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THE EFFECT OF pH ON THE METAL BINDING CAPABILITIES OF EXOPOLYMERIC SUBSTANCES FROM A MARINE BENTHIC DIATOM

BY

Vanessa June Maples O'Donnell
B.S. Western Washington University, 2008

THESIS

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Vanessa June Maples O'Donnell: Thesis Defense

The Effect of pH on the Metal Binding Capabilities of Exopolymeric Substances From a Marine Benthic Diatom

We, the undersigned agree that the above thesis has been successfully defended on the 29th of March, 2013.:

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7th June 2013

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Professor of Chemistry
Date

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DEDICATION

My thesis is dedicated to a group of people that have inspired me, encouraged me and guided me. They probably don't recognize the magnitude of their influence. I have been fortunate enough to meet my husband at a young age and he has been my partner in following our dreams together. My life would not be nearly as fun without him and without all the fun it's hard to stay focused. He is my balance. I would like also like to mention Mary Kozub who nurtured and mentored me. She was my teacher, which introduced me to my potential, and myself … allowing me to feel confident for the first time. Giselle Muller-Parker has also been a constant source of inspiration to me, a professor from my undergraduate that gave me my first exposure to algae and advanced microscopy. She introduced me to the process of graduate school and it was the first time I thought it was a possibility. I will always be thankful for her detail, organization, and discussions on the alignment of science and art. Paul Frazey, a chemistry professor of mine who always acted as an advocate and a major support system to me, gave me endless advice and encouragement. Larry Mayer who taught me the "real" process of science research, I will always use him as an example of what a scientist can be and accomplish. I feel privileged to have met him. I would also like to dedicate this to the children my husband and I will have together. I can't wait to teach you all about nature, science and art.

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ABSTRACT

THE EFFECT OF pH ON THE METAL BINDING CAPABILITIES OF EXOPOLYMERIC SUBSTANCES FROM A MARINE BENTHIC DIATOM

by Vanessa June Maples O'Donnell

University of New England, April, 2013

The marine sediment-water interface is a dynamic microenvironment containing diatoms, which produce exopolymeric substance (EPS). EPS has various functions for individual cells and for marine ecosystems. EPS substance is a species-specific composition that has strong absorptive qualities and is able to bind metals from even a very dilute aqueous solution. As industrially derived metals tend to accumulate in marine mudflats, where diatoms are the major EPS producing organisms, it is important to determine how marine benthic diatom EPS will bind with metals. To address the metal binding properties of diatom EPS, *Cylindrotheca closteriums'* EPS was isolated by tangential flow filtration, exposed to Cu and Cr at a range of pH levels and analyzed using a flame atomic absorption spectrometer. Results indicated that pH was a significant factor in the determination of bound Cu to diatom EPS under all treatments. Maximum bound Cu was 61.7 µg mg-EPS^{-1} at a pH of 6.0 in a buffered system and 17.1 µg mg-EPS^{-1} at a pH of 6.0 in an unbuffered system. Cu was preferentially bound over Cr and precipitation of metals occurred above a pH of 6.5. Diatom EPS binding with metals at a near neutral pH is a significant result and has not been shown before in the literature, but metal concentrations used in this study were unnaturally high, reducing practical implications. More research within this area of marine aquatics is needed to understand the greater global ramifications of pH shift effects on estuaries and fate of metals in the marine ecosystem.

INTRODUCTION

The study of microbial exopolymeric substance (EPS) is multi-disciplinary and can be found in climatology, biochemistry, oceanography, sedimentology, environmental science, toxicology, dentistry, medicine and biotechnology. Microalgae and bacteria are responsible for producing large quantities of EPS (Decho, 1990). Diatoms are a diverse class of unicellular microalgae, called Bacillariophyceae, which have raphae, and are encased in a frustule (siliceous wall); many Bacillariophyceae are benthic (Wetherbee *et al*., 1998).

Benthic diatoms live at the sediment-water interface and have important roles in marine ecosystems such as marine food web dynamics and metal absorption (Decho, 1990). Motility is crucial for phytobenthic diatoms, which live in the sediment but migrate to and from the photic zone (Underwood *et al.,* 2004). Diatoms do this by producing EPS from one end of the raphe (Pickett-Heaps and Wetherbee, 1986; Wetherbee *et al*., 1998; Zhang *et al*., 2008). Organic substances, such as EPS, are released from diatoms during all phases of growth, with EPS comprising up to 80–90% of the total extracellular release (Myklestad, 1995), but the quantity of EPS produced is phase dependent (Staats *et al*., 1999). Decho (2000) describes EPS as a group of large microbially secreted molecules that have diverse physical and chemical properties with a variety of biological roles. Marine benthic diatoms excrete large amounts of EPS in response to environmental conditions and motility requirements (Underwood *et al.,* 2004). EPS surrounding the organism can act as a buffer to physical environmental stresses, acting as a protective layer for the cell. For example, production of EPS could reduce the effects of desiccation (Decho, 2000). Diatom EPS is mostly composed of carbohydrate-rich polymers (Stal and de Brouwer, 2003; Underwood, *et al*., 2004). EPS has charged functional groups, which can act as binding sites (Bhaskar and Bhosle, 2006). A major component of diatom EPS is a polyglucuronic acid, (Figure 1) which has one carboxylic acid functional group per monomer unit and is a known chelator (Bhaskar and Bhosle, 2006; Escandar and Sala, 1992; Tajmir-Riahi, 1986). In addition, EPS has absorptive and adhesive qualities that can form mats in surface sediments; these mats do not just lay upon the sediments but are integrated with the sediment surface (Bhaskar and Bhosle, 2006).

EPS has a dynamic nature, able to take on different physical forms and chemical compositions according to the environmental conditions. Some of those forms include capsules, gels, loose slime and dissolved organic carbon (Decho, 2000). Each of these forms influences the function of EPS in the aquatic ecosystem. There are also types of EPS associated with any particular EPS producing organism; which include non-attached, attached and intracellular. The different types of EPS can play various environmental roles. For example, EPS that is closely associated to the cell can be more important for binding while the non-attached EPS would most likely be washed out from the sediment and not contribute to binding (Decho, 2000).

