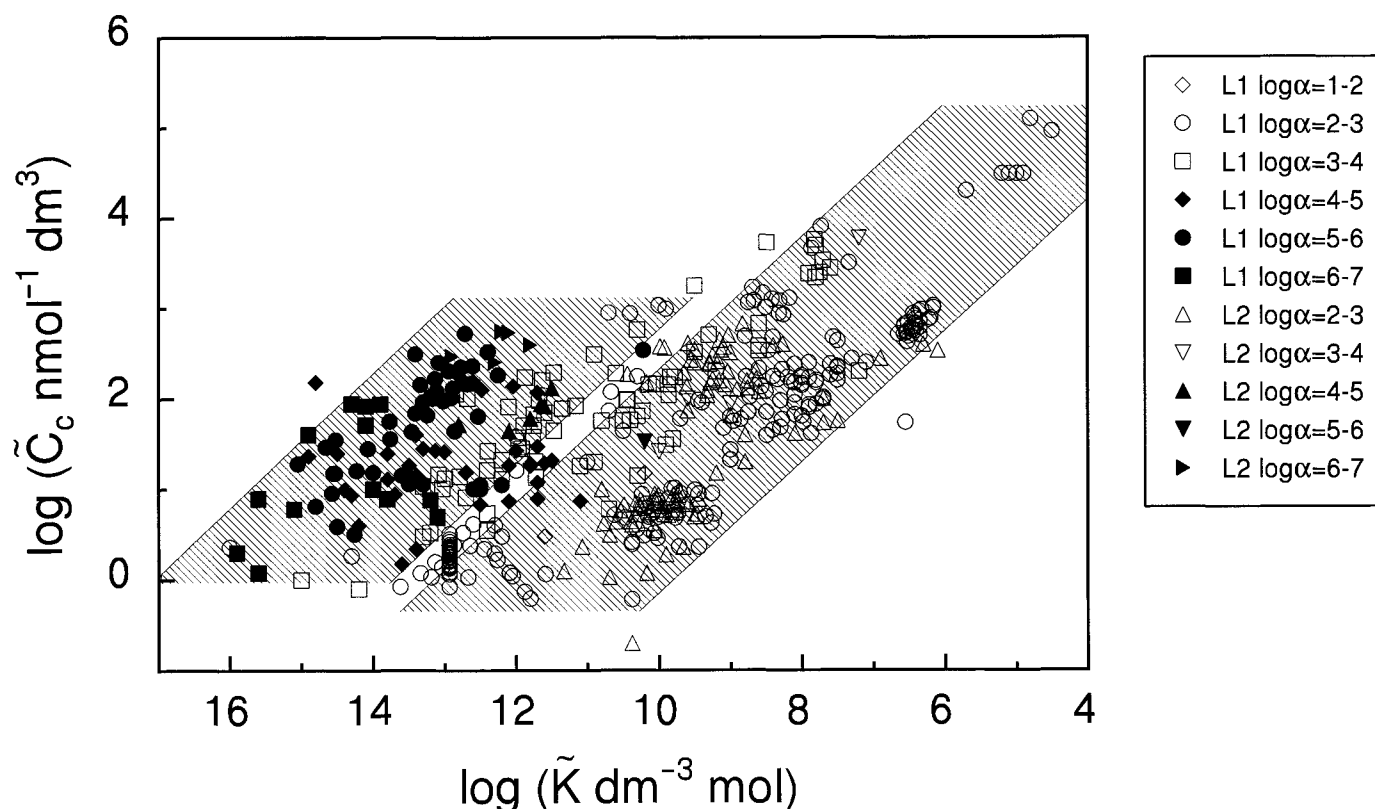


Fig. 7. Effect of the analytical detection window, α , on binding parameters ($\log \tilde{C}_c - \log \tilde{K}$) determined for Cu(II). Data for L1 and L2 ligands are grouped according to the $\log \alpha$ value for the analytical technique employed. The shaded region on the left encompasses most data in the $\log \alpha$ range above 4; shading on the right encompasses $\log \alpha < 4$ data.

under different experimental conditions or by using different analytical methods with different detection windows.

