# Organic complexation of copper in surface waters of Galveston Bay

*Degui Tang,*[1] *Kent W. Warnken, and Peter H. Santschi*
Texas A&M University, Department of Oceanography, 5007 Ave U, Galveston, Texas 77551

## Abstract

The importance of colloidal ligands in the organic complexation of Cu was elucidated in the estuarine waters of Galveston Bay by use of a number of instrumental techniques. Ultrafiltration was conducted to isolate the ultrapermeate fraction from the filter-passing fraction and competitive ligand equilibration/adsorptive cathodic stripping voltammetry measurements were conducted by use of varying amounts of catechol as the competitive ligand. Greater than 99.9% of Cu was bound by strong organic ligands with $\log[K'$ $(M^{-1})]$ values of $12.3 \pm 0.15$ and $11.1 \pm 0.29$ in the filter-passing and ultrapermeate fractions, respectively. The concentrations of Cu complexing ligands were lower in the ultrapermeate than in the filter-passing fraction, and the calculated $K'$ values for the colloidal ligands ($\log K' = 12.9$) were 60 times greater than those for the ultrapermeate fraction, suggesting that good separation of the colloidal fraction during ultrafiltration was achieved. Total Cu concentrations in the filter-passing fraction ranged from 2.27 to 12.9 nM and were fairly constant at salinity <20 but decreased at salinity >20. The calculated free Cu concentrations (0.05–0.49 pM) showed an overall increasing trend with salinity, possibly resulting from decreasing concentrations of Cu complexing ligands toward the seawater endmember. The concentration of Cu complexing ligands (21–54 nM) in the estuarine regions of Galveston Bay was approximately equal to the concentration of total reduced sulfur species (20–60 nM) in the filter-passing fraction, suggesting that reduced sulfur species could account for most of the Cu-complexing ligands in these waters.

The chemical and phase speciation of trace elements is an important aspect of their biogeochemical cycles in natural waters. Microorganisms can modify the speciation of trace metals by excreting metal-binding ligands (e.g., Moffett and Brand 1996). It has been shown that a number of trace metals are bound by biogenic ligands in open ocean surface seawater (Donat and Bruland 1995) and in estuarine water (Apte et al. 1990; Muller 1996; Wells et al. 1998). However, the chemical properties of these ligands, e.g., their structure, elemental composition, and functional groups, are not well known. Furthermore, the specific manner in which these organic ligands affect the biogeochemistry and bioavailability of trace metals in aquatic environments is still a matter of contention because of difficulties in the determination of their concentrations as well as their chemical speciation.

Copper is one of the most extensively studied trace metals in natural waters. It has been reported that the strong organic ligands ($L_1$) are mostly responsible for Cu complexation in open ocean surface waters (Coale and Bruland 1988), whereas the weaker ligands ($L_2$) can dominate Cu speciation in coastal waters (Donat et al. 1994). In waters with high phytoplanktonic activities, the strong specific ligands dominate at low Cu concentrations, whereas fulvic or humic substances, a major portion of bulk organic matter in fresh water

systems, can be important at elevated Cu concentrations (Xue and Sigg 1999). In addition, Cu complexing ligands can be released from sediment pore waters in estuarine and coastal environments (Skrabal et al. 2000). Ligand information inferred from model calculations, however, cannot be taken as proof for the formation of a given metal-organic complex. This is due to the complicated nature of organic matter in aquatic environments (Buffle 1990) and differences in the "detection windows" of the applied methods of detection (Apte et al. 1988; van den Berg and Donat 1992).

The organic ligands responsible for Cu complexation are part of the bulk organic matter pool and have sometimes shown a positive relationship with bulk organic carbon concentrations (Gerringa et al. 1998). The Cu-binding ligands in the colloidal fraction have relatively higher stability constants than the soluble ligands, which has led to new questions about the nature of the colloidal ligands, their specific or nonspecific metal-binding strengths, and the metal-scavenging behavior of organic matter (Muller 1996; Wells et al. 1998). Trace metal detoxification by organisms involves the release of organic compounds with functional groups containing reduced sulfur (Mason and Jenkins 1995). Recently, studies focusing on specific metal complexing compounds in controlled culture experiments or in waters where the organic matter is mostly newly formed have drawn increasing attention. Strong Cu-binding ligands can be released by cyanobacteria in both seawater (Moffett et al. 1990) and freshwater (McKnight and Morel 1979) environments. Leal et al. (1999) reported that thiols released by the algae *Emiliania huxleyi* can account for almost all of the Cu complexation ligands. This is because the sulfhydryl group is one of the strongest complexing ligands for B-type metals as well as for many transition metals, including Cu as Cu (I) (Leal and van den Berg 1998; Al-Farawati and van den Berg 1999).

Dissolved sulfides are present at nanomolar concentrations in seawater (Luther and Tsamakis 1989) and are likely sta-

bilized by complexed metals (Rozan et al. 1999). Similarly, thiols, e.g., glutathione (GSH), are also present at similar nM concentrations in seawater (Le Gall and van den Berg 1993, 1998). Sulfhydryl-containing ligands, e.g., metallothionein-like proteins and phytochelatins, could also be present in colloids and would have relatively higher stability constants compared with simple thiolic compounds, because of the extra conformational advantages of these macromolecules for metal binding. Other extracellular polymers, e.g., acidic polysaccharides or humic/fulvic materials, could also impact the speciation of trace metals in natural waters, especially if present at elevated concentrations. To date, efforts attempting to directly link experimental ligand information from various measurement techniques to actual occurrence of these ligands in the environment remains difficult.

In this paper, we discuss the organic complexation of Cu in the estuarine waters of Galveston Bay apparent from competitive ligand equilibration/adsorptive cathodic stripping voltammetry (CLE-aCSV) and stirred-cell ultrafiltration experiments to separate Cu in the "ultrapermeate" fraction from that in the "filter-passing" fraction. Differences in the complexation of Cu by macromolecular and low-molecular-weight organic ligands, as well as the possible relationship between these Cu complexing ligands and reduced sulfur compounds, are also addressed.

## Materials and methods

*Sample collection and stirred-cell ultrafiltration*—Sample collection in the field and processing in the laboratory are described in detail elsewhere (Tang et al. 2000). Briefly, ultraclean sampling procedures (Wen et al. 1996) were used to collect samples along a salinity gradient in Galveston Bay during 16–19 August 1999 (Fig. 1). Samples were peristaltically pumped, using C-Flex tubing and in-line filtration, through 0.45 $\mu$m MSI prefilters and filters into 500-ml low-density polyethylene bottles or 1-liter Teflon bottles. These samples, referred to as the "filter-passing fraction," were double bagged (inner bag acid-cleaned) and stored in a cooler while in the field and transferred to a refrigerator (4°C) once in the laboratory.

The ultrafiltration experiments were conducted using an Amicon 8200 stirred-cell ultrafiltration device with a YM1 (1 kDa) membrane. Here, the size fraction with a nominal molecular weight <1 kDa is termed the "ultrapermeate fraction" and contains the low-molecular-weight molecules, and the fraction >1 kDa but <0.45 $\mu$m is referred to as the "colloidal fraction" and contains the macromolecules. Generally, 130 ml of sample water was concentrated to about 20 ml, yielding concentration factors between 6 and 9.3. Both the filter-passing and ultrapermeate fractions were subsequently used for the Cu titration experiments. The colloidal separation and the Cu titration experiments were conducted in Class-100 clean benches.

