THE EFFECT OF SALINITY ON BINDING OF CD, CR, CU AND ZN
TO DISSOLVED ORGANIC MATTER

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ABSTRACT

Salinity can affect binding of metals to humic acid and thus affect bioavailability, however, the effects are poorly understood. The fraction of Cu, Zn, Cd and Cr (at 100 μg/L) complexed with Suwannee River Humic Acid (SRHA) was measured at salinities of 0, 1, 3, 10, and 15 psu using 1000 Dalton ultrafiltration membranes. In distilled water, 10 mg SRHA/L bound at least 40% of each metal. Except for Cu, metal binding decreased to less than 20% at salinities between 1 and 15 psu. Copper binding decreased to 27% at 3 psu, but increased to approximately 60% bound at 15 psu. Copper complexation with natural dissolved organics from a local estuary was also measured and ranged from 25 to 100%. Published by Elsevier Science Ltd

INTRODUCTION

The importance of metal speciation and metal-organic interaction has been pointed out by several investigators [1-3]. Frimmel [2] also pointed out the need to conduct investigations of humic-metal interactions under natural conditions. Environmental conditions can have dramatic effects on the binding of chemicals (especially metals) to dissolved organic matter (DOM) [3-9]. Much of this research has been conducted in freshwater and has been concerned with the effects of pH or competition from ions common to freshwater (i.e., Ca and Mg). Studies of the effect of humic acid (HA) in the marine and estuarine environments have been less common [10-13], and much remains to be learned about the effects of salinity on binding and bioaccumulation of chemicals bound to HA.

This research was undertaken to characterize the effect of salinity on the complexation of HA with Cd, Cr, Cu, and Zn. These metals are frequently introduced to river and estuarine environments through

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anthropogenic sources. According to the Toxic Chemical Release Inventory System [14], Cr, Cu, and Zn are among the top ten toxic chemicals released into the Gulf of Mexico. Copper is particularly common as a pollutant in the estuarine environment due to its use in antifouling paint and in treated wood [15].

The binding of metals to DOM is ligand specific and dependent on the number and type of ligands on the organic molecules and is, therefore, also dependent on the concentration of all metal ions and the competition between them. In addition, in marine and estuarine waters, inorganic ligands (i.e., Cl⁻) are also formed and make the measurements more complex. The use of dialysis provides a general separation into organic and inorganic forms. It is important to understand the metal binding characteristics of DOM because it can affect the bioavailability of metals to marine organisms. Knowledge of the bioavailability is necessary in order to make better predictions of the environmental risk of various metal pollutants. In this study, the well-defined and characterized Suwannee River Humic Acid (SRHA) [16] was used as a standard for humic binding measurements. In addition to the effect of salinity on the binding of Cd, Cr, Cu, and Zn to SRHA, the binding of Cu to DOM in water samples from Perdido Bay was also investigated. These samples represent a variety of DOM sources commonly found in estuaries.

MATERIALS AND METHODS

Laboratory binding experiments

Metal-humic binding experiments were carried out in 50 mL plastic centrifuge tubes (Falcon # 2098, Beckton Dickereson, Lincoln Park, NJ)[2] with 50 mL of humic solution in artificial seawater at different salinities with a final metal concentration of 100 μg/L. Artificial seawater used in all laboratory binding experiments was prepared using the Marine Biological Laboratory (MBL) formula [17] at half strength and was adjusted to the desired salinity as needed. Binding affinity measurements were made using salinities of 0, 1, 3, 10, and 15 psu. The 100 μg/L metal concentration was used in these experiments and was necessary to be able to accurately measure the metal concentration in the dialysis cell effluent since most of the metal was retained in the dialysis cell. Triplicate samples of each metal solution were prepared and allowed to equilibrate with the HA for approximately 24 hours before being subjected to dialysis. The pH of the samples was allowed to change with salinity and was measured in only one set of the samples since all samples were prepared from the same solutions (Table 1).

An Amicon® (Amicon Inc., Beverly, MA) stirred dialysis cell (model 8050) with YM1 membranes having a nominal pore size of 1000 Daltons was used for all dialysis experiments. The YM1 ultrafiltration membranes used in these experiments were determined to be permeable to each of the dissolved metals used

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[2] Mention of trade names does not constitute endorsement by the US Environmental Protection Agency.

