ROLE OF NATURAL ORGANIC MATTER IN GOVERNING THE BIOAVAILABILITY OF TOXIC METALS TO AMERICAN OYSTERS (*Crassostrea virginica*)

A Thesis

by

JENNIFER MARCELLE HAYE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2005

Major Subject: Oceanography

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Approved as to style and content by:

Peter H. Santschi (Chair of Committee)	John R. Schwarz (Member)
Luis A. Cifuentes (Member)	Robin L. Autenrieth (Member)
Sammy M. Ray (Member)	Wilford D. Gardner (Head of Department)

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ABSTRACT

Role of Natural Organic Matter in Governing the Bioavailability of Toxic Metals to American Oysters (*Crassostrea virginica*). (May 2005)

Jennifer Marcelle Haye, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Peter H. Santschi

Colloidal macromolecular organic matter (COM), which makes up a large portion of the bulk dissolved organic matter (DOM) in marine environments, has the capability to modify the bioavailability of potentially toxic metals to aquatic organisms. In order to better understand the bioavailability of some of these metals to estuarine bivalves, American Oysters (*Crassostrea virginica*) were exposed to different types of natural colloidal (COM) and model (alginic acid, carrageenan, and latex particles) organic biopolymers, tagged with gamma-emitting radioactive metal ions (^{110m}Ag, ^{109}Cd, ^{57}Co, ^{51}Cr, ^{59}Fe, ^{203}Hg and ^{65}Zn) or ^{14}C (to sugar OH groups). Natural COM was obtained from Galveston Bay water by 0.5μm filtration, followed by cross-flow ultrafiltration, using a 1kDa ultrafilter, diafiltration and freeze-drying. COM and DOM model compounds were used in the bioavailability experiments at 2 ppm concentrations. Separate 16-hour experiments using varying sizes of latex particles assessed the lowest size of colloids that can be filtered from the water. Results showed that filter-feeding bivalves could efficiently remove particles as small as 0.04μm (40nm) in diameter, with removal half-times of 2.5 to 5.5 hours, equivalent to filtration rates of about 50±15 ml/hour, or about 3 L d^{-1} g^{-1}, which are typical values for these oysters. Results of the 20-hour bioavailability

experiments demonstrated that oysters could effectively filter metals bound to COM, with the metals bound to alginic acid COM being removed at the highest rates from the water. However, the metals bound to alginic acid were not found in oyster meat in the highest amounts: it was the metals associated with the carrageenan COM. The ^{14}C labeled biopolymer data also showed alginic acid to be removed from the water at the highest rate and, contrary to the metals, was also present in the meat in the greatest amounts. Thus, while previous experiments suggested that the quantity (i.e., concentration) of natural organic matter is important for metal bioavailability, it was shown here that the "quality", i.e., the type of natural organic matter, is also a factor for controlling bioavailability, removal and incorporation rates of metals to oysters.

DEDICATION

This work is dedicated to David Graham Haye. May you achieve all your hopes and aspirations and may all doors be wide open on your road to success.

ACKNOWLEDGEMENTS

This work was funded by Seagrant ("Role of Natural Organic Matter in Governing the Bioavailability of Toxic Metals to Estuarine Bivalves" to TAMUG).

I would like to thank Dr. Peter H. Santschi, my committee chair, for his insight, expertise, ongoing support and encouragement. Thank you for your time and effort to see me through this life changing time. I would also like to thank the rest of my committee, Dr. L. Cifuentes, Dr. R. Autenrieth, Dr. J. Schwarz and Dr. S. Ray. Thank you all for your helpful and thought provoking comments.

I am eternally grateful to my husband Sheridon Haye for his steadfast love, support, encouragement and sacrifice for me to get my Master's Degree. Without you I would not be obtaining this goal. Thank you for believing in me.

I would also like to recognize my parents, Pauline and Vincent Forbes, for their enthusiasm, encouragement, love and gift of their time. Thank you for a strong foundation and for paving the way for your children to be whatever they wanted to be.

Finally, I want to thank David, my son, whose appearance was a wonderful surprise and a motivation to complete this objective.

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CHAPTER I

INTRODUCTION

Background

Toxic heavy metals are well known pollutants in many coastal environments and impact the health of the estuarine ecosystem. These pollutants, many of them bound to organic matter, can become bioavailable, e.g., through ingestion of colloidal matter or adsorption of metals through permeable membranes (Wang and Guo, 2000), to filter feeding benthic invertebrates, such as bivalves. This is significant because benthic organisms are an important component of the estuarine environment and an essential link in the estuarine food chain. Bivalves, such as American oysters, filter large volumes of seawater and can concentrate pollutants in their bodies. Therefore, they are ideal pollution indicators and are frequently used in environmental assessment and monitoring programs. Once ingested, toxic metals have the potential to bioaccumulate in the bivalve and move across the food web. With more than fifty-three percent of the United States population living in coastal regions, which make up only ten percent of the contiguous United States land area, this movement across the food web may ultimately have an impact on human health. As coastal population continues to grow, so will the input and impact of anthropogenic pollutants to estuarine and coastal environments, and the need to understand the relationships between toxic heavy metals and organic matter.

Bioavailability for this study is defined as the degree to which a chemical is able to move into or onto an organism (Benson, et al., 1994). Metal bioavailability to marine organisms has been an increasingly important area of study over the past several years.

This thesis follows the style of Marine Chemistry.

This is so because trace metals are known to be widespread contaminants in coastal and estuarine waters. Recent studies have investigated various aspects of metal uptake, bioavailability and complexation with organic matter in marine waters. Previous belief held that metal toxicity would be reduced in the presence of filter-passing natural organic matter because metals would bind with the soluble organic matter and reduce the amounts of free metal ions for uptake. This assumed uptake of heavy metals was occurring mainly through passive surface processes, such as adsorption followed by transport through permeable membranes. While such passive processes are important metal uptake pathways, current research also supports the possibility of active processes such as direct ingestion of food particles. In recent studies, the effects of metal uptake by bivalves under differing organic matter conditions has been addressed.

Guo et al., 2001, found, by comparing treatments with high and low molecular weight (HMW and LMW) dissolved organic carbon (DOC), that as DOC concentrations increased, uptake rates by bivalves of metals bound to colloids generally increased also. Their research supported other recent studies showing that, in the presence of HMW DOC, zebra mussels had increased uptake of dissolved metals (Roditi, et al., 2000), and that with the addition of organic matter there was increased bioavailability of metals to estuarine bivalves (Lee, et al., 1998). The dry-weight concentration factors (DCF), which indicate bioavailability to the organisms, varied between metals, with the highest DCF values (and therefore higher bioavailability) associated with B-type metals, e.g., Ag, Hg, Zn and Cd. Guo et al., 2002, also compared LMW and HMW DOC treatments at the same DOC concentrations (0.5ppm), and found depressed metal bioavailability for HMW DOC. The proposed explanation was bivalves use differing uptake pathways depending

on DOC concentration, as DOC increases, uptake of free metal ions in solution is replaced by direct ingestion of colloidally bound metals.

This leads to another important question when studying the bioavailability of colloidally complexed metals to marine organisms. Are these toxic metals remaining associated with colloidal ligands after uptake experiments begin? This question was addressed in Guo, et al., 2002 where some metals were found to dissociate from colloidal matter after they were introduced to the LMW (<1kDa) seawater. The repartitioning of the metals appeared to occur quickly, and such behavior explained the observed metal uptake patterns by the oysters. These authors therefore postulated that the bioavailable fraction in these experiments was mainly low molecular. However, they found that the percentage of colloid bound metals was quite stable for the short-term (0.5 to 8 h) uptake experiments. They concluded therefore that even though the dissociation of metal from the colloids will occur, the association will be retained long enough, under experimental conditions, to be able to assess bioavailability of colloidally bound metals to aquatic organisms. This research builds on these previous studies and examines metal uptake and bioavailability to American oysters by examining how the composition of organic matter affects colloid uptake.

Organic Matter

One of the largest reservoirs of organic carbon in seawater is the operationally defined "dissolved" state (Buffle and van Leeuwen, 1992). This definition includes colloids, with a macromolecular colloidal organic carbon fraction size range of ~1nm-1μm (Stumm and Morgan, 1996). In spite of this large reservoir, it was not known until a few years ago that colloidal organic matter-rich food particles were bioavailable to filter feeding marine organisms, such as bivalves (Benoit, et al., 1994). However, DOM is known to be a potentially nutritious food source for marine bivalves and the bioavailability and biogeochemical cycling pathways of many trace metals may be affected by the presence of DOM (Guo et al., 2001). In marine environments, studies indicated that high molecular weight (HMW) colloidal organic matter (COM) has relatively high biological and chemical reactivity and short residence times in the water column (e.g., Santschi et al., 1995, 2003). Therefore, due to their high complexation capacities, marine organic colloids are important in the biogeochemical cycling of trace elements in the ocean (Wells, 2002).

Natural organic matter (NOM) is composed of humic matter, proteins, lipids and polysaccharides. Humic matter in aquatic systems is mostly derived from land erosion. Proteins, lipids and carbohydrates are mainly derived from aquatic plants and microorganisms, either through cell death and lysis, or through extracellular release. The most abundant compound class in aquatic systems is carbohydrates, composed of monosaccharides and polysaccharides (Buffle, 1990). The role of polysaccharides in living organisms is threefold: (1) Storage of energy reserves, (2) Construction of plant 'skeletons': these polysaccharides are the principal components of cell walls, (3)

Formation of a microfibrillar network rich in polysaccharides, on the outer surface of the organism, which can result in an appreciable modification in local conditions (Buffle, 1990).

Acid polysaccharides are a subset of polysaccharides that, due to their high surface reactivities, have a critical role in the formation of extracellular mucilages, floc formation, removal of trace metals from the water and the creation of biofilms and aggregation (Verdugo et al., 2004). COM in Galveston Bay is composed of about 20% of polysaccharides and about 2% of uronic acids, the main components of acid polysaccharides (Hung et al., 2001). It is possible that polysaccharides, especially acid polysaccharides (APS), are taken up more efficiently due to their fibrillar and surface-active nature (Santschi et al., 1998) and can enhance removal from the water column. Model acid polysaccharides (APS) such as alginic acid and carrageenan are negatively charged, highly hydrated molecules and strongly bind positively charged metal ions.

Bioavailability

In general, marine organisms such as bivalves take up trace metals from food, through solution phases or through filter feeding (Guo et al., 2002). Much more is known about the uptake of sediment-bound metals (e.g., Griscom and Fisher, 2004) than uptake associated with dissolved organic matter. Therefore, this research focuses on the affinity of positively charged trace metals for negatively charged functional groups of organic ligands which may make trace metals available for uptake during filter feeding. This affinity is enhanced by the ability of marine colloids to strongly bind trace metals through covalent, electrostatic or hydrophobic interactions (Buffle, et al., 1998). Because

colloidal organic matter comprises 20-60% of the bulk DOM in marine environments and is a food source for filter feeders, heavy metals have the potential to bioaccumulate (concentrate) in bivalves and biomagnify (increase in concentration) as they move through the food chain.

Metal Behavior/Chemistry

Most trace metals have been found to be significantly associated with organic ligands in seawaters (e.g., Donat and Bruland, 1995). In estuarine waters, many metals, such as Cu, Ag, Fe, Hg, and Zn, have been found to be largely present associated with colloidal macromolecular organic matter (Wen et al., 1997, 1999; Guo et al., 2004; Santschi et al., 1999, and references therein). In aqueous solutions, a cation may prefer one type of ligand to another type, depending on the electronic configuration of the cation. Class A metal cations have inert gas configuration and their electron sheaths are not readily deformable under the influence of electric fields, while Class B metal cations have higher polarizability and electron sheaths more readily deformable than the class A metals (Stumm and Morgan, 1996). Class A metals, such as Fe(III) and Cr(III), are characterized as hard spheres, meaning they preferentially participate in interactions resulting in a gain in entropy, and prefer ligands containing alkyl groups. Class A metals prefer to bind with oxygen or nitrogen containing ligands. Class B metals, such as Ag(I) and Hg(II), are characterized as soft spheres and form covalent bonds and result in a reaction more enthalpic in origin. Class B metals prefer ligands containing sulfur (Buffle, 1990). Transitional metals used in these experiments that can have characteristics of either soft or hard character are Co(II), Zn(II) and Cd(II).

Some metals have characteristics that make them essential trace elements for living organisms. Essential metals include Cr, Fe, Co and Zn. Chromium is required for proper metabolism of sugars in humans, Fe is essential in animals and is a vital component of all hemoproteins, Co is essential in small amounts in many organisms and Zn is an essential element in humans and most organisms, and is an element that is both essential for oyster health, but shows toxic effects at higher concentrations (Meiller and Bradley, 2002). While Cadmium has no known biological function in oysters it does demonstrate an affinity for biogenous particulate matter (Furness and Rainbow, 1990; Wotton, 2000). Furthermore, metals have ligand properties related to their A- or B-type (hard or soft) character, influencing their interactions with other particles. For instance, Cd, Hg, Zn, and Ag are B-type or transition metals preferring sulfur-containing ligands to N- or O-containing ligands (Stumm and Morgan, 1996).

Research Objectives

The purpose of this thesis was to evaluate the uptake of metals associated with natural and artificial dissolved organic matter (DOM) by bivalves, which are often used as biomonitors for toxic metals (e.g., Rainbow, 1995). The goals were to determine: 1) the lower particle size limit a filter feeder could remove from the water, 2) if the "quality" (i.e., the chemical nature) of organic matter (Newell and Jordan, 1983) affects colloid uptake, 3) how the fibrillar and surface-active nature of APS (Santschi et al., 1998) changes uptake efficiency, and 4) whether certain toxic metals are preferred during feeding.

The hypotheses that were tested are:

1. Bivalves uptake metals associated with COM because it is used as a food source
2. COM uptake is a function of size and composition and that acid polysaccharides modify metal uptake
3. Metals behave differently with model APS than they do with natural COM

CHAPTER II

MATERIALS AND METHODS

Experimental Approach

Metal uptake experiments were carried out in the presence and absence of oysters, and in the presence of colloidal HMW DOC. LMW DOC treatments were not carried out in this research, but were an essential part of that by Guo et al. (2001 and 2002), and their results are directly applicable to this research.

Latex Particles

Latex particles of varying sizes, 0.02μm – 1.0μm diameter (Interfacial Dynamics, Inc.), were used to test the lower size limits of colloidal organic matter in a series of uptake experiments.