The majority of results we present here were obtained by cathodic stripping voltammetry. With this technique, a specific chelating agent for the metal ion of interest is added to the sample and is allowed to equilibrate. The amount of metal bound to the added ligand is then quantified by adsorptive collection of the complex at a hanging-mercury drop electrode, followed by measurement of the cathodic (reduction) current. For this technique, the detection window is determined by $\alpha = K_{\text{MAL}}[L]_A$, where $[L]_A$ is the concentration of added ligand and K_{MAL} is its conditional stability constant for complexation of the metal, M, corrected for pH and side reactions with other cations (van den Berg et al. 1990). The resulting K values for the natural ligands determined by this method are conditional constants, valid under the experimental conditions employed (van den Berg et al. 1990; van den Berg and Donat 1992). Therefore, the corresponding detected concentration of natural ligands with this apparent stability represents an effective complexation capacity. It has been estimated that an analytical method (with a α value given by $K_{\text{MAL}}[L]_A$) can detect complexes in a sample that has $K[L]$ values that lie within one decade of this α (Apte et al. 1988; Nimmo et al. 1989).

Therefore, an analytical method with an analytical window centered on $\log \alpha = 6$ would detect natural ligands that are apparently more complexing than those detected by one

with a window centered at 3. $\log \tilde{C}_c - \log \tilde{K}$ data that are grouped according to detection window should thus show a series of parallel lines. This is indeed the case, as is shown for Cu(II) in Fig. 7. The data on the left-most side of the plots (as presented) represent the more stable complexes that are detected at higher α values.

The data shown in Fig. 7 form a subset of the data presented in Fig. 1, and they are grouped by α values within one decade range. When considering each $\log \alpha$ group individually, the dispersion in the $\log \tilde{C}_c - \log \tilde{K}$ plot is greatly decreased relative to Fig. 1, and a linear relationship is evident. The shift observed in this plot with $\log \alpha$ corresponds to binding sites that are detected over the entire range of apparent complexation stabilities that are accessible with the experimental techniques employed. When grouped on the basis of water type, there was no discernible difference between the parameters reported for the various systems (Fig. 2).

Two interesting points arise from the $\log \alpha$ dependence shown in Fig. 7:

First, the majority of data published are for Cu speciation, and often, specific Cu chelators are proposed (e.g., McKnight and Morel 1979; Gerringa et al. 1995; Moffett and Brand 1996). It is therefore of interest to compare the complexation behavior of Cu(II) with that of other trace metals. As noted above, valid interpretation requires comparison of binding parameters that were measured at the same metal