Table 1. Binding measurements made on Suwannee River Humic Acid (SRHA) with artificial seawater (MBL formula) and Cu. In the first experiment, Cu concentration was 100 μg/L, SRHA concentration was 10 mg/L, and pH was not adjusted. In the second experiment, Cu concentration was 15 μg/L and pH was adjusted to 7.0.

Salinity (‰)	pH	% Cu Bound (100 μg Cu/L)		% Cu Bound (pH 7.0, 15 μg Cu/L)
0	6.6	73		89
1	6.0	31		88
3	6.6	27		84
10	7.0	39		79
15	7.4	60		78

by passing a distilled water solution of the metal through the membrane and comparing the concentration before and after passage. Measurements of the organic carbon in dialysates indicate that less than 5% of the organic carbon from SRHA passed through the dialysis membrane. The binding of HA with metals was determined by alternately measuring the concentration of metal in test solutions before and after passage through the dialysis membrane. The concentration of metal in the solution after passage through the dialysis membrane is considered to be the "free" metal concentration. The use of the term "free" in these experiments is not limited to the ionic forms, but is applied here to all forms of the metals passing through the dialysis membrane. Both inorganic complexes and organic forms that are complexed with small (<1000 Dalton) organic molecules may be included in this fraction.

For each test solution analyzed, the dialysis cell was rinsed with a small amount of the test solution, filled with approximately 30 mL of solution, and then pressurized to approximately 350 kPa to begin collecting the effluent. The first 1-2 mL were discarded, then 10-15 mL were collected for measurement of the effluent metal content. The concentrations of the metals in the humic solution before dialysis and in the effluent were analyzed using a Varian Spectra 400® (Varian Associates, Sugarland, TX) flame Atomic Absorption Spectrophotometer (AAS). Detection limits for Cd, Cu, and Zn were below 5 μg/L; for Cr, the detection limit was approximately 10 μg/L (based on three times the standard deviation of the lowest standard).

An additional Cu/SRHA binding experiment was carried out using pH controlled solutions at the same salinities and SRHA concentration as was used above with only 15 μg Cu/L. Before the pH was adjusted to 7.0, the measured pH of each solution was similar to those shown in Table 1. The Cu

concentration in these samples were measured by Dr. Ralph Smith at Skidaway Institute of Oceanography using ICP-MS(method 200.8). Method detection limit for Cu was 20 ng/L.