*Cu speciation measurements with catechol by CLE-aCSV*—Copper titration experiments were conducted by use of varying concentrations of catechol, to compensate for dissolved organic matter concentration changes. The experimental details about the CLE-aCSV and the chemical equal-
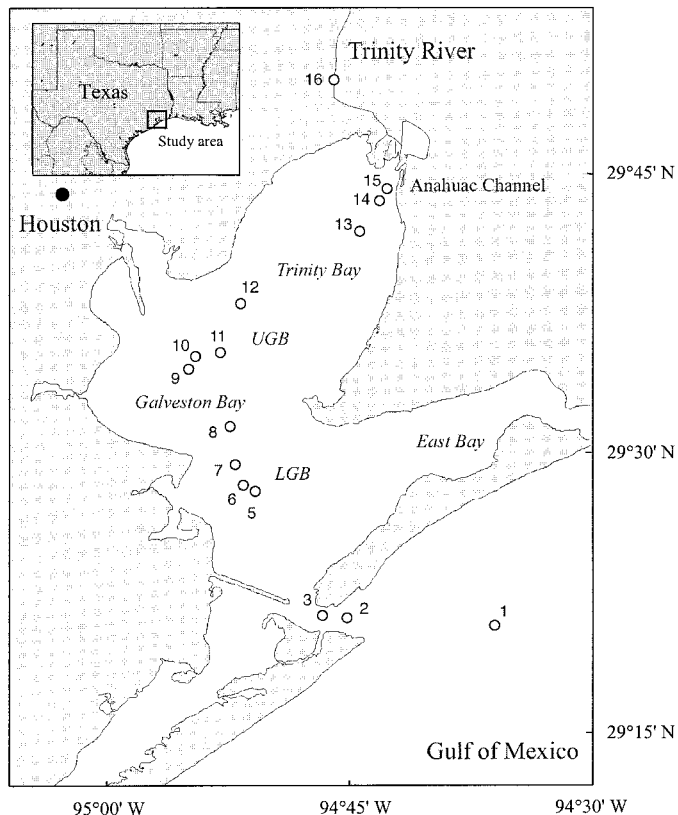


Fig. 1. Sampling locations in Galveston Bay Estuary and Trinity River on 16–19 August 1999. UGB is the Upper Galveston Bay; LGB the Lower Galveston Bay.

ibration reactions involved in this method have been reported elsewhere (van den Berg 1984; Xue and Sigg 1993). Briefly, subsamples were pipetted into a series of Teflon polarographic cells and buffered by use of a HEPES solution (*N*-2-hydroxyethylpiperazine–*N'*-2-ethanesulphonic acid) containing 1 M HEPES and 0.5 M NaOH. Incremental amounts of a Cu standard solution were spiked into all but one cell, giving an approximate concentration range of 5.0–500 nM for the "filter-passing" samples, whereas lower Cu concentrations of ≤200 nM were added to the "ultrapermeate" samples. Measurements were conducted by differential pulse cathodic stripping voltammetry in the same cell after equilibration overnight of the spiked sample at room temperature, using an EG&G PAR 384B polarographic system controlled by the 394 software, a 303A static mercury drop electrode, and a 305 stirrer. The working electrode was set in the hanging mercury drop (large) electrode mode (HMDE), and the reference electrode was a saturated calomel electrode (SCE) connected to the samples via a KCl salt bridge (>4 M). After first purging the sample for 6–8 min, 20 $\mu$l of catechol was added and followed by a 3-min equilibration period. The Teflon stirring bar was switched on simultaneously when a new Hg drop formed. The Cu-catechol complexes were then collected on the working electrode for 3 min at a potential at 0.0 V versus SCE. After collection, the stirrer was turned off, and 10 s later the voltage scan was started in the negative direction. The scan parameters included an initial po-

Table 1. Results of the calculation of the conditional stability constant ($K'$, M$^{-1}$), complexation capacity ($C_L$), and free ionic Cu concentration in the filter-passing fraction ($<$0.45 $\mu$m).

| Sta. No. | Salinity | $\alpha_{Cucat}$ | Total Cu (nM) | Log $K'$ | | $C_L$ (nM) | | Free Cu (pM) |
|---|---|---|---|---|---|---|---|---|
| | | | | Value | Error | Value | Error | |
| 1 | 36.4 | 2,300 | 2.27 | 11.3 | 0.07 | 46.3 | 2.2 | 0.284 |
| 2 | 31.9 | 1,980 | 5.81 | 12.5 | 0.44 | 20.5 | 1.8 | 0.115 |
| 3 | 29.0 | 1,830 | 6.31 | 12.3 | 0.41 | 21.8 | 1.7 | 0.223 |
| 5 | 24.9 | 5,620 | 8.41 | 12.4 | 0.09 | 29.4 | 1.1 | 0.163 |
| 6 | 22.0 | 5,620 | 11.32 | 12.5 | 0.09 | 31.1 | 1.1 | 0.162 |
| 7 | 19.5 | 4,780 | 10.18 | 11.9 | 0.11 | 52.8 | 3.7 | 0.279 |
| 8 | 17.6 | 6,440 | 11.47 | 12.3 | 0.13 | 49.1 | 3.1 | 0.165 |
| 9 | 16.2 | 7,880 | 11.13 | 11.8 | 0.12 | 45.0 | 3.5 | 0.491 |
| 10 | 13.5 | 15,800 | 10.47 | 12.3 | 0.13 | 51.3 | 4.0 | 0.122 |
| 11 | 11.4 | 26,700 | 11.10 | 12.7 | 0.13 | 53.6 | 3.9 | 0.058 |
| 12* | 9.3 | 24,200 | 11.20 | | | | | |
| 13 | 7.7 | 30,900 | 10.99 | 12.5 | 0.15 | 47.9 | 3.7 | 0.104 |
| 14 | 4.9 | 28,600 | 9.27 | 12.3 | 0.14 | 48.9 | 4.6 | 0.107 |
| 15 | 1.9 | 31,200 | 12.89 | 12.3 | 0.09 | 76.2 | 4.4 | 0.113 |
| 16 | 0.0 | 38,600 | 8.74 | 12.4 | 0.06 | 83.0 | 3.7 | 0.049 |
| Average | | | 9.44 | 12.3 | 0.15 | 49.6 | 3.0 | 0.174 |

* A missing sample.

tential of 0.0 V, a scan rate of 5 mV s$^{-1}$, a pulse amplitude of 25 mV, and a drop time 0.4 s. In addition to a manual determination of the signal peak height, voltammograms were also processed by the PAR 394 software.

The conditional stability constant for the Cu-organic ligand complex ($K_{CuLx}'$) and the Cu complexation capacity ($C_{Lx}$) were calculated from nonlinear curve fitting (using Kaleidagraph) of the total Cu concentration ($C_{Cu}$) given by the following equation (van den Berg 1984):

$$C_{Cu} = i_p/S + (i_p/S)$$
$$\times \sum \{K_{CuLx}'C_{Lx}/(\alpha_{Cu} + \alpha_{Cucat} + K_{CuLx}'i_p/S)\} \quad (1)$$

where $i_p$ is the peak current of the Cu-catechol species, $S$ is

the determination sensitivity of a titration, which is the slope in the linear region of the titration curve where the natural Cu-complexing ligands have been saturated by the added Cu. The side reaction coefficients, $\alpha_{Cu}$ and $\alpha_{Cucat}$, are for Cu complexation by inorganic ligands and catechol, respectively. The value of $\alpha_{Cu}$, 10.6 at pH 7.7 (van den Berg 1984), was negligible compared with $\alpha_{Cucat}$, $>$200 for all titrations conducted at pH values close to 7.5 (Tables 1 and 2), for both the filter-passing and ultrapermeate fractions. For most of the samples, the one-ligand assumption was adequate to describe the titration curve.