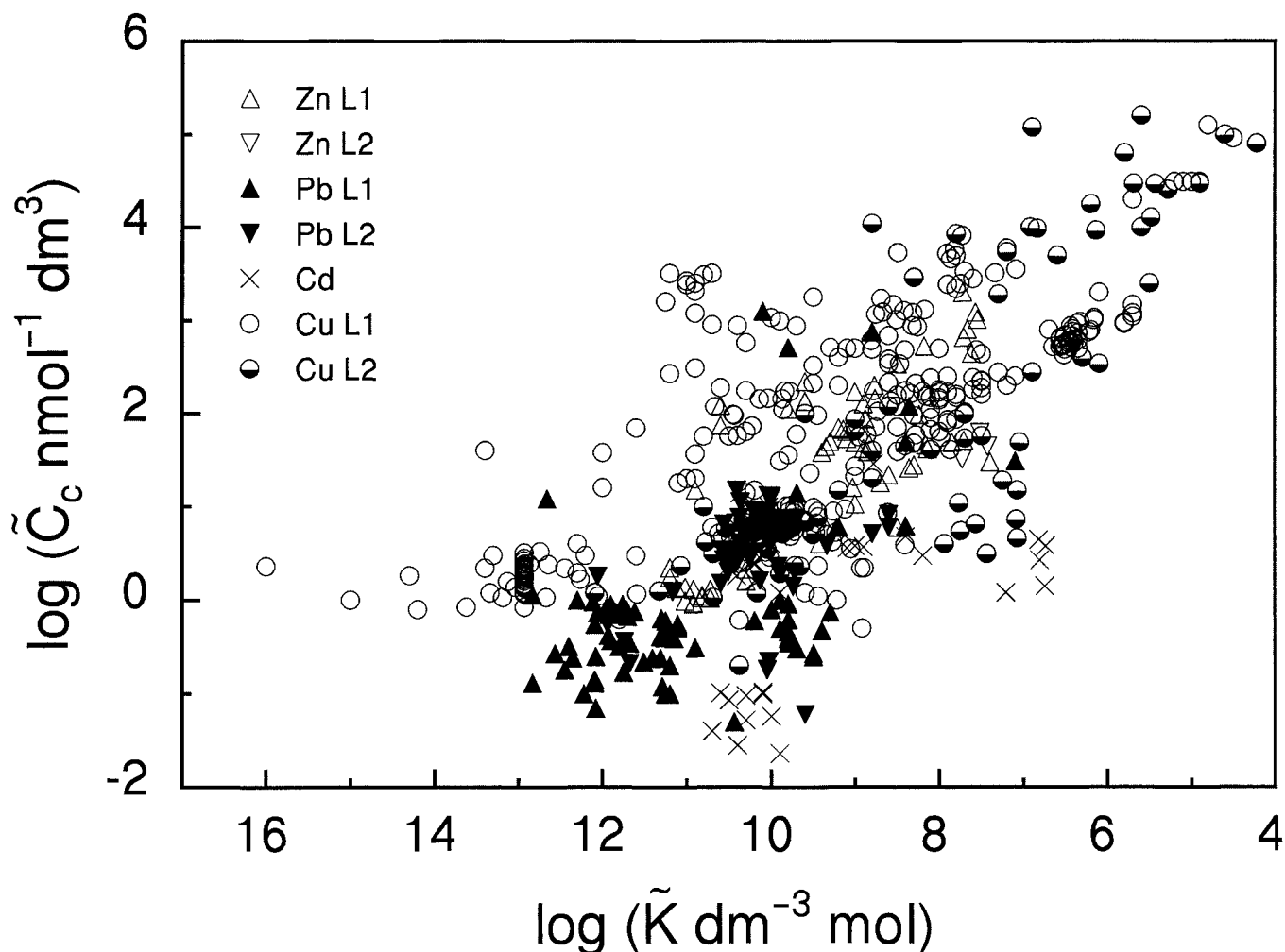


Fig. 8. Comparison of binding parameters ($\log \tilde{C}_c - \log \tilde{K}$) for Zn(II), Pb(II), Cd(II), and Cu(II), determined within an analytical detection window ($\log \alpha$) range of 1–3.

ion loading and with the same detection window. Under these conditions, Fig. 8 indicates that the behavior of Cu(II) is not significantly different from that of Zn(II), Pb(II), or Cd(II). The data presented in this figure are a subset of that data presented in Figs. 1 and 4 through 6, which correspond to a $\log \alpha$ range of 1–3. Again, there is no discernible difference between the L1 and L2 values either for each particular metal ion or across the different metal ions. This result is consistent with the presence of a range of different nonspecific ligands.

The second implication of the $\log \alpha$ dependence of reported binding parameters relates to the relative importance of organic complexation in contributing to the overall speciation of a particular metal. In recent years, many publications on Cu(II) speciation in aquatic systems have emphasized the importance of complexation by OM, with some authors proposing that greater than 90% of Cu exists in this form (Hering et al. 1987; Donat and van den Berg 1992; Mackey and Zirino 1994). However, evidence for the importance of organic ligands for Cu speciation is often based on calculations utilizing the $\log \tilde{K}$ and [L] values determined from Scatchard or van den Berg–Ruzic plots (e.g., van den

Berg 1984b; Apte et al. 1990a; Hirose 1994). Our analysis shows that this approach is not appropriate because (1) these $\log \tilde{K}$ values are dependent on metal ion loading (\tilde{C}_c), and (2) both the $\log \tilde{K}$ and effective \tilde{C}_c are dependent on the detection window of the analytical technique used to determine them.

By using a different approach, we can obtain more solid evidence for the importance of organic ligands in determining trace-metal speciation. A survey of publications on size-fractionated freshwater samples reveals that the amount of metal in each fraction correlates well with the DOC, as shown in Fig. 9 for Cu(II), Pb(II), Cd(II), and Zn(II). These data are from different sources than those from which the binding parameter values were obtained.

