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WILLIAM RAY LAMMELA

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A STUDY
OF THE METAL-BINDING ORGANIC CONSTITUENTS
IN GREAT BAY SEDIMENTARY SYSTEMS

BY

WILLIAM R. LAMMELA
B.S., Keene State College, 1977

A DISSERTATION

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Doctor of Philosophy
in
Chemistry

December, 1981
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Date
This dissertation is dedicated to my parents, Amos and Fran Lammela, whose unending love, encouragement, and support enabled me to complete this work.
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ABSTRACT

A STUDY OF THE METAL-BINDING ORGANIC CONSTITUENTS IN GREAT BAY SEDIMENTARY SYSTEMS

by

William R. Lammela

University of New Hampshire, December, 1981

The fact that the concentration of copper in pore fluids exceeds the values predicted by simple solubility products has led to the suggestion that the copper is solubilized by the formation of copper-organic matter complexes. Previous attempts by other workers to form these complexes have utilized material that may not be representative of organics naturally present in sediments.

Organic matter was isolated from anoxic sediments of Great Bay estuary in New Hampshire. Pore water, a double-deionized water extract, and an artificial seawater extract were used to isolate organic matter from the sediments. Dissolved organic carbon measurements revealed that the seawater and deionized water extractants removed considerably more material than the pore water isolation procedure. The process responsible for this increased amount of material was suggested to be abrasion of sediment particles during agitation, which resulted in the removal of organic coatings. Negligible effects were noted for temperature and microbial activity. Amicon ultrafiltration studies showed that, compared to pore water, the seawater and deionized water extracts had much more material with molecular weight greater than
nominally 500 amu. Reversed-phase liquid chromatography results showed that organics in pore water were more polar than material in the other extracts. It was found that the deionized water removed more organic matter than seawater, but the latter removed organics that were more non-polar.

Liquid chromatography in conjunction with atomic absorption spectrometry was employed with the Amicon ultrafiltration system to study copper binding to organic matter. Results indicated that copper complexed with non-polar fractions of all extracts. The quantity of copper retained by ultrafiltration, which was originally thought to be entirely bound to organics, was significantly larger than values obtained previously. It was felt that the discrepancy in binding capacities may be due to the retention of copper hydroxides by the Amicon system. Kinetic studies showed that copper complexation occurred within ten minutes after the addition of metal. EPR investigations revealed the presence of multiple copper(II) species in all samples.

Binding studies with iron(II) revealed that this metal complexed with the same fractions of organic matter as did the copper. This indicated that the copper-binding material could bind other trace metals as well.
CHAPTER 1

INTRODUCTION

Marine life depends on many different trace metals for normal growth, in a way analogous to terrestrial organisms (Riley and Chester, 1971; Lehninger, 1975). For example, copper is essential in many enzymes and, as a complexed ion, serves as a cofactor in oxidation-reduction cycles. Yet, at higher concentrations, copper can be extremely toxic, the toxicity being dependent on the form of copper present (Sunda and Guillard, 1976; McKnight, 1981). Because of the biological importance of this metal, a great deal of work has been done attempting to determine the speciation of copper in estuaries and oceans.

One species that is of particular interest from both a chemical and biological viewpoint is the chelation of copper with naturally occurring organic matter. Considerable work has been done on the elucidation of these organo-metallic complexes, and many of their physico-chemical properties have been well established for soil systems. Although the possibility of analogous complexes existing in estuarine sediments is not a new concept, their presence has never been demonstrated.

The possibility that trace metals, including copper, are complexed by organic matter in the sediments received serious consideration when various workers discovered that the concentrations of trace metals in pore fluids exceeded the values predicted by simple solubility products with inorganic anions (Brooks et al., 1968; Presley et al., 1972; Duchart et al., 1973; Rashid and Leonard, 1973; Nissenbaum and Swaine, 1976; Lyons, 1979). Brooks (1968) and Presley (1972) attributed these
increased levels to the formation of metal complexes with dissolved organic matter. Since then, much work has been done with both soil samples and natural waters, utilizing humic substances and other organic matter extracts in an attempt to isolate or synthesize these copper-organic matter species. However, as many studies have pointed out, metal-organic complexes have not been found in natural estuarine or marine sediment samples (Pocklington, 1977). Also, attempts to form these complexes in the laboratory have utilized material with questionable similarities to those organics naturally present in the sediments.

Copper in Estuarine Systems

The world average concentration of dissolved copper in incoming river water is about 7 ug/l (Turekian, 1969). When this dissolved metal enters an estuarine environment, the prevalent copper species is predicted thermodynamically to be a chelate with various inorganic ions, such as carbonate and hydroxide (Zurino and Yamato, 1972; Mantoura et al., 1978).

The major portion (90%-95%) of the copper entering an estuary is associated with discrete mineral particles with the copper generally being an integral part of the mineral lattice (Gibbs, 1973; de Groot et al., 1976). Pravdic (1970) found that this particulate material always has a net negative charge, but as the salinity increases, the net surface charge decreases. An inversion occurs at 2 o/oo salinity and a net positive charge appears at 6 o/oo salinity. The exact point of this reversal varies, and is dependent on the amount of organic matter in the system (de Groot et al., 1976). In addition,
as the ionic strength increases, the repulsive forces (which keep many of the suspended particulates from flocculating) decreases, and eventually the material precipitates out of solution.

Other common processes which contribute to the deposition of copper into sediment are:
1. Adsorption onto and incorporation into the oxides of iron and manganese.
2. Chelation of copper by organic matter (living organisms, detrital material, etc.).

Aston and Chester (1976) found that the world-wide average copper concentration of river-borne detrital material was 2500 ppm. Furthermore, in estuaries many organisms take up copper from the surrounding sea water and incorporate it into their skeletal structure and soft tissue. When these organisms die, their remains fall to the sediment, depositing the metal. Schmidt et al. (1978) determined that the total concentration of copper in San Francisco sediments is about 800 ug/l, with the soluble component not exceeding 5% of the total copper.

**Copper in Sedimentary Systems**

Once buried in sediments, the various forms of copper "hosts" undergo numerous processes: decomposition, oxidation-reduction, dissolution, destabilization, etc. The net result is that copper is distributed among several phases, much as it is in the water column (Gibbs, 1973). Jenne (1968) found that this distribution varies widely with geographical location of an estuary.

Much of the copper is associated with iron-manganese oxides as it is deposited into sediments. As sediments become anoxic (due to
bacterial activity), these oxides become unstable and release occluded copper to the pore water. The net observed effect is an increased concentration of dissolved copper with depth below the sediment-water interface (Duchart et al., 1973). At the same time, the concentration of reduced bisulfide is increasing because of the activity of sulfate-reducing bacteria (Berrer, 1980). Therefore, the solubility product of copper sulfide is exceeded close to the sediment-water interface, and precipitation occurs.

Clays could potentially play an important role in the copper chemistry of the sediments. Adsorption occurs within clays following the sequence:

\[
\text{Smectite} \rightarrow \text{Illite} \rightarrow \text{Kaolinite.}
\]

This is to be expected based on interstitial size considerations. However, as sediments are a conglomeration of clays, oxides, and organics, the influence of the clay particles themselves on the complexation of copper is expected to be minimal (Ermolenko, 1972).

It was found that humic materials were even better at binding copper than any clay (Reimer and Toth, 1970). Based on the Irving-Williams sequence (1953), copper is among the best trace metal ions for chelation to organic materials; therefore, the possibility of copper-organic complexes is not surprising. Willey and Fitzgerald (1980) found that more than 40% of the total copper was present in oxidizable and organic forms in Miramichi, New Brunswick estuarine sediments.
Organic Matter in Sedimentary Systems

Naturally occurring organic matter is commonly divided into two fractions based on particle size. That material which passes a 0.5 um filter is defined as dissolved, and that which is retained is particulate (Riley and Chester, 1971). The world average concentration of dissolved organic matter (DOM) content in river water is 10 mg/l (Beck et al., 1974), and in sea water the level ranges from 0.5-5 mg/l (Manheim et al., 1970). Intermediate values are found in estuaries for total organic carbon (TOC), but the distribution between various fractions may deviate from expected levels based on simple mixing considerations. In most aquatic environments, the amount of DOM is much greater than the amount of particulate organic matter (POM), but in estuarine areas, the levels are often similar. When acidic/neutral river waters mix with alkaline sea water of increased ionic strength, precipitation of many inorganic species occurs which removes dissolved organics (Head, 1976). Also, estuaries are areas of high biological productivity, where large amounts of POM and DOM are produced (POM > DOM) with a net conversion of DOM to POM. Organic matter found in estuaries is a mixture resulting from primary production within the estuary, and that which is input from adjacent ecosystems.

During mixing, removal of organic matter by coagulation is a well-established phenomenon (Sieburth and Jensen, 1969; Matson, 1968; Gardner and Menzel, 1974). Association also occurs between organic materials, clays, and ferric oxides. It has been estimated that up to 25% of humics in the sediments are deposited by this mechanism (Swanson et al., 1972). In estuaries, the majority of the organic material is terrestrially derived (Nissenbaum and Kaplan, 1972; Nissenbaum, 1974;
reaching the sea due to colloid formation around iron species (Sieburth and Jensen, 1968; Sholkovitz, 1976). Stephens et al. (1976) found that about half of the planktonic primary production was deposited on the bottom as organic detritus. During the winter, an equivalent amount of terrestrially derived material was deposited. Much material which is consumed by various organisms is recycled as fecal pellets which are deposited, as are the remains of the organism itself after it dies.

Organic Matter Complexes with Copper

Copper is the trace metal whose interaction with organic material has been most studied, particularly in soil systems. The order of stability between various metals and humic materials follows the Irving-Williams series:

$$\text{Pb}^{2+} > \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Cd}^{2+} > \text{Fe}^{2+} > \text{Mn}^{2+} > \text{Mg}^{2+}$$

therefore it seems probable, on a theoretical basis, that copper would complex with organic matter. Most work thus far has used "humic" substances (Schnitzer and Skinner, 1963; Kononova, 1966; Szalay, 1969; Rashid, 1971), and include a wide variety of chelating groups. These include salicylate (Gamble et al., 1970; Van Dijk, 1971; Stevenson et al., 1973; Buffle et al., 1977; Bresnahan et al., 1978), phthalate (Van Dijk, 1971; Manning and Ramamoorthy, 1973; Stevenson et al., 1973; Buffle et al., 1977), and groups with nitrogen donor atoms (Schnitzer and Khan, 1972; Stevenson and Arkakani, 1972).

Humics have been shown to be effective in influencing the speciation of copper when the metal is either an insoluble salt,
Gardner and Menzel, 1974). This material is severely degraded before it is buried in the sediments. This degradation in large part will be done by microbes, and therefore, the sediments will be enriched with material which is resistant to further decomposition. A significant portion of the organic matter is in the form of small colloidal particles, which are made up of polymeric material with strong resistance to biological and chemical degradation in the sedimentary environment (Eglinton and Barnes, 1976).

The organic matter in sediments consists of living and dead phytoplankton, zooplankton, meiofauna, fungi and bacteria together with fecal pellets, pollen grains, polymeric debris and inorganic particulates with adsorbed organic material. Once buried in the sediments, these various compounds undergo a great deal of transformation, mostly mediated by bacteria, but also due to the differences in chemical environment between open estuarine water and anoxic (oxygen-free) sediments. Gaskell et al. (1976) used $^{14}$C labeled fatty acids in estuarine sediments to show the decomposition of organic matter by bacteria. More recently, Orem (1981a) has done a study showing that bacteria have a significant effect on the dissolved organic carbon (DOC) content of Great Bay estuarine sediments.

The mechanisms for the deposition of organic matter in the oceans are somewhat different than those which occur in the estuarine environment. Two sources are indicated for marine humus; one from decomposition of marine plankton and the other from terrigenous organic matter. Nissenbaum and Kaplan (1972) determined that marine organic material is almost exclusively sea-derived and contains very little material from land. Soil humics are precipitated very rapidly upon
metallic cation, or mineral phase (Baker, 1973; Rashid and Leonard, 1973). Rashid (1974) found that humics bound copper by one of three mechanisms: chelation, cation exchange or surface adsorption. A wide range of metal to ligand ratios have been reported for Cu$^{2+}$-humate (or fulvate) complexes: 1:2 (Stevenson et al., 1973; Stevenson, 1977), 1:1 (Van Dijk, 1971; Stevenson, 1977), 2:1 (Schnitzer and Hansen, 1970; Bresnahan et al., 1978). Schnitzer and Skinner (1963) potentiometrically determined the stoichiometry for copper complexed with humic acids to be 1:1 (Cu:HA) at pH 3, and 2:1 (Cu:HA) at pH 5. He also determined that the binding was via carboxylate groups. Much of the variability is due to differences in the pH of the studies; Schnitzer and Hansen (1970) were the first to notice this effect. Buffle (1980) compiled a comprehensive survey of the studies done on the subject of copper-fulvic acid complexes. His work emphasizes three major points:

1. Complexation is dependent on the concentration of fulvic acid in a non-linear manner.
2. When looking at natural conditions (e.g. pH, ligand concentration), the possible formation of a precipitate of the hydrolyzed metal must be considered.
3. Mixed ligand complexes (e.g. with OH$^-$, CO$_3^{2-}$) must be considered.

Johnston (1964) demonstrated the importance of organic material in sea water as a chelator of various trace metals. Mills and Quinn (1981) have succeeded in extracting such complexes from sea water. In the interstitial fluids of sediments (pore water), copper is present in concentrations that are higher than the overlying water column (Brooks et al., 1968; Duchart et al., 1973). Brooks (1968) and Presley (1972) observed a 2-fold to 5-fold increase in copper concentration in
pore water relative to the overlying water column. Concentrations of copper in interstitial fluids of up to 380 \( \mu g/l \) have been reported by Duchart et al., 1973. These workers theorized that copper was being held in solution by an unknown complex, as the metal would normally be expected to precipitate as a sulfide under the environmental conditions present in the sediment. Krauskopf, in 1956, concluded that copper, along with other trace metals, has the greatest tendency to be concentrated in sediments rich with organic matter. Gibbs (1973) determined (based on extraction schemes) that between 3% and 6% of the copper in sediments associated with organic solids. This is one of only a few studies of copper in sediments, some others being done by Chester and Hughes (1967) and Engler (1974).

In the Rhine-Meuse and Ems estuaries (The Netherlands), it was found that the total concentration of copper in sediments had trends similar to those for the levels of organic matter: both concentrations decreasing from shore to deep ocean (de Groot et al., 1976). Schmidt et al. (1978) extracted organo-copper species from sediments in San Francisco Bay, and found that most of the copper in interstitial water was associated with organics of molecular weight less than 10,000 daltons, and up to 92% was complexed with organic compounds with molecular weights less than 500 daltons. Five classes of copper-organic compounds were isolated, ranging from ionic species to materials of molecular weights greater than 100,000 daltons. He also determined that, in San Francisco Bay sediments, the concentration of copper in pore waters did not exceed 5% of the total copper in the sediments.