*Total Cu determination*—Total Cu concentrations were determined by inductively coupled plasma mass spectrom-

Table 2. Results of the calculation of the conditional stability constant ($K'$, M$^{-1}$), complexation capacity ($C_L$), and free ionic Cu concentration in the ultra-permeate ($<$1 kDa) fraction separated by stirred-cell ultrafiltration.

| Sta. No. | Salinity | $\alpha_{Cucat}$ | Total Cu (nM) | Log $K'$ | | $C_L$ (nM) | | Free Cu (pM) |
|---|---|---|---|---|---|---|---|---|
| | | | | Value | Error | Value | Error | |
| 1 | 36.4 | 935 | 2.25 | 11.0 | 0.36 | 10.0 | 1.9 | 3.16 |
| 2 | 31.9 | 812 | 2.67 | 11.5 | 0.47 | 7.4 | 1.6 | 1.89 |
| 3 | 29.0 | 727 | 3.48 | 11.2 | 0.83 | 9.8 | 1.8 | 3.12 |
| 5 | 24.9 | 615 | 4.65 | 11.8 | 0.35 | 11.9 | 1.9 | 0.951 |
| 6 | 22.0 | 231 | 5.73 | 11.3 | 0.09 | 29.8 | 1.3 | 1.28 |
| 7 | 19.5 | 204 | 12.24* | | | | | |
| 8 | 17.6 | 224 | 6.02 | 10.6 | 0.12 | 42.6 | 3.2 | 3.97 |
| 9 | 16.2 | 280 | 5.96 | 10.2 | 0.23 | 43.6 | 8.3 | 10.3 |
| 10 | 13.5 | 501 | 4.20 | 11.1 | 0.19 | 34.2 | 3.8 | 1.05 |
| 11 | 11.4 | 501 | 5.57 | 10.9 | 0.19 | 32.9 | 3.9 | 2.54 |
| 12† | 9.3 | 575 | 4.89 | | | | | |
| 13 | 7.7 | 567 | 5.73 | 10.7 | 0.13 | 42.4 | 3.8 | 3.33 |
| 14 | 4.9 | 607 | 4.77 | 10.6 | 0.33 | 32.8 | 7.1 | 4.02 |
| 15† | 1.9 | | | | | | | |
| 16 | 0.0 | 812 | 1.46 | 10.8 | 0.23 | 35.8 | 5.9 | 0.602 |
| Average | | | 4.97 | 11.1 | 0.29 | 27.8 | 3.7 | 3.01 |

* Contaminated sample.
† Missing sample.

etry after online preconcentration, by using a VG PlasmaQuad II *Turbo* Plus instrument upgraded with a high performance interface (Warnken et al. 2000). Briefly, samples were acidified in the laboratory to pH <2 by addition of 1 ml of $HNO_3$ (Sea Star, Baseline) to 500 ml of water and UV-irradiated for a period of 24 h to destroy organic macromolecules that could interfere with the eventual extraction and analysis (Wen et al. 1999). During preconcentration, samples were buffered online at a pH value of ~8.80 by use of an ammonium-ammonium chloride buffer, before loading onto an imino-diacetate resin (Toyopearl AF-Chelate 650M) at a flow rate 2.4 ml min$^{-1}$. After sufficient rinsing with Nanopure water, extracted trace metals were eluted from the resin by use of 1M $HNO_3$ (SeaStar Baseline) and injected into the plasma after ultrasonic nebulization (CE-TAC U-5000AT). The transient signals were monitored by the Time Resolved Analysis application of PQ Vision 4.3d software.

*Total reduced sulfur analysis*—Total reduced sulfur (TRS) concentrations were determined by square wave cathodic stripping voltammetry (Le Gall and van den Berg 1993, 1998). A similar technique was used elsewhere to detect nanomolar concentrations of dissolved sulfides (Luther and Tsamakis 1989). Detection has involved the formation of Hg-SR precipitates on the electrode surface, which were then stripped away during a cathodic scan to a more negative potential (Florence 1979). Because cathodic stripping voltammetry has difficulties in identifying individual reduced sulfur species, acid titration was recently proposed to semi-quantitatively determine different metal-sulfide species in fresh waters (Rozan et al. 1999). Al-Farawati and van den Berg (1997) attributed the "sulfide-like" peak observed in surface waters to thiols such as thiourea or thioacetamide. In our study, we treated this "sulfide-like" peak, which includes both sulfides and thiols, as TRS.

In this TRS determination, the step involving the solid phase extraction of hydrophobic organic matter was not used (Le Gall and van den Berg 1993). Instead, samples with salinities <25 were diluted 4–5 times using UV-irradiated, ultrafiltered (<1 kDa) seawater from the deep Atlantic Ocean. Before measurement, 10 ml of sample was transferred into the Teflon polarographic cell and buffered by use of 100 $\mu$L of a borate solution (1 M borate with 0.4M NaOH, ultraviolet irradiated). For diluted samples, the seawater was first analyzed at the same conditions to check for possible contamination before actual samples of Galveston Bay water were added to a final volume of 10 ml. Between samples, the electrodes were rinsed with alternating solutions of Nanopure water, NaOH solution (0.01M), and HCl (1 M).

During analysis, the samples were purged by use of filtered (Class-100), water-saturated, ultrapure nitrogen for 6 min, to remove oxygen. The reduced sulfur species were deposited on HMDE for 120 s at a potential of $-0.25$ V. The Teflon-coated magnetic stirrer was automatically switched on during the purging and deposition period. After a 15-s quiescent period, a cathodic scan from $-0.25$ to $-0.9$ V using the square-wave mode, with a 2-mV step increment, a 50-Hz frequency, and a 25-mV pulse height, was performed. The reduced sulfur peak, occurring at about $-0.6$
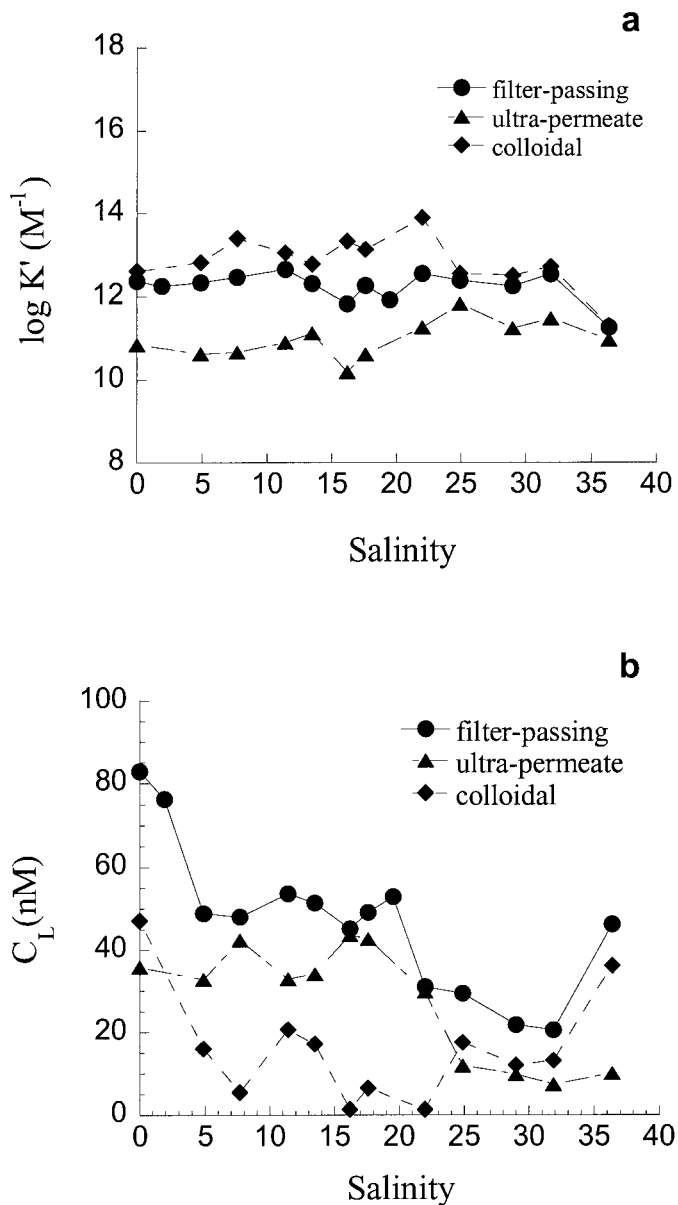