The large amount of EPS excreted by marine benthic diatoms is a response to 1) motility requirements and 2) environmental conditions (Underwood *et al*., 2004). EPS excreted by diatoms has been shown to have a wide range of ecologically significant functions in marine mudflats (Underwood *et al.,* 2004; Wolfstein and Stal, 2002). For example, EPS plays a useful role in sediment quality (Araújo, *et al.,* 2010) by forming extensive biofilms on the surface of intertidal mudflats, which stabilize sediment (Stal and de Brouwer, 2003; Widdows *et al*., 2000). Because benthic diatoms are the major constituents of the microbial community in intertidal areas, EPS production plays an important role in sediment dynamics (Staats *et al*., 1999). EPS is also capable of forming a microenvironment that stabilizes refugia populations (i.e. pathogenic bacteria) entering the marine environment from freshwater/terrestrial inputs (Duong *et al*., 2007). Diatom EPS is also important in the ecology of cells living in marine sediments because it can be used as a carbon source by bacteria, meiofauna, and macrofauna (Middleburg *et al.,* 2000). In addition to its biological necessity, EPS plays a key role in the ecology of aquatic environments and large scale processes, including cloud condensation nuclei (Bigg, 2007), sediment stability (Stal and de Brouwer, 2003; Widdows *et al*., 2000), food resource at the base of the aquatic food chain (Decho, 1990) and metal binding capabilities (Comte *et al*., 2008).

EPS is able to bind with heavy metals from even a very dilute aqueous solution (Ahluwalia and Goyal, 2005). The effectiveness of EPS for removal of metals from wastewater is made possible because of a property known as biosorption (Ahluwalia and Goyal, 2005), where the biomass can bind with metals, incorporating it. Because diatoms are an abundant source of food for aquatic food webs (Middleburg *et al.,* 2000, Duong *et al*., 2007), the associated EPS can act as a vector for metal bioaccumulation (Staats *et al*., 1999).

Most research has been conducted on EPS isolated from terrestrial and bacterial sources (Christensen, 1989; Decho, 1990; Helyer *et al*., 1993; Brown and Lester, 1980; Rudd *et al*., 1982; Underwood *et al*., 1995); however, bacterial EPS and diatom EPS can have various environmental purposes. For example, bacterial EPS high in the sugar arabinose can aid in bacteria aggregation (particle precipitation) where diatoms' EPS high in deoxy sugars can aid in flocculation (particle clumping); therefore, EPS composition is species specific and can alter environmental purpose (Bhaskar and Bhosle, 2005). The role that EPS plays in the marine sediment system is becoming of interest owing to the fact that diatoms compose up to 99% of the microbial biomass in marine mudflats (Underwood *et al*., 1995), linking EPS to the photoautotrophic community (van Duyl *et al*., 1999). Because diatom EPS binds with metals, determining the capacity of diatom EPS to bind with metals will aid in the understanding the fate of metals within aquatic settings and can better allow researchers to understand where metals end up in the environment.

The pH of estuary sediments has been shown to range from 5.0-7.8 (Ponnomperumo, 1972; van Cappellen and Wang, 1995; Stumm and Morgan, 1996). Benthic diatoms are found in highest densities in the upper 3 mm where photosynthesis occurs (Taylor and Patterson, 1997; Yallop *et al.,* 2000). Photosynthesis is the main driver of pH changes in surface estuary sediments on a diurnal timescale (Schneider and Campion-Alsumard, 1999; Albertano *et al*., 2000; Wolaver *et al*., 1986) but long-term pH changes can be due to sulfate reduction (Thorstenson, 1970; Gardner, 1973) and iron sulfide oxidation (Giblin and Howarth, 1984).

At the sediment-water interface, dynamic chemical competitions occur causing adsorption, precipitation, and remobilization processes of metal species (Yu *et al*., 2000). Cu entering into the ocean environment comes mostly from riverine particulates (Blossom, 2007), including anthropogenic sources. For example, biocide from marine antifouling paints (Blossom, 2007), high ship traffic (Daskalakis and O'Connor 1995) and combined sewer outflows (Iannuzzi *et al*. 1997) contribute significantly to Cu in marine sediments. However, the concentration of Cu in estuary sediment and pore water can vary greatly. Solid-phase sediment Cu concentrations in Halifax Harbor reached 250 ppm (Fader and Buckley, 1995; Buckley and Winters, 1992), while Singapore was 20-140 ppm (Goh and Chou, 1997) and Penobscot Bay, Maine ranged from 4.4 to 57.7 ppm, (Larsen *et al*., 1983). Sediment particulate Cu concentrations are significantly higher than those in sediment pore waters and can be released into the overlying water under acidic conditions. Most United States estuarine water concentrations of dissolved Cu ranged from 0.3–3.8 ppb (Kennish, 1998), but a higher dissolved Cu concentration (5.4 ppb) was found in San Diego Bay, mostly due to antifouling paints (Blossom, 2007). The EPA drinking water standards has set limits of less than 1.3 ppm for Cu and less than 0.1 ppm for Cr (EPA, 1991). Even at very dilute environmental concentrations, metals can be toxic to humans and ecosystems (EPS, 1991). Metals bioaccumulate in marine environments and potentially enter our food sources (Decho, 2000).

The binding of diatom EPS with metals varies according to, salinity, pH, metal concentration, concentration of competing ligands and binding capacity of the ligands (ATSDR, 2004). Knowledge of binding abilities of diatom EPS at the sediment-water interface aids in the understanding of the chemical fluxes, water column transport and/or sedimentary accumulation of toxic metals in the benthic environment. The processes above could have positive or negative effects but this dynamic system needs to be quantified before any conclusions can be drawn. This study examined binding of diatom EPS to metals with changes in pH and the relative binding of Cu and Cr. This study aims to 1) pH had a significant effect on diatom EPS binding with Cu and 2) whether Cu and/or Cr were preferentially bound at various pH levels.