Saxby (1969) has published one of the first reviews on the subject of trace metal-organic interactions in the sediments. In his review,
he stated that the form in which metals occur in sediment are uncertain, but the most prevalent theories are:

1. Complexation occurs via O, N, or S functional groups.
2. The metal is organically bound in "condensed" structures.

Saxby goes on to say that, "Metal-organic compounds could play a vital role in the formation of sedimentary sulfides, e.g. in the transport and accumulation of metals by organic materials and in the complex chemical reactions leading to metal sulfides by the action of sulfate-reducing bacteria." He further qualifies his remarks by stating, "Much of what has been said (and in geochemical literature as a whole) is full of uncertainties and speculations."

Buffle (1980) looked at the complexation under more natural conditions: pH (6-9), copper concentrations (1-100 uM), fulvic acid concentrations (2-100 mg/l), and calcium concentrations (0.1-5.0 mM). He determined that the largest variability between various authors was due to measurement and sampling artifacts, as opposed to initial differences in the samples.

**Manipulation of Samples**

**Environmental Considerations**— Once deposition has occurred in the estuary, diagenetic changes happen, many of which are bacterially mediated. In the surface layer of the sediment, aerobic bacteria rapidly deplete the dissolved oxygen to an undetectable level. Below this there is a thin layer where the bacteria *denitrifucans* reduces nitrate to ammonium ion, and below that is the zone of sulfate reduction. The organisms principally involved are *desulfovibrio* (Golhaber and Kapla, 1974). The net result of this bacterial activity is that the
sediments rapidly become anoxic and rich in reduced sulfur. Because of these environmental factors, special care must be taken to avoid exposing samples to laboratory atmosphere, which could lead to erroneous results (Loder et al., 1978; Templeton and Chasteen, 1981; Orem, 1981b). In the Great Bay estuary system, sulfate reduction has been documented as a dominant process (Lyons and Gaudette, 1979).

**Sampling and Handling Concerns**—Prior to 1973, many workers did not realize the importance of proper sample handling, thus studies done prior to this period are of questionable value because of possible alterations of the organic matter being investigated (Bray et al., 1973; Troup et al., 1974; Murray et al., 1978; Loder et al., 1978). This included two basic considerations: the procedures utilized to isolate the organic matter, and precautions necessary to prevent degradation after isolation.

Archard (1786) first reported the use of NaOH as an extractant. Oden (1919) defined humic acid as the alkali-soluble, acid insoluble organic component of soils, and fulvic acid as the component soluble at all pH values. These extractions were first criticized as being harsh by Shorey in 1930, and the question of these harsh extractants has been investigated by many workers since then (Dubach et al., 1963; Kononova, 1964; Schnitzer and Skinner, 1968; Schnitzer and Khan, 1972; Flaig et al., 1975; Cheshire et al., 1977; Stuermer and Harvey, 1978; Templeton and Chasteen, 1981). Sodium pyrophosphate was then used as a possible answer to this criticism and Kononova in 1966 reviewed this procedure.

Much controversy has developed as to the validity of using harsh extractants to isolate organic materials. Most of the studies to date
in this field still use operationally defined material (e.g. fulvic and humic acid) which is extracted under relatively harsh conditions. This procedure has been criticized as it gives results which may have little relevance to "real world" situations. (Schnitzer and Skinner, 1968; Schnitzer and Khan, 1972; Flaig et al., 1975; Templeton and Chasteen, 1981). Templeton (1980) showed quite conclusively that harsh extractants do severely alter the organic material obtained from anoxic sediments. In an attempt to prevent degradation, various organic solvents have been tried, as reported by Hayes et al. (1975). He also found that extractions performed anoxically yielded material with a higher carbon content than those done in the presence of oxygen.

Schmidt and co-workers (1978) studied interstitial water, heated water extracts, and ammonium acetate extracts, and determined that the heated water extract removed polysaccharide materials from the sediment grains. He also felt that elevated temperatures possibly caused artifacts due to aggregation of the organics. Kononova (1964) and Flaig (1968) have reviewed many of these extraction methods and have determined that many chemical and physical properties of humic materials vary with the method of isolation used.

Analysis of Organic Material and Binding Characteristics—Chemically mild extraction procedures often yield mixtures that are extremely heterogeneous, and analytically have provided little definitive data. A principal obstacle has been the lack of suitable separation techniques to yield fractions that are not degraded, yet are adequately resolved to isolate individual components, or classes of compounds. Reversed-phase liquid chromatography (RPLC) has been demonstrated to be effective in separating and isolating compounds of geochemical interest.
(Stewart and Wheaton, 1971; Hajibrahnam et al., 1978; McFadden et al., 1979; Saito and Hayno, 1979). Templeton (1980) developed a liquid chromatographic procedure which has proven to be a viable method for the separation of unaltered organic material into well resolved fractions. This method is an offshoot of that suggested by Johnson and Stevenson (1978).

Since it was known that harsh extractant yield products that are quite different from the material present in the natural environment, Templeton (1980) developed an isolation procedure using artificial seawater as an extractant. Picard and Felbeck (1976) reported that the solubility of organic matter in the sediments was enhanced in sea water relative to distilled water. Templeton (1980) reported that the amount of organic material extractable with artificial sea water was about 30 times greater than that obtained by pore water extraction procedure used by Lyons in the same location (Lyons, 1979). He suggested that the increase in DOM was due to the disruption of the organic coatings on sediment grains during shaking, but had no evidence to support this.

Ultrafiltration has been utilized extensively both as a concentration aid (Blatt et al., 1965; Pollak et al., 1968; Ellender and Sweet, 1972; Kahn and Thompson, 1976) and for the determination of binding parameters (Protein studies: Blatt et al., 1968; Handin and Cohen, 1976; Marine studies: Andren and Harriss, 1975; Guy and Chakrabarti, 1976; Smith, 1976). Ultrafiltration was first applied to marine systems by Barber in 1968. Since that time, many studies have utilized this separation and purification technique (Gjessing, 1970; Sharp, 1973; Ogura, 1974; Schindler and Alberts, 1974; Alberts et al.,

There has been considerable controversy regarding the proper method for the determination of binding parameters. Ion exchange has been used by a number of workers (Miller and Ohlrogge, 1958; Randhawa and Broadbent, 1965; Schnitzer and Skinner, 1966, 1967). Schnitzer and Hansen (1970) discussed this procedure, and the associated deficiencies. They demonstrated that a method of continuous variation was more reliable, but felt that the best approach was a combination of independent methods. Manning and Ranamooorthy (1973) used ion selective electrodes for their work, and this procedure has been used by others (Bresnahan et al., 1978; Saar and Weber, 1980). Truitt and Weber (1981) compared ISE with dialysis titration, and found few differences between the two methods. Weber's group (1975), among others, also have investigated the possibility of using anodic stripping voltammetry for the determination of stability constants for soil fulvic acid. (Matson, 1968; Chau and Lum-Shue-Chan, 1974; Batley and Florence, 1974, 1976; and Bresnahan et al., 1978).

Summary

Although a great deal of work has been done regarding copper-organic matter interactions in soil systems, and some research has been done in sea-water systems, very few studies have been attempted with copper-organic species in the sediments. Most of the investi-
gations of organic matter in the sediments have utilized procedures yielding analyte material which probably has few similarities to that actually present in the natural environment.

The overall goals for this research are:

1. To investigate various extraction procedures, with reference to the quantities and qualitative aspects of the organics isolated.
2. To elucidate the copper binding characteristics of this organic matter.
3. To observe competitive binding between cupric and ferrous ions; two metals very likely to complex with organic matter, and whose chemistry could be the most affected by these compounds.
CHAPTER 2

EXPERIMENTAL

Isolation of Material

A. Sampling Methodology—Sediment samples used in this research were taken from Adams Cove, a sub-tidal mudflat located on the west side of Great Bay estuary (Figure 2-1). This estuary is a drowned river valley, with an average tidal range of 1.85 m. Salinities vary from a few parts per thousand to those approaching open ocean values, about 35 parts per thousand (Armstrong et al., 1976).

Due to microbial activity, the sediment in this mudflat is anoxic very near the sediment-water interface. The actual location of the oxic/anoxic boundary varies seasonally, being 6-8 cm deep in the sediment in winter when bacterial activity is low, and less than 1 cm when the microbes are at maximum production of reduced sulfide. The depth of the boundary fluctuates with the amount of bioturbation and organic content of the sediments. The sediments, being rich in reduced sulfur, also contain metals in their reduced state: iron (2+) at 10-12 ppm (Loder et al., 1978), and manganese (2+) 1-2 ppm (Armstrong, 1981). Typical organic carbon content of these sediments is 1.9% for particulate organic carbon, and 30 ppm for dissolved organic carbon (DOC).

The sediments were sampled using an acid-washed, nitrogen flushed, plexiglass boxcore. The sampling site was about 30 cm below mean low water, in the central portion of the mudflat. An average core was approximately 17-20 cm in depth, with a volume of about 3500 cm$^3$. 
Figure 2-1: Map of Great Bay estuary in New Hampshire. Location of sampling site in Adams Cove designated by (x).
New Hampshire

Maine

Little Bay

GREAT BAY
All sediment samples were transported to the laboratory at ambient temperatures, 0-19°C, with water overlying the sediment. Transfer of sediment and subsequent extraction steps were conducted in a nitrogen flushed glovebox to prevent oxidation artifacts (Templeton, 1980).

B. Isolation Procedure—Once extruded into a nitrogen-flushed glovebox, the top oxic layer of sediments was removed and discarded. Each core was then divided into several sections to test various extraction procedures with the solid, wet sediment. In addition to analyzing pore water, artificial sea water (Kester et al., 1967) and double-deionized (D/D) water extracts were obtained and analyzed. Both extractants were purged with nitrogen, to remove dissolved oxygen, prior to use. In addition, sediment from core 3 was shaken without any added extractant. This sample was then processed in the same way as the other extracts.

Pore water contains organic material which is probably representative of that dissolved in the natural system. Organic material present in pore water may be at least partially responsible for the transport of trace metals in the sediments. Deionized water was chosen because this extractant may simulate the effects of ground water intrusion into the sediments. D/D extract analysis would also yield information as to the effects of ionic strength on the organic matter. This extractant would solubilize some material ordinarily coated on the sediment grains or trapped in sediment aggregates. Sedimentary organics could be involved in the transport of copper, assuming shifts in organic matter solubility; but more realistically represents compounds important for the storage of copper on the sediment grains.
Templeton's results (1980) showed that artificial seawater extraction procedure removed considerably more organic matter than is present naturally in the pore water by a factor of 4 to 8 times. He attributed at least some of this increase to the shaking process, which he felt removed organic matter from the sediment by physical abrasion. Subsequently, this "extra" organic material went into solution. However, as he had several variables in his procedure, such as shaking time, extractant, bacterial degradation; the specific influence of each individual component was never determined.

The goal was to correlate Templeton's results of extraction by artificial seawater with those obtained for pore water in this work. Sea water extractant was also expected to remove material from sediment grains. Material isolated by artificial seawater would also be expected to play a role in the storage of metals on sediment grains. This extractant may also reflect the effect of dilution by the overlying water on the organic content of pore water.

To promote the removal of organics, sediment samples were shaken on a platform shaker with extractant for ten to thirteen days. A volume of 250 ml of extractant was added to approximately 500 cm$^3$ of wet sediment. These sediment slurries were sealed, under nitrogen, in pre-cleaned polyethylene bottles. Finally, these bottles were sealed in glass jars, under nitrogen, to prevent gas diffusion. The extraction and isolation procedures are shown schematically in Figure 2-2.

Once a particular extraction procedure was completed, sediment slurries were placed in pre-cleaned, 250 ml centrifuge cones, under nitrogen, and centrifuged at 5000G for 1 hour at 0°C; the same param-
Figure 2-2: Schematic showing procedures used for the isolation of various organic matter extracts used in this research.
meters used by (Templeton 1980). Supernatant liquid was carefully
decanted and vacuum-filtered through glass-fiber filters (4.25 cm
diameter Whatman GF/C), and subsequently through 0.45μm Nuclepore
filters (2.5 cm diameter). All filters were pre-cleaned prior to use by
washing with dilute nitric acid followed by exhaustive washings with
D/D water. The first filtration removed sediment grains and was used
as a "crude" filtration: while the second filter removed some colloidal
material and smaller particulates. Analyses of the extracts for DOC
were done to determine losses of organics by filtration.

Concentration, if necessary, was accomplished using a 10 to 200
ml Amicon stirred-cell ultrafiltration system. Various concentration
factors were used of between 3 to 7 times, depending on the eventual
analysis of the prepared extract. A 5 l fiberglass reservoir was also
utilized. The cell and reservoir were pressurized with nitrogen at
60 PSI. A UM05 membrane with a nominal molecular weight cutoff of
500 daltons was used; either 25 mm in diameter for the 10 ml cell, or
62 mm in diameter for the 200 ml cell. These filters were pre-cleaned
prior to use according to the manufacturer's literature (Amicon Corp.,

C. Storage of Extracts-- To prevent bacterial degradation, mercuric
chloride or sodium azide solution was added to the extract (Hines,
1979). For early DOC and LC analyses, 1 ml of a solution of saturated
mercuric chloride was added to approximately 70 ml of extract to give
a final concentration of 1.3 mM. Because the mercuric ion could
potentially interfere with metal analyses, 2 ml of a 0.1 M solution of
sodium azide solution was added to each extract to give a final concen-
tion of about 0.8 mM.

For storage of up to 4 months, extracts were placed in pre-cleaned polyethylene bottles, under nitrogen, and placed in glass jars which were also sealed under inert atmosphere. The jars were then stored in a freezer at a temperature of -14°C, where bacterial and chemical degradation would be minimized.

Analytical Methodology

A. Amicon Ultrafiltration System— For many of these studies, a 10 or 200 ml Amicon stirred cell ultrafiltration system was utilized. Filters used were the UM05 membranes, with a nominal molecular weight cutoff of 500 daltons. These filters are made of a non-cellulosic polymer, and are anionic in nature (Amicon Corp., 1972). The cleaning procedure consisted of a preliminary wash with a 10% v/v sodium chloride solution to remove the glycerol coating, followed by exhaustive washings with D/D water. The apparatus itself was soaked in 10% v/v hydrochloric acid, and rinsed thoroughly with D/D water. This latter procedure was also used for the reservoir. Nitrogen was used to pressurize the cell at 60PSI and the final flow of effluent was about 0.8 ml/hr for the 10 ml cell, and 30 ml/hr for the 200 ml cell. A diagram of the set-up used for binding analyses including the fraction collector needed to obtain aliquots of the effluent is shown in Figure 2-3.

As the entire system was under nitrogen, minimal degradation should have occurred during the experiment.

B. Liquid Chromatography— All chromatographic separations were made in a Waters Associates Model ALC/GPC 202 equipped with a U6K injector. Detectors used were a 254 nm LCD-type differential UV detector, and a
Figure 2-3: Diagram of Amicon ultrafiltration apparatus used for binding analyses.
model 401 differential refractometer. Refractive index measurements proved to be much less sensitive than UV detection, and therefore, not useful for identifying fractions.