Fig. 2. The distribution of (a) conditional stability constants (log $K'$) and (b) Cu complexation capacities ($C_L$) in different size fraction of organic ligands in Galveston Bay waters.

V, was quantified by standard additions of GSH, yielding TRS as a GSH-equivalent concentration. The detection limit for an undiluted sample was ~1 nM, and the linear range was up to 60 nM GSH at these conditions.

## Results and discussion

*Distribution of the conditional stability constant and Cu complexation capacity*—The calculated parameters for Cu complexation are listed in Tables 1 and 2 for the filter-passing (<0.45 mm) and ultrapermeate (<1 kDa) fractions, respectively. The conditional stability constants [$K'$ (M$^{-1}$)] obtained for both fractions were similar across the salinity gradient in Galveston Bay (Fig. 2a). The log $K'$ for the filter-

passing fraction was $12.3 \pm 0.15$ and was higher than that determined for the ultrapermeate fraction of $11.1 \pm 0.29$. These values fall within the range reported for the strong ligand ($L_1$) in the ''dissolved'' phase ($<0.45 \ \mu m$) (see Coale and Bruland 1988; Xue and Sigg 1993). The Cu complexing ligand concentrations ($C_L$) in the filter-passing fraction decreased with increasing salinity (Fig. 2b), in agreement with the general pattern observed for other estuaries (van den Berg et al. 1987; Apte et al. 1990). In the Trinity River and Anahuac Channel regions (salinity $<5$), a large fraction of this complexation capacity was in the colloidal phase, evident from the differences in the Cu complexation capacity of the filter-passing and ultrapermeate fractions (Fig. 2b). The Gulf of Mexico seawater end-member (salinity 36.4) had a lower log $K'$ and a higher $C_L$ value and was anomalous when compared with the Galveston Bay stations. Some curvature was observed at lower Cu additions in the linearization curve of this sample, indicating the presence of more than one ligand. However, the two-ligand regression procedure did not converge and failed to provide more precise information on the nature of the ligands present in this sample.

The average log $K'$ value determined for the filter-passing fraction is one order of magnitude higher than that determined for the ultrapermeate fraction. This could result from good separation of organic ligands during the ultrafiltration procedure and the presence of colloidal ligands with a relatively high conditional stability constant in that fraction ($>1$ kDa and $<0.45 \ \mu m$), because the filter-passing fraction ($<0.45 \ \mu m$) contains both low- and high-molecular-weight species. Previously, differences in the reported log $K'$ values for various size fractions determined by anodic stripping voltammetry were relatively small ($<0.8$) (Muller 1996; Wells et al. 1998).

The conditional stability constant for the colloidal ligands can be calculated under the assumption that the ultrapermeate contains all the low-molecular-weight ligands ($L_p$) present in the filter-passing fraction and that the colloidal fraction contains all the colloidal ligands ($L_c$). Thus, the total ligand concentration in the filter-passing fraction ($L_f$) is equal to sum of $L_p$ and $L_c$. The ligand balance allows calculation of the concentration of the macromolecular ligand ($L_c$). From the mass balance of total metal concentrations, the following equation holds true in the filter-passing fraction:

$$K_c'[L_c] + K_p'[L_p] = K_f'[L_f], \qquad (2)$$

where $K'$ ($M^{-1}$) is the stability constant, subscripts $c$, $p$, and $f$ represent the colloidal ligands, the low-molecular-weight ligands, and the filter-passing ligands, respectively. For simplicity, the free ligand concentration ($L_x$) can be replaced by its total concentration ($C_{Lx}$) when $C_{Lx} > C_{Cu}$, as is the case here. The calculated stability constants and concentrations of the colloidal ligands are plotted in Fig. 2a and b, respectively. On average, log $K_c'$ values of 12.9 were ~4 times higher than those determined for the filter-passing fraction (log $K_f' = 12.3$) and 60 times higher than those determined for the ultrapermeate fraction (log $K_p' = 11.1$). However, it is important to note that this calculation could underestimate the conditional stability constant of the colloidal ligands ($K_c'$) and overestimate the concentrations of these Cu com-

plexing ligands ($C_{Lc}$), because not all low-molecular-weight ligands pass through the membrane during ultrafiltration (Guo et al. 2000). Interestingly, $L_c$ dominates the ligand pool at high salinity, whereas $L_p$ dominates in the low-salinity region of Galveston Bay.

Macromolecules seem to be responsible for the higher conditional stability constants observed in Galveston Bay samples as well as in other aquatic environments (Wells et al. 1998). This conclusion is further supported by studies that used affinity separation of natural organic matter with $C_{18}$ cartridges, since many macromolecules are relatively hydrophobic compounds extractable by $C_{18}$ resins (Paulson et al. 1994; Gerringa et al. 1998). However, Donat et al. (1986) showed that $L_1$ ligands responsible for Cu complexation were not retained on a Sep-Pak $C_{18}$ column. Additionally, recent studies have shown that most of the Cu complexation ligands were present in the low-molecular-weight range of natural organic matter (Muller 1996; Wells et al. 1998).

The presence of macromolecular ligands makes it more difficult to characterize the Cu complexing ligands, because macromolecules can exhibit a more complicated behavior in solution. In addition, their metal complexation constants show a broad distribution rather than discrete values (Buffle 1990), suggesting the presence of different functional groups with varying complexation constants. Metal titration techniques provide an effective way to determine the overall complexation strength and to predict the toxic level of a specific metal in natural waters by determining its free ion concentration (Apte et al. 1988). However, difficulties are encountered when linking the specific organic compounds to the model-derived complexation parameters, especially when these organic ligands are macromolecules. The colloidal macromolecular ligands could be refractory humic/fulvic materials or newly released high-molecular-weight intracellular biomolecules such as proteins (e.g., metallothioneins), peptides (e.g., phytochelatins) (Lee et al. 1996), or extracellular polysaccharides (Kaplan et al. 1987). The Cu complexing ligands in the ultrapermeate fraction could result from exudation of low-molecular-weight molecules by phytoplankton (Leal et al. 1999).