Radiotracers

Radiotracer experiments were run using artificial seawater of salinity = 15 $^{0}/_{00}$ which provides near optimal estuarine conditions with controls containing radiolabeled material and no oyster.

Gamma – emitting Metal Radioisotopes

Radioactive metals (Table 1), purchased from Perkin-Elmer Life Sciences for radioisotope labeled colloid experiments, included ^{110m}Ag(I), ^{109}Cd(II), ^{57}Co, ^{51}Cr(III), ^{59}Fe, ^{203}Hg and ^{65}Zn(II). Amounts of stable metals added to samples were similar to values reported by Carvalho et al. (1999). ^{109}Cd, ^{57}Co, ^{59}Fe and ^{65}Zn are transition metals, ^{51}Cr is an A-type metal and ^{110m}Ag and ^{203}Hg are B-type metals. Colloidal

organic matter was added to 200ml dH_2O and placed on a stir plate. The radioisotopes were then added to the COM solutions, and a pH between 10 and 12 was maintained by the addition of NaOH, and allowed to equilibrate overnight (12 hours). The next day, the solutions were transferred to stirred cell ultra-filtration flasks and the retentate concentrated by stirred cell ultrafiltration. The activity of the retentate was determined by gamma ( ) counting, and then added to the beakers containing seawater and oysters to obtain a COC concentration of 2ppm DOC for the uptake study.

Table 1
Radioisotope information. Isotope suppliers and isotope dilution activities used for uptake experiments.

Isotope	Supplier	Specific Activity (mCi/mg)	Reference Date	Amount of Stock Used (ml)	Volume of Acid for Dilution (ml)	Dilution Activity (mCi)
^{110m}Ag(I)	Isotope Products	480	8/15/02	0.5	0.5 of 0.1M HNO^3	0.05
^{109}Cd(II)	Perkin Elmer	317	7/22/02	0.05	0.95 of 0.5M HCl	0.25
^{57}Co(II)	Isotope Products	7000	8/15/02	0.05	0.95 of 0.5M HCl	0.5
^{51}Cr(III)	Perkin Elmer	267	7/22/02	0.05	0.95 of 0.5M HCl	0.5
^{59}Fe(III)	Perkin Elmer	18	7/22/02	0.05	0.95 of 0.5M HCl	0.25
^{203}Hg(II)	Isotope Products	0.572	10/15/02	0.05	0.95 of 0.5M HCl	0.15
^{65}Zn(II)	Isotope Products	35.4	8/15/02	0.05	0.95 of 0.5M HCl	0.25

Beta – emitting ^{14}C

^{14}C – dimethyl sulfate purchased from Sigma Chemicals, was used to radiolabel the sugar –OH group of the polysaccharide fraction of COM in the retentates of AA, C and COC. In a reaction vessel, COM was reacted with ^{14}C-dimethyl sulfate in 0.1M NaOH, mixing every ten minutes for 40 minutes. The labeled COM was then centrifuged and the retentate collected, and the activity determined using Liquid Scintillation Spectrometry, for use in time series experiments. The labeling process radiolabels both neutral and amino sugars by methylating the hydroxyl groups (Wolfinbarger et. al., 1983, Quigley et. al., 2002).

Oysters

American oysters (*Crassostrea virginica*) were obtained from one of two oyster houses located in West Galveston Bay. The oysters were collected by Dr. Sammy Ray at a time when spawning was not prevalent. Oysters were obtained and brought to the laboratory on the same day as collection. Oysters were cleaned of barnacles and other visible organisms and matter. Twenty to twenty-five oysters with similar weight (20-30g) and shape (4-6cm in length) were placed in beakers containing artificial or natural seawater, at a salinity of $15^{0}/_{00}$, and allowed to purge for 24 hours before experiments were run. Replicate purged oysters were each placed in a beaker with clean artificial or natural seawater, for each COM treatment, for the uptake experiments. Dry weights of individual oysters ranged from 0.15 to 0.73 g, with an average dry weight of 0.38g (Table 13).

Colloidal Organic Matter

Three types of biopolymeric organic matter were used in these experiments: Alginic Acid (AA), Carrageenan (C) -Type 1 or kappa and natural colloidal organic carbon (COC). While alginic acid has coagulant properties, and carrageenans have anticoagulant and antiviral properties (Libes, 1992), both are used as emulsifiers in the food and pharmaceutical industry. Radiolabeled C and AA, as well as natural COC, were added to the beakers with oysters at a concentration of 2ppm DOC.

Alginic Acid

Alginic acid (Figure 1) is an acid polysaccharide, purchased from Acros Organics, which has one carboxylic acid group per sugar monomer. Alginic acid is a naturally occurring hydrophilic colloidal acid polysaccharide obtained from the various species of brown seaweed (*Phaeophyceae*), with a chemical formula: $(C_6H_8O_6)_n$ (Buffle, 1990).

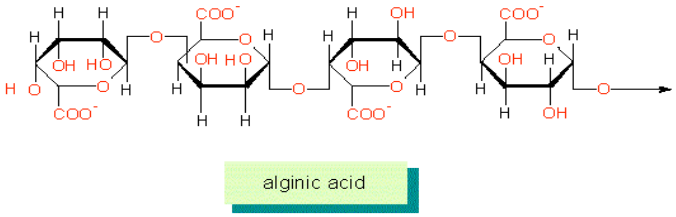


Fig.1. Alginic acid chemical structure.

Carrageenan

Carrageenan (Figure 2) is an acid polysaccharide, purchased from Sigma Chemicals, which has three sulfate ester groups per two sugar monomers. Carrageenan, a carbohydrate, is a family of linear sulfated polysaccharides obtained from red seaweeds. The carrageenan family has three main types named kappa, iota and lambda, with kappa

being used for these experiments. Kappa carrageenan was chosen because it has a double helical structure that allows it to form a gel (Buffle and Leppard, 1995).

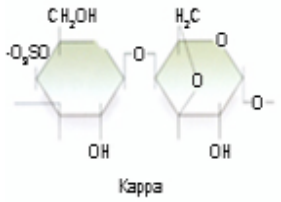


Fig. 2. Carrageenan chemical structure.

Natural Colloidal Organic Carbon

Natural colloidal organic carbon was extracted from Galveston Bay water. To obtain COC, bay water was collected and pre-filtered through a 0.5μm filter on the same day as collection. The pre-filtered water was then ultra-filtered using a 1kDa (kiloDalton) Amicon cross-flow ultra filter, and reduced from 10L to 100ml, and in subsequent sample filtration, from 20L to 155ml. The concentrated organic-rich sample was processed and analyzed for its organic carbon content by two different methods. One method was to measure the dissolved organic carbon content directly from the retentate using a TOC Analyzer. With this instrument, the content was determined to be 237 mg-C/L. The concentrated retentate was then diafiltered and freeze-dried for radio-labeling. The second method immediately diafiltered the retentate using a stirred cell ultrafiltration unit, and the retentate freeze-dried. The carbon content of the freeze-dried COM was determined using a CHN Analyzer and found to be 0.162mgC per mg COM. A concentration of 2ppm DOC, equivalent to 160μM-C of DOC, was then used in all experiments.

Uptake Experiments

Latex Particles

Five oysters, plus another for a control, were placed in Teflon beakers filled with 349.3ml of artificial seawater, with a salinity of 15ppm. Another five oysters, plus a control, were placed in Teflon beakers filled with 349.3ml of natural seawater of 15ppm salinity. With air being bubbled into the beakers, 0.7ml of latex particles were introduced to all beakers at a concentration of approximately 2ppm of latex particles, 1ml aliquots were taken at time intervals of 0, 0.5, 1, 2, 4 and 16 hours. These aliquots were then analyzed on an UV detector of an HPLC to determine their concentration (by absorbance, abs). Removal rates were then calculated by plotting absorbance as a function of time, according to equation 1:

$$\log[Abs(t)/Abs(t=0)] \qquad (1)$$

Where Abs(t=0) was the absorbance at time zero and Abs(t) was the absorbance at a given time interval after analysis began. The slope, λ, of the first-order removal was used to calculate the removal residence times as $1/\lambda$.

Metal Radioisotopes

For the first metal isotope experiment, five replicates of AA, C, and COC were included, with a total volume of 300ml (299.4ml artificial sea water plus 0.6ml radiolabeled organic matter) in the beakers, which resulted in a concentration of 2ppm DOC. Time series sampling began immediately after the addition of the labeled colloidal matter. 5ml sample aliquots were drawn at time intervals of 0,1, 2, 4, 6, 8, and 20 hours and counted on a gamma (γ) counter. For the second metal isotope experiment, the number of replicates for the AA, C and COC treatments was increased to eight, in

addition to controls, with a total beaker volume of 300ml (~299.3ml artificial sea water plus ~0.7ml labeled organic matter), to result again in 2ppm DOC. Sampling began after several minutes, after the labeled colloidal matter was dispersed throughout the beaker. 5ml sample aliquots were drawn at time intervals of 0, 0.5, 1, 2, 4, 6, and 8 hours from the 300ml in the beakers and counted on a gamma (γ) counter to determine the removal rate of the colloidal matter. After the time series was completed, the oysters were removed from the beakers and separated into body parts, fecal matter and shell. These were also gamma-counted separately to determine the percentage of activity in the meat and shell. Activities were corrected to 1ml solution geometry using appropriate correction factors determined in separate calibration experiments. After γ-counting, the oyster meat was freeze-dried and weighed for the determination of the dry-weight concentration factors (DCF), calculated using equation 2,

$$DCF(ml/g) = A_{ST}/A_W \qquad (2)$$

where A_{ST} is the activity concentration of a metal in the oyster meat (dpm/g dried tissue), and A_W the activity of a metal in the water (dpm/ml solution) (Wang et. al., 1996).

^{14}C

The initial ^{14}C experiment used five replicates of AA and COC, with beaker content consisting of 299.4ml artificial seawater plus 0.6ml labeled organic matter. 5ml sample aliquots were drawn at time intervals of 0,1, 2, 4, 6, 8, and 20 hours and counted on by Liquid Scintillation Spectrometry. For a second experiment, seven replicates of AA, C, and five replicates of COC were included for the experiment, with a total volume of 300ml (299.9ml artificial sea water plus 0.09ml labeled organic matter) in the beakers. The labeled colloidal matter in the beakers was at a concentration of 2ppm DOC for both

experiments. 5ml sample aliquots were drawn at time intervals of 0,0.5, 1, 2, 4, 6, and 8 hours from the 300ml in the beakers and counted on a Liquid Scintillation Spectrometer to determine ^{14}C concentration removal rate of the colloidal organic matter.

After the time series was completed, the oysters were removed from the beakers and separated into body, fecal matter and shell. These were counted separately to determine the percentage of activity in the meat and shell. The oyster meat was digested in 10ml of 6.25% tetramethyl ammonium hydroxide(TMAH) and sonicated until dissolved. For counting, 1 ml aliquots of the TMAH solution were added to the liquid scintillation cocktail (15ml of Scintaverse) and dilution corrected after counting. The shells for the ^{14}C treatment were leached in 100ml of 1% SDS (Dodecyl sulfate, sodium salt) before counting a 5ml aliquot by Liquid Scintillation spectrometry.

Standardization of Count Rates

Matrix Corrections

Geometry corrections were made in order to compare the water, shell and meat data. The only geometry correction needed for the radioactive metals was to the shell. The meat sample fit into a 5ml counting geometry, similar to the water samples. The oyster shell was counted on top of the well detector of the gamma counter. The correction factor for the shell is a ratio of counts, for each isotope, inside of and on top of the detector well, shown in equation 3:

(counts - inside of detector well / (counts – on top of detector well) (3)

For ^{14}C, geometry corrections were needed for both the shell and the meat. In this case, the correction is standardized to the seawater and solutions added to the scintillation fluid for counting on the Liquid Scintillation Spectrometer. The time series samples were in artificial seawater (ASW), the shells were leached in 1% SDS (Dodecyl sulfate, sodium salt) and the meat was digested in 6.25% TMAH (tetramethyl ammonium hydroxide). The geometry correction factors for the shell and meat are shown in equations 4 and 5:

$$(cpm - ASW) / (cpm - SDS) \quad (4)$$

$$(cpm - ASW) / (cpm - TMAH) \quad (5)$$

These factors were applied to the data after the background, quenching and dilution corrections were made.

Quenching Curve

By varying the ratio of radiolabeled ^{14}C oyster meat to scintillation cocktail, a quenching curve was generated in order to determine the quenching factor. The quenching factor, defined as

$$\frac{(Count\ rate\ without\ meat)}{(Count\ rate\ with\ meat)} \quad (6)$$

The cocktail/meat mixture was counted by Liquid Scintillation spectrometry, and the count-rate (cpm) vs. time was plotted. From the resulting quenching curve, a correction to ambient oyster meat in solution was made using the slope and y-intercept, shown in equation 7,

$$\text{y-intercept/y} \quad (7)$$

$$\text{where} \quad y = (\text{wt.oyster}*\text{slope})/\text{y-intercept}) \quad (8)$$

CHAPTER III

RESULTS

Latex Microsphere Experiment

Initial results from the latex particle experiments indicated that spherical particles as small as 0.04μm (40nm) in diameter were efficiently removed from the seawater as pseudofeces. Removal residence (1/λ) times ranged from 4 -10 hours, depending on oyster (Figure 3), equivalent to filtration rates of 50±20 ml/hr, or 125±50 ml hr^{-1} g^{-1}, assuming a 100 % efficiency of removing these colloids, and an average oyster dry weight of 0.4 g. Filtration rates of this magnitude are typical values for oysters (Newell et al., 2004), which would indicate that colloidal sized particles were efficiently removed during oyster filtration activities. Removal rates were not affected by the use of artificial seawater as opposed to pre-filtered natural seawater. These results show that nanoparticles are being filtered out of the water and therefore indicate that COM may be used as a food source for bivalves. Because of the inert nature of latex, the particles were ejected as pseudofeces.