Analytical-scale separations were achieved on a Waters C-18u BONDAPAK column (0.25m x 6.5mm) with a particle size of 10 μm. Injection volumes ranged from 20-40 μl. DOC values for these extracts varied from 10 mg C/l to 120 mg C/l. Preparative scale isolations were carried out using a Waters C-18 10u-Porasil-B column (4m x 10mm), with injection sizes ranging from 1.0-2.0 ml.

Solvents were purchased either from Burdick and Jackson (n-propanol) or Fisher (n-propanol, acetonitrile, and water). All solvents used were of chromatography grade, except for the Fisher n-propanol, which was a certified grade. Solvents were filtered prior to use through Millipore membranes (0.2 um pore size and 47 mm in diameter); fluoropore filters for the acetonitrile, and celotate for the other solvents. In addition to removing particulate, filtration also served as an initial degassing step. Final degassing was done by subjecting the solvent to ultra-high frequency sound for periods of up to 2 hours, as recommended in the manufacturer's literature (Waters).

Solvent systems varied from 20% v/v n-propanol in water to 20% v/v propanol, 20% v/v acetonitrile in water. Flow rates for analytical separations ranged from 1.0-1.5 ml/min with a pressure about 2000 PSI, and from 3.0-4.0 ml/min with a pressure about 600 PSI for preparative isolations. All analyses were performed at ambient temperatures.

C. Atomic Absorption Spectrometry (AAS)— A Varian Techtron Model AA-3 spectrometer modified with AA-5 electronics was employed for metal analyses. Experimental conditions employed are listed in Table 2-1.
Table 2-1: Listing of parameters used for the analysis of copper and iron by atomic absorption spectroscopy.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Copper</th>
<th>Iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical wavelength</td>
<td>324.7 nm</td>
<td>248.4 nm</td>
</tr>
<tr>
<td>Flame conditions</td>
<td>Lean</td>
<td>Lean</td>
</tr>
<tr>
<td>Fuel</td>
<td>Acetylene</td>
<td>Acetylene</td>
</tr>
<tr>
<td>Oxidant</td>
<td>Air</td>
<td>Air</td>
</tr>
<tr>
<td>Lamp current</td>
<td>5 ma</td>
<td>10 ma</td>
</tr>
<tr>
<td>Slit width</td>
<td>50 um</td>
<td>100 um</td>
</tr>
<tr>
<td>Working Range</td>
<td>0.5-10 ppm</td>
<td>0.5-5 ppm</td>
</tr>
</tbody>
</table>
A type AB-51 burner was used with a 14 cm slot-head and an air-acetylene flame. All standards were prepared from Fisher brand atomic absorption--1000 ppm standards and diluted with the same matrix as the samples to minimize interference effects; either the LC solvent system or HEPES buffer.

D. Dissolved Organic Carbon Analysis-- A Sybron-Barnstead PHOTOchem organic carbon analyzer was used for DOC determinations. An ultraviolet photo-chemical oxidation system was utilized by the DOC analyzer; a technique which was reviewed by Poirer and Wood (1978). Detection was accomplished by a conductivity cell which determined the specific resistance in the measuring chamber. Aliquots of 1.0 ml were injected, followed by 1.0 ml of 0.148 M phosphoric acid. Primary standard grade potassium acid phthalate (Mallinckrodt) was dissolved with photo-chem water, which was considered organic-free, and diluted to a final concentration of 100 ppm carbon. This was then used as the calibration standard. Diluted phosphoric acid was used as a blank in all experiments.

E. Carbon, Hydrogen, Nitrogen Analysis-- Carbon, hydrogen, nitrogen (CHN) analyses were performed on a Perkin-Elmer model 240-E elemental analyzer. Combustion at 800°C with 5 mg of tungstic anhydride occurred in pure oxygen under static conditions and the combustion products were analyzed in a thermal conductivity analyzer (Perkin Elmer, 1978). Cyclohexanone-2,4,dinitro-phenyl hydrazone (Perkin-Elmer) was used as a calibration standard. Solid sample sizes were in the range of 0.8 to 2.6 mg. Samples were obtained by freeze-drying 10 or 50 ml aliquots of extracted organic matter with a dry ice-acetone lypholyzation apparatus.
attached to a vacuum pump.

F. Electron Paramagnetic Resonance—X-band frequency (9.5 GHz) EPR spectra were measured on a Varian E-4 spectrometer fitted with a TE102 rectangular cavity, operated at 100KHz magnetic field modulation. A quartz solution flatcell with a volume of 300 ul was used for room temperature spectra and a quartz tube with an approximate sample volume of 350 ul (approximately 4 mm OD and 3 mm ID) was employed for frozen solution (77 K) and solid (powder) spectra. Nitrogen bubbling was minimized by the method of Chasteen (1977). Diphenylpicrylhydrazyl (g=2.0036) was used as a reference compound for determining g-values. The magnetic field was calibrated with a Newport Instruments Li/H nuclear magnetic resonance gaussmeter.

Project Methodology

A. Comparison of Extraction Procedures—By using the final filtrates from the isolation procedures discussed earlier (Figure 2-2), a series of experiments were carried out to determine the differences and similarities between the organic materials collected; both quantitatively and qualitatively.

DOC measurements were chosen as the means to assess the amount of material extracted by each procedure. The initial extract, before any filtrations, was first analyzed with subsequent determinations on solutions filtered through the glass fiber filters, through both filters, and concentrated by the Amicon system. For completeness, the eluents from the ultrafiltration procedure were also analyzed, and a total carbon balance was determined. This procedure was carried out for all extractants as a test of the efficiency of the given system employed.
To address the effect of shaking, extracts were analyzed initially then after 1 hour, 1 day, and 13 days on a platform shaker. This procedure was repeated for each extractant. An additional sample was left at room temperature, under nitrogen, for 13 days, with no shaking. The purpose of these procedures was to determine the effect, if any, temperature had on the extraction process, and to demonstrate conclusively the effect of agitation on the extraction efficiencies.

For qualitative measurements, three methods were employed: LC, CHN analyses, and DOC determinations. The liquid chromatography separations yielded information about the polarity of the material isolated. These determinations consisted of analyses on the analytical column, using 20% v/v n-propanol in water as the solvent system. Solvent was purged with nitrogen during use and was fed under pressure at 60 PSI. Injection size was 30 ul, fixed by a loop injection system. DOC values ranged from 10 to 120 mg C/l. Flow rate was held constant at 1.5 ml/min. Elemental analyses were done on solid samples to determine variations in the C, H, N composition of the various organics. These elemental analyses were compared to DOC measurements made earlier to determine total amounts of organic material (carbon) isolated. DOC measurements yielded information as to the molecular size class of the material extracted by each method.

B. Liquid Chromatography Optimization— For an effective separation to occur, the proper solvent system must be employed. As an initial step, the system of Templeton (1980) was chosen because the material he studied was similar to that investigated in this work. In an effort to improve the resolution, a third solvent was introduced and optimization of this ternary system was carried out. Methanol and aceto-
nitrile were selected as the third components to be examined. Using LC fractions collected earlier, a systematic variation of solvent composition was carried out on the ternary systems: water/n-propanol/methanol and water/n-propanol/acetonitrile. The water content was varied from 60-80%v/v, n-propanol from 5-20%v/v, and the third component (acetonitrile or methanol) from 0-20%v/v. A table of the actual combinations tried is found in Appendix I. All work on this project was performed on the analytical-scale column. The other parameter that was tested was flow rate, which was varied from 1.0-1.5 ml/min. Sample size was held constant at 30 ul by a fixed-loop injection system. Two different fractions were analyzed in an attempt to develop the best possible solvent system.

C. Preliminary Binding Studies— The purpose of these investigations was to determine if copper complexed with organic matter. Extract (10 ml) was placed in a nitrogen-flushed, pre-cleaned serum vial, and 10 to 40 ul of a 0.0505 M copper solution, prepared from solid CuCl₂ 2H₂O in water, was added. The final concentration of the cupric ion was either 6 or 24 ppm. After an equilibrium period of between 2 to 24 hours, the extract was placed in a 10 ml Amicon cell, which was attached to a reservoir. Nanopure water was initially used as a wash, but in later experiments, a 0.1 M solution of HEPES (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid, Aldrich Chemical) at a pH of 7.5 was used. The sample was flushed slowly with wash solution, and eluents collected until at least five volumes of initial extract were eluted. The residue and 0.5 to 1.5 ml aliquots of the ultrafiltrate were analyzed for copper by AAS. A total copper balance was calculated for the system. Blank samples of D/D water and 0.1 M HEPES buffer were run.
to ensure that there was no retention of copper by the Amicon system itself.

D. Kinetics—One of the primary considerations when beginning any binding study is the determination of the time necessary for equilibrium to be reached. To address this situation, 10 ml aliquots of various extracts were placed in an Amicon cell, and then spiked with 50 ul of a 0.0505 M cupric ion solution. The cell was immediately pressurized, and aliquots of the filtrate were collected at various times of between 10 min to 105 hours. These eluents were analyzed for copper by AAS. As the membrane cutoff size in the Amicon system was 500 daltons which was the same used for sample concentration, it was anticipated that very little organic matter would escape. Therefore, all copper eluted could be considered uncomplexed metal. Variables in this study were: sample concentration (DOC level of between 7-120 mg C/l), buffer presence (0-9.1 M buffer), and type of extractant used.

E. Binding Reactions—The objective was to determine what fractions of the organic material were responsible for binding copper, and if these fractions were identical between various extracts. This study was accomplished by first separating the material using the LC, followed by copper analysis with AAS. The flow diagram in Figure 2-4 illustrates the apparatus which involved collecting 1.5 ml fractions as they were eluted from the LC, and then analyzing each fraction (or sub-fraction) for copper by AAS. AAS conditions are listed in Table 2-1.

Extracts (10 ml) were spiked with 50 ul of a 0.0505 M cupric ion solution, and in some cases 0.1 M HEPES buffer (pH = 7.5) was added.
Figure 2-4: Diagram of liquid chromatograph and atomic absorption spectrometer used to study the binding of copper to various fractions of organic matter. Samples were first separated on the L.C., collected in the fraction collector, and subsequently analyzed by atomic absorption spectroscopy.
The preparative-scale C-18 Porasil-B column was used exclusively for this work. One or two milliliter aliquots were analyzed, and eluent was collected at the rate of 2 tubes/min. The flow rate was 3 ml/min. and the solvent system employed was 60% v/v water/ 20%v/v acetonitrile/ 20% v/v n-propanol; the solvent was degassed prior to use.

F. Binding Capacity— The major emphases in this study were to elucidate which fractions of the organic material bind copper, and to note any differences between various extracts.

Aliquots (10 ml) of the different extracts were placed in the Amicon cell. Then, 250 ml of a 10 or 20 ppm cupric solution, prepared by dilution of the 0.0505 stock solution with HEPES buffer (pH 7.5), was placed in the reservoir. This solution was flushed with nitrogen gas for at least 30 min. prior to addition to the sample. The system was purged with nitrogen and 0.5 to 1.5 ml aliquots of the filtrate were collected in the fraction collector to be subsequently analyzed for copper AAS. The process continued until the eluent copper concentration was identical to that in the reservoir.

G. Competitive Binding— The technique used to study the competition of cupric and ferrous ions for binding sites on organic matter was LC - AAS.

This procedure employed the apparatus illustrated in Figure 2-4. Extracts (2 ml--20-120 mg C/l) were fractionated on the LC at a flow of 4 ml/min. The solvent system used was either 60% water/20% n-propanol/20% acetonitrile v/v or 70% water/30% n-propanol v/v. Fractions were collected every min. The preparative-scale column was utilized in every case. Injection volumes of 2 ml were employed.
Samples run included the extract itself (no added metal), and with added copper or iron. Finally, to study the competitive nature of the metals, aliquots of extract were spiked first with copper and then iron, or vice versa. As a blank, 2 ml of 0.1 M HEPES buffer, which had been "fractionated" by the LC, was analyzed. In this way, using the LC to separate bound from free metal based on retention times, it was possible to determine relative binding characteristics of the two metals.
I. **Comparison of Extraction Procedures**

The purpose of these studies was to determine the polarity differences between various extracts and pore water. These extracts were meant to mimic different environmental conditions that could exist in sedimentary systems. Table 3-1 shows results for DOC measurements from two cores. Several cores were analyzed, and all showed similar trends as can be seen by the entries.

Addition of extractant, and subsequent shaking, solubilized considerably more material than was otherwise present in the pore water. D/D water extracted 4 to 56% more material than artificial SW extractant.

The radical difference in the amount of material extracted by each procedure was not totally surprising. The methods included a vigorous agitation step for up to 13 days, which has been hypothesized to remove material by physical abrasion (Templeton, 1980). Another possible mechanism is that the addition of extractant shifted solubility equilibria, resulting in some material dissolving from the sediment grains.

The two mechanisms mentioned above would result in the solubilization of organic material with different characteristics from that normally present in pore water (polarity, molecular weight, etc.). If a shift in equilibria was the dominant process, extra soluble material
Table 3-1: DOC content for various extracts of organic matter from anoxic sediment cores taken in August and April.

Core #2 (August)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average DOC (mg C/liter)</th>
<th>Total Volume (ml)</th>
<th>mg C/section</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW Extract</td>
<td>44.8</td>
<td>540</td>
<td>24.1</td>
</tr>
<tr>
<td>D/D Extract</td>
<td>66.9</td>
<td>560</td>
<td>37.5</td>
</tr>
<tr>
<td>Total Pore Water</td>
<td>11.6</td>
<td>193</td>
<td>2.25</td>
</tr>
<tr>
<td>Subsection 1-A*</td>
<td>12.4</td>
<td>35</td>
<td>0.43</td>
</tr>
<tr>
<td>1-B*</td>
<td>10.1</td>
<td>57</td>
<td>0.58</td>
</tr>
<tr>
<td>2-A*</td>
<td>14.5</td>
<td>39</td>
<td>0.57</td>
</tr>
<tr>
<td>2-B*</td>
<td>10.8</td>
<td>62</td>
<td>0.67</td>
</tr>
<tr>
<td><strong>Total amount of material extracted (as mg carbon)</strong></td>
<td><strong>63.8</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Core #3 (April)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average DOC (mg C/liter)</th>
<th>Total Volume (ml)</th>
<th>mg C/section</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW Extract</td>
<td>36.1</td>
<td>685</td>
<td>24.8</td>
</tr>
<tr>
<td>D/D Extract</td>
<td>48.7</td>
<td>530</td>
<td>25.7</td>
</tr>
<tr>
<td>Pore Water</td>
<td>45.9</td>
<td>145</td>
<td>6.7</td>
</tr>
<tr>
<td>Pore Water (shaken)#</td>
<td>87.0</td>
<td>252</td>
<td>21.9</td>
</tr>
<tr>
<td><strong>Total amount of material extracted (as mg carbon)</strong></td>
<td><strong>79.1</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---Core was divided into subsections (with location and depth in core) and analyzed for DOC. Results were pooled, and reported as total for pore water section.