For the data presented in Fig. 9, all the values refer to subfractions of the dissolved phase (i.e., $<0.45 \mu\text{m}$). Typically, an ultrafiltration process is employed to fractionate a water sample into various size ranges, and this process is followed by determination of the total metal and DOC within each fraction. Although different size fractionations were employed in each case, the large-colloidal, small-colloidal, and low-molecular weight (l.m.w.) size ranges correspond

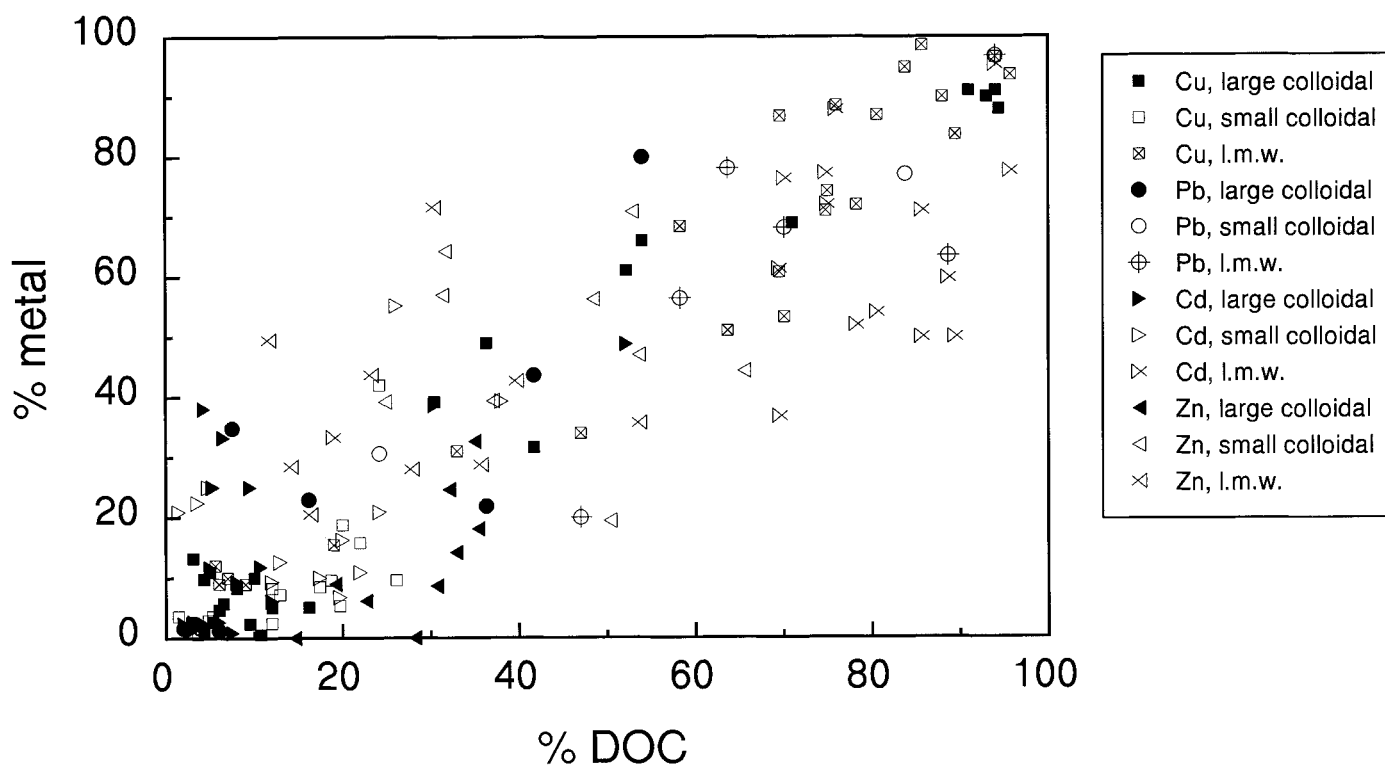


Fig. 9. Relationship between the distribution of Cu(II), Pb(II), Cd(II), and Zn(II) and DOC amongst size fractions of the dissolved phase. The large-colloidal, small-colloidal, and low-molecular weight (l.m.w.) size ranges correspond to components of approximately $>100,000$ Da, $10,000$ – $100,000$ Da, and $<10,000$ Da, respectively. Data from Baccini and Suter 1979; Hoffmann et al. 1981; Valentini et al. 1983; Whitehouse et al. 1990; Tanizaki et al. 1992*a,b*; Dai and Martin 1995; Dai et al. 1995; Martin et al. 1995; Powell et al. 1996; Camusso et al. 1998; Dupre et al. 1999; Eyrolle and Benaim 1999; and Wen et al. 1999.

to components of approximately $>100,000$ Dalton (Da), $10,000$ – $100,000$ Da, and $<10,000$ Da, respectively. The observation that the concentration of metal correlates with that of OM across these size ranges implies that the natural ligands are heterogeneous with respect to size (in addition to heterogeneity of binding-site types) and confirms the concept that specific functional groups are present in different size fractions.