*Distribution of free Cu species in Galveston Bay waters—* The calculated concentrations of free Cu in these estuarine waters were four and three orders of magnitude lower than the measured total Cu concentrations in the filter-passing and ultrapermeate fractions, respectively (Tables 1 and 2). In the filter-passing fraction ($<0.45 \ \mu m$), the total Cu concentrations (2.27–12.89 nM) were fairly constant at salinity $<20$ but decreased with increasing salinity $>20$. The free Cu concentrations (0.059–0.49 pM) showed an increasing trend with increasing salinity (Fig. 3a). This behavior could have resulted from the decreasing concentration of the natural organic ligands with increasing salinity (Fig. 2b). However, in the ultrapermeate fraction ($<1$ kDa), the free Cu concentrations followed a similar trend as the total Cu concentrations (Fig. 3b). In this fraction, free Cu was buffered by somewhat weaker complexing ligands (log $K' = 11.1$), with compounds being present within a narrow molecular weight range.
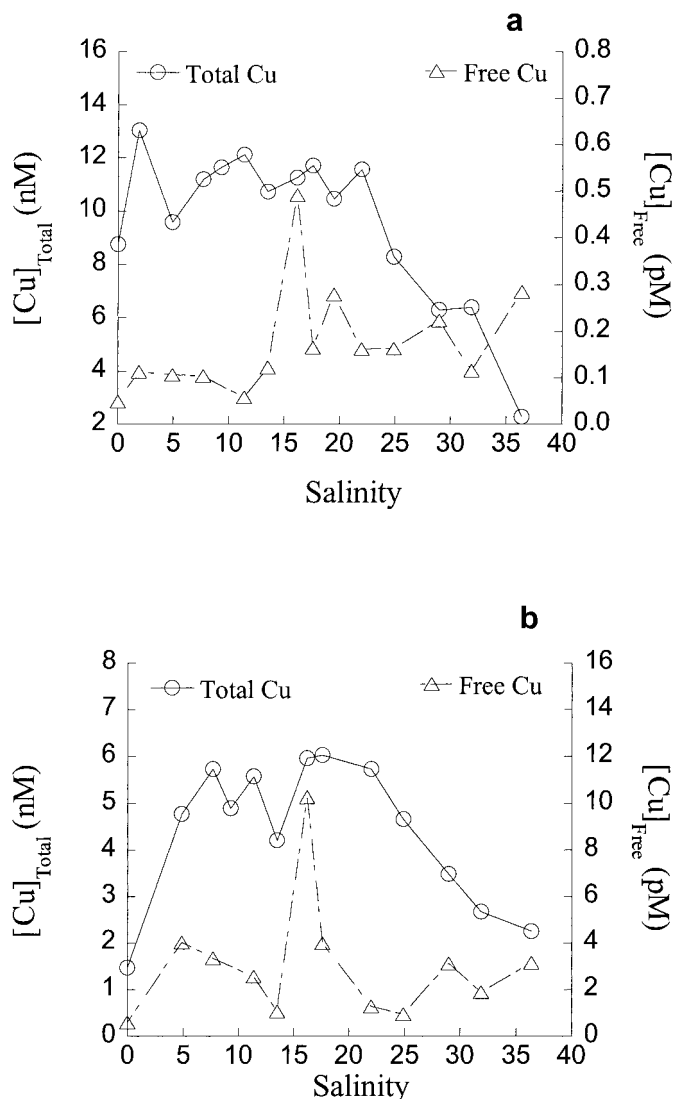
In Fig. 2b, Cu complexing ligands in the filter-passing

Fig. 3. Distribution of free and total Cu concentrations in (a) the filter-passing fraction (<0.45 µm) and (b) the ultrapermeate (<1 kDa).

fraction showed more variability in concentrations than in the ultra-permeate fraction. The conditional stability constants for the colloidal fraction in mid-Galveston Bay were higher than those from the river or seawater end-members (Fig. 2a). The ligands in the filter-passing fraction were higher in concentration in the Trinity River/Anahuac Channel region, where colloidal, macromolecular ligand concentrations made up a significant fraction of the complexation capacity (Fig. 2b). These non-conservative behaviors are consistent with the previous report that macromolecular organic matter is preferentially removed from waters in the low-salinity Trinity Bay region (Guo and Santschi 1997), where coagulation/flocculation processes are most important. A similar removal phenomenon was also observed in another estuary of the Gulf of Mexico (Powell et al. 1996).

Greater than 99.9% of the Cu in the filter-passing fraction, operationally termed the "dissolved" phase, was present as organic complexes in Galveston Bay waters. The ratio of the total Cu concentration in the ultrapermeate fraction to that in the filter-passing fraction was quite constant (0.49 ± 0.06), when the river and seawater end-members were excluded. This is in agreement with previous results for Galveston Bay (Wen et al. 1999) which showed that, on average, 45% of the dissolved Cu was present in the ultrapermeate fraction, whereas 55% was present in the colloidal fraction.

Different organic ligands in the different size fractions could be responsible for Cu organic complexation, so that the existence of macromolecular ligands would not make the low-molecular-weight ligands irrelevant for Cu speciation in the traditionally defined "dissolved" phase. The free Cu concentrations were effectively buffered and controlled different equilibrations of chemical reactions. Cu complexing ligands are likely present in excess amounts, compared with Cu concentrations in each size range, even after their separation into different size fractions by ultrafiltration. The free Cu concentration calculated in the ultrapermeate fraction indicates the effect of a physical technique on the chemical speciation by separation of organic ligands, which depends on both the dissociation kinetics of Cu organic complexes during ultrafiltration and the size distribution of these metal organic complexes. For some metals, e.g., Cd and Zn, most complexing ligands appear to be present in the ultrapermeate or low-molecular-weight fractions, whereas for others, e.g., Pb, the colloidal macromolecular complexing ligands can dominate their organic speciation (Wells et al. 1998; Muller 1996, 1998; Wen et al. 1999).

*Cu speciation and the possible influence of dissolved organic carbon and reduced sulfur species*—The concentrations of Cu complexing ligands in both the filter-passing and ultrapermeate fractions increased with increasing dissolved organic carbon (DOC) concentrations (Fig. 4a). However, Cu-complexing ligands are not present in constant proportion in DOC. The ligand concentrations in the ultrapermeate fraction appear to level off at higher DOC concentrations (>150 µM). Fractions of the Cu complexing ligands in each DOC pool in the ultrapermeate and colloidal fractions were inversely related to each other (Fig. 4b), reflecting changes in the type of organic matter relevant for Cu speciation in these fractions. If there is no separation of organic ligands during ultrafiltration, the ligand-to-carbon ratio should be similar in both fractions. The higher values of the Cu complexing ligands-to-carbon ratios in the colloidal fraction than in the ultrapermeate fraction also coincides with higher conditional stability constants of the colloidal ligands.