---A separate section of core was shaken on platform shaker (in an analogous fashion to the samples with added extractant) and then pore water was isolated in the normal fashion.
would likely be similar chemically to that already present in the pore water. Abrasion, however, could well remove compounds from the sediment coatings that are larger, less polar, and contain less functionality than organics from pore water. Therefore, by looking at the composition of extracts, it should be possible to infer something about removal mechanisms. With an increased amount of liquid in contact with sediments, a greater amount of material could remain in solution. Also, the liquid provided a more mobile phase for the movement of sediment grains than the extraction procedure where no liquid was added, promoting abrasion and removing coatings of organic matter. Seawater, because of dissolved salts, is less able to solubilize organic matter and keep it in solution (Rothbart, 1973). The D/D water, with its low ionic content, is better able to hydrate organic compounds. This is an example of the "salting out" phenomenon often seen in organic chemistry (Lehman, 1981). The reason for this loss of organic matter solubility is that salt changes the structure of water itself. Interactions between salt ions and water molecules are stronger than those between water and organic compounds, which results in these weaker bonds being destroyed, and organic matter precipitates. In D/D water, however, these weak interactions do occur, and the material is held in solution.

Table 3-2 presents data for extracts that were ultrafiltered and subsequently analyzed for DOC. In pore water samples, the amount of organic material in fractions of greater, or less than, a nominal molecular weight of 500 daltons was similar. The extracts, although considerably higher in total DOC values, were quite similar to pore water with respect to amounts of low molecular weight material. This
<table>
<thead>
<tr>
<th>Sample</th>
<th>DOC (mg C/liter)</th>
<th>Volume (ml)</th>
<th>mg C/subsection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pore water</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-total</td>
<td>31.2</td>
<td>64.</td>
<td>2.0</td>
</tr>
<tr>
<td>conc.*</td>
<td>155.6</td>
<td>17.</td>
<td>2.7</td>
</tr>
<tr>
<td>UF*</td>
<td>114.0</td>
<td>39.</td>
<td>4.5</td>
</tr>
<tr>
<td>B-total</td>
<td>UND</td>
<td>UND</td>
<td>UND</td>
</tr>
<tr>
<td>conc.</td>
<td>92.6</td>
<td>19.</td>
<td>1.8</td>
</tr>
<tr>
<td>UF</td>
<td>74.4</td>
<td>21.</td>
<td>1.6</td>
</tr>
<tr>
<td>C-total</td>
<td>UND</td>
<td>UND</td>
<td>UND</td>
</tr>
<tr>
<td>conc.</td>
<td>45.5</td>
<td>14.</td>
<td>0.6</td>
</tr>
<tr>
<td>UF</td>
<td>25.6</td>
<td>27.</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Pore water (shaken)</strong>#</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-total</td>
<td>81.4</td>
<td>130.</td>
<td>10.6</td>
</tr>
<tr>
<td>conc.</td>
<td>127.8</td>
<td>38.</td>
<td>4.4</td>
</tr>
<tr>
<td>UF</td>
<td>41.1</td>
<td>75.</td>
<td>3.1</td>
</tr>
<tr>
<td>II-total</td>
<td>93.0</td>
<td>122.</td>
<td>11.4</td>
</tr>
<tr>
<td>conc.</td>
<td>156.5</td>
<td>35.</td>
<td>5.5</td>
</tr>
<tr>
<td>UF</td>
<td>20.9</td>
<td>50.</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>D/D Extract</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-total</td>
<td>UND</td>
<td>320.</td>
<td>UND</td>
</tr>
<tr>
<td>conc.</td>
<td>117.6</td>
<td>96.</td>
<td>11.3</td>
</tr>
<tr>
<td>UF</td>
<td>15.2</td>
<td>215.</td>
<td>3.3</td>
</tr>
<tr>
<td>II-total</td>
<td>UND</td>
<td>300.</td>
<td>UND</td>
</tr>
<tr>
<td>conc.</td>
<td>107.2</td>
<td>87.</td>
<td>9.3</td>
</tr>
<tr>
<td>UF</td>
<td>14.1</td>
<td>130.</td>
<td>1.8</td>
</tr>
</tbody>
</table>
Table 3-2: (continued)

<table>
<thead>
<tr>
<th>Sample</th>
<th>DOC (mg C/liter)</th>
<th>Volume (ml)</th>
<th>mg C/ subsection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SW Extract</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-total</td>
<td>33.9</td>
<td>335.</td>
<td>11.4</td>
</tr>
<tr>
<td>conc.</td>
<td>84.8</td>
<td>104.</td>
<td>8.8</td>
</tr>
<tr>
<td>UF</td>
<td>10.6</td>
<td>200.</td>
<td>2.1</td>
</tr>
<tr>
<td>II-total</td>
<td>38.3</td>
<td>350.</td>
<td>13.4</td>
</tr>
<tr>
<td>conc.</td>
<td>94.8</td>
<td>93.</td>
<td>8.8</td>
</tr>
<tr>
<td>UF</td>
<td>14.3</td>
<td>200.</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Totals for each section expressed as mg C per sections of equivalent size.

Pore Water                          7.78
Pore Water (shaken)                 21.93
D/D Extract                         25.72
SW Extract                          24.77

*conc.—that material retained by a 500 MW cutoff Amicon filtration membrane.
*UF----that material which passed through a 500 MW Amicon filtration membrane.
#-----this material was isolated from a section of a sediment core that was shaken for 10-13 days on a platform shaker, and then processed in the normal fashion to isolate the pore fluids.
UND----undetermined.
suggests that this added material is larger than 500 molecular weight. This extra material may be a polymeric species (e.g. humic substances), as hypothesized by Krom and Sholkovitz (1977).

For PW-I and PW-II samples, there is a discrepancy in that the amount of organic material in the two fractions; concentrate (greater than 500 MW) and ultrafiltrate (less than 500 MW), which do not add up to the total of material initially present. The most likely reason for this is that precipitation of the organics occurred during the ultrafiltration process. This observation has been made several times previously during the course of this research and has been noted by others as well (Templeton, 1980; and Orem, 1981b). For the PWA sample, the only explanation is that some contamination occurred during the ultrafiltration process. A possible source is the glycerine that is used to coat the ultrafiltration membrane during manufacture. If this was not completely removed during the cleaning process, it is conceivable that this material could have leached into the sample resulting in the higher DOC values observed.

Table 3-3 shows the results of an experiment designed to address the question as to why the extraction procedure results in such a dramatic increase in DOC. The data shows quite conclusively that as time of shaking increases, DOC levels also increase for both pore water samples and seawater extracts. For PW (t = 13 days) sample, it appears as if contamination occurred from the GF/C filter resulting in the increased DOC level observed.

Bacterial activity could provide an explanation for increased DOC level in extracts. Since the extracts are shaken for prolonged periods of time (10-13 days), bacteria would have ample opportunity to
Table 3-3: Results of study on the effects of variable sediment shaking time on DOC determinations.

All values are reported as DOC content of extract in units of mg C/liter

<table>
<thead>
<tr>
<th>Sample</th>
<th>Unfiltered extract</th>
<th>Filtered extract (GF/C)*</th>
<th>Filtered extract (GF/C and 0.45u)#</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pore water</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 0</td>
<td>31.1</td>
<td>30.3</td>
<td>30.4</td>
</tr>
<tr>
<td>t = 1 hour</td>
<td>UND</td>
<td>7.8&amp;</td>
<td>16.2&amp;</td>
</tr>
<tr>
<td>t = 1 day</td>
<td>42.1</td>
<td>45.8</td>
<td>31.3/32.6ç</td>
</tr>
<tr>
<td>t = 13 days</td>
<td>33.8/36.3ç</td>
<td>47.9</td>
<td>42.3</td>
</tr>
<tr>
<td></td>
<td>(unshaken)</td>
<td>25.5</td>
<td>21.4</td>
</tr>
<tr>
<td><strong>SW extract</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 1 hour</td>
<td>21.3</td>
<td>21.3</td>
<td>16.3</td>
</tr>
<tr>
<td>t = 1 day</td>
<td>32.8</td>
<td>25.4</td>
<td>20.2</td>
</tr>
<tr>
<td>t = 13 days</td>
<td>45.9</td>
<td>48.4</td>
<td>37.9</td>
</tr>
</tbody>
</table>

*----this set of samples was filtered through pre-cleaned glass fiber filters only.

#----this set of samples was filtered first through pre-cleaned glass fiber filters and subsequently through pre-cleaned 0.45 micron Nucleopore filters to remove colloidal material.

&----These samples were diluted with overlying water and therefore have a lower DOC content than would normally be expected.

ç----this represents duplicate samples; two sections of a core processed identically but independently through the entire experiment.
alter the organic material in the sediment sample. It has been proposed that microbes break down large complex molecules on the sediment grains into smaller compounds (Head, 1976) which are more soluble. This hypothesis, however, can be discounted using the data in Table 3-2. In the extracts labeled "I," sodium azide was added to kill bacteria, yet the DOC concentrations increased the same as those extracts where no preservative was added. If bacterial activity was significant cause for the increased amount of organic matter in the extracts, these samples with the preservative should have had lower DOC values.

Another possibility considered was that elevated temperature of the laboratory compared to the field was a significant factor in the increased amount of material extracted as temperatures could have increased the solubility of organic matter in pore fluids. Schmidt et al. (1978) found that heating an extract altered what organic materials were extracted. Furthermore, it has been observed by Hulburt and Brindle (1975), that the amount of trace metal dissolved in pore fluids was also altered at higher temperatures.

The effect of temperature on the amount of DOC extracted was examined by analyzing a sample left at ambient temperature for thirteen days, and comparing these DOC results with those for an extract processed by the same procedure immediately after sampling. The data in Table 3-3 shows that the DOC level of the pore water sample left for 13 days was lower than the control (t = 0), strongly suggesting that temperature was not the cause of an increased level of material removed by the extractants.

A possible explanation for a reduced DOC content for unshaken pore water sample (t = 13 days) when compared to the control (t = 0)
is that the prolonged delay allowed the system to come to equilibrium. It is possible that sediments are constantly undergoing change, and therefore never come to true equilibrium. Because this sample was removed from this dynamic system, equilibrium could be achieved. This may involve precipitation of organic matter which has a limited solubility in pore fluids. This is consistent with the observation that organics precipitate during the ultrafiltration process. The results presented here do not address this possibility, and further work would need to be done to answer this question.

It can be seen in Table 3-1 that the DOC values were lower for core 2 obtained in April than for core 3 obtained in August. Also, the total amount of DOC for the same amount of sediment was lower for the April core. This is logical considering microbial activity is greater in the summer. The increased activity would produce small organic molecules, which would most likely be soluble and hence would show up as DOC in the pore water. This result is consistent with the results obtained by Orem (1981b).

The overall conclusions that can be drawn from this quantitative study are:
1. D/D water and artificial SW extractants remove significantly higher concentrations of organic matter compared to that present in pore water. Abrasion of sediment grains during the shaking process is the most likely reason for this increase. It was also demonstrated that temperature and bacterial factors had negligible effect on the results obtained in this study.
2. D/D water removes more material than SW; most likely due to differences in solubility of OM in the two extractants.
3. The amount of DOC in pore water is greater in August than in early April, probably due to seasonal microbial activity variation. This is supported by the work of others (Orem, 1981b).

4. The "extra" material (that isolated by the two extracts as compared to PW) removed by the extraction process is in the greater than 500 molecular weight size class.

B. Qualitative Studies—Reversed-phase LC was the primary method used for the determination of compositional differences in the extracts. Figures 3-1 through 3-3 give typical chromatograms for pore water concentrate and the two extract concentrates.

The pore water chromatogram was relatively simple, with two principal peaks quite close to the void volume as well as lesser peaks with longer retention volumes. The D/D extract is more complex, with several peaks superimposed on a broad signal. These compounds were also significantly more non-polar than those for the pore water.

The seawater (SW) extract also had some peaks with slightly longer retention times than those for the D/D extract. The broad signal was not observed for the SW chromatogram and the resolution was much improved. This chromatogram had three distinct peaks, with smaller shoulders evident.

These chromatograms also support the idea that the material in the extracts is a complex mixture of compounds probably present originally on the sediment grains as coating. This extracted material would be expected to be non-polar, larger, and therefore less soluble than organic matter in pore fluids. The excess material that is removed by the D/D extract is an extremely heterogeneous group of com-
Figure 3-1: Reversed phase liquid chromatogram of pore water extracted from anoxic sediments.

Liquid chromatogram of pore water isolated from anoxic sediments in Great Bay. Liquid Chromatography parameters: Column; analytical-scale column packed with c-18 u-Bondapak on 10 u particles; Sample, pore water from section 1-A in core #2; Solvent, 20% v/v n-propanol in water; solvent flow rate, 1.5 ml/min.; Detector, UV at 254 nm; Detector attenuation, X4; Sample size, 30 ul.
Figure 3-2: Reversed phase liquid chromatogram of D/D extract of organics from anoxic sediment.

Liquid chromatogram of D/D extract of sediments from Great Bay. Liquid chromatography parameters: Column, analytical-scale column packed with C-18 u-Bondapak on 10 u particles; Sample was D/D extract #2 from core #2; Sample size, 30 ul; Solvent, 20% v/v n-propanol in water; Solvent flow rate, 1.5 ml/min.; Detector, UV at 254 nm; Detector attenuation, X8.
Increased Polarity

UV Absorbance 254 nm

Retention Time
Figure 3-3: Reversed phase liquid chromatogram of SW extract of organics from anoxic sediment.

Liquid chromatogram of SW extract of sediments from Great Bay. Liquid chromatography parameters: Column, analytical-scale column packed with C-18 u-Bondapak on 10 u particles; Sample was SW extract #1 from core #2; Sample size, 30 ul; Solvent, 20% v/v n-propanol in water; Solvent flow rate, 1.5 ml/min.; Detector, UV at 254 nm; Detector attenuation, X8.
UV Absorbance 254 nm

Retention Time

↑ Increased Polarity
pounds that appear in the broad band of the LC. This is the material that is not removed by the SW extract (as observed by LC) and accounts for the different levels of DOC between the two extracts.

To address this possibility, further experiments are required. Suggestions for future work are presented in the next chapter.

C, H, N elemental measurements were made in an attempt to detect qualitative differences between the organic matter in pore water and that in the extracts. The results for these analyses (Table 3-4) reveal a great lack in the consistancy of the data. The reason for the low amounts of organic matter in the solids is that a great deal of inorganic salts were present; either from the pore water itself or added with the SW extractant.

Analysis of variance was used to test for significance between the ratios; C/H, C/N, and H/N. The only significant difference were in the C/H ratios. As no differences were seen in the C/N ratios, which is a better indication of organic matter variation, it appeared as if there was no significance in the organic matter in the three cores. The variation in the C/H ratios could be due to differences in the bicarbonate content of the samples, or the water content; both of which would affect the C/H ratio.

Elemental (C,H,N) analyses were also used to verify the accuracy of the DOC measurements. If both techniques are accurate, the amount of carbon determined by each method of analysis should be equal. Generally, the results for elemental analyses, shown in Table 3-5, were higher than that for DOC determinations. This suggests that perhaps the DOC analyzer is not totally efficient in oxidizing, and therefore measuring, the organic material present in the extracts.
Table 3-4: Elemental analyses for extracts of organic matter from different cores with and without added copper.