Biota as a possible source of L1—Biota have often been proposed as the source of the putative L1 ligands, which may be generated to maintain constant low levels of trace metals (van den Berg et al. 1979; Gerringa et al. 1995). Supporting evidence for this suggestion comes from studies on isolated biota in which exopolymer production increases in the presence of metal ions (e.g., Fisher and Fabris 1982; Lumsden and Florence 1983; Gonçalves et al. 1988; Xue and Sigg 1990; Maršálek and Rojíčková 1996). Indeed, production of a strong ligand ($\log K$ ca. 12–13, obtained from Scatchard analysis of titration data) in an approximate 1:1 metal:exopolymer ratio has been reported (Moffett and Brand 1996), and Cu(II)-induced release of thiol compounds by a laboratory culture of marine microalgae has been observed (Leal et al. 1999). However, this is not a universal observation, and this mechanism may not be available to all biota (McKnight and Morel 1979). Furthermore, although such a defense mechanism may be effective when metal con-

centrations are relatively low, there are many reports in which the concentration of metal exceeds that of the L1 sites (Hirose et al. 1982; Donat et al. 1994; Capodaglio et al. 1995).

It is therefore of interest to further investigate the possible relationship between metal ion concentration and generation of “L1” ligands by biota. We compiled the reported concentrations of “L1” and “L2” present in the water column and the corresponding concentration of metal ions (from 47 studies). For the literature data, only the total dissolved ($<0.45 \mu\text{m}$) metal concentrations are widely reported. Figure 10 shows a plot of L1 and L2 as a function of total dissolved Cu(II) concentration for seawater and freshwaters. No trend is observable in these data, and no distinction can be made between the behavior of L1 versus that of L2 ligands. Although this observation does not preclude a dynamic relationship between the total dissolved metal concentration and the effective ligand concentration, it indicates that such a mechanism is not dominating Cu speciation in these waters. Similar results were obtained for Zn(II), Pb(II), and Cd(II) (data not shown). We also found no relationship between $\log K$ and the total dissolved metal concentration (data not shown). Thus, even if very strong chelators are being excreted in response to elevated metal concentrations, they do not dominate the resultant metal speciation in most systems.