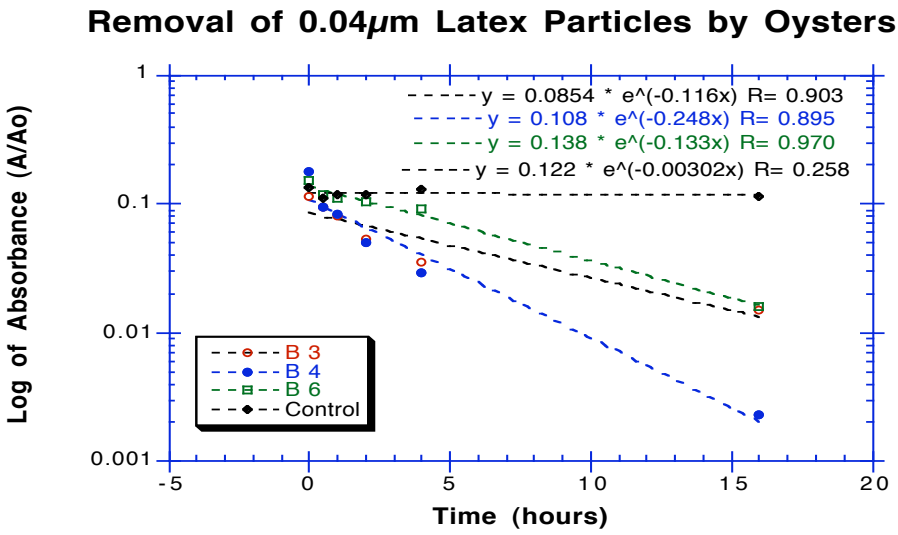


Fig. 3. Latex particle removal. Removal times ranged from 4-10 hours.

Metal Radioisotopes and ^{14}C Experiments

These experiments tested how the "quality" of COM (COC vs. Acid Polysaccharides(APS)) affects metal uptake by bivalves. The results showed a higher removal rate from the water of AA compared to C and natural COC, with COC having the slowest removal rate for both ^{14}C and metal isotopes.

Metal Radioisotopes

Results from the first experiment showed that most of the COM was removed from the water within the first 10 hours. In most cases, there appeared to be one oyster that had greater pumping efficiency than the rest (Figure 4).

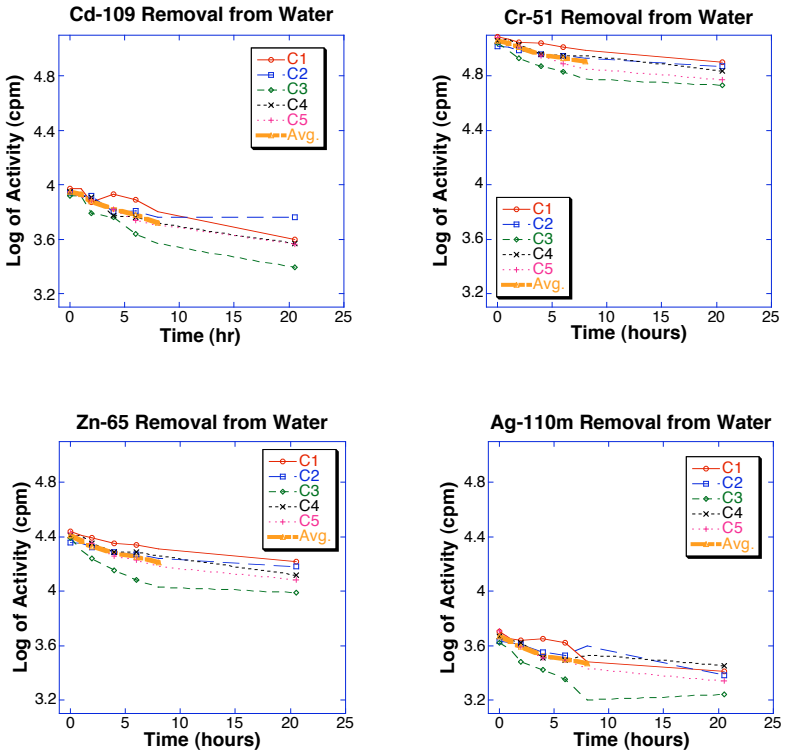


Fig. 4. Example of outperforming oyster. Oyster C3 appears to be filter at a higher rate than rate than others. Dark orange line is average of all oysters.

The curves of these plots flatten out after about 10 hours, indicating approach to steady state between removal and regeneration processes. As the time series progressed, and more of the colloidal matter was filtered out of the water or lost to the beaker walls, the removal rates appear to slow down, or level off. By focusing on the first 10 hours of the experiment, a fairly linear removal curve is apparent.

In an effort to increase the ability to choose a more representative group of oysters, decrease the variability in removal rates within that group and decrease loss to the beaker walls, the number of replicate oysters was increased for subsequent experiments, and the duration of time series was shortened.

Mass balance determination for Experiment 1 is shown in Figure 5 for all three treatments (COC, AA, C). Cadmium and Zn appeared to be present in the meat in greater percentages than Cr or Ag, with all of them having a significant portion on the shell. Mass balance calculations also showed that there was loss of the labeled organic matter to the beaker walls. Mass balance was determined by,

$$\left[\frac{A_f}{A_t}\right] x100 \qquad (9)$$

, with A_f the activity of a fraction at the completion of the time series and A_t the total activity for all fractions at the final time for the time series.

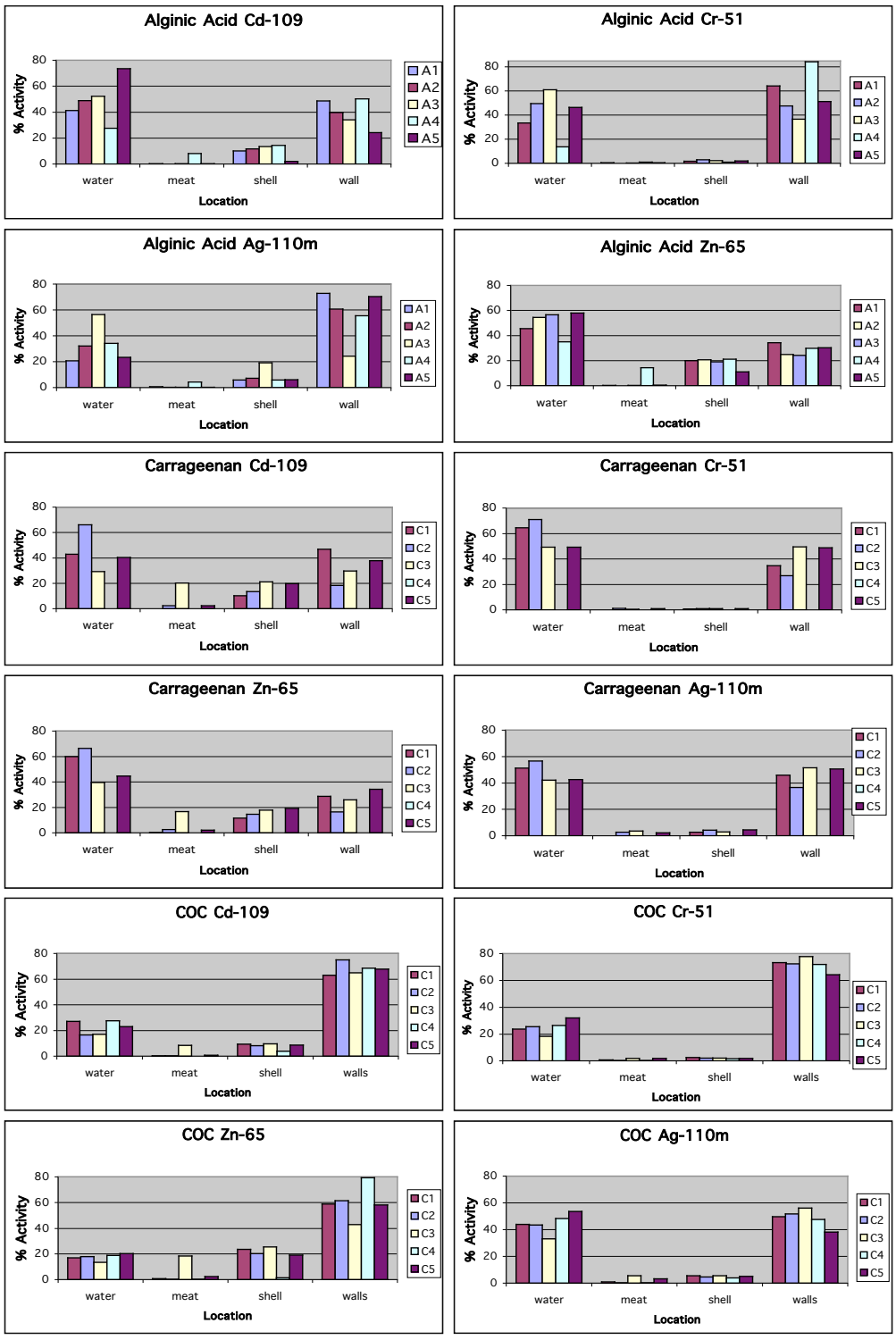


Fig. 5. Experiment 1 mass balance. Mass balance for metals associated with three different types of colloidal matter. Two model biopolymers, alginic acid, carrageenan, and natural colloidal organic carbon.

For Experiment 2 (Figure 6), mass balance analysis showed the C treatment had the highest activities in the meat, followed by the COC treated organic matter while AA had the lowest amounts in the meat. Cadmium (17.2 for C, 11.2 for COC and 0.89 for AA), Zn (6.0 for C, 8.5 for COC, 0.46 for AA), Hg (4.3 for C, 3.5 for COC, 1.7 for AA), and Ag (3.7 for C, 3.5 for COC, 0.63 for AA) were found in the meat in the greatest percentages for all three treatments. Based on the percent activity removed from the water for experiment 2 results showed, for all three treatments (COC, AA, C), that Hg, Ag and Zn were removed in the highest amounts from the water (Figure 7), with a higher percentage of AA and C being removed than COC. Cobalt and Cd had the lowest removal percentages from the water.

The labeled COC was removed in higher amounts than AA or C, with Zn and Cr with the highest removal percentages (Figure 7). Removal percentages for COC associated Zn and Cr were 58.8 and 59.8, respectively. Percentages for AA and C associated Zn and Cr were 50.1 and 47.5, respectively, for Zinc, and 51.3 and 55.8, respectively, for Cr.

A

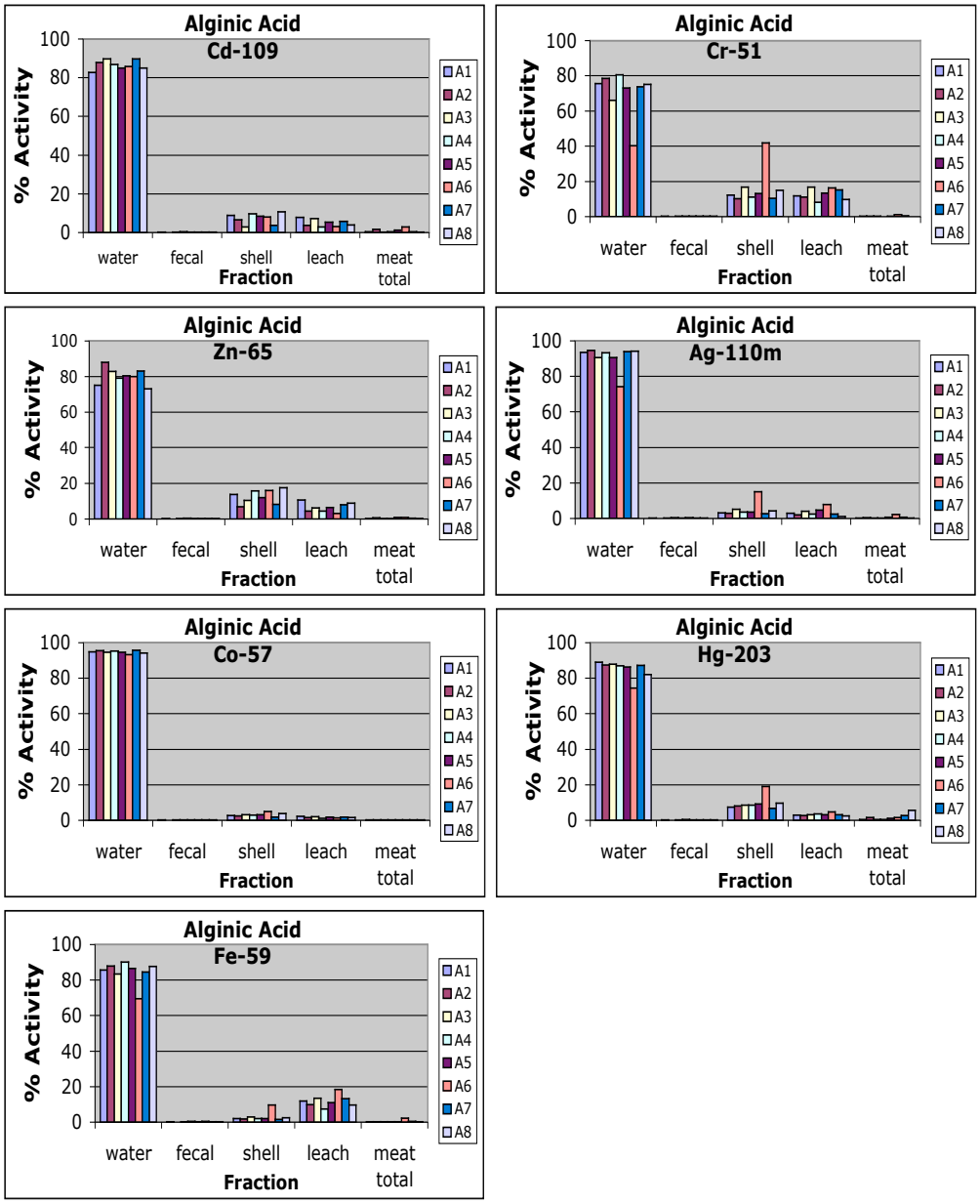


Fig. 6. Experiment 2 mass balance. (A) Mass balance for metals associated with alginic acid.