Core #2, no added copper

<table>
<thead>
<tr>
<th>Sample of Extract#</th>
<th>%C</th>
<th>%H</th>
<th>%N</th>
<th>mole C/mole H</th>
<th>mole C/mole N</th>
<th>mole H/mole N</th>
</tr>
</thead>
<tbody>
<tr>
<td>D/D (D1)</td>
<td>17.42</td>
<td>2.56</td>
<td>1.86</td>
<td>0.57</td>
<td>10.9</td>
<td>19.3</td>
</tr>
<tr>
<td>D/D-I (T1)</td>
<td>6.11</td>
<td>1.43</td>
<td>0.66</td>
<td>0.36</td>
<td>10.8</td>
<td>30.3</td>
</tr>
<tr>
<td>D/D-I (T2)</td>
<td>5.46</td>
<td>1.30</td>
<td>0.52</td>
<td>0.35</td>
<td>12.3</td>
<td>35.0</td>
</tr>
<tr>
<td>D/D-I</td>
<td>34.17</td>
<td>6.76</td>
<td>9.81</td>
<td>0.42</td>
<td>4.1</td>
<td>9.7</td>
</tr>
<tr>
<td>SW-II (S1)</td>
<td>26.13</td>
<td>3.59</td>
<td>2.66</td>
<td>0.61</td>
<td>11.5</td>
<td>18.9</td>
</tr>
</tbody>
</table>

Core #3, no added copper

<table>
<thead>
<tr>
<th>Sample of Extract#</th>
<th>%C</th>
<th>%H</th>
<th>%N</th>
<th>mole C/mole H</th>
<th>mole C/mole N</th>
<th>mole H/mole N</th>
</tr>
</thead>
<tbody>
<tr>
<td>D/D-II (Filt.)</td>
<td>0.80</td>
<td>0.20</td>
<td>0.28</td>
<td>0.33</td>
<td>3.3</td>
<td>10.0</td>
</tr>
<tr>
<td>D/D-II (Unfil.)</td>
<td>1.34</td>
<td>1.19</td>
<td>0.27</td>
<td>0.09</td>
<td>5.79</td>
<td>61.7</td>
</tr>
</tbody>
</table>

Core #3, with added copper

<table>
<thead>
<tr>
<th>Sample of Extract#</th>
<th>%C</th>
<th>%H</th>
<th>%N</th>
<th>mole C/mole H</th>
<th>mole C/mole N</th>
<th>mole H/mole N</th>
</tr>
</thead>
<tbody>
<tr>
<td>D/D-I (K3)</td>
<td>3.53</td>
<td>1.42</td>
<td>0.51</td>
<td>0.21</td>
<td>8.1</td>
<td>39.0</td>
</tr>
<tr>
<td>D/D-I-Cu</td>
<td>2.46</td>
<td>1.58</td>
<td>BDL*</td>
<td>0.12</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>D/D-I-Cu</td>
<td>2.68</td>
<td>1.68</td>
<td>BDL</td>
<td>0.13</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>SW-I-Cu</td>
<td>1.74</td>
<td>1.01</td>
<td>0.26</td>
<td>0.14</td>
<td>7.8</td>
<td>54.4</td>
</tr>
<tr>
<td>SW-II-Cu(K2)</td>
<td>28.05</td>
<td>6.05</td>
<td>8.08</td>
<td>0.39</td>
<td>4.1</td>
<td>10.5</td>
</tr>
<tr>
<td>PWA-Cu</td>
<td>34.82</td>
<td>6.73</td>
<td>10.17</td>
<td>0.43</td>
<td>4.0</td>
<td>9.3</td>
</tr>
<tr>
<td>FWT-Cu</td>
<td>0.68</td>
<td>1.15</td>
<td>BDL</td>
<td>0.04</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>PWT-Cu</td>
<td>0.59</td>
<td>1.46</td>
<td>BDL</td>
<td>0.03</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

Accuracy for CHN analysis (for standards): 0.3% (95% confidence limits)

Precision for CHN analysis (for standards): 0.2% for carbon,
0.1% for hydrogen,
0.1% for nitrogen.

* D/D------double distilled water extract
SW------artificial seawater extract
PW------pore water
sample names refer to extract-section-metal (if added)

# BDL------below detection limits of instrument
Table 3-5: Comparison of carbon determination by DOC analyzer and CHN analyzer.

<table>
<thead>
<tr>
<th>Sample</th>
<th>mg C/sample DOC analyzer (1)</th>
<th>mg C/sample CHN analyzer (2)</th>
<th>Ratio (2)/(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfiltered D/DII</td>
<td>0.99</td>
<td>1.93</td>
<td>1.95</td>
</tr>
<tr>
<td>Filtered D/DII</td>
<td>0.78</td>
<td>1.13</td>
<td>1.45</td>
</tr>
<tr>
<td>D/DI (K3)</td>
<td>5.88</td>
<td>6.47</td>
<td>1.10</td>
</tr>
<tr>
<td>SWII (K2) *</td>
<td>4.74</td>
<td>56.97</td>
<td>12.02</td>
</tr>
<tr>
<td>D/DI-Cu *</td>
<td>1.18</td>
<td>0.86</td>
<td>0.73</td>
</tr>
</tbody>
</table>

It is felt that the samples labeled with * were mislabeled. If this was the case, and the results recalculated, the difference is as follows:

<table>
<thead>
<tr>
<th>Sample</th>
<th>mg C/sample DOC analyzer (1)</th>
<th>mg C/sample CHN analyzer (2)</th>
<th>Ratio (2)/(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWII (K2)</td>
<td>4.74</td>
<td>9.42</td>
<td>1.99</td>
</tr>
<tr>
<td>D/DI-Cu</td>
<td>1.18</td>
<td>5.22</td>
<td>4.42</td>
</tr>
</tbody>
</table>

All calculations were done assuming that the entire extract was processed (freeze-dried, and analyzed) without loss.
and pore water. The other possibility is that the C, H, N, determinations measured some inorganic carbon, thus giving the higher values obtained. For the two starred values, the discrepancies are unexplainable. It is curious to note that if the results for elemental analyses are exchanged for these two samples and calculations redone, the values are closer to expected results. This suggests that perhaps a simple mislabeling occurred.

The important information obtained by this qualitative study was:

1. LC demonstrates significant differences in polarity between the organic matter in pore water, D/D extract and SW extract. The pore water shows a simple chromatogram, while those of the extracts are more complex, and more non-polar. The SW extract has the most non-polar component. The chromatogram of the SW extract is better resolved than those obtained for the PW and D/D samples.

2. The results from C, H, N elemental analyses suggest that there is no difference in the organic matter between the cores taken in April and August. Also, no significant differences were observed between the analyses for PW organic matter and the results for the organics in the extracts.

II. Liquid Chromatography Optimization

The goal of this study was to develop a solvent system that would:

1. Achieve better separation of the organic material in pore water and the extracts.
2. Further resolve fractions isolated from these initial chromatographic runs.

The solvent system initially used, 80% water/20% n-propanol v/v, was developed by Templeton(1980). However, it was observed early in this research that resolution was poor (see Figures 3-1 to 3-3), and that it would not be feasible to isolate fractions because of significant cross-contamination.

The first attempt to improve the separation was to change the water/n-propanol ratio from 80/20% v/v to 60/40% v/v in stages. The propanol component was increased in all investigations because it was noted that a very non-polar component often remained on the column after the separation was seemingly completed. As the propanol concentration increased, the retention of the final peak became less of a problem. Figure 3-4 shows four sample chromatograms for a progression of increasing propanol composition. The very non-polar peak (off-scale on the 20% run) can be seen to migrate toward the void volume. The resolution is greatly improved, especially in the 30% n-propanol case. However, it was hoped to further fractionate the sample and resolve the peaks. Also, these chromatograms are for diluted samples; for the actual extract, a poor separation was seen, possibly due to column overloading. However, later studies were done where the sample volume was varied, but the resolution was unchanged.

Flow rates were varied from 1.0 ml/min to 2.0 ml/min, to observe the effect on the resolution. Figure 3-5 shows a comparison of three flow rates. From this data, and other results not shown, a rate of 1.5 ml/min. appeared to be the best for this system. The higher flow rate had poorer resolution compared to that rate chosen.
Figure 3-4: Effect of n-propanol concentration on LC chromatogram of D/D extract.

Liquid chromatogram of D/D #1 extract diluted 1:10 with D/D water.
LC parameters: Column, analytical-scale column packed with C-18 u-Bondapak on 10 u particles; Flow rate, 1.5 ml/min.; Solvent, variable (see below); Detector, UV at 254 nm; Detector attenuation, ×2; Injection volume, 30 ul.

A: Solvent, 20% v/v n-propanol in D/D water.
B: Solvent, 25% v/v n-propanol in D/D water.
C: Solvent, 30% v/v n-propanol in D/D water.
D: Solvent, 40% v/v n-propanol in D/D water.
Increased Polarity

UV Absorbance 254 nm

Retention Time →
Figure 3-5: Effect of flow rate on LC chromatogram of SW extract.

Liquid chromatogram of SW-I extract diluted 1:10 with artificial SW.
LC parameters: Column, analytical-scale column packed with C-18 u-Bondapak on 10 u particles; Flow rate, variable (see below); Solvent, 25% v/v n-propanol in D/D water; Detector, UV at 254 nm; Detector attenuation, X2; Injection volume, 30 ul.

A: Flow rate, 1.0 ml/min.
B: Flow rate, 1.5 ml/min.
C: Flow rate, 2.0 ml/min.
Increased Polarity

Retention Time

UV Absorbance

inj
The system, at this stage, was still not considered adequate to separate the initial extracts. All work done in previous studies utilized the analytical-scale column. However, as it was hoped to collect fractions of eluent in later experiments, it was important to find a solvent system that would provide adequate resolution for the semi-preparative-scale column. A ternary system was tried, with either methanol or acetonitrile as the third component. Figure 3-6 shows a comparison of methanol and acetonitrile as the third component. It was observed that the system of 60/20/20 % v/v water/n-propanol/acetonitrile gave a much better separation than the methanol system. Therefore, it was chosen as the third solvent.

The overall composition of the solvent was also varied and in Figure 3-6, a few of the chromatograms are presented. It was apparent that the 60%/20%/20% (v/v) water/n-propanol/acetonitrile was the best of those solvent systems tested. A mixture with greater than 20% v/v for either organic solvent was not tested. This system was deemed satisfactory, and the project was ended at this point.

Appendix I is a table of various separation schemes tried. Variables tested included all of those mentioned above, in addition to trying different extract fractions.

The conclusions that can be draw from this study are:

1. The dual component solvent system of 80%/20% (v/v) water/n-propanol is not adequate for resolving the organic constituents of the extracts. A system of 30% n-propanol in water is the optimum for a two-component system, but the ternary system of 60% water/20% n-propanol/20% acetonitrile is much better.

2. Although flow rate is not as critical in this case as solvent
Figure 3-6: Effect of solvent on LC chromatogram of SW #1 extract.

Liquid chromatogram of fraction #2 of SW #1 extract. LC parameters: Column, Analytical-scale column packed with C-18 u-Bondapak on 10 u particles; Flow rate, 1.5 ml/min; Solvent, variable (see below); Detector, UV at 254 nm; Detector attenuation, X2; Injection volume, 30 ul.

A: Solvent, 80/5/15% v/v water/n-propanol/acetoneitrile.

B: Solvent, 80/5/15% v/v water/n-propanol/methanol.

C: Solvent, 80/10/10% v/v water/n-propanol/acetoneitrile.

D: Solvent, 60/20/20% v/v water/n-propanol/acetoneitrile.
composition, a flow of 1.5 ml/min was found to be best for all extracts when using the analytical-scale column. For the semi-preparative-scale column, a flow rate of 4.0 ml/min. was best.

III. Preliminary Binding Investigations

This study was undertaken to test the hypothesis that copper binds to organic matter in sediments, and that further investigations into the details of this complexation were warranted. The experiment entailed adding copper to an aliquot of pore water, or extract, and letting the sample equilibrate. These samples were subsequently placed in the Amicon ultrafiltration system, and the free metal eluted.

The results from these preliminary binding studies are presented in Table 3-6. After six washes, there was still large amounts of copper which was retained by the Amicon membrane. As these did not occur when deionized water was in the cell, it suggested that the organic matter was complexing copper. Similar results were obtained for both extracts and pore water.

A refinement of this procedure (given in section C in "Project Methodology") used a buffer to wash out the excess copper as indicated by the results in Table 3-7. Because of the relatively large amounts of copper retained by the Amicon system, it appeared that organics were again binding the metal. Determinations were run without extract to assure that no (or minimal) copper was retained by the Amicon ultrafiltration system itself.

Because significant amounts of copper were eluted, it was thought that either the binding was quite weak, or the kinetics of copper binding were slow. The fact that different equilibration times were used
Table 3-6: Results of Amicon binding study of copper to various anoxic sediment extracts using D/D water as a wash.

**Sample #1**—SW-II extract, 10 ml sample volume, DOC = 45.3 mg C/1.

<table>
<thead>
<tr>
<th>Eluent #</th>
<th>ppm Cu</th>
<th>Aliquot volume (ml)</th>
<th>ug Cu/ aliquot</th>
<th>%Total Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>0.3</td>
<td>5.2</td>
<td>1.6</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>BDL*</td>
<td>5.0</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>#3</td>
<td>BDL*</td>
<td>5.0</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>#4</td>
<td>0.2</td>
<td>5.3</td>
<td>1.1</td>
<td>4.4</td>
</tr>
<tr>
<td>#5</td>
<td>0.3</td>
<td>5.1</td>
<td>1.5</td>
<td>6.0</td>
</tr>
<tr>
<td>#6</td>
<td>1.1</td>
<td>5.0</td>
<td>5.5</td>
<td>21.9</td>
</tr>
<tr>
<td>Concentrate</td>
<td>4.8</td>
<td>3.2</td>
<td>15.4</td>
<td>61.3</td>
</tr>
<tr>
<td>Total</td>
<td>25.1</td>
<td></td>
<td></td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Sample #2**—D/D-II extract, 10 ml sample volume, DOC = 73.4 mg C/1.

<table>
<thead>
<tr>
<th>Eluent #</th>
<th>ppm Cu</th>
<th>Aliquot volume (ml)</th>
<th>ug Cu/ aliquot</th>
<th>%Total Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>0.3</td>
<td>6.3</td>
<td>1.9</td>
<td>5.9</td>
</tr>
<tr>
<td>#2</td>
<td>0.5</td>
<td>4.6</td>
<td>2.3</td>
<td>7.1</td>
</tr>
<tr>
<td>#3</td>
<td>0.6</td>
<td>6.3</td>
<td>3.4</td>
<td>10.5</td>
</tr>
<tr>
<td>#4</td>
<td>0.6</td>
<td>5.0</td>
<td>3.0</td>
<td>9.3</td>
</tr>
<tr>
<td>#5</td>
<td>0.6</td>
<td>4.9</td>
<td>2.9</td>
<td>9.0</td>
</tr>
<tr>
<td>#6</td>
<td>0.8</td>
<td>5.0</td>
<td>4.0</td>
<td>12.4</td>
</tr>
<tr>
<td>Concentrate</td>
<td>2.6</td>
<td>5.7</td>
<td>14.8</td>
<td>45.8</td>
</tr>
<tr>
<td>Total</td>
<td>32.3</td>
<td></td>
<td></td>
<td>100.0</td>
</tr>
</tbody>
</table>

BDL*—Below detection limits, 0.1 ppm Cu.
Table 3-7: Results of Amicon binding study of copper to various anoxic sediment extracts using 0.1M HEPES buffer (pH = 7.5) as a wash.