It has been shown elsewhere that the concentrations of Cu complexation ligands increased with increasing total Cu concentrations, close to a 1 : 1 molar ratio (van den Berg et al. 1987; Kozelka and Bruland 1998). Here, this ratio clustered around a 5 : 1 molar ratio (Fig. 4c), suggesting that not all of the Cu complexation ligands are actually bound by Cu in Galveston Bay waters. This reflects the possibility that, in estuarine waters, the organic ligands are not specific to Cu but can be complexed by other trace metals, such as Zn and Ni, which have similar concentrations in these waters (Tang 2000). The ligand concentrations in natural waters, which have been termed the complexation capacity (Buffle 1990), are often quantified by various titration methods. However,
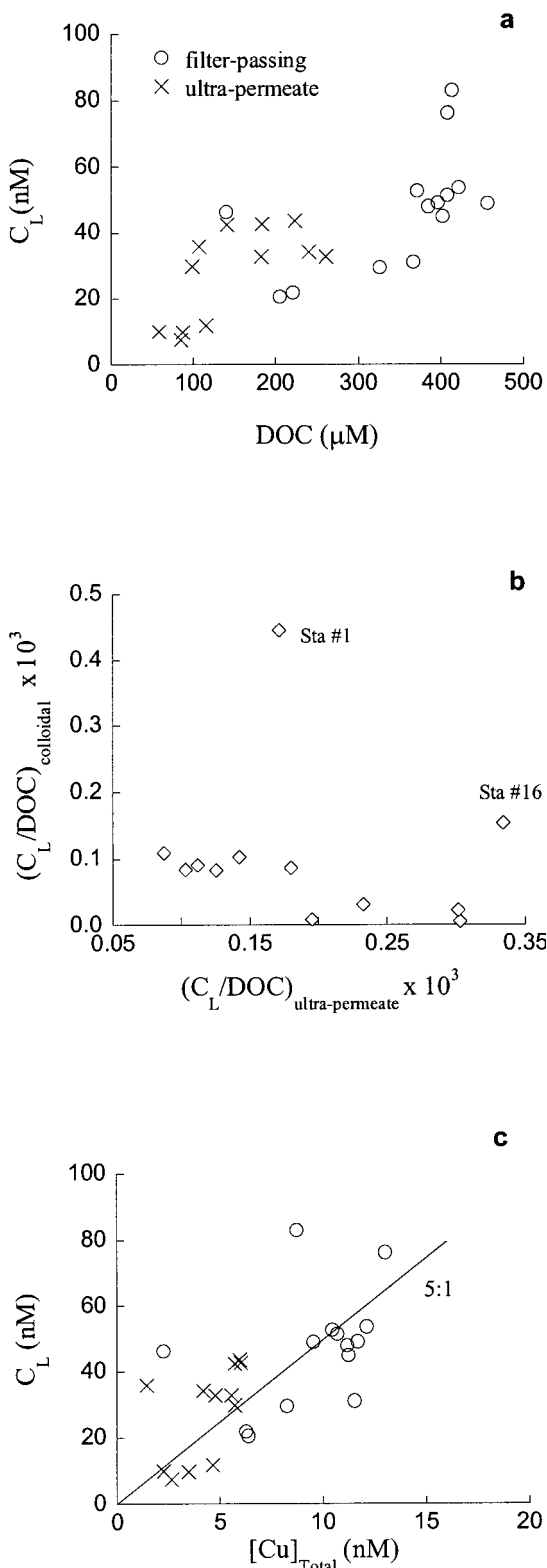
Fig. 4. (a) Cu complexing ligands ($C_L$) increase with dissolved organic carbon (DOC) in both the filter-passing and the ultrapermeate fraction. (b) Ratios of $C_L$ to DOC in the colloidal fraction are inversely related to those in the ultrapermeate fraction. (c) Relationship between concentrations of $C_L$ and total Cu.
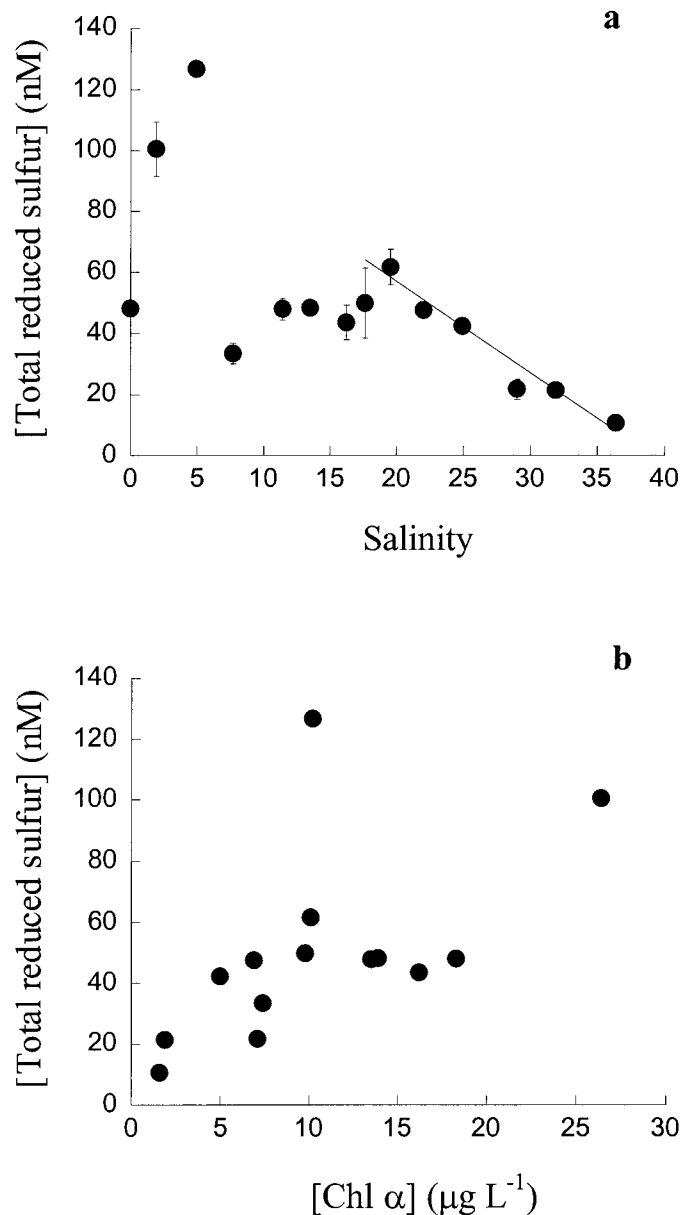


Fig. 5. (a) Total reduced sulfur (TRS) species distribution versus salinity in Galveston Bay. Error bars represent analytical errors of duplicate measurements. (b) Relationship between TRS concentration (as glutathione-equivalent) and chlorophyll *a* concentration in Galveston Bay.

the fact that these ligands are detected with one metal does not necessarily mean that all of the ligands are bound by that specific metal.

The distribution of TRS species, as a function of salinity, is shown in Fig. 5a. The concentrations of TRS decreased linearly with increasing salinity in lower Galveston Bay but were somewhat more evenly distributed in upper Galveston Bay. The concentrations of TRS in Galveston Bay ranged from 10 nM at the seawater end-member (salinity 36.4) to ~130 nM in the Anahuac Channel (salinity 4.9). These values are much higher than the dissolved sulfide concentrations (4.3 ± 0.6 nM) determined within 1 month, i.e., Sep-

tember 1999 (Tang and Santschi 2000), suggesting that TRS in Galveston Bay waters must be composed mostly of thiolic compounds. The concentrations measured here are comparable to those found in the surface waters of Mersey estuary, England, where a conservative distribution of GSH was also observed (Le Gall and van den Berg 1993). The conservative behavior of TRS in lower Galveston Bay suggests a lower reactivity of TRS than free GSH, which can be very reactive in seawater (Le Gall and van den Berg 1998). As a consequence of the quasiconservative behavior of TRS, its turnover time must have been longer than the water mixing time.