METHODS

Organism and Culture Conditions

Strains of *Cylindrotheca closterium* species (obtained from the Culture Collection of Algae and Protozoa, CCAP Argyll, Scotland) were grown in f2 medium at 16 °C. Cultures were illuminated at an incident irradiance of 60 micromol photons m^{-2} s^{-1}, over a light: dark cycle of 14:10 h. Culturing methods used are from Staats *et al*. (1999). Sea sand was collected and sieved using a 50-micron sieve. The sieved sea sand was then acid washed and autoclaved. The diatom cultures were grown on the purified sand substrate at a salinity of 30 PSU in 1000 mL glass Erlenmeyer flasks. Sterility of the culture was assessed to ensure they were axenic by plating on Lysogeny broth agar medium and observing with a compound microscope. Cultures were discarded if contaminated (Bhaskar and Bhosle, 2006).

Isolation of EPS

Methods of EPS extraction and isolation for *Cylindrotheca closterium* were modified from Staats *et al*. (1999). Briefly, the diatom cultures were harvested in exponential growth at the same time of day (11 am - 3 pm). Cells were homogenized by gently swirling prior to harvest. Suspended cells were poured off and centrifuged for 15 min at 4000 *g* at 10 °C. The supernatant was reserved and the pellet was re-suspended in tap water for 1 hr at 30 °C. The suspension was subsequently centrifuged for 30 min at 4700 *g* and 10 °C, yielding the attached EPS (Figure 2). Because nonattached EPS is present in the culture supernatant, this EPS fraction was pre-concentrated by ultrafiltration using tangential flow filtration (TFF) (Pellicon XL Ultrafiltration Module Biomax 5 kDa) (Figure 2). Attached and nonattached EPS were combined and EPS was precipitated using reagent grade ethanol in 80% (v/v). Ethanol was then evaporated off and evaporation speed was increased by flowing nitrogen gas over the solution surface. Dry weight was measured using an analytical balance prior to compositional analysis.

Experimental Setup

Experimental methods were adapted from Bhaskar and Bhosle (2006). Briefly, metal stock solutions of Cu and Cr were prepared by dissolving appropriate quantities of copper sulfate and chromium (III) acetate in deionized water within a range of pH (specified in experiments below) (Table 1). A known amount of EPS was dissolved in a known volume of water (amounts varied by experiment and are indicated in Table 1). EPS solution was placed in dialysis bags with molecular weight 3.5 kDa cutoff membrane and suspended in acid-cleaned containers with the metal solutions at various pH. If a buffer was used it was added to the solution and mixed well, prior to adjusting pH. The pH was adjusted using NaOH and measured using a pH meter (Hanna HI322001). The dialysis bags and metal solutions were equilibrated with constant agitation. After equilibration, the bags were removed from the metal-containing solution. Blanks at each pH level were performed, in which the diatom polymer solution was replaced with an equal volume of deionized water and then equilibrated. Similarly, to ensure there was no metal being introduced, EPS without any metal treatment was equilibrated in deionized water and used as a control with five replicates. Dialysis bags of all treatments and blanks were retrieved after 140 min of exposure to metal solution and analyzed for metal bound to EPS.

Optimization of Chemical Kinetics

Kinetic analysis of EPS and Cu binding was performed using the methods described above. Cu (5.6 ppm) was exposed to EPS (6.3×10^{-5} g) and the EPS analyzed for bound Cu at pH 6 at a range of times (0.5, 0.8, 1.3, 1.8, 2.3, 3.0, 4.8, 6.0, 17 and 24 h) to determine the optimal time of metal binding. The system was buffered using 3-(N-morpholino) propanesulfonic acid (MOPS).

Effect of pH/Metal

Three experiments were performed to test for the role of pH and the comparative uptake of Cu and Cr. Experimental setups were done in replicates of five (control and at each pH treatment). All blanks from each pH treatment were averaged and subtracted from the sample value; any concentration above the averaged blank was assumed to be metal bound to EPS.

pH 3-7 Experiment

EPS (1.68 x10^{-4} g) was exposed to Cu solution (15 ppm) and analyzed for bound Cu, at a broad pH range (3-7). The system was not buffered (Table 1). This experiment was completed to determine a general trend of how pH affects EPS and Cu binding.

pH 6-8 Experiment

Cu (5.6 ppm) was exposed to EPS (6.3 x10^{-5} g) and analyzed for bound Cu at a pH range (6-8). The system was buffered using 3-(N-morpholino) propanesulfonic acid (MOPS) (Table 1). This experiment was completed to determine understanding of how pH affects EPS and Cu binding in marine sediments by looking at actual sediment pH range.

Cu vs. Cr Experiment

An EPS binding experiment was conducted where Cu (15ppm) and Cr (12.3 ppm) were co-dissolved and exposed to EPS (1.68 x10^{-4} g) and bound metals were measured to determine if metals are preferentially bound to EPS.

Determination of Metals Bound to EPS

Unknown Sample Solution

3.5 mL of the EPS solution (n=5) or controls (n=5) was pipetted out of the dialysis tubing and placed in a 10 mL volumetric flask. Distilled deionized (DI) water was filled to the mark and mixed thoroughly. Note: All glassware was acid washed to eliminate cross contamination.

Metal Solutions

Cu standard stock solutions (0.1, 0.5, 1, 2 and 10 μg/mL) and Cr standard stock solutions (0.1, 0.5, 1, 5 and 10 μg/mL) were prepared with care to ensure accuracy and precision. Appropriate amounts of reagent grade metal solutions (1000 μg/mL single-element stock solutions in 2-10% nitric acid) were placed in 100 mL volumetric flasks. Distilled DI water was filled to the mark and mixed thoroughly.