**Sample #1**—PW-1B extract, 10 ml sample volume, DOC = 10.1 mg C/l.

<table>
<thead>
<tr>
<th>Eluent</th>
<th>ppm Cu</th>
<th>aliquot volume (ml)</th>
<th>ug Cu/ aliquot</th>
<th>%Total Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>BDL*</td>
<td>15.0</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>#2</td>
<td>1.2</td>
<td>14.0</td>
<td>16.8</td>
<td>59.6</td>
</tr>
<tr>
<td>#3</td>
<td>0.7</td>
<td>5.6</td>
<td>3.9</td>
<td>13.8</td>
</tr>
<tr>
<td>#4</td>
<td>0.4</td>
<td>8.2</td>
<td>3.3</td>
<td>11.7</td>
</tr>
<tr>
<td>#5</td>
<td>0.2</td>
<td>9.3</td>
<td>1.9</td>
<td>6.7</td>
</tr>
<tr>
<td>Concentrate</td>
<td>0.4</td>
<td>5.7</td>
<td>2.3</td>
<td>8.2</td>
</tr>
</tbody>
</table>

**Total** 28.2 100.0

**Sample #2**—SW-II extract, 10 ml sample volume, DOC = 45 mg C/l.

<table>
<thead>
<tr>
<th>Eluent</th>
<th>ppm Cu</th>
<th>aliquot volume (ml)</th>
<th>ug Cu/ aliquot</th>
<th>%Total Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>0.6</td>
<td>12.5</td>
<td>7.5</td>
<td>12.9</td>
</tr>
<tr>
<td>#2</td>
<td>0.7</td>
<td>7.0</td>
<td>4.9</td>
<td>8.4</td>
</tr>
<tr>
<td>#3</td>
<td>0.8</td>
<td>10.0</td>
<td>8.0</td>
<td>13.8</td>
</tr>
<tr>
<td>#4</td>
<td>0.7</td>
<td>12.0</td>
<td>8.4</td>
<td>14.4</td>
</tr>
<tr>
<td>#5</td>
<td>0.6</td>
<td>7.5</td>
<td>4.5</td>
<td>7.7</td>
</tr>
<tr>
<td>Concentrate</td>
<td>3.1</td>
<td>8.0</td>
<td>24.8</td>
<td>42.7</td>
</tr>
</tbody>
</table>

**Total** 58.1 100.0

**Sample #3**—PW-II extract, 10 ml sample volume, DOC = 156 mg C/l.

<table>
<thead>
<tr>
<th>Eluent</th>
<th>ppm Cu</th>
<th>aliquot volume (ml)</th>
<th>ug Cu/ aliquot</th>
<th>%Total Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>3.8</td>
<td>4.7</td>
<td>17.9</td>
<td>24.5</td>
</tr>
<tr>
<td>#2</td>
<td>2.4</td>
<td>5.5</td>
<td>13.2</td>
<td>18.1</td>
</tr>
<tr>
<td>#3</td>
<td>0.8</td>
<td>5.5</td>
<td>4.4</td>
<td>6.0</td>
</tr>
<tr>
<td>#4</td>
<td>0.6</td>
<td>6.0</td>
<td>3.6</td>
<td>4.9</td>
</tr>
<tr>
<td>Concentrate</td>
<td>8.3</td>
<td>4.1</td>
<td>34.0</td>
<td>46.5</td>
</tr>
</tbody>
</table>

**Total** 73.1 100.0
Table 3-7: (continued)

Sample #4—SW-I extract, 10 ml sample volume, DOC = 85 mg C/l.

<table>
<thead>
<tr>
<th>Eluent</th>
<th>ppm Cu</th>
<th>Aliquot volume (ml)</th>
<th>ug Cu/ aliquot</th>
<th>%Total Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>0.1</td>
<td>5.7</td>
<td>0.6</td>
<td>3.0</td>
</tr>
<tr>
<td>#2</td>
<td>0.3</td>
<td>6.2</td>
<td>1.9</td>
<td>9.5</td>
</tr>
<tr>
<td>#3</td>
<td>0.2</td>
<td>7.5</td>
<td>1.5</td>
<td>7.5</td>
</tr>
<tr>
<td>#4</td>
<td>0.5</td>
<td>1.3</td>
<td>0.7</td>
<td>3.5</td>
</tr>
<tr>
<td>Concentrate</td>
<td>4.8</td>
<td>3.2</td>
<td>15.4</td>
<td>76.6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>20.1</td>
<td></td>
<td>100.0</td>
</tr>
</tbody>
</table>

*BDL*—Below detection limits, 0.1 ppm Cu.
(ranging from 10 min to 24 hours), and yet the same trends were observed tends to disprove the hypothesis that kinetics is a controlling factor. The following study was performed to determine the kinetics of the complexation process.

IV. Kinetics

The objectives of this study were:

1. To elucidate how rapidly the copper was taken up by the organic matter.

2. If the kinetics were slow, to determine the equilibration time necessary for subsequent binding investigations.

Earlier studies produced a plot shown in Figure 3-7. At first, this was extremely disturbing, as the gradual rise in free copper concentration in the eluent was opposite to that normally expected; a gradual decline, or no change in free copper concentration (Amicon Corp., 1977). The explanation is that a relatively substantial dead volume was present between the membrane, where the bound copper was being retained, and the port where the eluent was sampled. This led to mixing of samples and therefore erroneous results.

Later analyses were performed on a larger scale to prevent significant concentration changes in organic matter. Also, the dead volume was virtually eliminated so that eluent was being sampled right below the membrane. Once these corrections were made, the results were closer to that expected for a kinetically rapid system (Figure 3-8).

As the majority of the copper was bound before the first aliquot of eluent was sampled (a maximum of ten minutes elapsed time), it can
Figure 3-7: Results of kinetic study of copper binding to D/D-II extract of anoxic sediment without experimental modifications.

Experimental parameters: Sample, D/D-II extract; Sample volume, 10 ml; DOC, 107.2 mg C/l; Initial copper concentration, 10.0 ppm; pH adjustment, none; Initial pH, 8.8.
Figure 3-8: Results of kinetic study of copper binding to D/D-II extract of anoxic sediment using experimental modifications.

Experimental parameters: Sample, D/D-II extract; Sample volume, 10 ml; DOC, 107.2 mg C/l; Initial copper concentration, 9.1 ppm; pH adjustment, to 7.6.
be assumed, with little hesitation, that the binding is quite rapid and therefore not a problem in equilibrium analyses.

It is interesting to note that none of the review papers that discuss organic matter-trace metal binding recognize the possibility that kinetics could play a significant role in the complexation process observed in the laboratory (Saxby, 1969; Schnitzer and Hansen, 1970; Rashid, 1971; Schnitzer and Khan, 1972; Guy and Chakrabarti, 1976; Nissenbaum and Swaine, 1976; Pocklington, 1977; and Buffle, 1980). Most authors seem to have their own preference for equilibration times ranging from several minutes to days, yet no explanation is given as to the reason why the particular intervals were chosen. Buffle (1980) in his review casually mentions that there are variations in elapsed times used (between mixing of the metals and organic matter, and the time of measurement), but dismisses this, without any justification, as being unimportant.

V. Binding Fractions

Once it was known, from the preliminary binding studies, that copper was being complexed by the organic matter, it was desired to determine in which fractions the bound metal was located.

A typical chromatogram for pore water with its corresponding metal analysis, is shown in Figure 3-9. From this comparison, several points should be noted:

1. No free copper is seen, as the uncomplexed metal would appear at the void volume. When copper standard was injected into the LC, it came out as a single sharp peak, very near the void volume. Therefore, it appears that all copper was bound in some form,
Figure 3-9: Liquid Chromatography and Atomic Absorption plots of copper-binding to fractions of Pore water extract.

Results of copper binding to PWA extract after separation by liquid chromatography and copper analysis by atomic absorption.

LC parameters: Flow rate, 3 ml/min.; Column, semi-preparative scale; Column packing, C-18 Porasil-B on 10 μ particles; Sample size, 2 ml; Detector, UV at 254 nm; Detector attenuation, X2; Solvent, 60/20/20% v/v water/n-propanol/acetonitrile; Sampling rate of LC eluent, 0.5 min/aliquot.

Copper was added as 50 ul of 0.0505 M CuCl₂ solution in D/D water.
Copper L.C.-A.A.

Injection point

A_{a,a.} \rightarrow A_{i,c.}
possibly to organic matter.

2. The most polar peak, labeled A, has an undetectable corresponding copper peak, less than 0.1 ppm copper. If this material retained as much copper on a relative basis as fraction B, the amount of copper observed should have been approximately 0.2 ppm. Because there is very little copper present in the material in this fraction, one of two processes could be occurring. Either the organic matter does not bind copper, or if binding does occur, it is much weaker than the other fractions which contained metal after the LC analysis.

3. The second peak on the LC (labeled B) has a large corresponding copper peak. This fraction seems to bind significant amounts of copper quite strongly.

4. The third LC peak (labeled C) may bind a small amount of copper, but it cannot be determined for certain as the corresponding copper signal would be buried beneath the broad envelope seen.

It is interesting to note that one large copper absorbing peak apparent by AAS (labeled I), has no corresponding peak on the LC chromatogram. There are two possible explanations for this:

1. First, it could be that the LC peak for this copper-binding constituent is hidden under the broad envelope seen.

2. The second possibility is that this particular organic component does not absorb in the UV. There are several types of compounds which could be present, and yet not absorb at 254 nm: aliphatic hydrocarbons, alcohols, ethers, simple carboxylic acids, fluorinated or chlorinated compounds, primary or secondary amines, mercaptans, nitriles, or molecules with non-conjugated double bonds. (Sixma
The other extracts showed similar types of profiles. The SW extract is shown in Figure 3-10. In this example, the first two smaller peaks on the LC chromatogram in Figure 3-10 fail to show any copper binding. Again, it is the more non-polar fractions that are complexing the metal.

The fact that the first two peaks complex very little copper is surprising as it would be expected that the more polar fractions, most likely having a fair amount of functionality, would bind trace metals quite readily. The more non-polar substances, however, perhaps because of fewer substituent groups, would bind less of the metal.

A possible explanation is that the fractions that bind copper, although more non-polar than the fractions which do not bind copper, contain more functional groups, such as salicylate or phthalate, which would preferentially bind copper. Another possibility is that the first fractions do bind copper, but are prevented in this case either by a kinetically slow process (relative to the other sites), or a weaker thermodynamic equilibrium constant. The result in either instance would be that the other, non-polar peak (labeled B in Figure 3-9) would be the preferred complexation site and therefore bind all available metal. If this site were saturated, then perhaps binding in the other, less-desired locations, would be observed.

One interesting observation is that all extracts tested are relatively close to pore water in terms of the qualitative aspects of the copper binding, i.e. the same fractions appear to be complexing metal. Some possible explanations for this are:

1. All, or most, of the material that will bind copper is in the
Figure 3-10: Liquid Chromatography and Atomic Absorption plots of copper-binding to fractions of artificial sea water extract.

Results of copper binding to SW-I extract after separation by liquid chromatography and copper analysis by atomic absorption.

LC parameters: Flow rate, 3 ml/min.; Column, Semi-preparative scale; Column packing, C-18 Porasil-B on 10 μ particles; Sample size, 2 ml; Detector, UV at 254 nm; Detector attenuation, X2; Solvent, 60/20/20% v/v water/n-propanol/acetonitrile; Sampling rate of LC eluent, 0.5 min/aliquot.

Copper was added as 50 μl of 0.0505 M CuCl₂ solution in D/D water.
pore water and very little change occurs in this binding potential with removal of the coatings. This would be the case if the compounds being solubilized had no affinity for copper binding.

2. Material that is on the coatings is similar, if not identical, to that in the pore water. Therefore, when the coatings solubilize, there is the same type of material binding the copper, only more of it.

3. The extraction procedure does indeed pull off different organic material that can complex copper, but the UV absorbance of this additional material is not seen as it is "buried" beneath the broad envelope present in the chromatogram.

The second hypothesis can be discounted for a number of reasons:

1. DOC values show that the extracts have a much higher percentage of high molecular weight material. The ratios of high MW/low MW would be similar if the amount of organic material in pore water were governed by solubility only, and therefore the coatings were very similar to the compounds dissolved in solution.

2. The LC chromatograms (Figure 3-1 to 3-3) clearly demonstrate that there is a qualitative difference in the organic material in the extracts.

Looking at the chromatograms (Figures 3-1 to 3-3) in more detail, the third possibility seems most likely. The amount of organic matter in the envelope which is responsible for metal binding changes from extract to extract, and in a manner that is not consistent with the DOC levels. This indicates that different material is present in the extracts, with different molar absorptivities.

Coupling this observation with the LC and DOC data mentioned
earlier, the hypothesis of different Cu-binding organic compounds being extracted during the various isolation processes is plausible. To fully test the first concept mentioned (all metal-binding compounds are in the pore water initially), quantitative metal-binding experiments were performed.

VI. Binding Capacity

The liquid chromatography results revealed very little difference in metal binding characteristics between pore water and the two extracts. The quantitative aspects of the organic matter's ability to complex copper will be discussed below.

The procedure published by Amicon was used for all calculations to determine the amount of copper bound (Amicon Corp., 1977). Figure 3-11a is a diagram of an idealized study, and Figure 3-11b shows the results from a study done with pore water. The apparatus used is illustrated in Figure 2-3.

To calculate the amount of copper bound, mass balance calculations were employed. Because the system was theoretically closed (no loss or copper contamination), the amount of copper added to the cell should equal the amount in the effluent plus the quantity remaining in the cell. The amount of metal added can be calculated by multiplying the concentration of copper in the reservoir by the volume of reagent added. In practice, it is difficult to measure the volume of solution added as the reservoir is pressurized throughout the experiment, therefore the volume of eluent was measured, and the two volumes, titrant and eluent, were assumed to be equal.

Once added, copper was present in one of two forms: free or
Figure 3-11: Results of Binding Capacity Study (idealized and actual) of copper with pore water.


3-11B: Binding results of pore water binding copper. Experimental parameters: DOC of extract, 30.4 mg C/l; Reservoir copper concentration, 15 ppm (C_{res}) in 0.1 M HEPES buffer, pH = 7.5; Nitrogen pressure, 60 PSI; Sampling rate, 99 min/aliquot; average filtrate volume, 0.9 ml; Sample volume, 10.0 ml.
bound. The "bound" copper was assumed to be complexed to organic matter, and the amount of bound metal was the unknown in this case. The free metal was a combination of that metal collected as eluent, and that which remained in the cell. A blank run (no organic material present) demonstrated that free copper was not being retained by the cell. Therefore, it was assumed that the concentration of free metal inside the cell was the same as in the eluent at any given time.