In general, DOC : TRS molar ratios fell between 5,000 and 20,000 (Tang 2000), which are far above ratios generally observed for humic or fulvic acids. For instance, Urban et al. (1999) showed that C : S ratios in lake humic acids varied from 14 to 78, with 40%–60% of the humic acid sulfur in an "oxidized" form (e.g., sulfate esters and sulfonates) and a significant portion present as reduced sulfur (e.g., thiols or organic sulfides). Concentrations of TRS increased with increasing concentrations of chlorophyll $a$ in Galveston Bay (Fig. 5b). The presence of low-molecular-weight thiols in the particulate fraction was related to the presence of different species of phytoplankton in Galveston Bay waters at this time (Tang et al. 2000). Laboratory studies have shown that different phytoplankton species could produce different amounts of thiolic compounds in cultures (Rijstenbil and Wijnholds 1996), which could explain the scatter in the data.

Reduced sulfur species are nonspecific metal complexation ligands that can be strongly complexed by various trace metals in natural waters (Al-Farawati and van den Berg 1999). Because Cu-thiol complexes are relatively strong (Leal and van den Berg 1998), the weaker metal-thiol complexes (i.e., Zn-thiol complexes) could become Cu complexing ligands under severe Cu stress, such as during a titration experiment or in highly contaminated waters, by displacing the more weakly bound metals. If most of the Cu-complexing ligands were thiolic compounds, one would expect the TRS and Cu complexing ligand ($C_L$) concentrations to be similar. In Fig. 6, $C_L$ and TRS data from the filter-passing fraction in the mid–Galveston Bay samples were very close to the 1 : 1 molar ratio line (with the exclusion of the seawater and Trinity River/Anahuac Channel endmember samples) with a level of confidence $(1 - p) > 0.98$. This suggests that reduced sulfur species could account for most of the Cu complexing ligands in these waters. Since both GSH (2–6 nM) (Tang et al. 2000) and dissolved sulfide (4.3 $\pm$ 0.6 nM) (Tang and Santschi 2000) concentrations were low in Galveston Bay surface waters, other thiols must play an important role in the organic complexation of Cu.

In natural waters, a continuum of ligands exists with a variety of functional groups (Buffle 1990). Recent culture studies have shown that a direct relationship exists between the production of thiols, some of the strongest ligands for Cu, and the release of Cu complexing ligands (Leal et al. 1999). The apparent conditional stability constant (log $K'$) previously obtained for Cu-thiol complexes was 11.12 for cysteine and 10.88 for GSH, when the data were treated as a 1 : 1 complex (Leal and van den Berg 1998). The average conditional stability constant obtained here for the ultrapermeate fraction (log $K' = 11.1$) was similar to these values,
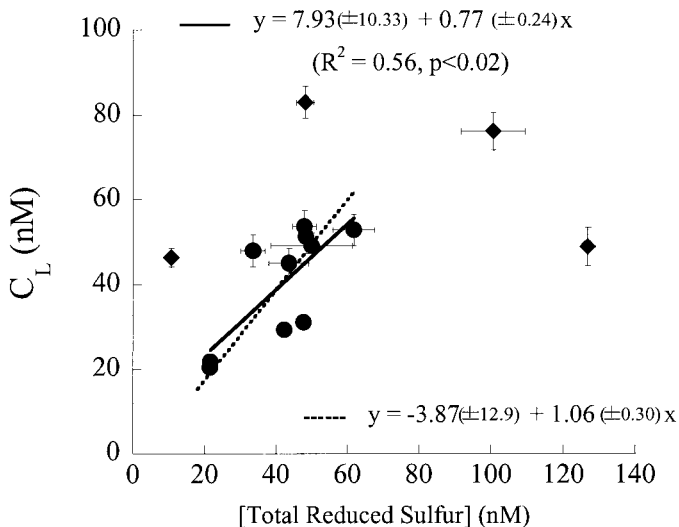


Fig. 6. Relationship between concentrations of the Cu complexing ligands ($C_L$) and total reduced sulfur (TRS) in the filter-passing fraction. The seawater end-member and Trinity River/Anahuac Channel samples (solid diamonds) are excluded in the regression. The solid and dashed lines represent results from the ordinary and orthogonal regressions, respectively.

whereas $K'$ for the filter-passing fraction was higher by an order of magnitude (log $K' = 12.3$). This is not unexpected, because of the presence of the colloidal ligands (log $K' = 12.9$) in the filter-passing fraction and most of the low molecular weight thiols being present in the ultrapermeate fraction (Tang et al. 2000).

## Summary

This study has shown the importance of colloidal ligands in the complexation of Cu, both evident from the elevated values of the conditional stability constant calculated for the colloidal fraction and the higher concentrations of Cu-complexing ligands in the filter-passing ($<0.45$ $\mu$m) fraction, compared with the ultrapermeate ($<1$ kDa) fraction. However, the existence of macromolecular ligands does not make the low-molecular-weight ligands irrelevant for Cu speciation in the traditionally defined "dissolved" phase. Greater than 99.9% of Cu was still bound by strong organic ligands in both the ultrapermeate and filter-passing fractions; however, these organic ligands were not saturated with Cu in situ. This work has also provided preliminary evidence for the importance of the sulfhydryl functional groups as Cu complexing ligands in the estuarine waters of Galveston Bay, although the concentrations of the total reduced sulfur species in these waters were much lower than those of DOC.

## References

AL-FARAWATI, R., AND C. M. G. VAN DEN BERG. 1997. The determination of sulfide in seawater by flow-analysis with voltammetric detection. Mar. Chem. **57:** 277–286.
———, AND ———. 1999. Metal-sulfide complexation in seawater. Mar. Chem. **63:** 331–352.
APTE, S. C., M. J. GARNDER, AND J. E. RAVENSCROFT. 1988. An

evaluation of voltammetric titration procedures for the determination of trace metal complexation in natural waters by use of computer simulation. Anal. Chim. Acta **212:** 1–21.

———, ———, AND ———. 1990. An investigation of copper complexation in the Severn Estuary using differential pulse cathodic stripping voltrammetry. Mar. Chem. **29:** 63–75.

BUFFLE, J. 1990. Complexation reaction in aquatic systems—an analytical approach. Ellis Horwood.

COALE, K. H., AND K. W. BRULAND. 1988. Copper complexation in the North Pacific. Limnol. Oceanogr. **33:** 1084–1101.

DONAT, J. R., AND K. W. BRULAND. 1995. Trace elements in the oceans, p. 247–281. *In* B. Salbu and E. Steinnes [eds.], Trace metals in natural waters. CRC.

———, AND OTHERS. 1986. An evaluation of a C-18 solid phase extraction technique for isolating metal-organic complexes from central north Pacific Ocean waters. Mar. Chem. **18:** 85–99.

———, K. A. LAO, AND K. W. BRULAND. 1994. Speciation of dissolved copper and nickel in South San Francisco Bay, a multi-method approach. Anal. Chim. Acta **284:** 547–571.

FLORENCE, T. M. 1979. Cathodic stripping voltammetry, part I. Determination of organic sulfur compounds, flavins and porphyrins at the sub-micromolar level. J. Electroanal. Chem. **97:** 219–236.

GERRINGA, L. J. A., H. HUMMEL, AND T. C. W. MOERDIJK-PO-ORTVLIET. 1998. Relations between free copper and salinity, dissolved and particulate organic carbon in the Oosterschelde and Westerschelde. Netherlands J. Sea Res. **40:** 193–203.