Natural humic binding studies

To examine the binding affinity of natural humic material for Cu, a series of water samples were collected on June 30, 1994 from a local estuary (Perdido Bay, AL/FL - Figure 1), following heavy rains which produced dark brown color in the water (11.5 inches of rainfall were recorded in Pensacola between
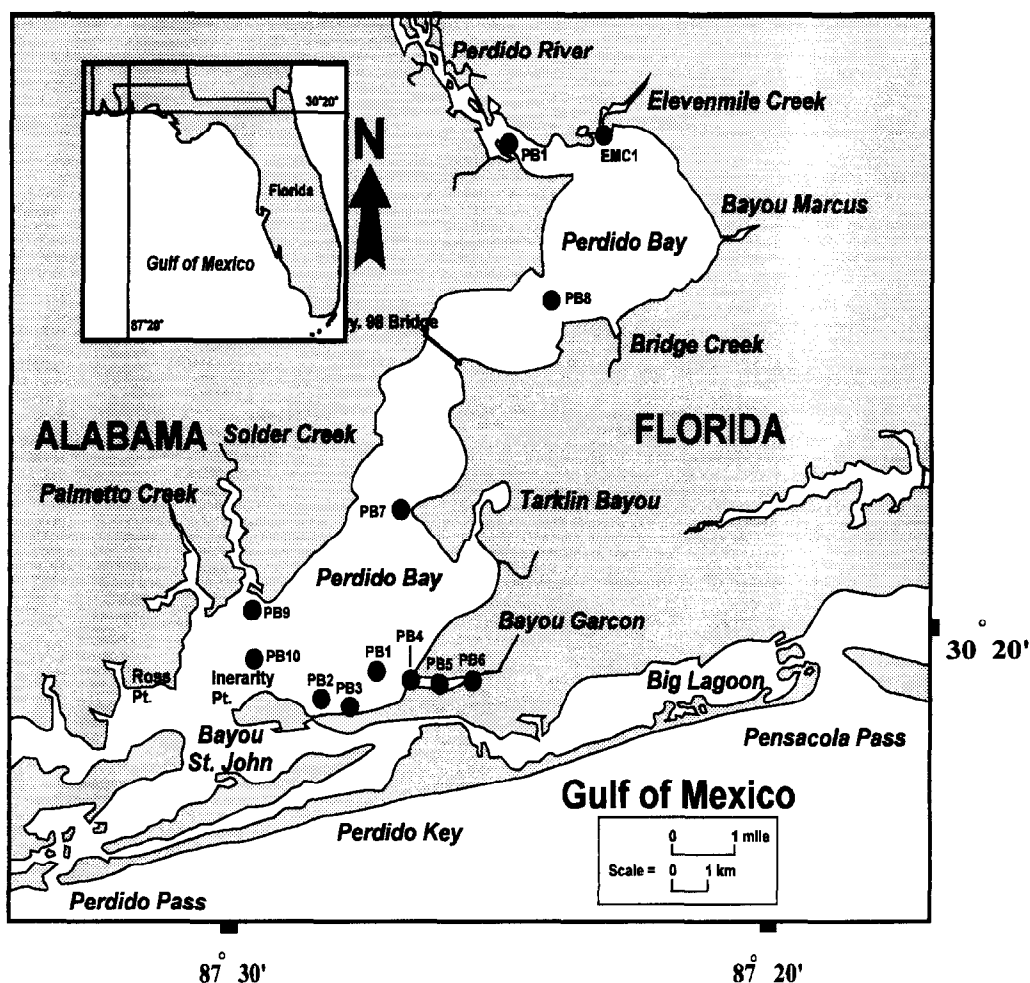
Figure 1. Sampling stations for water collected from Perdido Bay and used in Cu binding experiments. Samples were collected on 6/30/94 after heavy rains which colored the water dark brown.

6/20/94 and 6/25/94 by the National Weather Service). Surface water samples were taken in 1L glass sample jars (not pre-combusted) at each site, except at PB-6 (Figure 1) where a bottom (depth , 1.2 m) sample was also taken. Samples were placed on ice and then stored at 4°C on return to the laboratory. Surface to bottom water quality profiles including pH, dissolved oxygen content, temperature, and salinity were taken at the surface and at 1.0 m intervals at each site using a Hydrolab H-20 DataSonde® (Hydrolab Corp., Austin, TX). In addition, Secchi disc transparency depth was also measured at each site (Table 2).

Table 2. Water quality data from measurements taken on site with Hydrolab DataSonde H20® and Secchi disc depth of transparency for samples collected from Perdido Bay 6/30/94. See Figure 1 for location of sampling sites. Dissolved organic carbon (DOC) concentration of samples and copper binding expressed as ratio of Cu retained in dialysis cell relative to DOC concentration.

Site	pH	Salinity (‰)	Depth of Secchi (m)	Dissolved Organic Carbon (mg/L)	Cu/C Ratio (μg Cu/mg C)
PR-1	8.4	0.2	0.70	26	0.89
EMC-1	8.1	0.4	0.60	34	2.6
PB-1	7.7	8.7	0.90	8.9	5.8
PB-2	7.7	8.7	0.55	9.3	5.4
PB-3	7.7	8.2	0.60	22	2.4
PB-4	7.4	4.8	0.60	12	6.8
PB-5	5.7	1.7	0.50	47	1.6
PB-6S†	4.8	1.8	0.55	41	1.7
PB-6B†	5.9	10.2	NA	41	1.8
PB-7	8.3	5.0	0.55	29	1.4
PB-8	8.1	1.4	0.50	22	3.1
PB-9	8.5	5.2	0.75	18	4.2
PB-10	8.2	8.1	0.80	sample lost	NA

† A surface and a bottom sample were taken from this site, the bottom sample was taken

from a depth of 1.2 m.