B

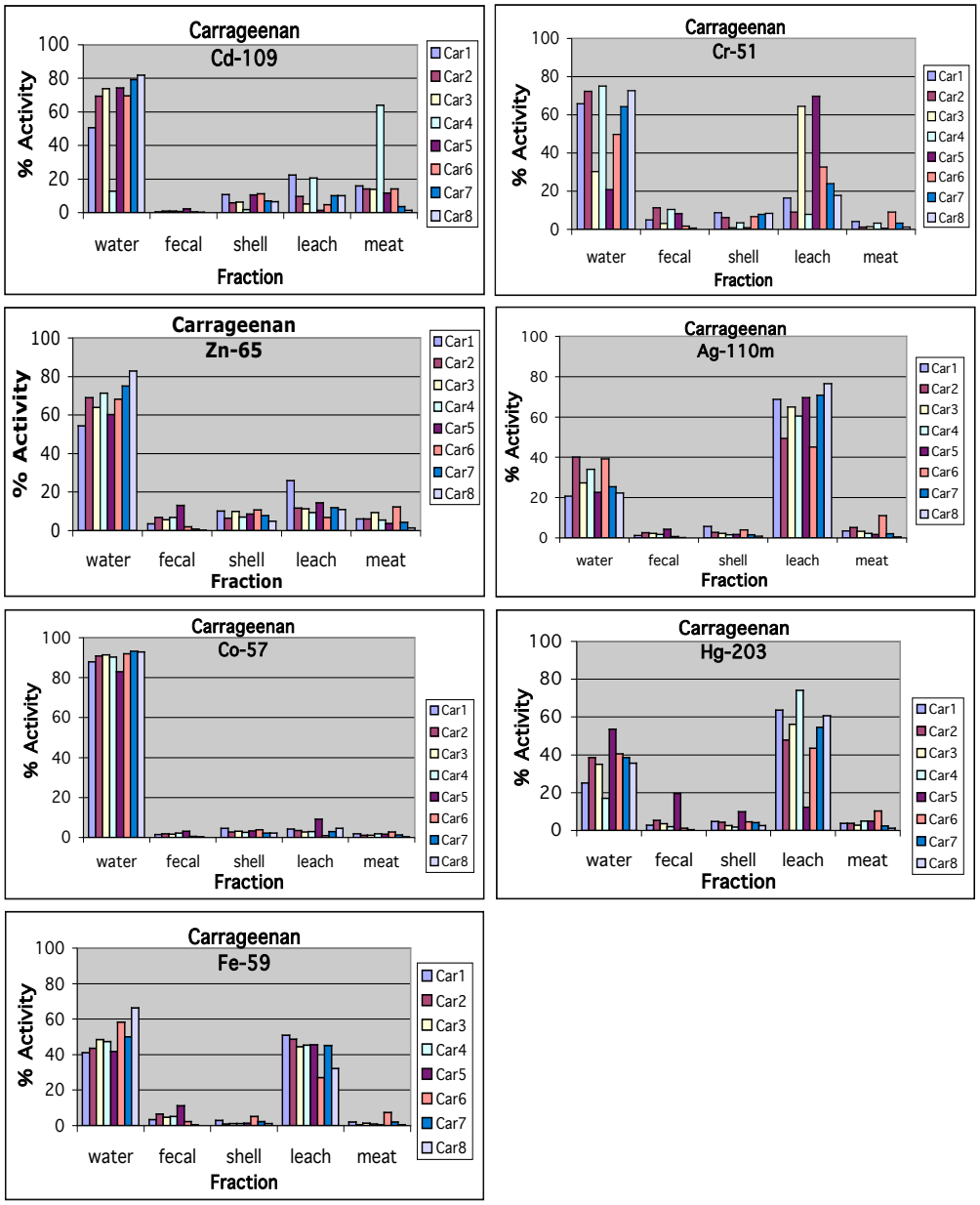


Fig. 6. Continued. (B) Mass balance for metals associated with carrageenan.

C

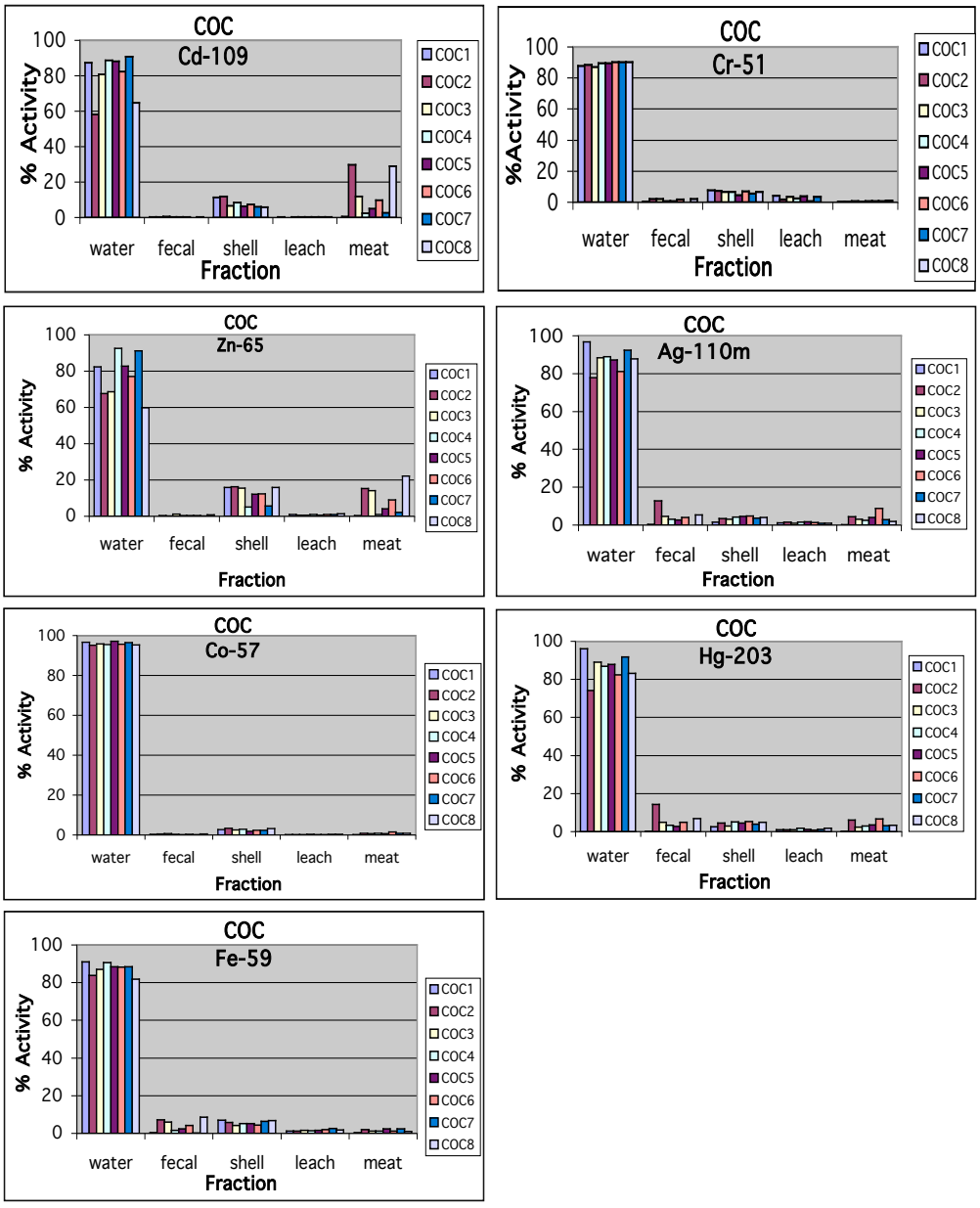
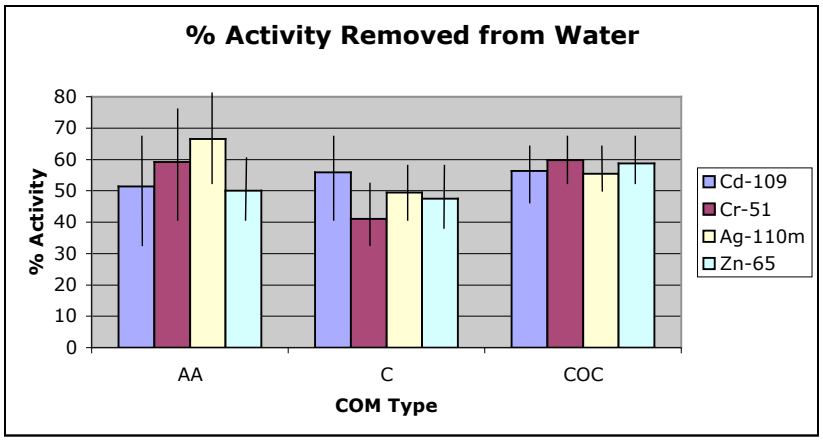


Fig. 6. Continued. (C) Mass balance for metals associated with colloidal organic carbon.

A



B

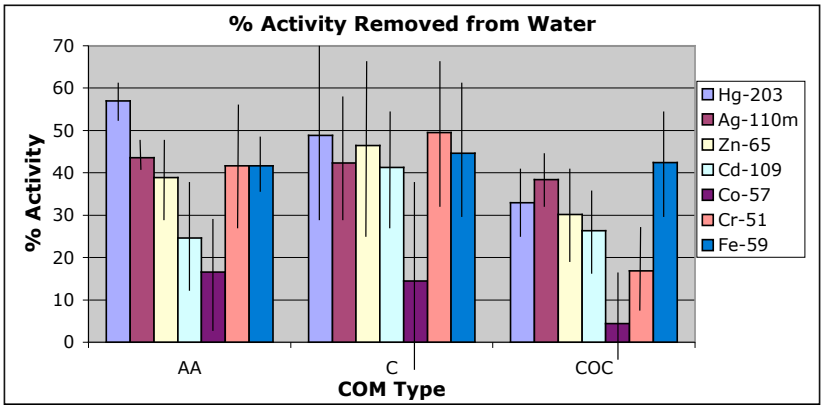


Fig. 7. Metal experiments - Percent activity removed from water. (A) Experiment 1; (B) Experiment 2.

For experiment 2, the loss of activity to the beaker walls for the AA and COC treatments was much lower than in the previous experiment. However, for the C treatment, the loss of activity to the beaker wall was in some instances higher than for experiment 1 and quite significant for some of the metals, as can be seen in Figures 5 and 6.

Oysters were dried and weighed after the experiment was completed to calculate dry weight concentration factors, DCF. The average DCF was calculated for Experiment 1 (Figure 8, Table 2) and Experiment 2 (Figure 9, Table 3).

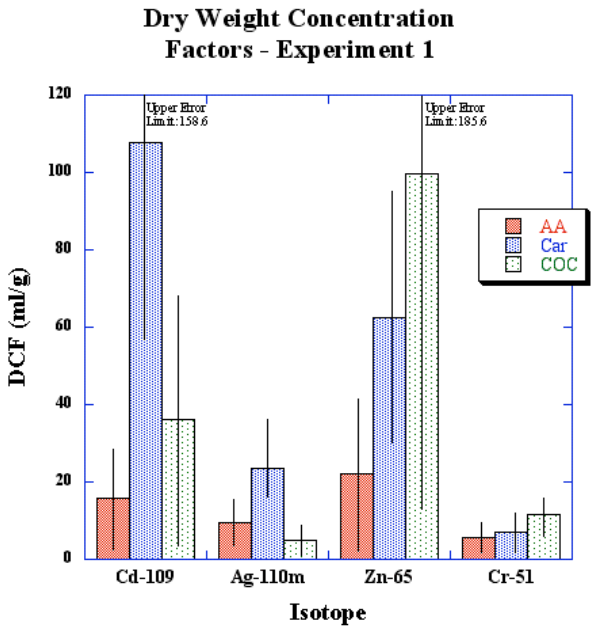


Fig. 8. Metal experiment 1 dry weight concentration factors.

Table 2
Metal experiment 1 dry weight concentration factor (ml/g) values. Values +/- standard deviation.

AA	**DCF**			
	Cd-109	**Ag-110m**	**Zn-65**	**Cr-51**
	15.7 +/- 10	9.4 +/- 5	22 +/- 18	5.5 +/- 3
C	**DCF**			
	Cd-109	**Ag-110m**	**Zn-65**	**Cr-51**
	108 +/- 62	25 +/- 7	63 +/- 28	7.1 +/- 5
COC	**DCF**			
	Cd-109	**Ag-110m**	**Zn-65**	**Cr-51**
	36 +/- 29	4.8 +/- 4	100 +/- 83	11.7 +/- 5

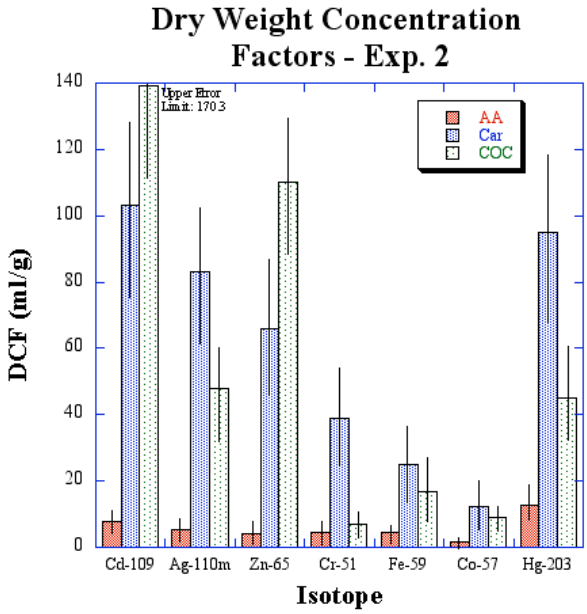


Fig. 9. Metal experiment 2 dry weight concentration factors.

Table 3
Metal experiment 2 dry weight concentration factor (ml/g) values. Values +/- standard deviation.

AA	**DCF**						
	Cd-109	**Co-57**	**Hg-203**	**Cr-51**	**Ag-110m**	**Fe-59**	**Zn-65**
	8.0 +/- 3	1.6 +/- 1	12.9 +/- 5	4.5 +/- 2	5.3 +/- 2	4.5 +/- 2	4.1 +/- 3
Carrageenan	**DCF**						
	Cd-109	**Co-57**	**Hg-203**	**Cr-51**	**Ag-110m**	**Fe-59**	**Zn-65**
	103 +/- 25	12.5 +/- 6	95 +/- 20	39 +/- 17	83 +/- 19	25 +/- 7	66 +/- 19
COC	**DCF**						
	Cd-109	**Co-57**	**Hg-203**	**Cr-51**	**Ag-110m**	**Fe-59**	**Zn-65**
	139 +/- 24	9.1 +/- 4	45 +/- 14	6.8 +/- 3.4	48 +/- 18	17 +/- 6	110 +/- 22

The highest DCF's for both experiments were associated with the Carrageenan and COC treatments, especially for ^{109}Cd and ^{65}Zn as well as ^{110m} Ag for Experiment 2. These higher DCF values correspond to an increased bioavailability to the oyster. In fact, the highest amounts of radioisotopes in the meat for experiment 1 (Figure 10, Table 4) and experiment 2 (Figure 11 and Table 5) were for ^{109}Cd, ^{65}Zn and ^{110m} Ag in the Carrageenan treatment. The DCF values were lowest for all the metals in the Alginic Acid treatments, corresponding to lower levels in the meat. The DCF values from Exp. 2 were compared to results from a previous study (Figure 12, Table 6). The comparison shows a significant difference between the calculated values, however the DCF's for Experiment 2 do fall within the range of DCF's of the Experiment 1. The DCF averages for these two studies are typically lower than the previous results (Guo, et al., 2001), which may be due to increased wall adsorption of the radioisotopes during Experiments 1&2.