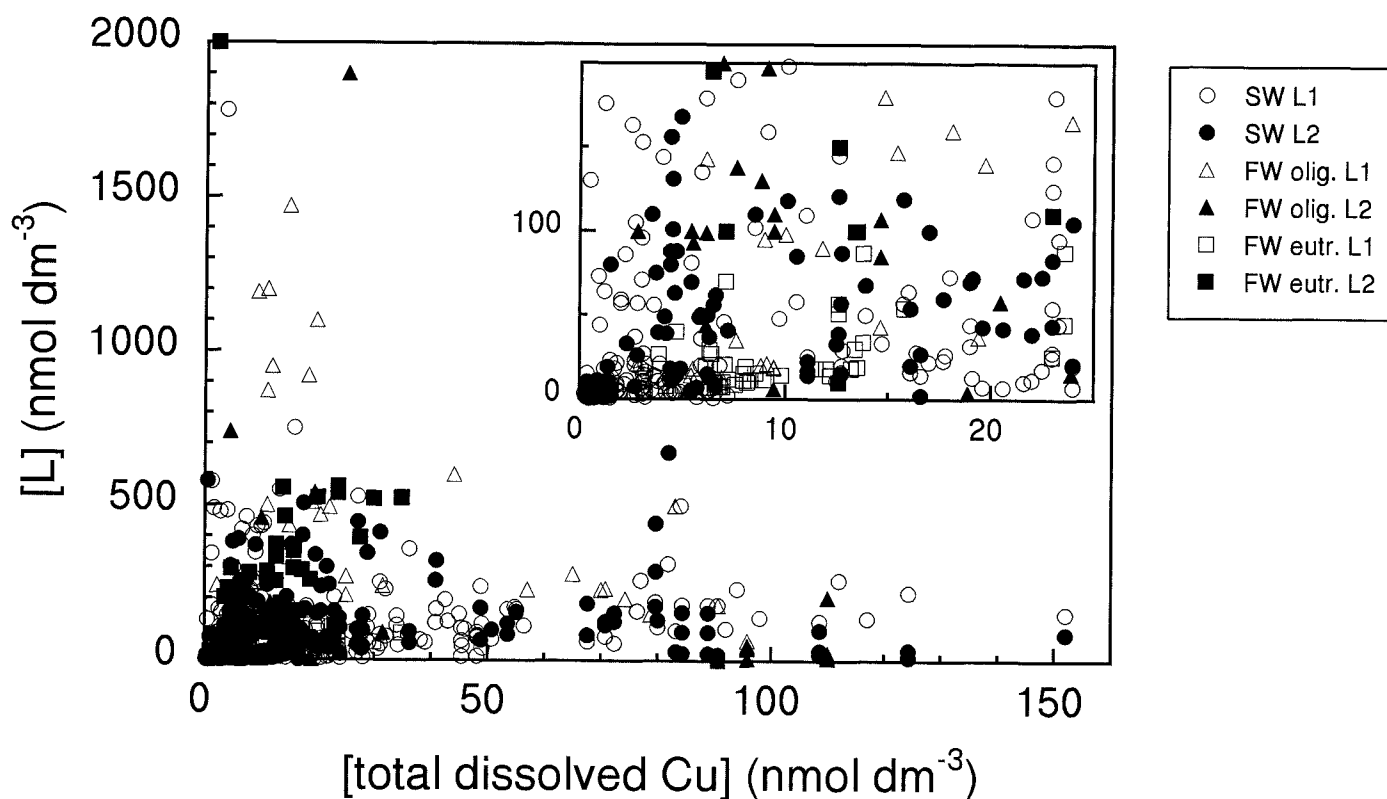


Fig. 10. Relationship between the analytically determined concentration of Cu(II) binding sites ($[L]$) and the total dissolved metal concentration. Data from: Gächter et al. 1978; Sunda and Hanson 1979; Hart and Davies 1981; Hirose et al. 1982; Sunda and Ferguson 1983; Anderson et al. 1984; Hart and Jones 1984; van den Berg 1984*a,b,c*; Buckley and van den Berg 1986; Kramer 1986; van den Berg and Rebello 1986; van den Berg et al. 1986; Hering et al. 1987; Moffet and Zika 1987; Sunda and Hansen 1987; van den Berg et al. 1987; Coale and Bruland 1988; Hanson et al. 1988; Apte et al. 1990*a,b*; Coale and Bruland 1990; Midorikawa et al. 1990; Moffett et al. 1990, 1997; Gardner and Ravenscroft 1991; Sunda and Hunstman 1991; Donat and van den Berg 1992; Midorikawa et al. 1992; Scarano et al. 1992; van den Berg and Donat 1992; Xue and Sigg 1993; Campos and van den Berg 1994; Donat et al. 1994; Midorikawa and Tanoue 1994; Miller and Bruland 1994; Capodaglio et al. 1995; Xue et al. 1995, 1996; Gerringa et al. 1996; Muller 1996, 1998; Achterberg et al. 1997; Verweij and Ružić 1997; Xue and Sunda 1997; Knauer et al. 1998; and Kozelka and Bruland 1998.

Comparison of binding parameters for whole waters and those for isolated OM fractions—In a whole natural water, the total amount of OM and the relative amounts of aquagenic versus pedogenic OM present will be system dependent (and may vary seasonally). In open oceans, the OM is predominantly aquagenic, but in coastal areas, pedogenic contributions may be significant (Anderson et al. 1984). For rivers with DOC levels of several mg dm^{-3} , the dissolved OM is mostly pedogenic; the pedogenic component of DOC decreases with increasing stream size and decreasing stream velocity (Buffle 1988). Lakes represent more complex mixtures of OM (Buffle 1988), as the composition of OM varies seasonally and is also influenced by local events, such as heavy rainfall in the watershed (Zumstein and Buffle 1989; Biber et al. 1996; Fukushima et al. 1996).