Instrumentation and Settings

The flame atomic absorption spectrometer (AAS) (932 Plus, GBC Scientific) used air/acetylene flame. The instrument was calibrated to ensure accurate results by doing the following 1) setting up parameters specific to the metal as noted in the instrumentation manual, 2) burner alignment, 3) using a proper hollow-cathode lamp either Cu (λ= 324.7) or Cr (λ= 357.9) (Athanasopoulos, 2002). Deionized water was aspirated for 30 sec between each sample.

Measurement Method Using Flame AAS

Samples, including stock solutions, were gently inverted 3x prior to analysis and then sampled using flame atomic absorption spectrometer. Each sample was tested for 3 seconds and sampled 3 times, and then an average absorbance was determined by instrumentation software. A calibration curve was used to determine the unknown sample concentrations (Figure 3).

Saturation Calculation

Saturation state of the solutions with respect to precipitation of $Cu(OH)_2$ was calculated using activities rather than concentrations to incorporate reactivity of ion species (Harris, 2002). Ionic strength (I) of the solution was calculated using the concentrations of experimental ions under various pH conditions [1]. Because $I < 0.01$, the activity coefficient (γ_i) was then determined using the Debye-Huckel Limiting Equation, [2] (Harris, 2002).

Ionic Strength = $I = 1/2 \, \Sigma \, M_i Z_i^2$ [1]

where M_i is the concentration (M in mol l^{-1}) of i^{th} ion and Z_i is its ionic charge

Debye-Huckel Limiting Equation:

$\log \gamma_i = - A Z_i^2 I^{1/2}$ [2]

where A is a constant (0.51)

Activity = $a = \gamma_i M_i$ [3]

where, M_i = concentration i^{th} ion (mol / L) and γ_i = activity coefficient of i^{th} ion

The activities of ions were then calculated using equation [3] and used to calculate the Ion Activity Product (IAP) by multiplying activies by one another. The IAP was then used to determine if (1) dissolved ions are below saturation in solution (IAP < Ksp), where Ksp is the solubility product and for $Cu(OH)_2$ equals 4.8E-20) (2) the solution is at saturation (IAP=Ksp), or (3) if ions are at concentrations above saturation and therefore should precipitate (IAP > Ksp) (Harris, 2002).

RESULTS

Kinetics Experiment

The maximum binding of EPS occurred at 140 min (Figure 4), which indicates the potential of metal binding conditions within the buffered, experimental system.

pH 3-7 Experiment

EPS binding with Cu increased from a pH of 3 to a pH of 6, and then binding decreased from a pH of 6.5 until a pH of 7 (Figure 5). Maximum bound Cu was 17.1 µg mg-EPS^{-1} and occurred at a pH of 6.0. IAP becomes greater than Ksp above pH 6.5, which indicates that precipitation of $Cu(OH)_2$ should occur above pH of 6.5 while Cu ion should remain completely dissolved below pH 6.5 under these experimental conditions (Figure 6). Visual observation of metal solution showed a blue tint at a pH of 7.

pH 6-8 Experiment

EPS binding of Cu decreased from pH of 6 to 8 , where binding of 61.7 µg bound Cu mg^{-1} was highest at pH of 6 (Figure 7). Visual observation of metal solution showed a blue tint starting at a pH of 7 and increasing in color intensity until pH of 8.

Cu vs. Cr Experiment

EPS preferentially bound with Cu over Cr throughout the experiment pH range. Binding of both metals slightly increased from a pH of 4 to a pH of 5, where Cu binding was highest at 10.4 µg bound Cu mg^{-1}, and Cr binding was highest at 5.4 µg bound Cr mg^{-1}. Then binding for both metals decreased from a pH of 5 until pH of 8 (Figure 8). Visual observation of metal solution showed a blue tint at a pH of 6 and increasing in color intensity until pH of 8.

DISCUSSION

Photosynthesis is a major driver of diurnal pH change in marine sediment. As sediment pH decreases, metals tend to change species from a complexed to a free form (Raven *et al*., 2005). Lower pH is of concern due to its influence on dissolved metal speciation (Sudhanandh *et al*., 2011). Even at very low concentrations, metals can be toxic in aquatic environments. This study aimed to address the dynamic relationship between EPS-metal binding and pH as it relates to this environmentally relevant issue.

EPS harvested from *C. closterium* displayed maximal Cu binding at 2.2 h (Figure 4). This finding is similar to that found for Cu binding to bacterially derived EPS that maximized at 2 h under similar experimental conditions (Bhasker and Bhosle, 2006). The difference of 0.2 h in maximal binding could be due to Bhasker and Bhosle's (2006) use of higher MW cutoff (6-8 kDa) dialysis tubing as opposed to the 3.5 kDa cutoff used in this study. Metals could also equilibrate faster, reaching all the binding sites more quickly with 6-8 kDa dialysis tubing. There is also a slight decrease after maximum binding occurs in both this study and Bhasker and Bhosle's (2006) (Figure 4). The slight decrease could be due to the breakdown of EPS into smaller molecular weight decreasing the bound EPS or breaks down small enough to escape through the dialysis tubing.

EPS maximum uptake in this study was 61.7 µg bound Cu mg-EPS^{-1} in the buffered system (Figure 7) while the unbuffered system yielded 17.1 µg bound Cu mg-EPS^{-1} (Figure 5). The difference in bound Cu is potentially due to variability in EPS binding ability. EPS composition may have varied in each batch culture, and separate batches of isolated EPS were used for each experiment. Batches were also harvested at different times during the day accounting for variation in EPS and binding capabilities. EPS composition variability is common among species, for instance, *C. closterium* EPS composition varies on diurnal scales (de Brouwer and Stal, 2002) and with temperature and irradiance (Wolfstein and Stal, 2002). The binding capacity range determined in this study for diatom EPS is consistent with binding capacity found for bacterial EPS in the literature. Bound Cu to bacterial EPS was 189 µg mg-EPS^{-1} (Bhasker and Bhosle, 2006), 16.8 µg mg-EPS^{-1} (Ford *et al*., 1987) and 114 µg mg-EPS^{-1} (Mittelman and Geesey, 1985).