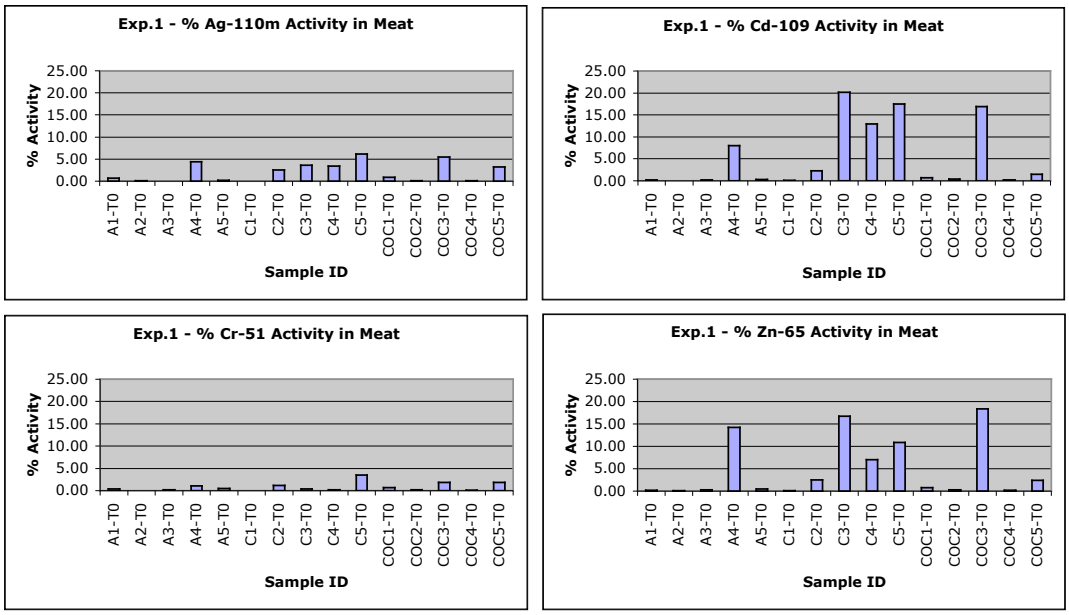


Fig. 10. Percent activity in meat for metal experiment 1.

Table 4
Values for percent activity in meat for metal experiment 1

% Act. in Meat				
Sample	Cd-109	Cr-51	Ag-110m	Zn-65
A1-T0	0.21	0.41	0.71	0.24
A2-T0	0.04	0.05	0.12	0.05
A3-T0	0.24	0.20	-0.03	0.26
A4-T0	8.00	1.05	4.37	14.2
A5-T0	0.26	0.46	0.21	0.50
C1-T0	0.11	0.04	0.02	0.13
C2-T0	2.29	1.13	2.57	2.50
C3-T0	20.1	0.41	3.60	16.7
C4-T0	12.9	0.21	3.43	6.97
C5-T0	17.4	3.54	6.20	10.8
COC1-T0	0.65	0.67	0.85	0.75
COC2-T0	0.36	0.21	0.14	0.33
COC3-T0	16.8	1.81	5.45	18.3
COC4-T0	0.19	0.13	0.15	0.23
COC5-T0	1.44	1.88	3.19	2.45

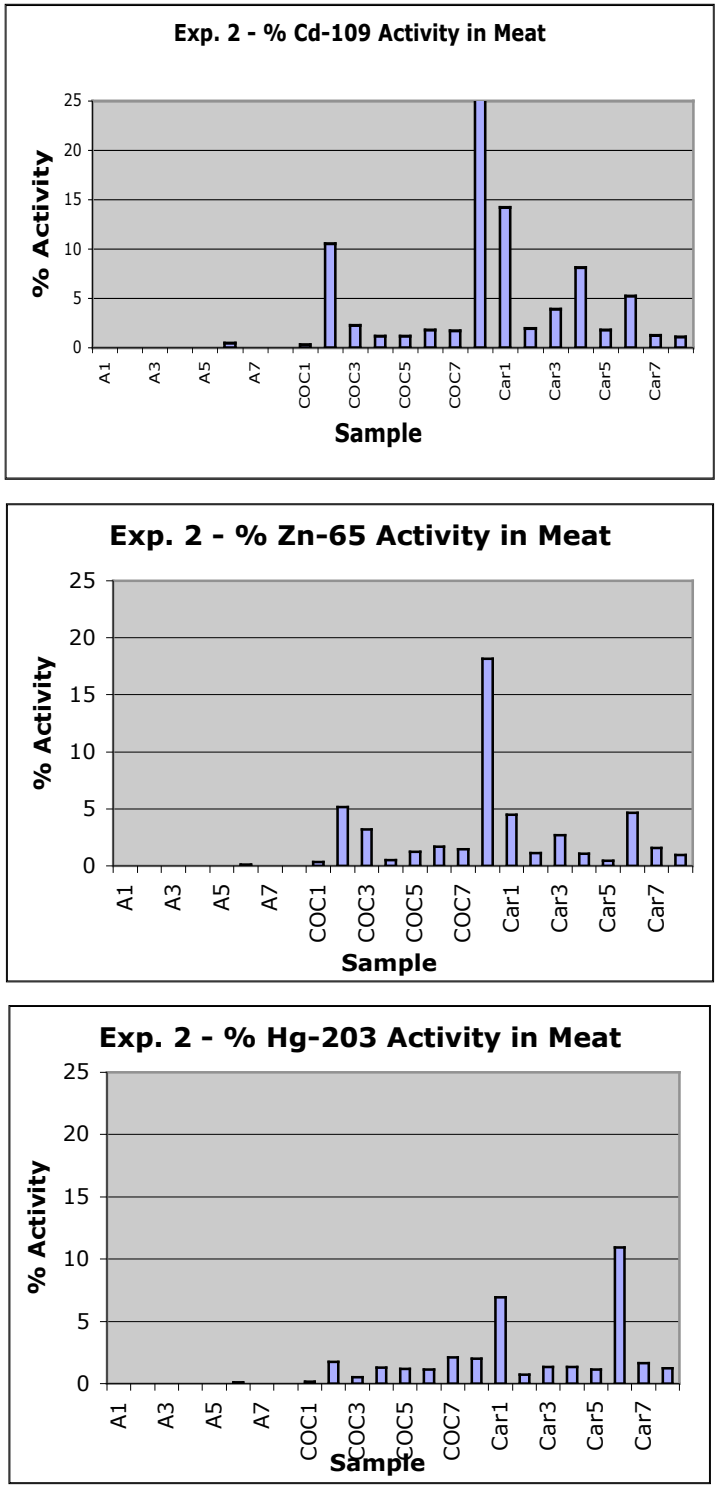
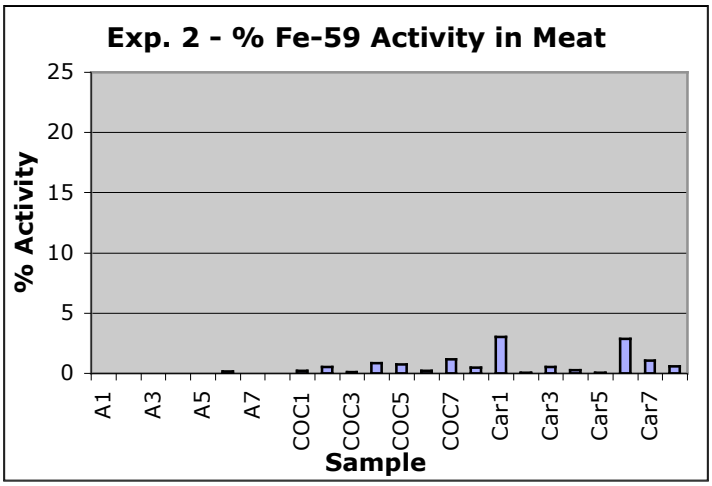
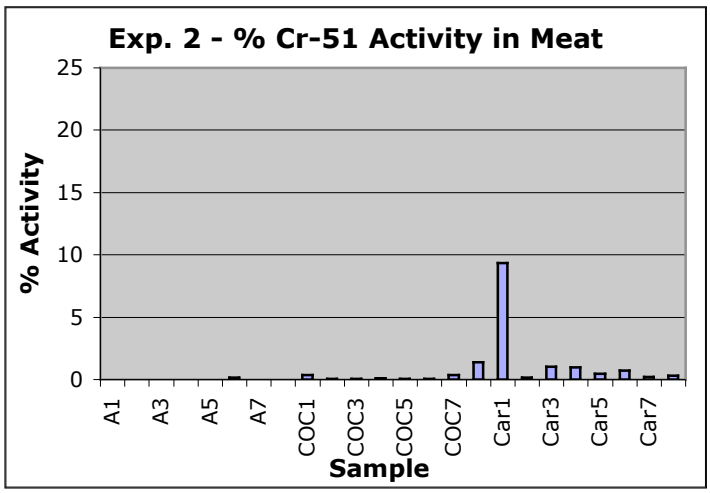


Fig. 11. Percent activity in meat for metal experiment 2.





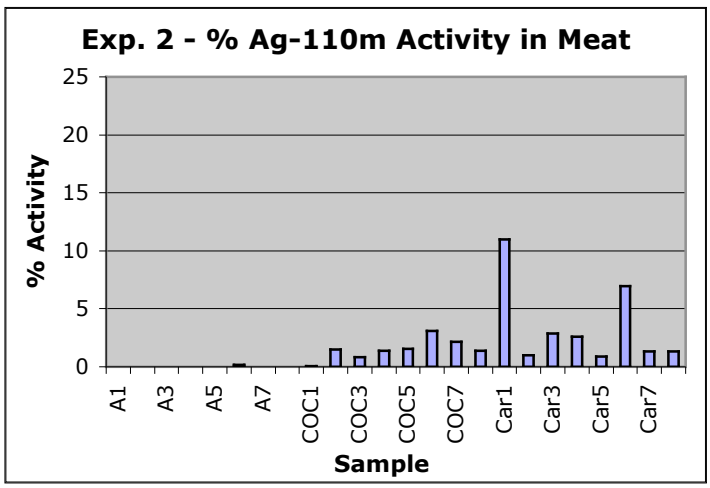


Fig. 11. Continued.

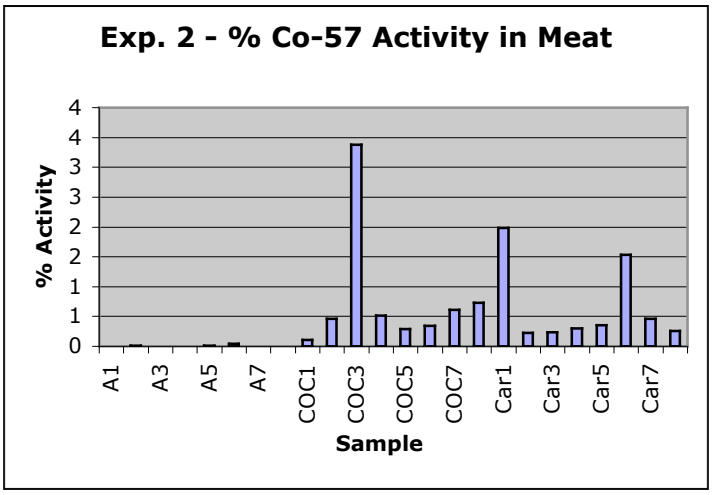


Fig. 11. Continued.

Table 5
Values for percent activity in meat for metal experiment 2

% Act. in Meat							
Sample	Cd-109	Co-57	Hg-203	Cr-51	Ag-110m	Fe-59	Zn-65
A1	0.005	0.003	0.005	0.002	0.003	0.002	0.003
A2	0.033	0.007	0.016	0.007	0.007	0.007	0.013
A3	0.002	0.002	0.002	0.001	0.002	0.001	0.002
A4	0.007	0.003	0.007	0.003	0.003	0.003	0.005
A5	0.024	0.007	0.013	0.008	0.010	0.007	0.018
A6	0.46	0.042	0.11	0.13	0.14	0.14	0.11
A7	0.003	0.002	0.010	0.005	0.003	0.004	0.003
A8	0.002	0.001	0.006	0.001	0.001	0.001	0.002
COC1	0.33	0.11	0.15	0.36	0.03	0.19	0.34
COC2	10.5	0.46	1.76	0.06	1.50	0.52	5.14
COC3	2.24	3.38	0.54	0.05	0.81	0.12	3.22
COC4	1.20	0.51	1.28	0.11	1.35	0.82	0.53
COC5	1.20	0.29	1.16	0.05	1.53	0.74	1.23
COC6	1.80	0.34	1.15	0.06	3.07	0.21	1.67
COC7	1.71	0.61	2.13	0.35	2.18	1.15	1.43
COC8	25.7	0.73	1.98	1.40	1.36	0.48	18.1
Car1	14.1	1.99	6.91	9.35	10.9	3.04	4.51
Car2	1.95	0.22	0.71	0.15	1.02	0.05	1.09
Car3	3.90	0.24	1.34	1.03	2.85	0.56	2.71
Car4	8.16	0.31	1.32	0.96	2.61	0.27	1.08
Car5	1.78	0.36	1.13	0.47	0.88	0.05	0.42
Car6	5.21	1.53	10.9	0.72	6.94	2.88	4.67
Car7	1.23	0.46	1.62	0.19	1.35	1.07	1.56
Car8	1.07	0.26	1.23	0.28	1.33	0.58	0.97

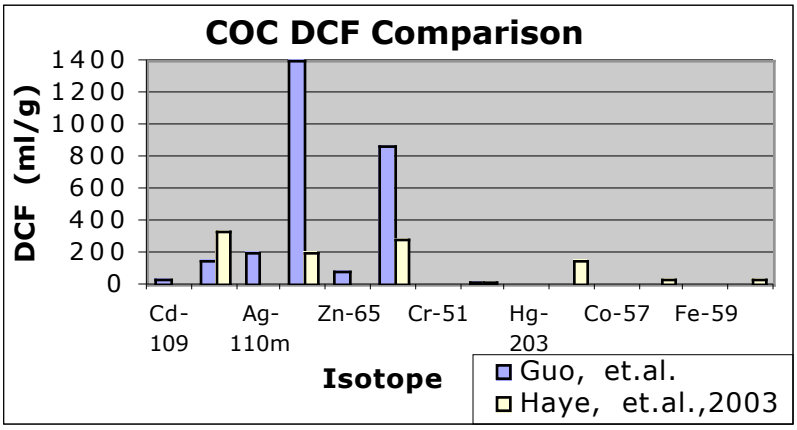


Fig. 12. Dry weight concentration factor comparison.