Figure 11 compares the $\log \tilde{C}_c - \log \tilde{K}$ data reported for seawaters and freshwaters with that reported for isolated humic substances (Buffle et al. 1984) and biota (algae and bacteria and their components) (Filella and Town unpubl. data). As noted previously, ligand concentrations determined for whole-water samples are typically reported in nmol dm^{-3} units. To facilitate comparison of the seawater data with

those for the isolated OM components, conversion of these units to mmol g C^{-1} was conducted with the assumption of a DOC concentration of $850 \mu\text{g dm}^{-3}$. This value is representative of surface seawater concentrations, viz. $700\text{--}1,000 \mu\text{g dm}^{-3}$ (Guo et al. 1995; Guo and Santschi 1997). Consideration of higher DOC concentrations would shift these data to apparently lower $\log \tilde{C}_c$ values (e.g., assuming a maximum DOC of $2,500 \mu\text{g dm}^{-3}$ [Buffle 1988] results in a shift in $\log \tilde{C}_c$ of ca. -0.4 relative to the values shown). The seawater data presented in Fig. 11 correspond to an analytical window of 1–3, since this is consistent with the methods generally used to determine binding by humic substances (see above discussion on the role of the analytical detection window).

The freshwater data shown in Fig. 11 were converted to mmol g C^{-1} units using the actual DOC concentrations reported in the original data sources. For these freshwater samples it was verified that the correlation coefficient for the $\log [L] (\text{nM}) - \log \tilde{K}$ plot, for an analytical window of 2–3, was very similar to that for $\log \tilde{C}_c \text{ mmol g C}^{-1} - \log \tilde{K}$. This result validates our approach of using the reported ligand concentrations as analogues for the effective complexation capaci-