The resulting equation, substituting in the assumptions made above, becomes:

\[(\Sigma V_i)(Cr) = (V_c)(C_{fi}) + \Sigma((V_i)(C_{fi})) + Ab\]  

(1)

where

\[
\begin{align*}
\Sigma V_i & \quad \text{Total volume of eluent} \\
V_i & \quad \text{Volume of eluent in each aliquot} \\
Cr & \quad \text{Concentration of copper in the reservoir} \\
V_c & \quad \text{Volume of cell (10 ml)} \\
C_{fi} & \quad \text{Concentration of free copper in each aliquot} \\
Ab & \quad \text{Amount of bound copper}
\end{align*}
\]

Rearranging the above equation to solve for amount of bound copper:

\[Ab = (\Sigma V_i)(Cr) - (V_c)(C_{fi}) - \Sigma((V_i)(C_{fi}))\]  

(2)

This equation was then utilized for all data obtained.

This technique was applied to several samples, and the results are listed in Table 3-8, along with results for a blank study. Although
the organic matter was not completely saturated with copper in these experiments, some conclusions can be drawn from the results.

From the results of the first set of experiments shown in Table 3-8, it appears as if the D/D extract is not only higher in organic content, but also has a greater capacity to bind copper. As all of these earlier experiments ended at a different point, i.e. varying amounts of titrant had been added, therefore the organic matter had been saturated to different degrees. Because of this, it was not possible to directly compare the results. In an attempt to overcome this obstacle, a normalization procedure was used. This involved re-calculating the results on the basis of the same amount of titrant added (16.3 ml). Therefore, all samples would have had the same amount of copper added. At this point, any difference in the amount of metal bound should represent differences in the copper-binding characteristics of the organic matter in the various sample.

Based on these later results as presented at the bottom of Table 3-8, it is apparent that for a given volume of titrant, the D/D extract complexed more copper. For this particular set of experiments, the concentration of organic material in the D/D sample was less than that present in the other runs (PW-1 and SW-1) as is shown in Table 3-8.

The SW extract shows the poorest binding, despite the fact that the concentration of organics was less than that for the D/D extract sample for this same study. As this extract was second only to the PW-1 sample in amount of organic material used in the experiment, this is a bit surprising. One possible explanation is that the material extracted during the isolation process with D/D water had more sites
Table 3-8: Results of binding capacity study of anoxic sediment extracts for copper.

<table>
<thead>
<tr>
<th>Sample (Extract)</th>
<th>DOC (mg C/l)</th>
<th>Bound Cu (ug)</th>
<th>Total volume of eluent (ml)</th>
<th>mmole Cu/g OM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank (D/D Water)</td>
<td>0.0</td>
<td>-1.4</td>
<td>34.0</td>
<td>---</td>
</tr>
</tbody>
</table>

**Partial Saturation**

<table>
<thead>
<tr>
<th>Sample (Extract)</th>
<th>DOC (mg C/l)</th>
<th>Bound Cu (ug)</th>
<th>Total volume of eluent (ml)</th>
<th>mmole Cu/g OM</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWI-Cu</td>
<td>127.8</td>
<td>44.8</td>
<td>80</td>
<td>18.6</td>
</tr>
<tr>
<td>SWI-Cu</td>
<td>84.8</td>
<td>96.2</td>
<td>56</td>
<td>16.3</td>
</tr>
<tr>
<td>D/D-II-Cu</td>
<td>78.3</td>
<td>771.3</td>
<td>88</td>
<td>43.6</td>
</tr>
</tbody>
</table>

**Saturated**

<table>
<thead>
<tr>
<th>Sample (Extract)</th>
<th>DOC (mg C/l)</th>
<th>Bound Cu (ug)</th>
<th>Total volume of eluent (ml)</th>
<th>mmole Cu/g OM</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWA-IV</td>
<td>30.4</td>
<td>296.3</td>
<td>45</td>
<td>86.1</td>
</tr>
</tbody>
</table>

Normalizing all results to the values of bound copper cotained at an elution volume of 16.3 ml yields the following results:

<table>
<thead>
<tr>
<th>Sample (Extract)</th>
<th>DOC (mg C/l)</th>
<th>Bound Cu (ug)</th>
<th>Total volume of eluent (ml)</th>
<th>mmole Cu/g OM</th>
</tr>
</thead>
<tbody>
<tr>
<td>D/D water</td>
<td>0.0</td>
<td>-2.4</td>
<td>16.3</td>
<td>---</td>
</tr>
<tr>
<td>PWI-Cu</td>
<td>127.8</td>
<td>41.4</td>
<td>83</td>
<td>16.3</td>
</tr>
<tr>
<td>SWI-Cu</td>
<td>84.8</td>
<td>96.2</td>
<td>56</td>
<td>16.3</td>
</tr>
<tr>
<td>D/D-II-Cu</td>
<td>78.3</td>
<td>303.1</td>
<td>93</td>
<td>16.3</td>
</tr>
<tr>
<td>PWA-IV</td>
<td>30.4</td>
<td>158.3</td>
<td>64</td>
<td>16.3</td>
</tr>
</tbody>
</table>

For all studies, reservoir was a buffered solution of 0.1 M HEPES at pH = 7.5. Volume of extract used was 10.0 ml, and the apparatus used is illustrated in Figure 2-3.

**Abbreviations:**
- D/D---double deionized water.
- DOC---dissolved organic carbon.
- OM----organic material.
- PW----pore water.
- SW----artificial seawater extract.
that were capable of complexing copper.

From the completed study with pore water (Table 3-8 and the plot given in Figure 3-12), it can be concluded that pore water has an enormously high capacity to bind copper, 75 mmole Cu/g organic matter. Templeton (1980) in his work with sedimentary organic matter did not find enough carboxylate, or other functional groups capable of binding copper to account for such a high binding capacity.

One possible explanation for the seemingly high results is that not all the metal retained was actually bound to the organic matter. Flaig and co-workers (1975) noted that co-precipitation occurred between organic materials (in his case humic substances) and metal hydroxides. Perhaps in this case, the organic material coagulated the copper hydroxide, and binding to organic matter occurred to a much small extent.

Binding capacities obtained previously substantiate the concept that all this retained copper was not bound to organic material. Schnitzer used soil organic matter, and obtained a maximum of 61 umole of copper bound for each gram of organic matter (Schnitzer et al., 1965). One large difference was that his investigations were conducted at pH 5.0, which would result in fewer deprotonated groups capable of binding metal. Rashid (1971) had results that were closer to the values obtained in this work; 1.5 mmole of metal/ g of organic matter. His later work (1974) elevated this value to 10 mmole/g OM. Also, the pH was closer to this work (7.0 vs. 7.5 for this research). This still is significantly lower than values obtained for the partial saturation of the D/D extract (15mmole/g), and substantially lower than that for the completed pore water study (75 mmole/g OM).
Figure 3-12: Plot of bound copper versus filtrate volume for binding capacity study of pore water.

Plot of bound copper versus filtrate volume for PWA-IV. Bound copper calculated using equation #2 in text.
Experimental parameters: DOC, 30.4 mg C/l; other parameters as in Figure 3-11B.
Another possible explanation for the discrepancy in binding capacities is that copper could be being retained for some other reason, for example, polymerization of the hydroxide (coagulation). This is unlikely, however, as a blank study showed that no metal was retained by the cell under similar experimental conditions. The determination of the actual cause of the unusual results mentioned above requires further investigation.

Despite the difficulties encountered in these studies, some conclusions can be made.

1. There appeared to be a quantitative difference in the extracts in terms of their binding capacity, based on preliminary investigations. D/D seemed to complex copper more efficiently, and the SW extract was poorest for binding metal.

2. A great deal of copper appeared to be binding to the organic matter, both in the preliminary and completed studies. As this quantity was substantially greater than had been reported by other workers, it seemed likely that organic matter complexation alone was not sufficient to explain the retention of copper by the Amicon cell. A possible explanation for this discrepancy is that aggregates of copper hydroxides were being retained. These copper hydroxides would be the dominant inorganic copper species pH greater than 7.2. Below that Cu(CO$_3$) or Cu$^{2+}$(aq) species would be dominant. These polyhydroxide species could be retained in the Amicon system because of the net negative charge on the membrane surface.
VII. **Comparison of Binding between Fe (2+) and Cu (2+) Ions**

The goals of this study were as follows:

1. The determination of which fractions of the various extracts and pore water would bind ferrous (Fe (2+)) ions, and compare these results with those for cupric (Cu(2+)) ions.

2. The investigation of competitive binding between ferrous and cupric ions to organic matter.

A typical profile showing the binding of both metals (Fe (2+) and Cu (2+)) to the organic matter is presented in Figure 3-13 and Table 3-9. It was readily apparent that ferrous ions were complexed in the same fractions of organic material as cupric ions. This suggests that the same components of the extracts were complexing both metals, and also the same binding sites in the individual compounds could be binding both metals.

The presence of copper in all samples in Table 3-9 is because the acetonitrile used in the LC solvent had a trace level of copper present (1.1 ppm). This was then scavenged by the organics in the samples, and subsequently appeared in the analyses. In a later experiment, the solvent system was changed to prevent any possible influence of contaminated solvent. The results were similar to those obtained here.

It is interesting to note that the iron signal is lower in samples where both metals were present, as compared to those samples that contained only iron. There were two possible explanations:

1. Copper displaced the organically complexed iron, which was then eluted elsewhere in the run, or hydrolyzed and precipitated prior to analysis.
Figure 3-13: Liquid Chromatography and Atomic Absorption plots of copper and iron binding to fractions of pore water.

Results of copper and iron binding to PWA-IV after separation by liquid chromatography and metal analysis by atomic absorption.

LC parameters: Flow rate, 4 ml/min.; Column, Semi-preparative scale; Column packing, C-18 Porasil-B on 10 u particles; Sample size, 2 ml; Detector, UV at 254 nm; Detector attenuation, X2; Solvent, 60/20/20% v/v water/n-propanol/acetonitrile; Sampling rate of LC eluent, 1.0 min/fraction.

Metals were added to achieve a final concentration of 20 ppm.

Sample had a DOC of 30.4 mg C/l.
Table 3-9: Numerical results of copper and iron binding to fractions of pore water.

**Sample #1—no metals added**

<table>
<thead>
<tr>
<th>LC eluent</th>
<th>Copper conc. (ppm)</th>
<th>Iron conc. (ppm)</th>
<th>LC Absorbance+ (relative units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-6</td>
<td>BDL*</td>
<td>BDL</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0.12</td>
<td>BDL</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>0.51</td>
<td>BDL</td>
<td>11</td>
</tr>
<tr>
<td>9</td>
<td>1.13</td>
<td>BDL</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>1.63</td>
<td>BDL</td>
<td>51</td>
</tr>
<tr>
<td>11</td>
<td>1.63</td>
<td>BDL</td>
<td>56</td>
</tr>
<tr>
<td>12</td>
<td>1.16</td>
<td>BDL</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td>0.81</td>
<td>BDL</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>0.12</td>
<td>BDL</td>
<td>1</td>
</tr>
</tbody>
</table>

**Sample #2—copper added**

<table>
<thead>
<tr>
<th>LC eluent</th>
<th>Copper conc. (ppm)</th>
<th>Iron conc. (ppm)</th>
<th>LC Absorbance+ (relative units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4</td>
<td>BDL</td>
<td>BDL</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0.12</td>
<td>BDL</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>BDL</td>
<td>BDL</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>BDL</td>
<td>BDL</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>BDL</td>
<td>BDL</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>0.22</td>
<td>BDL</td>
<td>26</td>
</tr>
<tr>
<td>10</td>
<td>0.88</td>
<td>BDL</td>
<td>48</td>
</tr>
<tr>
<td>11</td>
<td>1.82</td>
<td>BDL</td>
<td>57</td>
</tr>
<tr>
<td>12</td>
<td>2.91</td>
<td>BDL</td>
<td>105</td>
</tr>
<tr>
<td>13</td>
<td>1.35</td>
<td>BDL</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>BDL</td>
<td>BDL</td>
<td>60</td>
</tr>
<tr>
<td>15</td>
<td>BDL</td>
<td>BDL</td>
<td>185</td>
</tr>
</tbody>
</table>

**Sample #3—iron added**

<table>
<thead>
<tr>
<th>LC eluent</th>
<th>Copper conc. (ppm)</th>
<th>Iron conc. (ppm)</th>
<th>LC Absorbance+ (relative units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>BDL</td>
<td>BDL</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>BDL</td>
<td>BDL</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>BDL</td>
<td>BDL</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>BDL</td>
<td>BDL</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>BDL</td>
<td>BDL</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>BDL</td>
<td>BDL</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>0.12</td>
<td>0.10</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>0.77</td>
<td>0.41</td>
<td>26</td>
</tr>
<tr>
<td>10</td>
<td>2.06</td>
<td>1.48</td>
<td>150</td>
</tr>
<tr>
<td>11</td>
<td>3.53</td>
<td>3.52</td>
<td>179</td>
</tr>
<tr>
<td>12</td>
<td>3.36</td>
<td>4.65</td>
<td>185</td>
</tr>
<tr>
<td>13</td>
<td>0.73</td>
<td>0.47</td>
<td>235</td>
</tr>
<tr>
<td>14</td>
<td>BDL</td>
<td>BDL</td>
<td>10</td>
</tr>
<tr>
<td>15</td>
<td>BDL</td>
<td>BDL</td>
<td>7</td>
</tr>
</tbody>
</table>
Table 3-9: (continued)

Sample #4—copper and iron added

<table>
<thead>
<tr>
<th>Aliquot of LC eluent</th>
<th>Copper conc. (ppm)</th>
<th>Iron conc. (ppm)</th>
<th>LC Absorbance+ (relative units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BDL</td>
<td>BDL</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>BDL</td>
<td>BDL</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>BDL</td>
<td>BDL</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>BDL</td>
<td>BDL</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>BDL</td>
<td>BDL</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>BDL</td>
<td>BDL</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>BDL</td>
<td>BDL</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>0.12</td>
<td>0.10</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>0.68</td>
<td>0.26</td>
<td>11</td>
</tr>
<tr>
<td>10</td>
<td>2.27</td>
<td>2.01</td>
<td>53</td>
</tr>
<tr>
<td>11</td>
<td>4.36</td>
<td>3.74</td>
<td>129</td>
</tr>
<tr>
<td>12</td>
<td>3.36</td>
<td>2.33</td>
<td>180</td>
</tr>
<tr>
<td>13</td>
<td>BDL</td>
<td>BDL</td>
<td>30</td>
</tr>
<tr>
<td>14</td>
<td>BDL</td>
<td>BDL</td>
<td>9</td>
</tr>
<tr>
<td>15</td>
<td>BDL</td>
<td>BDL</td>
<td>6</td>
</tr>
</tbody>
</table>

BDL*—Below detection limits (0.1 ppm for both metals).
LC+—UV absorbance at 254 nm.
#—a peak was located at this point.
2. Copper facilitated the oxidation, and subsequent hydrolysis of iron which caused the metal to precipitate and therefore not be eluted by the LC.