Table 6
Values for dry weight concentration factor (ml/g) comparison.

	Alginic Acid	Carrageenan	COC	
	This work	This work	Guo, et al. (2000)	This work
Cd-109	11.0 +/- 21	105 +/- 89	32	100 +/- 137
			145	
Ag-110m	6.9 +/- 10	60 +/- 59	200	31 +/- 61
			1400	
Zn-65	11 +/- 28	65 +/- 55	78	106 +/- 102
			875	
Cr-51	4.9 +/- 7.3	27 +/- 38	15	8.7 +/- 3.4
Fe-59	4.5 +/- 7.4	25 +/- 32		17 +/- 8.4
Co-57	1.6 +/- 0.8	13 +/- 9.0		9.1 +/- 7.9
Hg-203	13 +/- 11	95 +/- 74		45 +/- 42

^{14}C

Two experiments were also run for ^{14}C. The initial study included only AA and natural COC, with the following study including C as well as AA and COC. Similar to the metal experiments, the majority of the removal from the water occurred within the first eight hours (Figures 13 and 14).

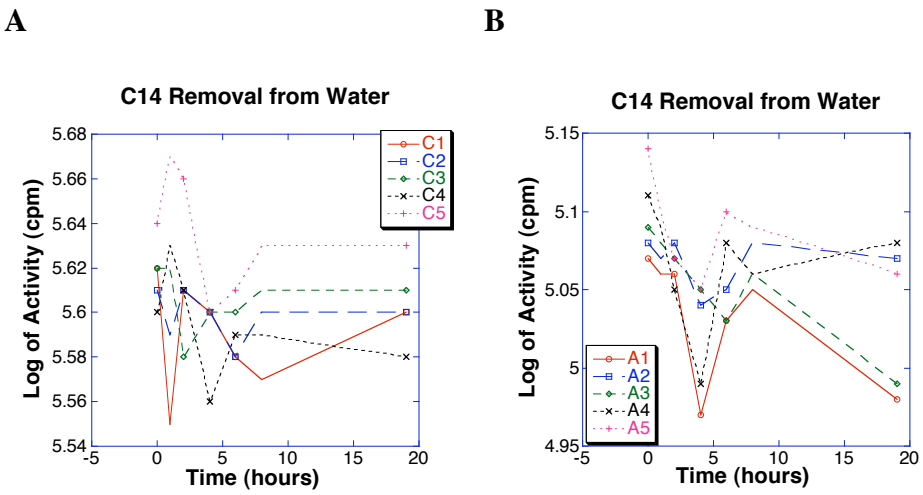


Fig. 13. ^{14}C Experiment 1 – Removal from water. (A) ^{14}C labeled colloidal organic carbon; (B) ^{14}C labeled alginic acid.

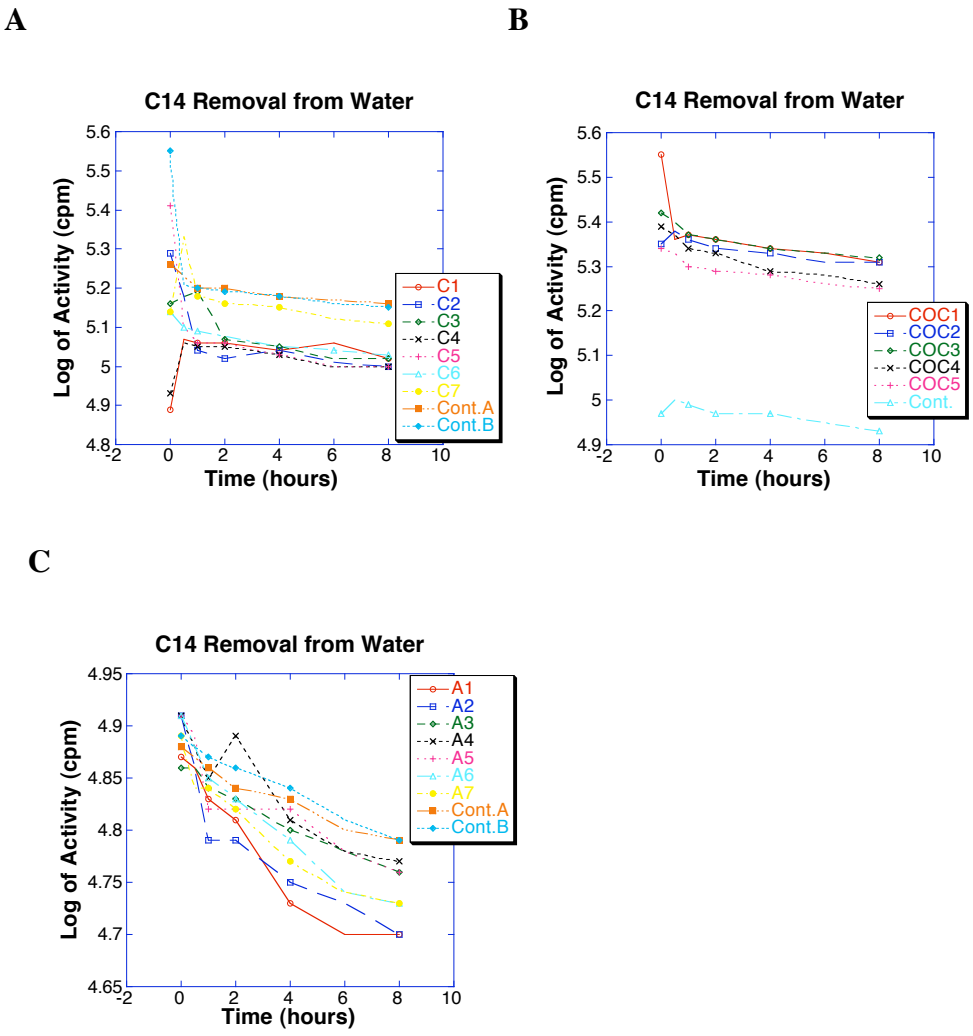
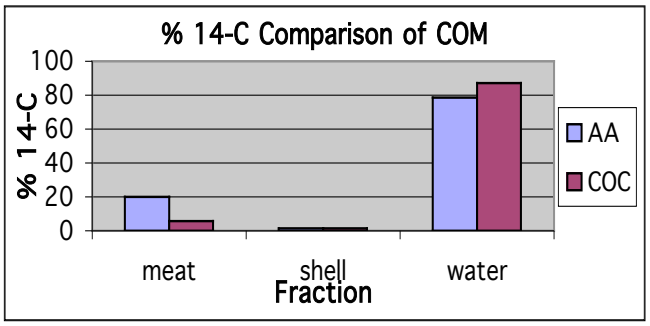


Fig. 14. ^{14}C Experiment 2 – Removal from water. (A) ^{14}C labeled carrageenan; (B) ^{14}C labeled colloidal organic carbon; (C) ^{14}C labeled alginic acid.

For both Experiment 1 and 2, the mass balance calculations showed increased meat uptake of the AA as opposed to the natural COC (Figure 15). Even though the labeling of the colloidal matter for experiment 1 was not very efficient, the results from Experiment 2 confirmed these results. For Experiment 2, greater uptake was indicated by both the meat (Figures 16) and the water data (Figure 17).

A



B

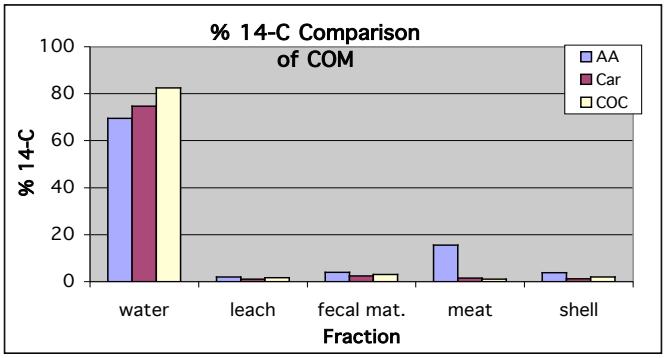
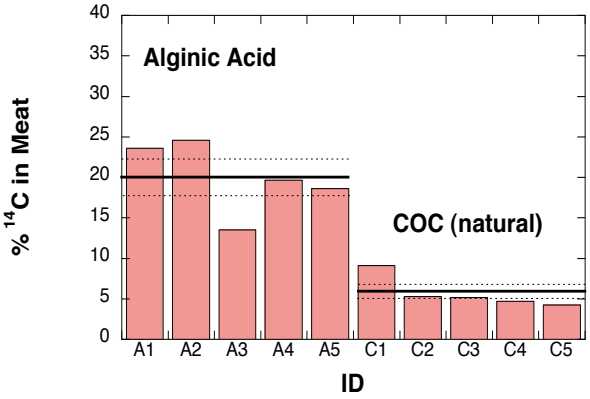


Fig. 15. ^{14}C Mass balance. (A) Experiment 1; (B) Experiment 2.

A



B

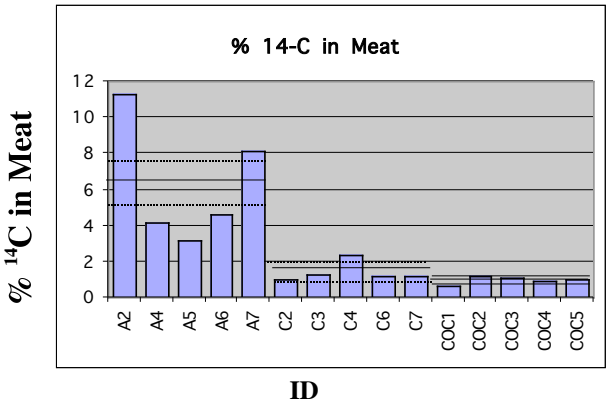


Fig. 16. Percent ^{14}C in meat. (A) Experiment 1; (B) Experiment 2. Average line shown +/- standard deviation.

A

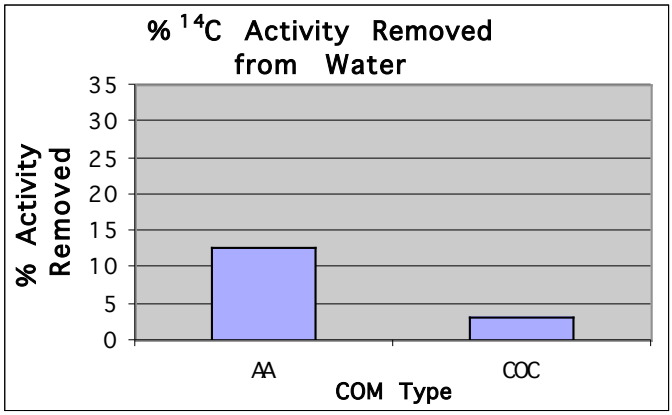


Fig. 17. Percent ^{14}C activity removed from water. (A) Experiment 1; (B) Experiment 2.

B

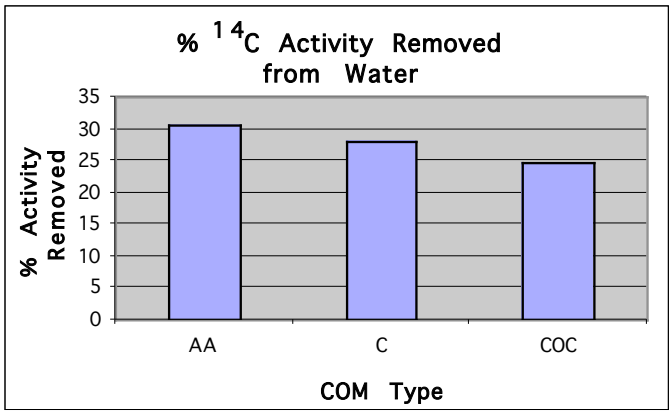
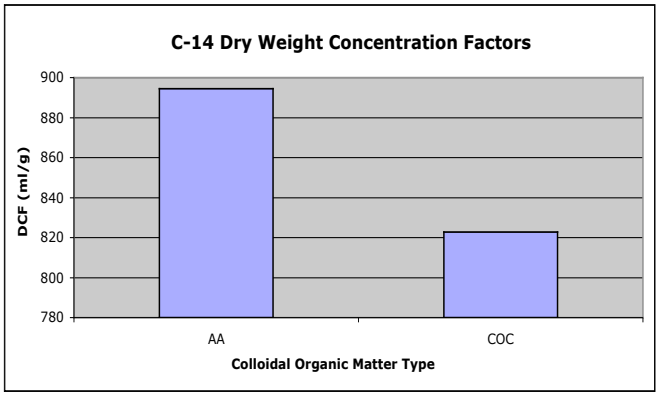


Fig. 17. Continued.

For both Experiment 1 & 2, the DCF values calculated (Figure 18 and Table 7) show the greatest uptake from AA, supporting the results from the removal of ^{14}C from the water and the % ^{14}C in the meat. Several corrections were made to the radioactive counts in the meat to generate the numbers used in Figure 16: background, quenching, dilution and matrix corrections. Mass balance calculations indicate a small but significant percentage of activity unaccounted for (Figure 15). Future experiments should attempt to locate the source of the unaccounted loss of activity by digesting fecal matter, increasing the strength of leach solution and increasing the leach time of beakers walls. Although the percentage of ^{14}C in the meat from Experiment 1 was less than Experiment 2 (i.e., 6% vs. 20% for AA, and 1% vs. 5 % for COC), AA still proved to yield a higher uptake than COC (Figure 16 and Table 7).