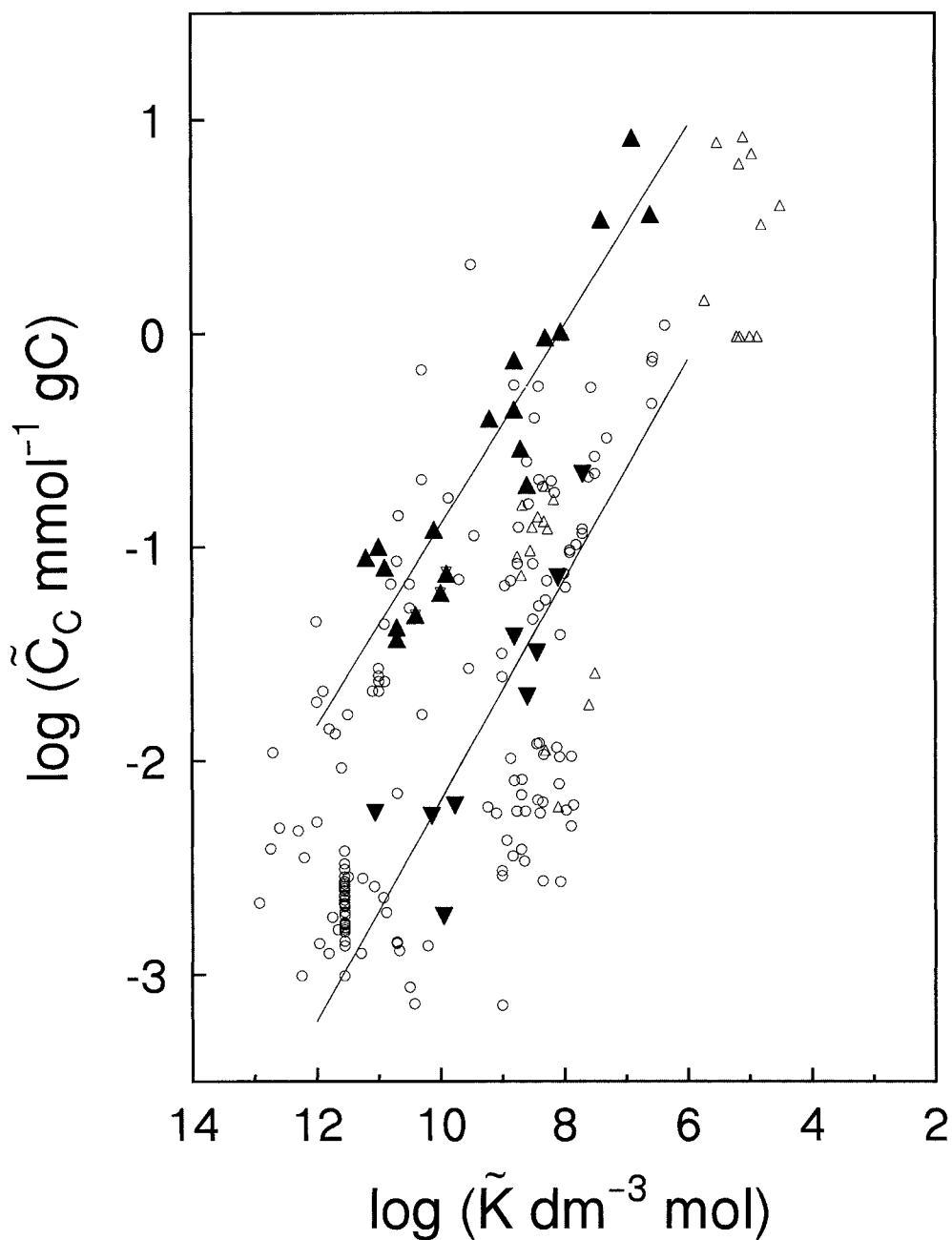


Fig. 11. Comparison of binding curves ($\log \tilde{C}_c - \log \tilde{K}$) for isolated aquatic fulvic substances, ▲ (Buffle et al. 1984) and biota, ▼ (Filella and Town unpublished) with that for natural waters. Analytical window, $\log \alpha = 1-3$. Data converted to mmol g C^{-1} scale (see text for details). ○ seawater; △ freshwater pH 5.7-7.4; ▽ freshwater pH 8-8.3. Seawater data were converted from values presented in Fig. 7(b); freshwater data calculated from: Ramamoorthy and Kushner 1975; Shuman and Woodward 1977; Srna et al. 1980; Hart and Davies 1981; Hoffman et al. 1981; Pardo et al. 1994; Antelo et al. 1998.

ties. The pH range (mostly 6–7) is slightly lower than that for the other samples shown (at lower pH, the $\log \tilde{C}_c - \log \tilde{K}$ plot will shift to apparently weaker complexing; Buffle 1988).

In natural waters, the complexing material present will be a combination of that derived from terrestrial sources and

that formed in situ in the water column. Taking isolated fulvic acids and biota as representatives of each of these OM sources, Fig. 11 shows that the degree of metal ion complexation observed for whole seawaters and freshwaters lies between the binding curves for these two groups of compounds. This is an important observation in itself, given the

difficulties and limitations associated with measurement of binding parameters for each of these milieu. To further our understanding of metal ion speciation in aquatic systems, we need to discern the relative importance of complexation by biota (and their products) relative to that by refractory OM present in the bulk of the water body. This question is especially important, since biota effects are local in terms of their scale (as well as dynamic), whereas refractory OM is influenced by more global scale properties that emerge far from the vicinity (and direct influence) of organisms. However, although this work has highlighted some interesting features of complexation parameters reported for whole natural waters, any further resolution of the data will require more selective speciation techniques.

Conclusions

Several important conclusions arise from our critical analysis of published binding parameters for whole natural waters: Metal ion behavior in natural waters can be explained by the presence of many different types of binding sites (ligands) with different complexation strengths and concentrations. At present, we do not have sufficient information to speculate on the overall nature of these ligands.

Any given parameters are "valid" for the conditions under which they were measured; however, in order to develop a more global understanding of metal complexation in natural waters, the possible effect of the measurement conditions on the resulting binding parameters must be considered. Any comparisons and resulting interpretations must consider data obtained at the same metal ion loading and with the same detection window. Under these conditions, (1) remarkably similar behavior is observed for all trace metals across diverse water types, and (2) there is no detectable difference in the binding curves ($\log \tilde{C}_c - \log \tilde{K}$) for Cu(II) compared to other trace metals [Zn(II), Pb(II), Cd(II)]. However, our analysis makes no assumptions as to the specific nature of the binding sites, and this similarity in the binding curves does not necessarily imply that the same sites are involved in complexation of each metal ion. The degree of metal ion complexation observed for whole waters is comparable to that observed for biotic and humic ligands.

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Received: 22 November 1999

Accepted: 25 April 2000

Amended: 31 May 2000