NA - Not Applicable

In the laboratory, subsamples for DOC were filtered through pre-combusted Whatman GF/F filters into pre-combusted glass vials the day after collection and stored at -70°C until analysis. The DOC content

was measured on a Shimadzu TOC-5000® (Shimadzu Corp., Columbia, MD) total organic carbon analyzer (Table 2). The detection limit was below 1.0 mg/L and the coefficient of variation for all samples and standards was less than 3%.

The measurement of the Cu binding affinity of the natural dissolved organics in these samples was also begun the day after collection without any alteration of the samples except filtration through 0.45 μm filters. A 50 mL subsample was fortified with 100 μg Cu/L and allowed to equilibrate for at least two days. The fraction of Cu bound to the dissolved organics was determined by dialysis as with the SRHA.

Calculations

The simplest way to express binding is as a percentage of the total amount of a chemical in solution. The percentage of a metal bound is calculated as:

$$\% \; Bound \; = \frac{C_T - C_F}{C_T} \, x \, 100 \tag{1}$$

Where: C_T = total concentration of the metal before dialysis (bound and free); C_F = concentration of the metal in the dialysis effluent (free). The problem with expressing binding in this simple form is that it only applies to a single DOC concentration and does not provide any predictive capability for other DOC concentrations. More complex methods used by physical chemist involve titration of the DOM. Specific metal binding relationships can be determined by titration of the ligands on DOM while measuring true metal ion concentrations (i.e., Cu^{++}) using ion specific electrodes. These methods can determine the binding affinity and binding capacity of the various functional groups on the DOM (e.g., [4]), however, these methods are beyond the scope of this study.

RESULTS AND DISCUSSION

Binding of Cu, Zn, Cd, and Cr to SRHA were all similar in deionized/distilled water with approximately 40-70 % of the metal bound in each case (Figure 2). There was a sharp decrease in the binding of all of the metals with the first addition of the artificial seawater. As salinity increased Cu binding reached a minimum of approximately 28 % bound at 3 psu; however, the binding then increased to approximately 60 % bound as the salinity was increased to 15 psu. For all other metals, the binding remained around 0-10% as the salinity increased to 15 psu. The pH of the samples used in the binding measurements with 100 μg metal/L were not adjusted, but was allowed to change with salinity and showed a minimum at 1 psu (Table 1). The drop in pH at 1 psu is curious, since it was caused by the addition of artificial seawater with a pH > 7.0. However, since there was no buffer present, it is presumed that the addition of cations in the seawater