A



B

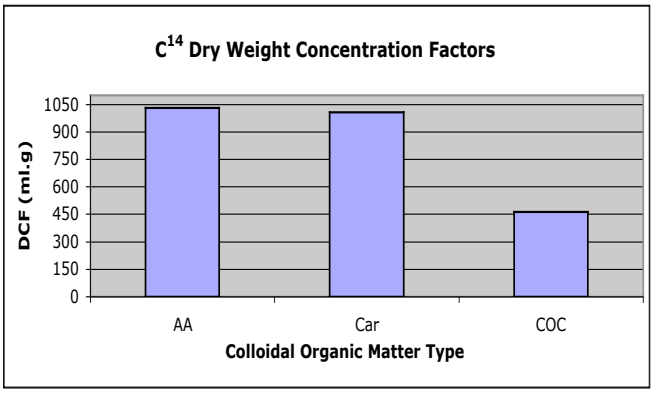


Fig. 18. ^{14}C Dry weight concentrations factors. (A) Experiment 1; (B) Experiment 2.

Table 7
Values for ^{14}C dry weight concentration factors. (A) Experiment 1; (B) Experiment 2

A

C-14 Exp. 1	DCF
AA	894 +/- 80
COC	801 +/- 9

B

C-14 Exp. 2	DCF
AA	1032 +/- 78
Car	1006 +/- 466
COC	463 +/- 81

DCF values were also calculated for the ^{14}C treatment and the geometric means (GM) for all DCF's, and upper and lower 95% confidence limits (CL) (2 standard deviations) were determined (Table 8). This type of transformation is often calculated for experiments with biological components, which show log-normal distributions as in these experiments, and which can thus assist in getting a more evenly distributed variance. The geometric means were useful in assessing the dry weight concentration factors as they reduced the apparent error and thus improved the ability to determine the relative bioavailability of metals and organic matter. As mentioned previously for the metals, AA was removed from the water in greater amounts, yet the highest DCF's were found in the Carrageenan treatments. For the ^{14}C experiment, AA was again removed from the water in the greatest amounts, however the DCF's were also greatest for AA. For the metals, the DCF's of C were highest for Cd, Zn and Ag. The GM of ^{109}Cd was 71, with upper and lower 95% CL of 199 and 25, respectively. For ^{65}Zn, the GM was 51 with upper and lower CL of 114 and 23, and for ^{110m}Ag the GM was calculated to be 65, with upper and lower CL of 139 and 30. For ^{14}C, the highest DCF values were associated with the AA treatment with a GM of 11 and upper and lower CL of 26 and 5. Carageenan had the next highest DCF, followed by COC. These results closely relate to the concentrations of ^{14}C found in the oyster meat.

Table 8
Geometric means for dry weight concentration factors.

C-14	Geometric Mean	Upper 95% Conf. Limit (2 s.d.)	Lower 95% Conf. Limit
AA	10.94	25.82	4.64
Car	1.69	3.54	0.81
COC	0.57	0.87	0.37
Co-57	Geom. Mean	Upper 95% Conf. Limit	Lower 95% Conf. Limit
AA	1.43	2.29	0.90
Car	9.79	20.74	4.62
COC	6.93	15.40	3.11
Hg-203	Geom. Mean	Upper 95% Conf. Limit	Lower 95% Conf. Limit
AA	9.07	22.65	3.63
Car	71.82	159.61	32.32
COC	30.27	89.43	10.24
Cr-51	Geom. Mean	Upper 95% Conf. Limit	Lower 95% Conf. Limit
AA	2.26	6.71	0.76
Car	27.09	63.42	11.57
COC	6.05	10.36	3.53
Cd-109	Geometric Mean	Upper 95% Conf. Limit	Lower 95% Conf. Limit
AA	4.21	13.78	1.29
Car	70.96	198.95	25.31
COC	72.04	294.53	17.62
Ag-110m	Geom. Mean	Upper 95% Conf. Limit	Lower 95% Conf. Limit
AA	3.18	8.01	1.26
Car	64.82	138.85	30.26
COC	25.24	103.54	6.15
Fe-59	Geom. Mean	Upper 95% Conf. Limit	Lower 95% Conf. Limit
AA	2.28	6.66	0.78
Car	15.34	40.49	5.81
COC	14.10	27.62	7.19
Zn-65	Geom. Mean	Upper 95% Conf. Limit	Lower 95% Conf. Limit
AA	3.25	6.58	1.60
Car	51.40	113.85	23.21
COC	57.17	231.59	14.11

Experimental results indicated that more ^{14}C-labeled AA was removed from the water than labeled C or COC (Figure 17; Appendix Table 10), corresponding to the relative values of DCF's in the oyster meat (e.g., 1032 ml/g, 1006 ml/g, 463 ml/g, respectively, Figure18 and Table 7). However, trace metal removal rates and DCF values did not necessarily correspond to this sequence. In the first experiment, COC had the largest percent of activity removed from the water for ^{109}Cd and ^{65}Zn, while ^{110m}Ag had the highest removal with AA (Figure 7). The only DCF corresponding to the removal from the water was for the ^{65}Zn associated with COC (Figure 8 and Table 2). The rest of the DCF's for the oyster meat had no correlation. In the second experiment, COC had the lowest removal from the water for all metals (Figure 4b & Table 4b). The AA had the greatest removal for ^{203}Hg, ^{110m}Ag and ^{57}Co with the greatest removal for ^{65}Zn, ^{109}Cd, ^{51}Cr and ^{59}Fe associated with C (Figure 7). The DCF values showed AA with the lowest values for all metals (Figure 9 & Table 3). The highest DCF's were associated with the C treatment for the majority of metals (Figure 9 & Table 3).

CHAPTER IV

DISCUSSION AND CONCLUSIONS

Discussion

Bioavailability of metals may be altered by 1) the formation of aggregates of colloidal matter that increase the overall size of the particle being filtered, aided by the stickiness of some of its components (Wen, et al., 1997); 2) uptake at the gills after release from the carrier molecule (Wang and Guo, 2000); 3) uptake in the digestive system after release from the food particles through the action of enzymes and surfactants (Roditi, et al., 2000).

The variability in meat and fecal matter inventories, for both the metals and ^{14}C, displayed in this research could be due to the difference between "super performers" as opposed to "under performers". This means that some oysters filter at much higher or lower rates than the majority. The difference in filtering rates may be attributed to distinctive feeding strategies of the oysters. Part of the active feeding mechanism of oysters is to capture food particles and colloidal matter using a "mucus web", which oysters use in conjunction with cilia to move food particles into the mouth (Wotton, 2000). Though a mucus web can be used during active feeding, it is not consistently used. An oyster might use the mucus web at one feeding and not at another, or may only use the mucus web for part of an active feeding session. Furthermore, while one oyster may be feeding using the mucus web, another may not be ingesting any food at all. The experiments used for this study were not designed to test whether or not an oyster was actively feeding during the time series experiment or whether the mucus web was being used. Instead, oysters were kept unfed overnight and allowed to purge to observe viable

specimens for use in the time series experiments. It is likely that some of the replicate oysters for the different treatments were using the mucus web system to feed while others were not, contributing to the large variability seen in the meat and fecal inventories. In addition, during uptake from food, heavy metals adsorb to the mucus web used in ciliary feeding and therefore, "super-performers" would be ingesting greater amounts of metals and increasing the amounts available for assimilation into the gut and fecal matter.

An important uptake route for marine invertebrates is from solution (Rainbow, 1990). Uptake from solution is a passive process, in contrast to uptake by particle feeding, requiring no extra expenditure of energy by the oyster. This uptake occurs at permeable surfaces of the body, such as the gills, as well as in the alimentary tract when food is swallowed. Oysters that are open and feeding during the time-series experiments would have greater exposure to, and therefore greater uptake of, metals, which could also explain the variability in the meat and fecal inventories between oysters.

While this study did find that COM was ingested by the bivalves, due to the presence of ^{14}C in the oyster meat, uptake of the associated metals was not supported for all treatments. The largest inconsistency is for the alginic acid colloidal organic matter, which had the highest amounts of ^{14}C in the oyster meat and the highest DCF values for ^{14}C, yet had the lowest metal percentages in the meat and lowest metal DCF values. Therefore, metals appear to have been remobilized after ingestion and not sufficiently assimilated into the oyster meat. The carrageenan ^{14}C-DCF values were close to those of alginic acid (1006 ml/g and 1032 ml/g, respectively), but many of the metals associated with carrageenan were found to have the highest metal DCF's and highest percentages in the meat. It appears some metals, particularly ^{110m}Ag, ^{203}Hg, ^{109}Cd and ^{65}Zn, may be more

bioavailable than ^{57}Co, ^{59}Fe and ^{51}Cr based on DCF values and percentage activity in oyster meat. However, the question remains, are these metals present in greater amounts simply because they are bound to the organic matter ingested by the oyster or are they selected by the oyster because they are essential to oyster health.

For COM uptake as a function of composition, the ^{14}C experiments revealed that the APS alginic acid was present in the oyster meat in greater percentages than natural COC. More importantly, the DCF values for ^{14}C indicated a greater bioavailability of the APS to the oyster than the natural COC. This result appeared to support the hypothesis that the composition of the organic matter was important to the uptake. In spite of this, the metal experiments were much less conclusive and did not present a clear-cut indication of uptake relating to COM composition. If the composition of APS were influencing the uptake of metals, we would expect to find C and AA having comparable results. Instead, all metals associated with AA had the lowest meat activities, in some cases with values one or two orders of magnitude lower, and lowest DCF values, typically at least one order of magnitude lower, compared to either C or COC. It was C and COC that had similar percentages of metals in the meat, and similar DCF values. For instance, DCF values for C and COC were: 71 & 72ml/g for ^{109}Cd, 51.4 & 57.2 ml/g for ^{65}Zn, 71.8 & 30.3ml/g for ^{203}Hg and 64.8 & 25.2ml/g for ^{110m}Ag, respectively. Although the ^{203}Hg and ^{110m}Ag values were higher for C, they are the same order of magnitude as COC. This research provides some evidence that APS modify metal uptake based on the knowledge that AA can be taken up by oysters (from ^{14}C experiment results), yet metals associated to AA are not making it into the oyster meat. In addition, C treatments did have greater uptake of metals than natural COC, albeit not by very much for some metals.

The important questions as to the exact mechanisms by which APS modify metal uptake could not be answered by this research. A method to determine if APS are forming fibrils during uptake experiments would be important for future research, and if so, what are the implications they have to bioavailability of the toxic metals.

From these experiments, it is evident that metal behavior differs for varying types of colloidal matter, however it was not possible to state that the difference lies between model APS and natural COM. The B-type metals had higher percentages in the oyster meat and higher DCF values than A-type metals, but this was true for all organic matter treatments. While one model APS, carrageenan, did show an overall higher bioavailability of metals to oysters compared to natural COC, the other model APS, alginic acid, was much less bioavailable than the natural COC. We know from the ^{14}C experiments that oysters do take up AA, so further studies are needed to assess why and how metals are being dissociated from the AA colloidal matter. Furthermore, the metal character (B-type or A-type) did not appear to prefer one type of ligand to another. A predicted outcome would have been to find the highest levels of A-type metals, ^{59}Fe and ^{51}Cr, in the meat of oysters fed the alginic acid COM treatments, as these metals prefer to bind with O-containing or N-containing ligands. However, none of the metals seemed to prefer the AA treatments and both A-type and B-type metals were found to be associated with the C COM. Most metals appear to be more bioavailable in the C treatments compared to natural COM, with the exception of ^{109}Cd and ^{65}Zn. Therefore, this research did elucidate that metals behave differently with different types of organic matter, but did not increase our understanding of how these metals are interacting with the functional groups of organic ligands, and how metals are incorporated into oyster meat.

Conclusions

These oyster uptake experiments were carried out to improve our understanding of the mechanisms and pathways that control metal uptake, thus increasing our ability to use bivalves as pollution indicators. From the results of short-term (8-hour) oyster bioassays that utilized a series of radioactive metals and ^{14}C bound to natural colloidal macromolecular organic matter or model acid polysaccharides alginic acid and carrageenan, we arrived at the following conclusions:

1) American oysters can remove nanoparticles with diameters as small as 0.04μm from the water through filter feeding,

2) Alginic acid, but not associated metals, appeared to be taken up rapidly into the meat but seemed to have a protective effect on metal uptake.

3) Carrageenan was taken up much less, only slightly more than natural COM, but associated metals were taken up much more than with alginic acid, and to a similar extent as with natural COM.

4) The sequence of metal uptake was similar to that previously observed, i.e., Zn ~ Cd > Hg ~ Ag > Cr ~ Fe ~ Co.

5) Further generalizations cannot be made because of the large variability in uptake rates whereby the large variability was mostly due to an "outperforming" individual oyster.

6) It is possible that the decoupling of (acid) polysaccharide and metal uptake in these oyster bioavailability experiments may be due to aggregate or gel formation in the water aiding the filtration from the water, followed by metal

remobilization in the digestive tract of the bivalve.

7) While it could be shown that metals behaved differently when associated with APS compounds than when associated with natural COM, the hypothesis that bioavailability of metals bound to organic matter was modified because APS or natural COM is used as a food source could not be sufficiently tested because of possible metal release, e.g., during digestive activities of the oyster, as well as possible uptake by the gills of metals dissociated from the colloids (Guo et al., 2002).

8) The hypothesis that the "quality aspect" of organic matter can be important, e.g., that COM uptake by oysters is a function of COM composition, was shown to be true, and metal uptake by oysters was different for different organic matter types.