As no free iron was ever seen, it is unlikely that the substitution of copper for iron occurred. Also, the fact that the material was undersaturated with respect to total metal binding capacity (as evidenced by the earlier results) supported the argument that no substitution occurred.

VIII. EPR Spectroscopic Studies of Complexes

The goal of this series of investigations was to obtain information about the specific details of copper binding to organic matter.

This included:

1. Attaining further evidence of copper-organic complex formation and information about the presence of single or multiple species.

2. Potential identification of the ligands on the organic material responsible for metal binding.

3. Determination of differences between metal-organic matter complexes in pore water and the extracts.

Solid sample and frozen solution (-14°C) spectra are shown in Figures 3-14 through 3-16 and demonstrate the presence of copper (2+) as opposed to copper (1+). Table 3-10 lists the calculated g and a-hyperfine values. Solid samples yielded the best spectra because the samples of organic matter (and complexed metal) were greatly concentrated, and therefore greater signal/noise was achieved.

Several spectra were obtained for different samples (solution and solid). All showed the presence of multiple species and traces
Figure 3-14: First derivative, frozen (-140°C), X-band, EPR spectrum of copper bound to D/D extract.

First derivative, frozen (-140°C), X-band, EPR spectrum of D/D I extract (10 ml, 117.6 mg C/l) with 10 ppm copper.

EPR parameters: Modulation Amplitude, 16 mT; Scan rate, 4T/16 min.; Time constant, 1 sec; Microwave power, 10 mW; Microwave frequency, 9.17 GHz.
Figure 3-15: First derivative, room temperature, X-band, EPR spectrum of solid sample of copper bound to pore water.

First derivative, room temperature, X-band EPR spectrum of solid PWA extract (10 ml, 155.6 mg C/l) with 10 ppm copper added prior to freeze drying.

EPR parameters: Modulation amplitude, 10 mT; Scan rate, 4T/16 min.; Time constant, 3 sec; Microwave power, 20 mW; Microwave frequency, 9.52 GHz.
Figure 3-16: First derivative, frozen (-140°C), X-band, EPR spectrum of copper bound to SW extract.

First derivative, frozen (-140°C), X-band, EPR spectrum of SWII (10 ml, 94.8 mg C/l) extract with 10 ppm copper.

EPR parameters: Modulation amplitude, 16 mT; Scan rate, 4T/16 min.; Time constant, 1 sec; Microwave frequency, 9.17 GHz.
Table 3-10: Calculated $g$ and $a$ values for EPR spectra shown in Figures 3-14 through 3-16. For explanation of samples, see figure captions.

<table>
<thead>
<tr>
<th>Figure</th>
<th>Sample</th>
<th>$g_1$ (ave.)</th>
<th>$g_{ll}$ (ave.)</th>
<th>$a_{ll}$ (ave.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-14</td>
<td>D/D-I-Cu</td>
<td>2.05</td>
<td>2.42</td>
<td>18 mT</td>
</tr>
<tr>
<td>3-15</td>
<td>PWA-Cu</td>
<td>2.05</td>
<td>2.39</td>
<td>18 mT</td>
</tr>
<tr>
<td>3-16</td>
<td>SWII-Cu</td>
<td>2.04</td>
<td>UD</td>
<td>UD</td>
</tr>
</tbody>
</table>

D/D-----double deionized water extract
PW------pore water extract
SW------artificial seawater extract
UD------unable to determine
of iron, probably as colloidal iron hydroxide. The presence of multiple species was not surprising. It was felt that an equilibrium probably existed between free and bound copper. Also, as each copper species had a unique EPR spectrum, the net result would be a composite of the two individual spectra. In addition, since the organic material extracted is not a single compound (or even a single class of compounds), but a heterogeneous mixture, there are expected to be several organo-copper species, each with an individual spectrum.

It was observed that spectra taken from similar samples (i.e. same extraction procedure) from different cores often showed marked differences. The reason for these differences between different cores could be due to the variation in the organic matter present in different cores.

Because of the large variation seen in supposedly similar samples, no conclusions could be drawn with regards to the ligands responsible for binding, or differences between the complexes formed with material isolated by the various extraction techniques. Perhaps future work could prove more fruitful in this area.
CHAPTER 4

CONCLUSIONS AND FUTURE STUDIES

I. **Organic Matter Isolation**

One of the most important factors to be considered when working with any environmental sample is, "How does this sample relate to the material present in the natural system?" If this basic issue is not addressed, the results are of dubious value. The first part of this dissertation work was meant to give some insight into the relationship between extracted material and organics present in undisturbed sediments.

Templeton (1980) was one of the first to show the importance of proper sample handling precautions when working with organic matter in anoxic samples. However, he never questioned how his seawater extracts related to the material initially present in the natural environment. This research has emphasized the simultaneous comparison of PW, SW extract and D/D extract. In this way, it was felt that a comparison could be made between these various isolation techniques. These conclusions, theoretically, could then be extended to encompass other work that Templeton did, e.g. metal-binding, degradation studies, physico-chemical characterization of the organic matter, and research done by others investigating pore water systems.

The procedure used to obtain pore water (Figure 2-2) was thought to yield material that was representative of that actually present in the interstices of the sediment grains. Yet, it was entirely possible...
that the various steps involved (exposure to nitrogen, sectioning, centrifugation and filtration) may have altered that organic matter, or added material that was not in the interstitial fluids originally. However, as this was the most representative material available to date for Great Bay sediments, it will be used as the "natural, unaltered" standard for comparison purposes.

The D/D extract, because of the higher amount of organic material isolated when compared to interstitial fluids, must include not only that organic matter present in the pore water, but some from other sources as well. It is quite possible that this added material is derived from organic coatings on the sediment grains, substances that were originally associated with sediment aggregates, or materials present as organic floc in the sediment. Therefore, any conclusions drawn from data obtained for this material must deal with the fact that this extract is a heterogeneous mixture of compounds.

The D/D extraction procedure also gave information as to the effect of ionic strength on this organic material. Because the salt content in the pore fluids has been substantially diluted by the addition of the water extractant, the final extract will have a reduced ionic strength compared to PW. This procedure also resulted in an increase in the amount of material removed for a given amount of sediment, when compared to the seawater (SW) extract. Since the only difference between the two procedures is the extractant used, the variation can be attributed to the difference in ionic strength.

In the natural system, it is possible to have a ground water influx into the sediments (Orem, 1981b). This ground water will dilute the salt content of the pore fluids, resulting in a system of lower
ionic strength. This parallels the model system using D/D water as the extractant. Therefore, it might be possible to extrapolate the results for the D/D extract to any type of situation where salinity changes occur in the sediments.

The organic matter in the D/D extract is of an intermediate polarity when compared to the SW extract or pore water. Much of the material is more polar than that present in PW, but not as non-polar as some components of the SW extract. Polarity differences between the two extracts, as determined by liquid chromatography, is likely caused by the ionic strength differences between the two extractants.

The artificial SW extract could be representative of the situation where interstitial fluids are diluted by overlying water. Results indicate that increased ionic strength of the extractant solubilized less organic material. Dissolved material would be more likely to flocculate and precipitate out of solution. This hypothesis is consistent with the results of this work.

From the data presented earlier (Table 3-3), it is evident that one major reason for the increased amount of organic matter in the extracts was the the shaking step in the extraction procedure. The effects of bacteria and temperature on the amount of material extracted, although important in qualitative aspects of the organic matter, were masked by the effect of the agitation process.

Since the extracts are so different in polarity and amount of material extracted from PW, what possible relevance could they have to the natural sedimentary system? To answer that question, it is important to recall the sources of organic matter in the extracts. A majority of this material is probably present in the sediments as
organic coatings, the rest as dissolved material in the interstitial fluids. The organic constituents in pore water are most likely small (based on the dissolved organic carbon (DOC) and ultrafiltration data), and relatively polar compounds (liquid chromatography results). These pore water organics, being dissolved in the fluids, are relatively mobile in the sedimentary system. Much of the material in the extracts, however, is likely to be larger and more non-polar, therefore much less mobile.

If organic materials do interact with metals, the organics in pore water could be involved with trace metal transport. The organics on the sediments are more likely involved in the storage of metals onto sediment particles. It has been established in results presented earlier, that organic matter in the sediment, both pore water samples and sedimentary materials, interacts with copper, and is capable of binding a large quantity of the metal (10+ mmole of metal per gram of organic matter).

By noting the similarities (metal-binding properties) and differences (polarity and amount of organic material extracted) between pore water and extracts, the results of Templeton (1980), and Lyons and co-workers (1980) with organic matter in the sediments can be coupled to the results from this research to give a more complete picture of the sedimentary system. Templeton also observed copper binding to organic matter, but to only one fraction. The results from this research demonstrate that at least two fractions in the pore water bind copper. The same is true for the SW extracts. The discrepancies could be explained by the fact that Templeton used partially oxidized material, which could have altered his results. Oxidation could have
Ill degraded one of the binding constituents, and therefore render it incapable of complexing the metal. The other possibility is that the "missing" copper-binding fraction in Templeton's work could have precipitated upon exposure to oxygen, a phenomenon seen in this research, and by others (Orem, 1981b).

The above discussions demonstrate how results using pore water data can be correlated with results for extracts. Because of the extra amount of material obtainable through extraction procedures, these processes are often desirable when investigating sedimentary systems. Since comparisons are possible, results using sedimentary extracts are of value, and can be used to explain processes in the sediments, particularly those differences between sedimentary and dissolved organic matter. This is especially true when analyses are done simultaneously with pore water measurements for comparison purposes.

II. Metal Binding to Organic Matter

Perhaps the single most important conclusion reached during this research was that the unaltered organic matter isolated from anoxic sediments does bind copper, and binding occurs to significant extent. This complexation occurs in all extracts, as well as in pore water. The process is kinetically quite rapid, occurring in less than 10 minutes. It is possible that these organic compounds stabilize copper in pore waters and prevent the metal from precipitating in the form of a metal-sulfide as is predicted by the limited thermodynamic data available.

An interesting aspect of this research is that the most polar component(s) of these samples does (do) not bind copper. A couple of possibilities exist to explain these results.
1. The first fraction observed on the LC chromatogram could consist of small, polar compounds: for example, straight chain fatty acids such as acetic acid. These compounds may not be capable of binding metal, or if complexation does occur the stability constant is extremely small. These binding sites on the non-polar compounds are quite possibly salicylate and phthalate groups which are especially good chelators of copper. This is consistent with results reported by others studying trace metal binding to organic matter (Gamble et al., 1970; Van Dijk, 1971; Manning and Ramamoorthy, 1973; Stevenson et al., 1973; Buffle et al., 1977; and Breshnahan et al., 1978).

2. Binding could be governed by the kinetics of the system, as opposed to the thermodynamics. It has been shown by this work that the kinetics of binding are quite rapid (less than 10 minutes for complete complexation). Possibly the complexation of copper by the first, polar fraction, is kinetically slower, and therefore no bound metal is observed for these constituents under the experimental conditions used here.

This study, unfortunately, does not prove conclusively that these organic fractions from anoxic sediments complex copper in the natural system. The results indicate that the potential is there, and that binding could occur, but whether it does or not is an issue that this research does not address.

Other species which could conceivably account for the increased solubility of copper in pore fluids include polysulfides, a suggestion first made by Gardner (1974). However, these copper-polysulfidic species have yet to be isolated from sediments, or formed in the
Several workers have extracted what they call organic-copper species from sediments (Nissenbaum and Swaine, 1976; Kitano et al., 1980). However, these studies were based on indirect evidence from various extraction techniques. No direct evidence has been given as to the existence of these species, in the natural system. Until such evidence is presented, no final conclusions as to the presence of these species in the sediments can be made.

III. Overall Conclusions of Research

This research was carried out to obtain information about metal-binding organic constituents in estuarine sediments. Specific conclusions reached through results presented in this dissertation are as follows:

1. The extraction procedures used, while relatively mild in nature, removed both dissolved and sedimentary organic matter.

2. Ionic strength has a definite effect on organic matter. High ionic strength media yielded components that were more non-polar than either pore water organics or those extracted with D/D water extractant.

3. Unaltered organic matter in sediments do have the capability of binding copper. This is true for pore water and sedimentary organic materials.

4. Not all fractions of this material will bind copper but those which do bind the metal have a large binding capacity compared to humic and fulvic substances.

5. The kinetics of these processes are quite rapid with binding
occurring in less than 10 minutes.

6. The same fractions that will bind copper also bind ferrous ions (and potentially other metals). This suggests that perhaps similar binding sites are involved, as well as similar mechanisms and indicates that organic matter could be involved in the chemistry of other trace metals.

IV. Suggestions for Future Work

From this work, it is readily apparent that additional research is warranted in three areas: further investigations on the organic matter itself, field-oriented studies on metal-binding, and additional work in the area of metal-binding characteristics.

A. Organic Matter Investigations—Some fractionation of this material has been accomplished, but additional work is needed. The determination of differences between pore water organics and sedimentary organic materials is especially important. This could include further liquid chromatography investigations, as well as nuclear magnetic resonance work and further studies with elemental analysis. It is also important to identify potential and actual metal-binding sites within the organic matter, and to quantify the number of such sites.

It is also critical to keep in mind the sample being analyzed and to be aware of how these results relate to the natural system. Further studies are needed in the determination of just what "pore water" represents and how closely it is related to the material that is actually present in the sediments. A comparison of the present technique for isolation of pore water should be examined and compared with others available (squeezing procedures, dialysis, etc.).
would allow the validity of the assumption that no modifications occur during sampling and subsequent processing.

B. Field Studies—All work done on this project has utilized samples obtained at Adams Cove near Jaskson Estaurine Laboratory. The question remains whether or not these results are applicable to other locations around the Great Bay system, or perhaps to other estuaries. Also, it is important to determine whether metal-binding characteristics vary seasonally. It is known that microbial activity changes with season of the year. Therefore, it is reasonable to expect that chemical equilibria will be shifted. This may have a profound effect on fate of metals in pore water and on the sediment coatings.

C. Metal-Binding Characteristics—This area of work has barely been touched. To fully understand the sedimentary system, it is important to understand how the organic matter is complexing the metal (copper, as well as other). This may give a clue to other interactions in the sediments (e.g. exchange of metals in porphyrins and the eventual fate of metals during diagenesis).

It would also be interesting to investigate some competitive binding studies with reduced sulfides (bisulfide), polysulfides, and other species in the pore fluids to see if binding between the trace metals and organics still occurred.

Since microbial processes are important for many of the diagenetic processes that occur in the pore fluids, it would be interesting to investigate the effect of bacteria, not only on the organic matter, but on the trace metal chemistry in general.
On course, repeating the studies presented here with other metals (e.g. Fe (2+), Ca (2+), Mg (2+)) is important for the attainment of a complete understanding of the overall influence of organic matter in pore water chemistry.
REFERENCES
References


**APPENDIX 1**

**SOLVENT SYSTEMS TESTED**

**FOR**

**LIQUID CHROMATOGRAPHY OPTIMIZATION**

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