GUO, L., AND P. H. SANTSCHI. 1997. Isotopic and elemental characterization of colloidal organic matter from the Chesapeake Bay and Galveston Bay. Mar. Chem. **59:** 1–15.

———, L.-S. WEN, D. TANG, AND P. H. SANTSCHI. 2000. Re-examination of cross-flow ultrafiltration for sampling marine colloids: Evidence from molecular probes. Mar. Chem. **69:** 75–90.

KAPLAN, D., D. CHRISTIAEN, AND S. ARAD. 1987. Chelating properties of extracellular polysaccharides from *Chlorella* spp. Appl. Environ. Microbiol. **53:** 2953–2956.

KOZELKA, P. B., AND K. W. BRULAND. 1998. Chemical speciation of dissolved Cu, Zn, Cd, Pb in Narragansett Bay, Rhode Island. Mar. Chem. **60:** 267–282.

LEAL, M. F. C., AND C. M. G. VAN DEN BERG. 1998. Evidence for strong copper (I) complexation by organic ligands in seawater. Aquatic Geochem. **4:** 49–75.

———, M. T. S. D. VASCONCELOS, AND C. M. G. VAN DEN BERG. 1999. Copper induced release of complexing ligands similar to thiols by *Emiliania huxleyi* in seawater cultures. Limnol. Oceanogr. **44:** 1750–1762.

LEE, G. J., B. A. AHNER, AND F. M. M. MOREL. 1996. Export of cadmium and phytochelatin by the marine diatom *Thalassiosira weissflogii.* Environ. Sci. Technol. **30:** 1814–1821.

LE GALL, A.-C., AND C. M. G. VAN DEN BERG. 1993. Cathodic stripping voltammetry of glutathione in natural waters. Analyst **118:** 1411–1415.

———, AND ———. 1998. Folic acid and glutathione in the water column of the North East Atlantic. Deep-Sea Res. (Part I) **45:** 1903–1918.

LUTHER, G. W., III, AND E. TSAMAKIS. 1989. Concentration and form of dissolved sulfide in the oxic water column of the ocean. Mar. Chem. **37:** 165–177.

MASON, A. Z., AND K. D. JENKINS. 1995. Metal detoxification in aquatic organisms, p. 479–608. *In* A Tessier and D. R. Turner [eds.], Metal speciation and bioavailability in aquatic systems. Wiley.

MCKNIGHT, D. M., AND F. M. M. MOREL. 1979. Release of weak and strong copper complexing agents by algae. Limnol. Oceanogr. **24:** 823–837.

MOFFETT, W. J., AND L. E. BRAND. 1996. Production of strong, extracellular Cu chelators by marine cyanobacteria in response to Cu stress. Limnol. Oceanogr. **41:** 388–395.

———, R. G. ZIKA, AND L. E. BRAND. 1990. Distribution and potential sources and sinks of copper chelators in the Sargasso Sea. Deep-Sea Res. **37:** 27–36.

MULLER, F. L. L. 1996. Interactions of copper, lead and cadmium with the dissolved, colloidal and particulate components of estuarine and coastal waters. Mar. Chem. **52:** 245–268.

———. 1998. Colloidal/solution partitioning of metal-seletive organic ligands, and its relevance to Cu, Pb and Cd cycling in the Firth of Clyde. Estuar. Coastal Shelf Sci. **46:** 419–437.

PAULSON, A. J., H. C. CURL, AND J. F. GENDRON. 1994. Partitioning of Cu in estuarine waters. 1. Partitioning in a poisoned system. Mar. Chem. **45:** 67–80.

POWELL, R. T., W. M. LANDING, AND J. E. BAUER. 1996. Colloidal trace metals, organic carbon and nitrogen in a southeastern U.S. estuary. Mar. Chem. **55:** 165–176.

RIJSTENBIL, J. W., AND J. A. WIJNHOLDS. 1996. HPLC analysis of nonprotein thiols in planktonic diatoms: Pool size, redox state and response to copper and cadmium exposure. Mar. Biol. **127:** 45–54.

ROZAN, T. F., G. BENOIT, AND G. W. LUTHER III. 1999. Measuring metal sulfide complexes in oxic river waters with square wave voltammetry. Environ. Sci. Tech. **33:** 3021–2026.

SKRABAL, S. A., J. R. DONAT, AND D. J. BURDIGE. 2000. Pore water distribution of dissolved copper and copper-complexing ligands in estuarine and coastal marine sediments. Geochim. Cosmochim. Acta **64:** 1843–1857.

TANG, D. 2000. Organic complexation of trace metals in the estuarine waters of Galveston Bay: The importance of reduced sulfur species. Ph.D. dissertation, Texas A&M University.

———, C.-C. HUNG, K. W. WARNKEN, AND P. H. SANTSCHI. 2000. The distribution of biogenic thiols in surface waters of Galveston Bay. Limnol. Oceanogr. **45:** 1289–1297.

———, AND P. H. SANTSCHI. 2000. Sensitive analysis of dissolved sulfide in estuarine water by solid phase extraction and HPLC detection of methylene blue. J. Chromatogr. A **883:** 305–509.

URBAN, N. R., K. ERNST, AND S. BERNASCONI. 1999. Addition of sulfur to organic matter during early diagenesis of lake sediments. Geochim. Cosmochim. Acta **63:** 837–853.

VAN DEN BERG, C. M. G. 1984. Determination of the complexing capacity and conditional stability constans of complexes of copper (II) with natural organic ligands in seawater by cathodic stripping voltammetry of copper-catechol complex ions. Mar. Chem. **15:** 1–18.

———, AND J. R. DONAT. 1992. Determination and data evaluation of copper complexation by organic ligands in sea water using cathodic stripping voltammetry at varying detection windows. Anal. Chim. Acta **285:** 281–291.

———, A. G. MERKS, AND E. K. DUURSMA. 1987. Organic complexation and its control of the dissolved concentrations of copper and zinc in the Scheldt estuary. Estuar. Coastal Shelf Sci. **24:** 785–797.

WARNKEN, K. W., D. TANG, P. H. SANTSCHI, AND G. A. GILL. 2000. Performance optimization of a commercially available imino-diacetate resin for the determination of Mn, Ni, Cu, Cd and Pb by on-line preconcentration ICP-MS. Anal. Chim. Acta. **423:** 265–276.

WELLS, M. L., P. B. KOZELKA, AND K. W. BRULAND. 1998. The complexation of ''dissolved'' Cu, Zn, Cd and Pb by soluble and colloidal organic matter in Narragansett Bay, RI. Mar. Chem. **62:** 203–217.

WEN, L.-S., P. H. SANTSCHI, G. A. GILL, AND C. PATERNOSTRO.

1999. Estuarine trace metal distributions in Galveston Bay: Importance of colloidal forms in speciation of the dissolved phase. Mar. Chem. **63:** 185–212.

———, M. C. Stordal, D. Tang, G. A. Gill, and P. H. Santschi. 1996. An ultraclean cross-flow ultrafiltration technique for the study of trace metal phase speciation in seawater. Mar. Chem. **55:** 129–152.

XUE, H. B., AND L. SIGG. 1993. Free cupric ion concentration and Cu(II) speciation in a eutrophic lake. Limnol. Oceanogr. **38:** 1200–1213.

———, AND ———. 1999. Comparison of the complexation of Cu and Cd by humic or fulvic acids and by ligands observed in lake waters. Aquatic Geochem. **5:** 313–335.