Although more research needs to be done to understand the binding of EPS at various pH, EPS binding at near neutral pH is a significant result because this is the natural pH (5.0-7.8) of marine sediments (Ponnomperumo, 1972; van Cappellen and Wang, 1995; Stumm and Morgan, 1996). EPS binding with the metals Cu^{2+} and Cr^{3+} is pH-dependent and under these experimental conditions, the data suggest that EPS binding with Cu is optimal between a pH of 6-6.5 (Figures 5 and 7) at the Cu concentrations used here. It has been previously shown that glucuronic acids form complexes with metal ions at these pH levels (Cook *et al*., 1986; Escander and Sala, 1992; Jeon *et al*., 2002; Kohn, 1987; Rudolf, 1987). At higher pH levels, the amount of Cu used in these experiments forms a precipitate of $Cu(OH)_2$ (s) that is unavailable to bind with EPS (Harris, 2002). As pH decreases from 8 to 6, the Cu is more soluble and Cu^{2+} ions are increasingly available for EPS binding. However, more research is needed to better understand and make conclusions at pH treatments greater than 6 (Figure 5 and 7). These conclusions were similar to those reported by Ferris *et al*. (1989), Loaëc *et al*. (1998) and Lores and Pennock (1998). With decreasing pH levels below 6, there is less Cu-EPS binding due to higher proton concentrations competing for binding sites in acidic solutions. EPS becomes protonated as pH decreases and binding with metals decreases. This finding is consistent with Cook *et al*. (1986), who found that glucuronic acid dissociated from metals below a pH of 5.

Metal pollution in coastal waters has a positively correlated relationship to bioaccumulation in fish, which is a food source for many (Metwally and Fouad, 2008). EPS binding of metal is the highest at a near neutral pH in the sediment-water interface, where metal bound to EPS is consumed by bacteria and their grazers (Decho, 1990), possibly contributing to bioaccumulation.

EPS binds more with Cu^{2+} than Cr^{3+} (Figure 8). Abdullah et al. (2007) found that although Cr (water and sediment) concentrations were higher than Cu, Cu was bioaccumulated in mussels over Cr in all cases. Cu has higher electronegativity than Cr, which may explain why EPS can preferentially bind Cu over Cr. The greater affinity of EPS for Cu could protect the diatoms from Cu but the fate of the Cu would vary due to this dynamic system. For example, if the EPS saturated Cu was consumed by another organism, bioaccumulation of Cu could occur. EPS binding with Cu could possibly lead to metal sedimentation. It is important to note there are differences in Cu and Cr IAP curves and precipitation of Cu occurs at a higher pH of 6.25 while that of Cr occurs at a pH of 5.25 (Figure 9).

For future experiments, work should be completed with lower dissolved metal concentrations to mimic a more environmentally realistic environment, perhaps using graphite furnace to achieve necessary detection limits. Metal concentrations found in ambient mudflat sediments are much lower than this study used, this study metal concentrations ranged from 5.6-15 ppm.

This study demonstrates that 1) EPS could selectively bind Cu over Cr with metals over a wide pH range and potentially influence metal fate/distribution and 2) pH affects the binding capacity of diatom EPS. More research is needed to understand metal absorption at the dynamic sediment-water interface in marine mudflats, as affected by controlling factors such as microbial communities, EPS composition, type of sediment, cations present, and metal species (Petruzzelli *et al*., 1985; Zhu and Alva, 1993).

FIGURES

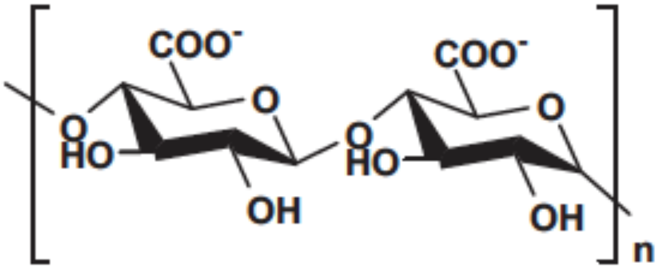

Figure 1. Molecular structure of polyglucuronic acid, with molecular formula of $C_6H_{10}0_7$. *(Elboutachfaiti et al., 2011).*