Cd/HA BINDING

Cr/HA BINDING

Cu/HA BINDING

Zn/HA BINDING
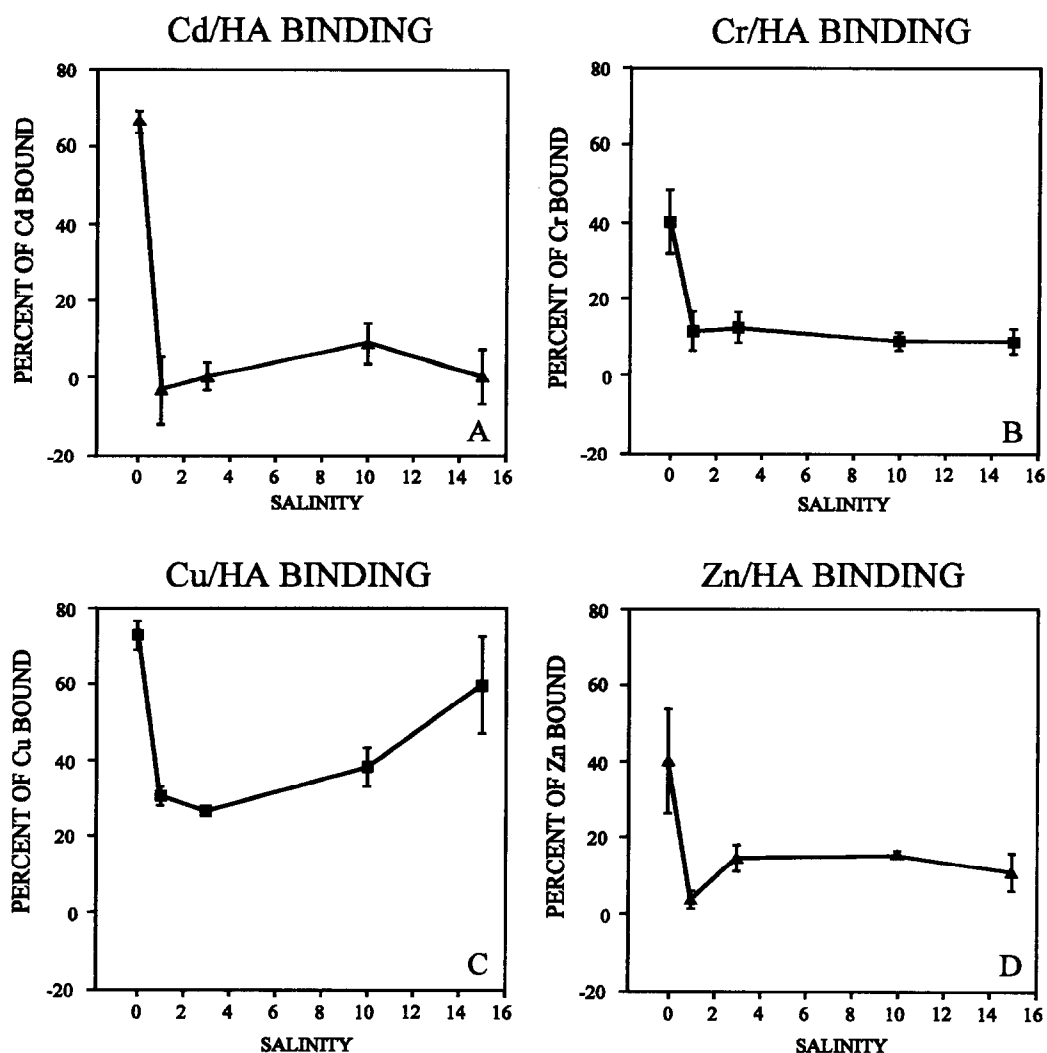
Figure 2. The effect of salinity on the binding affinity of Suwannee River Humic Acid (SRHA) for: a) Cd, b) Cr, c) Cu, and d) Zn expressed as the percent bound to the humic acid. These measurements were obtained using a dialysis membrane with a 1000 Dalton pore size. The metal concentration was 100 μg/L and the SRHA concentration was 10 mg/L for all tests.

may have displaced protons from the humic acid. In the later experiment to measure the effect of salinity on Cu binding at a constant pH, the pH of the prepared solutions was recorded before adjusting to pH 7.0. The initial pH in the DI water with 10 mg SRHA/L was 7.1, the initial pH of the 1 psu treatment was 6.2. The

initial pH in the remaining treatments increased with salinity as before. When the pH was held constant at 7.0 and the binding measurements were made using 15 μg Cu/L, the binding decreased with increasing salinity and the overall binding was higher due to the lower Cu concentration. Table 1 shows the % of Cu bound to SRHA as a function of salinity for both of these experiments.

The effects of salinity on the binding of Cu in these studies indicate a complex interaction between HA, salinity, pH and Cu. The concentration of 100 μg/L for the metals in this experiment is above expected environmental concentrations and may have exceeded the capacity of certain types of binding sites for some metals, but the higher concentration was needed to accurately measure the metals in the dialysis cell effluent by AA spectrophotometry. The drop in Cu binding at low salinity in the experiment with 100 μg Cu/L was unexpected. Whether the lower binding at low salinity is due to the competitive interaction of other ions, involvement of weaker ligands at higher Cu/HA ratios, interactions of saltwater and humic acids causing changes in pH or to changes in the inorganic form of the Cu (i.e., $CuCl+$, $CuCl_2$) is uncertain. Based on models of the effects of pH and ionic strength [4, 7, 8, 18-21] much of that interaction was probably due to the pH changes, since the pH decreased to 6.0 at low salinities then increased to 7.3 at higher salinity, and the constant pH samples analyzed by Skidaway did not show the same minimum in binding. In the experiment at a constant pH of 7.0 and 15 μg Cu/L, Cu binding decreased slightly with increasing salinity (Table 1) and the % Cu bound was also higher at all salinities, indicating that the measurements made with 100 μg Cu/L did saturate some of the binding sites on the SRHA. Some increased Cu binding with salinity was reported by Fukushima [20], who suggested that at least part of the increase in binding is likely due to changes in the conformation of the humic molecules exposing more Cu binding sites. Changes in the dissociation constants of competitive cations and interactions with various other ions in seawater and on HA are likely involved as well.

The water samples from Perdido Bay showed a range of binding affinities for Cu (Figure 3). The percentage of Cu bound ranged from 30% to 100%. The lowest overall binding was found in the sample from Perdido River (PR-1 in Figure 1), while the highest overall binding was found in the samples from Elevenmile Creek (EMC-1) and the mouth of Bayou Garcon (PB-4). Binding is also expressed as the ratio of Cu retained in the dialysis cell to the DOC concentration in Table 2. The results from the water quality and the DOC measurements for the samples from Perdido Bay are also shown and reflect the high levels of dissolved organics (8.9 to 47 mg C/L) which resulted from the heavy rains. The pH in these samples ranged from 4.8 to 8.5, while the salinity ranged from 0.2 to 8.7 at the surface and was 10.2 for the bottom sample from upper Bayou Garcon. Since these samples were taken after an unusually heavy rain event, they may not represent the normal conditions in the bay, but since the Cu concentration were high for analytical purposes, these high DOM concentrations provide a more realistic ratio of Cu to DOM than would be possible under normal conditions.
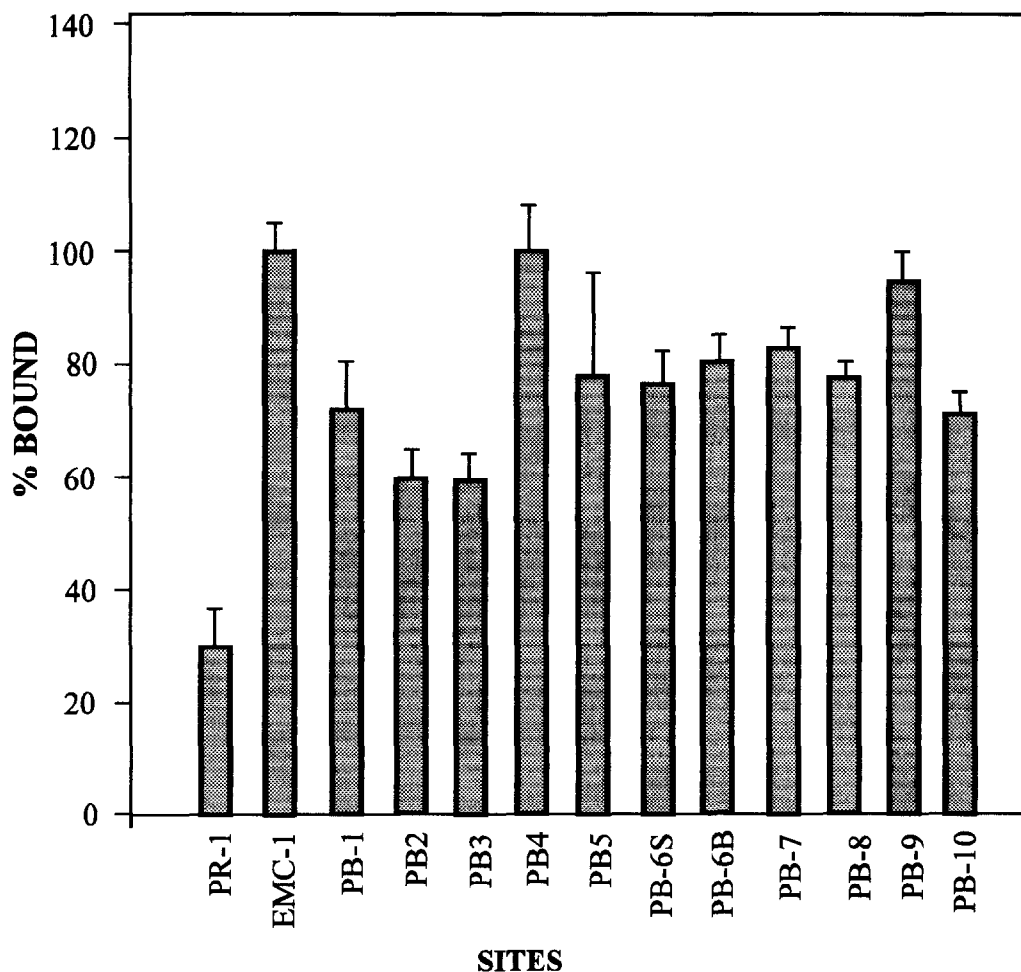
Figure 3. The binding of Cu by natural DOM from Perdido Bay. Binding is expressed as the % bound. The sites correspond to Figure 1. Error bars represent standard deviation. The water quality measurements and total dissolved carbon values are shown in Table 1.