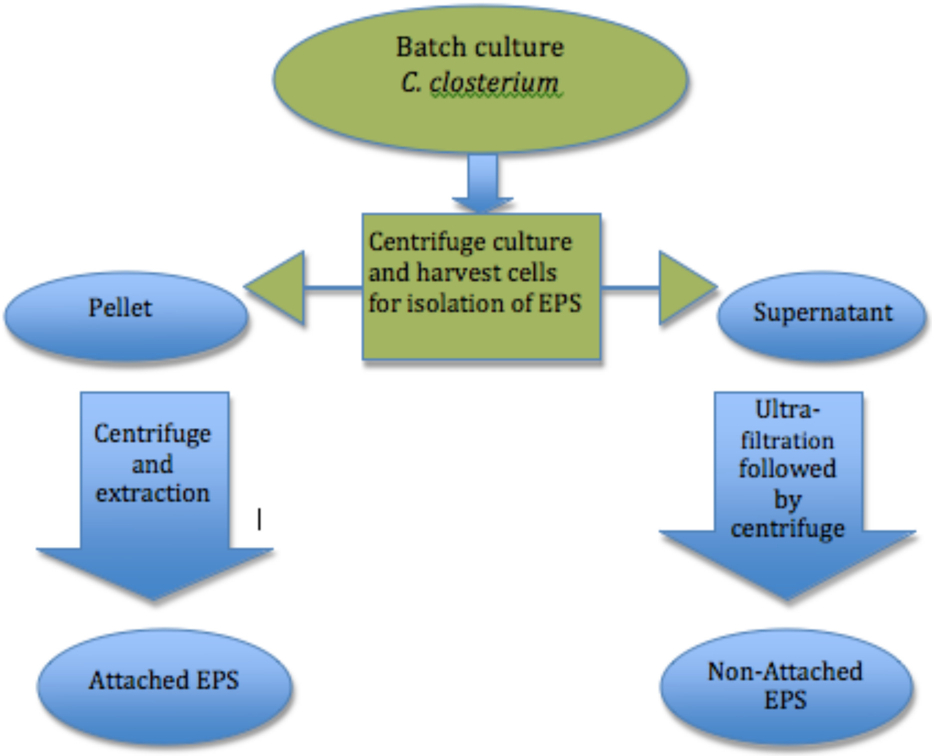

Figure 2. Extraction and isolation of marine diatom EPS

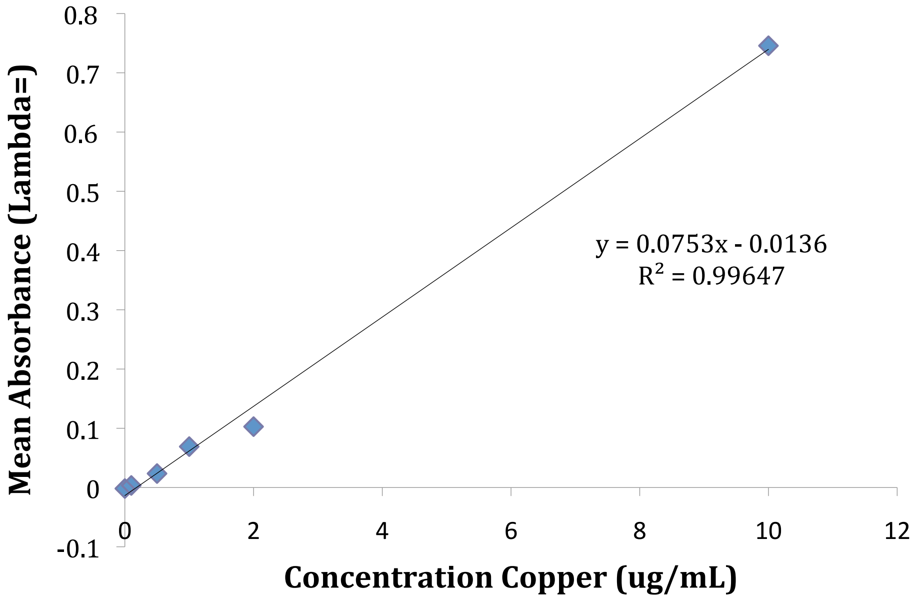

Figure 3. Sample Calibration curve of Cu standard stock solutions used to determine metal concentrations of unknown samples.

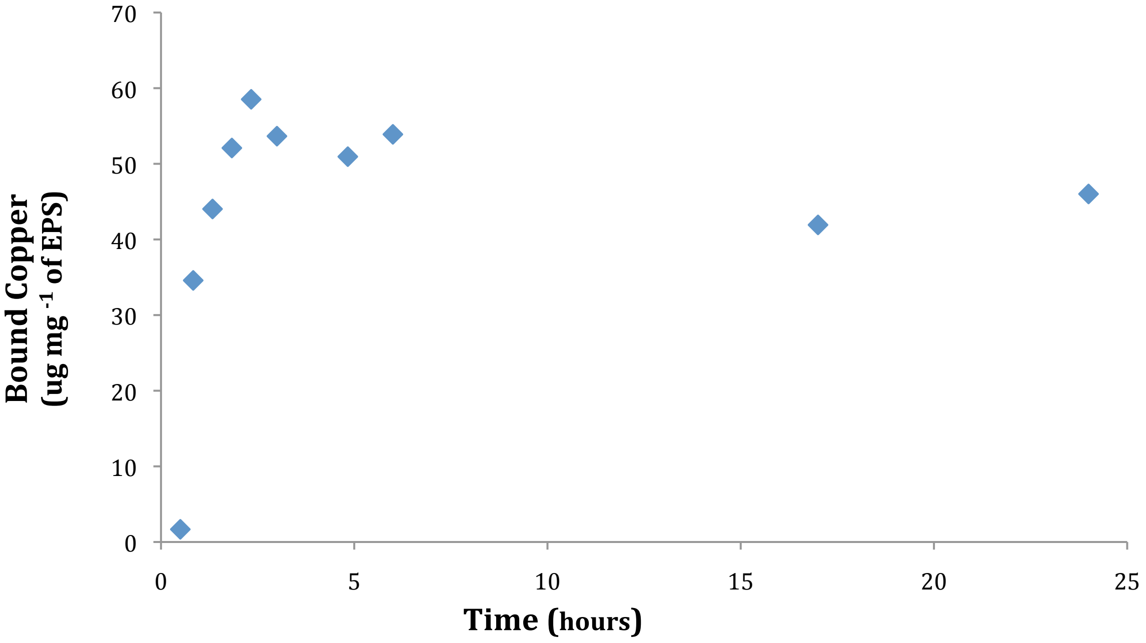

Figure 4. Chemical kinetics of EPS binding with Cu over a 24-hour period. Optimal binding occurs at 2.2 h at pH of 6 (n=1).