The dissolved organics in Perdido Bay result from both natural and anthropogenic sources. These organic mixtures include humic acids as well as other organic materials that may not be involved with Cu binding. The organics entering through Perdido River are predominantly from riverine bottomland and freshwater swamps. The dissolved organics entering from Elevenmile Creek are predominately from pulp waste from paper manufacturing process. The dissolved organics from PB-4, 5, and 6 (Bayou Garcon) are

predominately of a saltmarsh origin. Those samples taken from the middle of the bay represent a mixture of the various sources above as well as possible sources of dissolved organics from the intracoastal waterway. The wide variety of dissolved organic sources in Perdido Bay make it difficult to see patterns in Cu binding associated with pH and salinity. However, when the binding affinity of the samples is compared based on sample location, some patterns do appear. The low binding of Cu by DOM in the sample from the mouth of Perdido River (PR1) is similar to the low binding by SRHA at low salinities. Both are primarily natural riverine material, although SRHA is an isolated HA, while the DOM from PR1 is bulk material. When the samples from Bayou Garcon are compared with each other, the patterns found in the laboratory experiments emerge. As the pH increases from 4.8 at PB-6 to 7.4 at PB-4 in the mouth of Garcon Bayou, the binding increases. Binding also increases with salinity when one compares PB-6S with PB-6B or PB-4. The generally high binding in the saltmarsh samples may be related to structural characteristics common to humics from this type of environment. Studies on binding and functional characteristics of estuarine and near-coastal marine humics [11, 22] found a higher nitrogen to carbon (N/C) ratio in these humics. Alberts et al.[11] demonstrated a correlation of Cu binding with the N/C ratio of the DOM, and nitrogenous functional groups also have a high binding affinity for Cu. The high binding by the sample from Elevenmile Creek is expected, since it is primarily sulfolignin from pulp mill waste and these high molecular weight molecules are known to have high binding affinities [23]. The remaining samples are all mixtures of types of DOM and the differences in binding affinities could be related to the type of material or to the environmental conditions.

The DOC levels in these samples are the result of an episodic event, and the nature of the DOC may be different from that found under more normal conditions. However, from an ecotoxicological perspective, these binding studies indicate the importance of DOM in establishing realistic risk assessment for Cu in the marine environment. When considering these binding measurements, it is important to realize that they apply only to a particular metal binding with a specific DOM (or type of DOM) under a specific set of environmental conditions. Predictions of the effects of DOM on bioavailability must be based on our knowledge of the type of DOM and how binding changes with environmental parameters such as pH and salinity. There are numerous different functional groups associated with humic molecules with various binding affinities, and the number and type of functional groups changes with the source of the material [24-26]. More specific binding measurements correlated with DOM sources and characteristics under a variety of environmental conditions are needed before realistic predictions of the effects of DOM on bioavailability can be established for chemicals in the marine and estuarine environment. However, direct measurement of the binding may be useful for site-specific predictions.