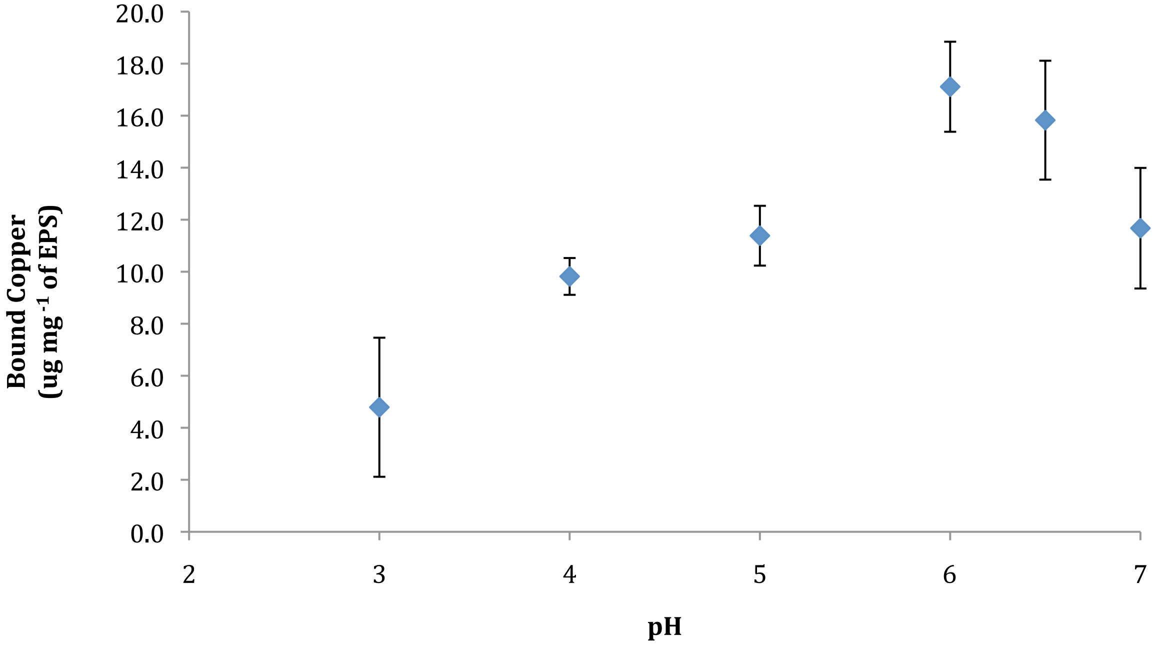

Figure 5. Influence of pH on EPS binding with Cu in an unbuffered system. Within this pH range, EPS bound Cu maximizes (at 17.1μg mg^{-1}) at pH of 6.0-6.5 (n=5).

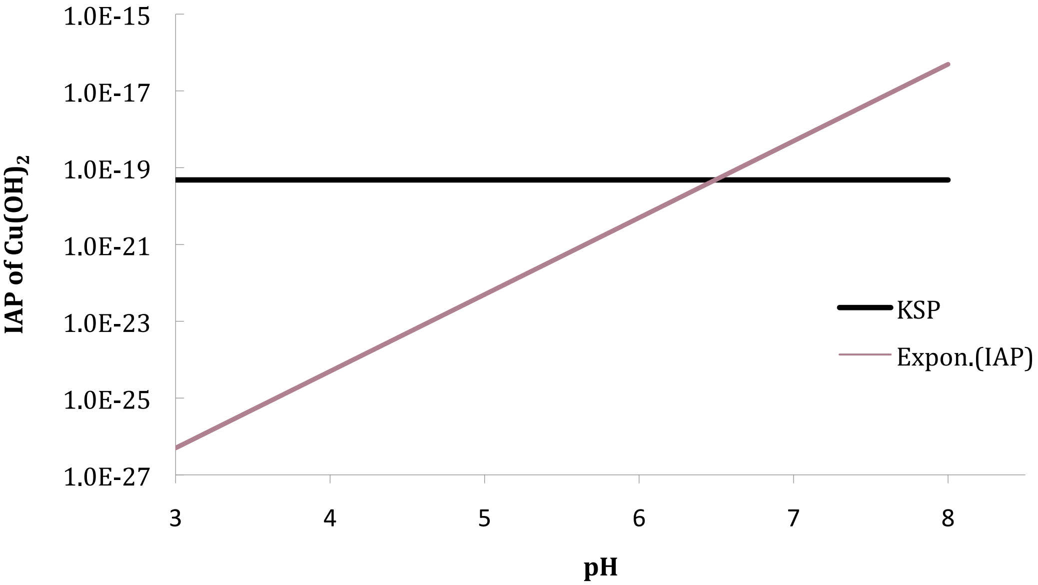

Figure 6. Calculated IAP of Cu(OH)$_2$, which exceeds the Cu(OH)$_2$ Ksp (horizontal line) at pH of 6.5.

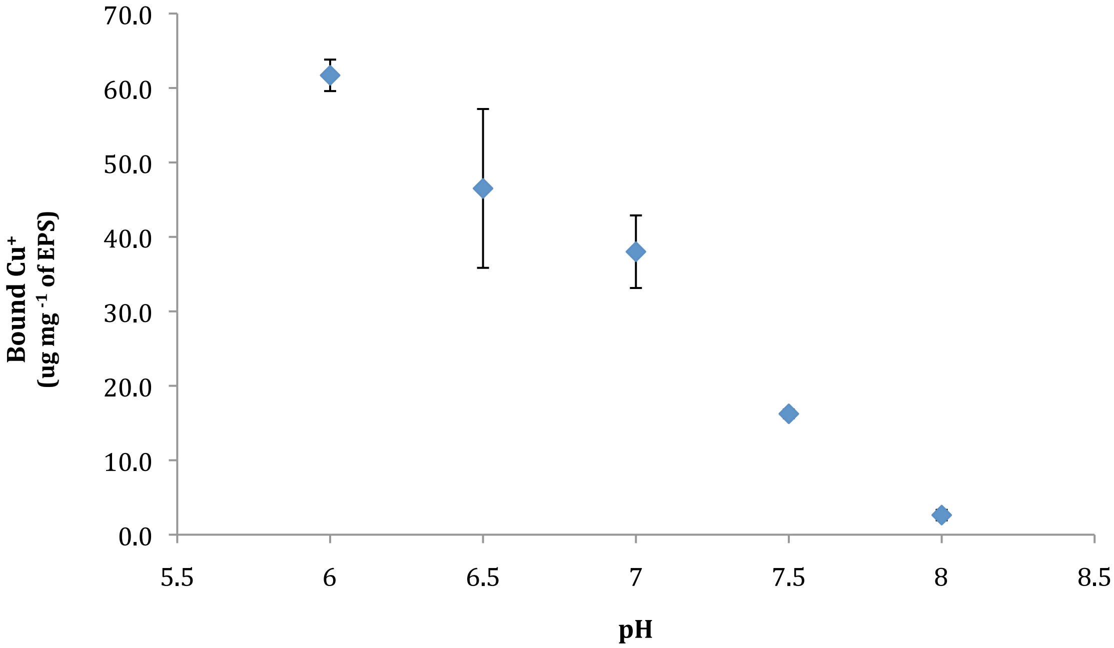

Figure 7. Influence of pH on EPS binding with Cu in a MOPS buffered system. EPS bound Cu maximizes at 61.7 µg mg^{-1} of diatom EPS at pH of 6.0 (n=5).

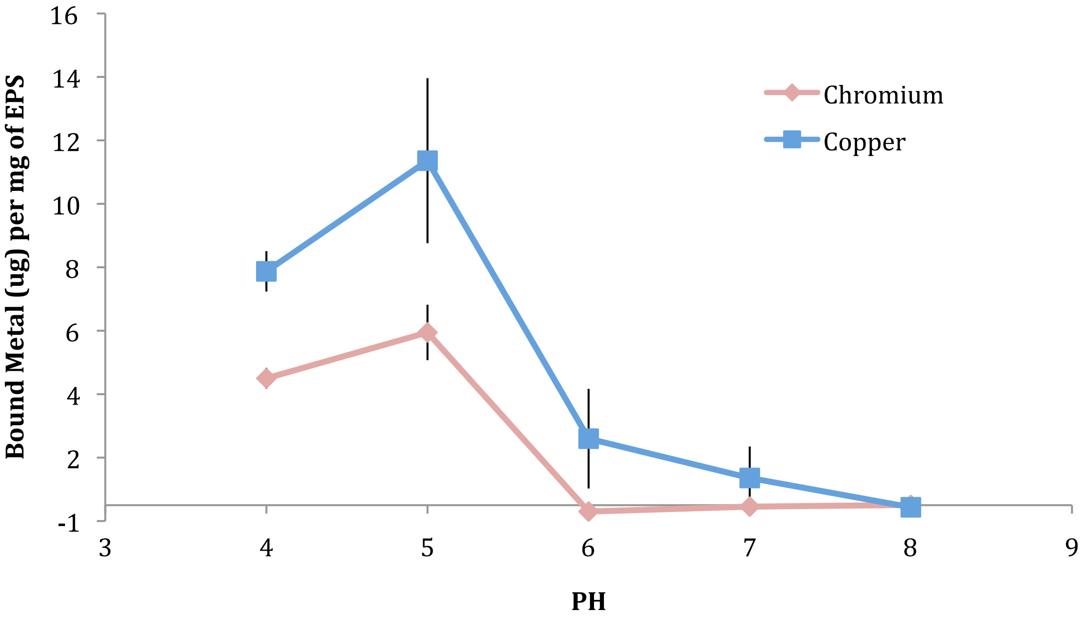

Figure 8. Influence of pH on EPS binding of Cu and Cr (4-8) (n=5).

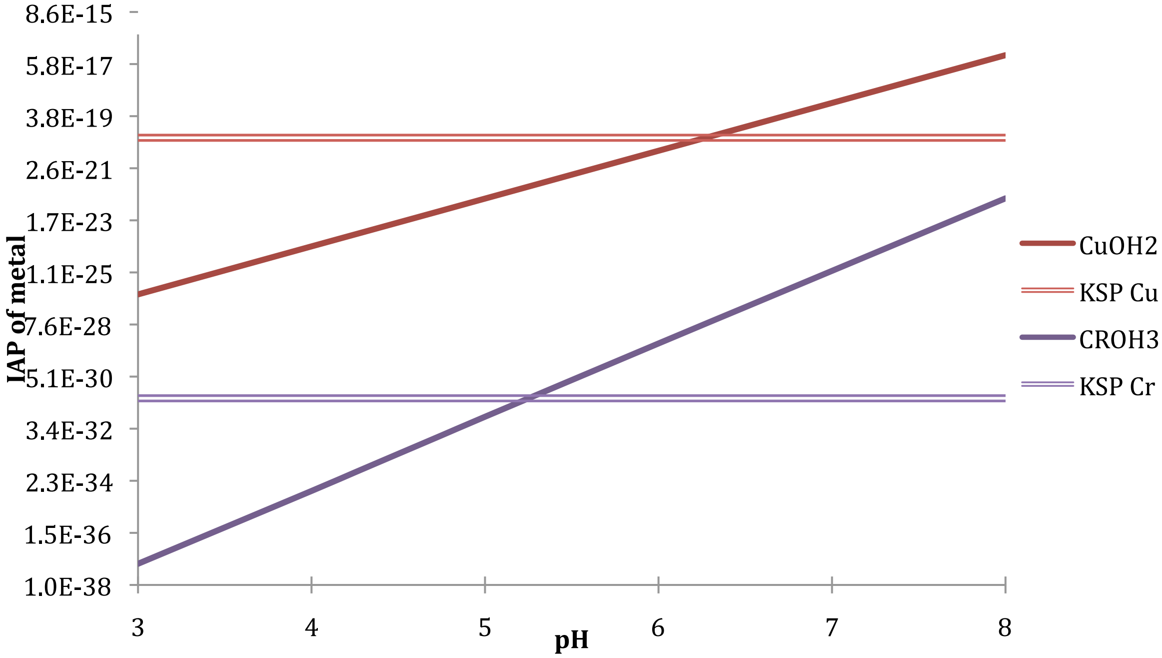

Figure 9. Calculated IAP of Cu(OH)₂, which exceeds the Cu(OH)₂ Ksp at pH of 6.25 and calculated IAP of Cr(OH)₃, which exceeds the Cr(OH)₃ Ksp at pH of 5.25.

Table 1. Experimental design of pH effects on diatom EPS binding capacity.

pH		**EPS(g): Metal (ppm)**	**EPS (g)**	**Temp. (˚C)**	**Buffer**
Range	Increments				
3-7	1.0	1: 8.9 E-4	1.68 E-4	30	No
6-8	0.5	1: 8.9 E-4	6.31E-5	23.4	Yes

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