Substantial complexation of Cu in seawater has been observed by others [27-29]. Williams [29] found up to 28% of the Cu in natural waters around San Diego was organically complexed. In studies on the organic complexation of Cu and Zn in natural waters at salinities of approximately 5 to 15 psu, van den Berg

et al. [28] found that significant portions (generally greater than 50 %) of the Cu was complexed with organic ligands in natural waters of the Scheldt Estuary, (generally less than 50% of the Zn was complexed).

The release of bound metals from DOM as these materials mix with marine waters could have implications of increased risk from metals that are entering estuaries through riverine sources. For most metals in this study the results are similar to those found by Comans and van Dijk [30] who found that increasing salinity caused the release of Cd from particulates as river water is mixed with marine water. They suggest that the release is due to competition between the particles and the chloride ions for the Cd^{++} ions. As they pointed out, when river waters enter an estuary, these released metals could become available to estuarine organisms. Similar processes are to be expected for DOM and other metals. More research is needed on changes in availability of chemicals associated with DOM as they undergo changes related to estuarine conditions, especially in the oligohaline regions.

The complex behavior of humic binding at various salinities described in these experiments should enhance our understanding of this phenomenon and improve the assessment of site-specific environmental risk for metals. The complexity of the binding of metals to DOM under marine and estuarine conditions may explain why in some cases binding to DOM results in a decrease in availability, while in others the binding is associated with an increase in availability. Changes in the environmental conditions such as pH and salinity, as well as changes in the concentration and sources of DOM, can have dramatic effects on the bioavailability of these bound materials. One of the major environmental consequences of humic-metal binding is the transportability of the humic bound metals. Humic complexes may form with metals in sediments, especially in paraders. As a result, metal-humic complexes can then be transported into the water column and can release the complexed metals as they establish equilibrium with the water. The free metal is then available for uptake by aquatic organisms.

If the bioavailability of metals can be related to the concentration of free metal (as determined by dialysis), then the use of dialysis to determine the free metal concentration can also be used to improve environmental risk assessment. There are many interactions involved as aqueous metals mix with estuarine waters. The metals that are complexed with organic molecules (DOM and particulates) undergo displacement as cations from seawater such as Na^+, K^+, Ca^{++}, and Mg^{++} are encountered. The ionic forms of dissolved metals (.ie., Cu^{++}, Cd^{++}) form inorganic complexes with anions in seawater (i.e., $CuCl_2$ and $CdCl_2$). The metals that are complexed with small organic molecules and the inorganic complexes will probably pass through the dialysis membrane. Although the bioavailability of all these dialyzable forms is not clearly understood, they can dissociate into the ionic forms that are generally recognized as bioavailable and the dissociation rate of the inorganic forms are generally much faster than the macromolecular organic complexes that are retained by the dialysis membrane. Therefore, dialysis can provide a "first cut" that significantly improves our ability to assess bioavailability of metals.

CONCLUSIONS

These metal binding experiments indicate that DOM is a very important environmental factor to be considered in the assessment of metal bioavailability in the marine environment. In the oligohaline region of estuaries, as humic-bound metals are mixed with seawater, organically complexed metals can be released and could become available. At a salinity 15 psu, SRHA concentration of 10 mg/L bound over 50 % of the Cu. This binding of Cu to DOM under estuarine conditions will probably reduce the uptake or bioavailability for most estuarine organisms, protecting them from adverse effects. For the other metals, Cd, Cr, and Zn, the experiments demonstrate that DOM will have less impact on the bioavailability of metals in the marine environment. However, there is reason to consider the results of these experiments in areas where there may be a significant freshwater input of humic bound metals. In these cases, the displacement of bound metals by competitive ions in estuarine and marine waters may make these metals more available for uptake, especially in the upper regions of estuaries.

The variations in binding affinity by various types of DOM indicate the complexity of this issue. Continued efforts to measure the binding affinity of a wide variety of DOM types may ultimately produce patterns that will be more obvious. Additional studies are needed to correlate these findings with the bioavailability of metals to marine and estuarine organisms.

ACKNOWLEDGMENTS

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