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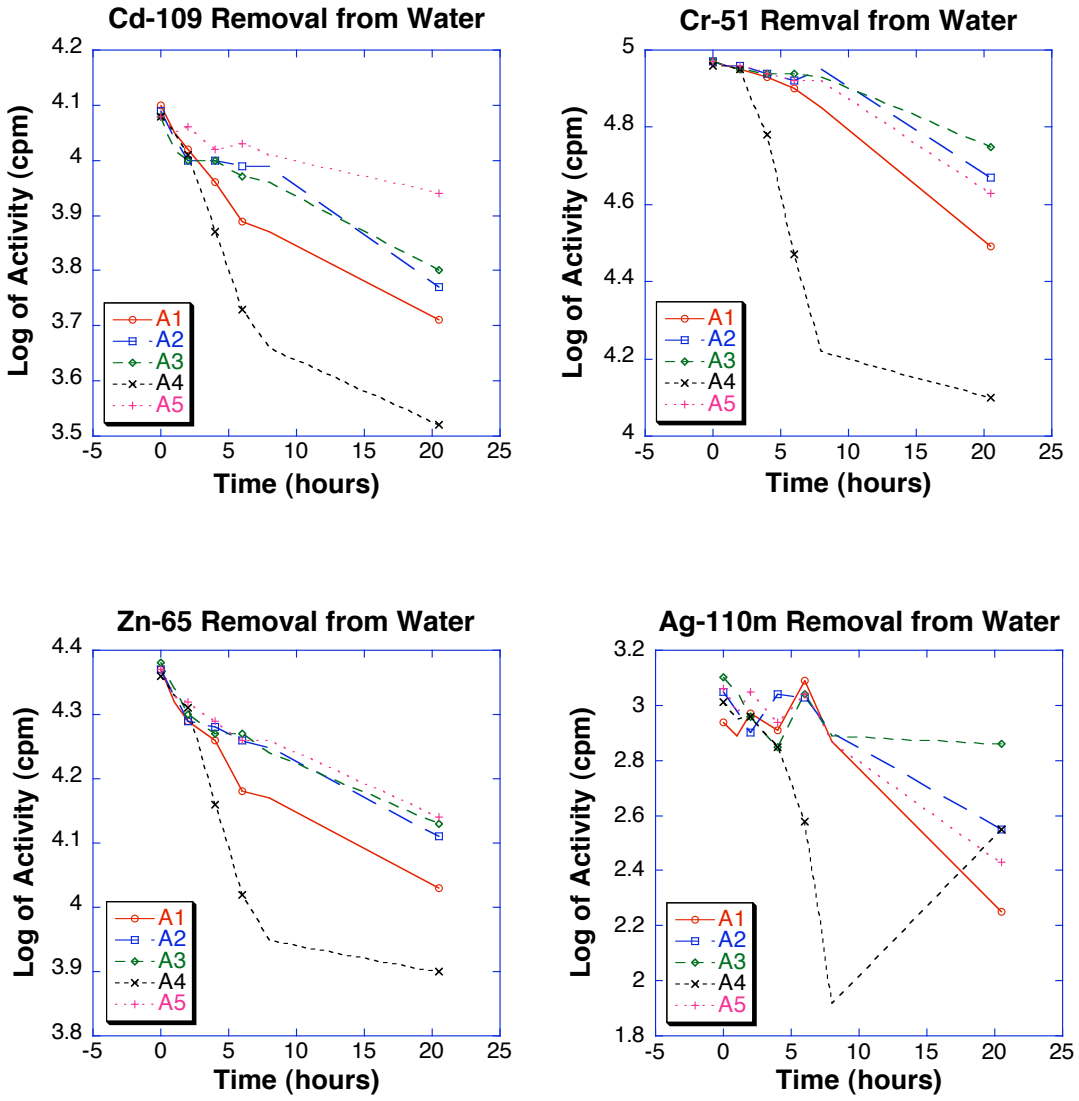
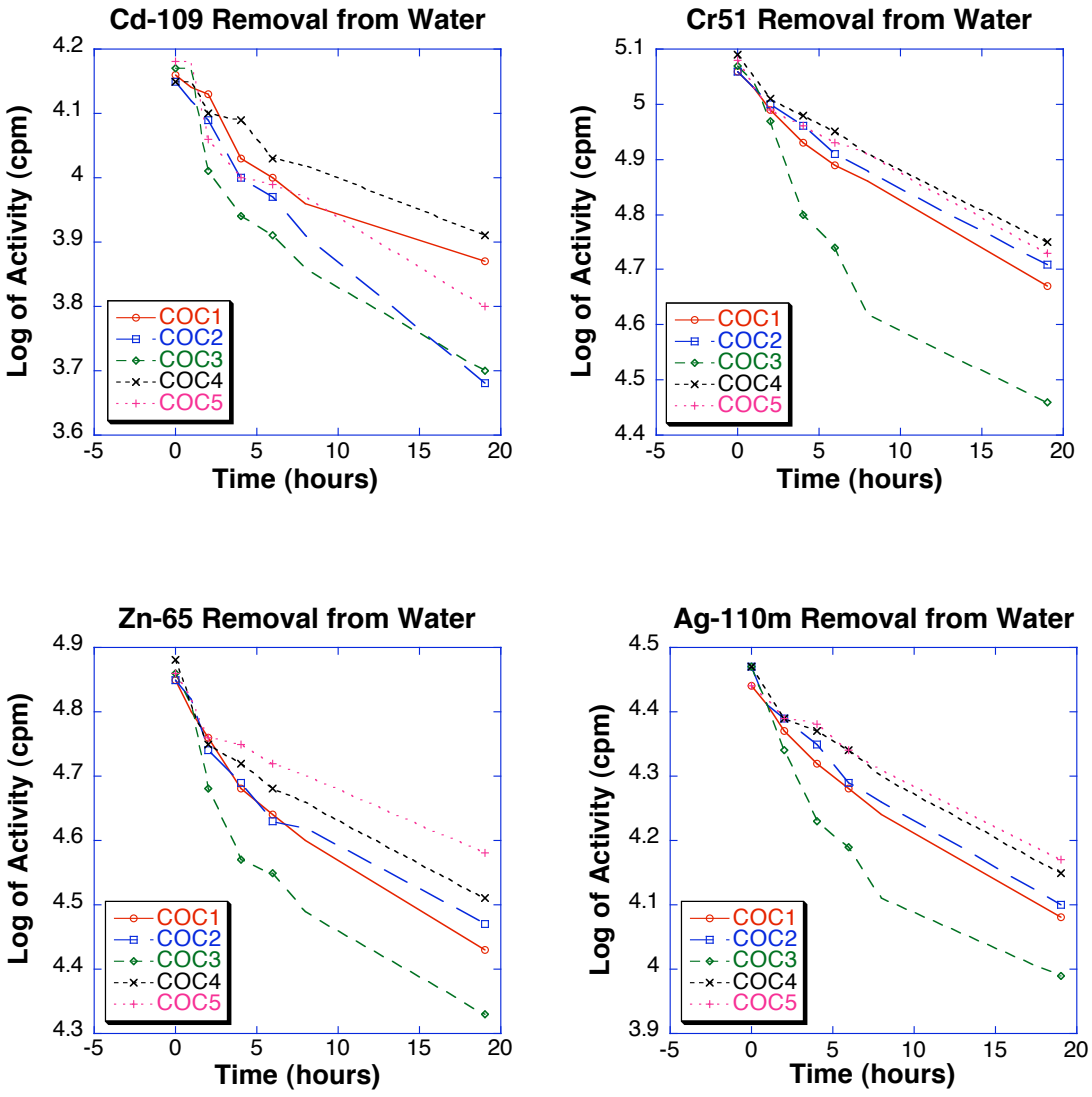
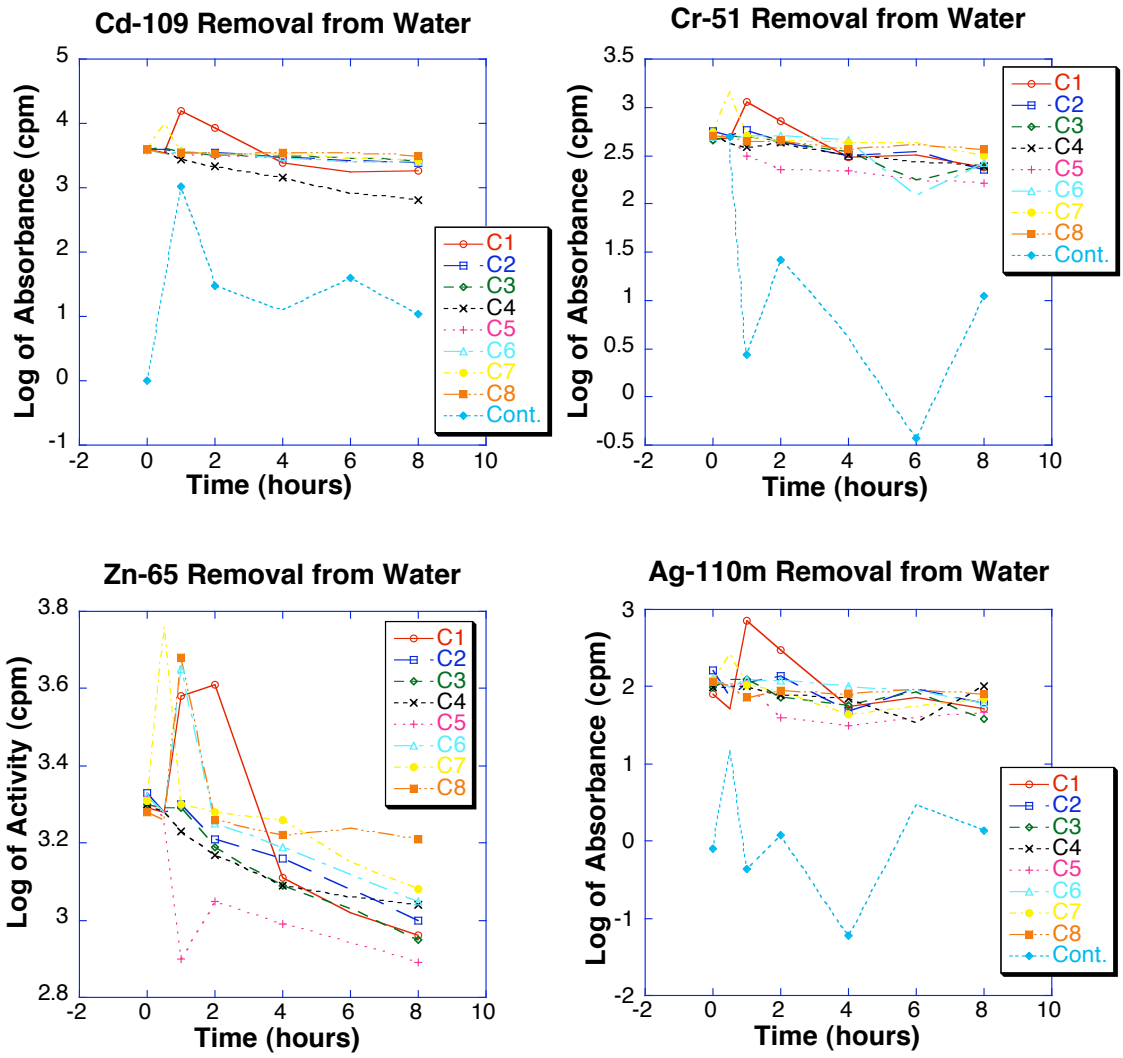
APPENDIX



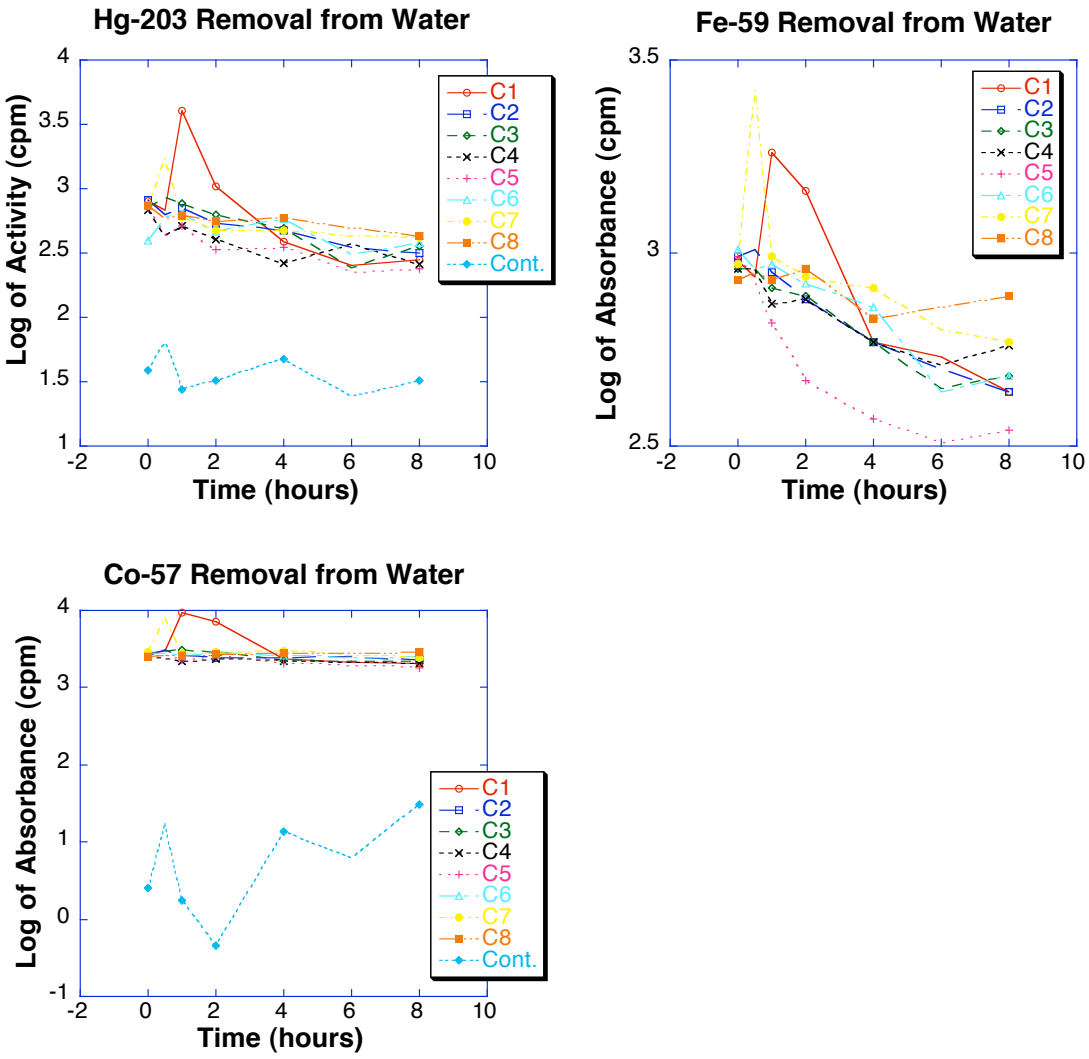
Appendix Fig. 1. Metal experiment 1 – alginic acid colloidal matter removal from the water.



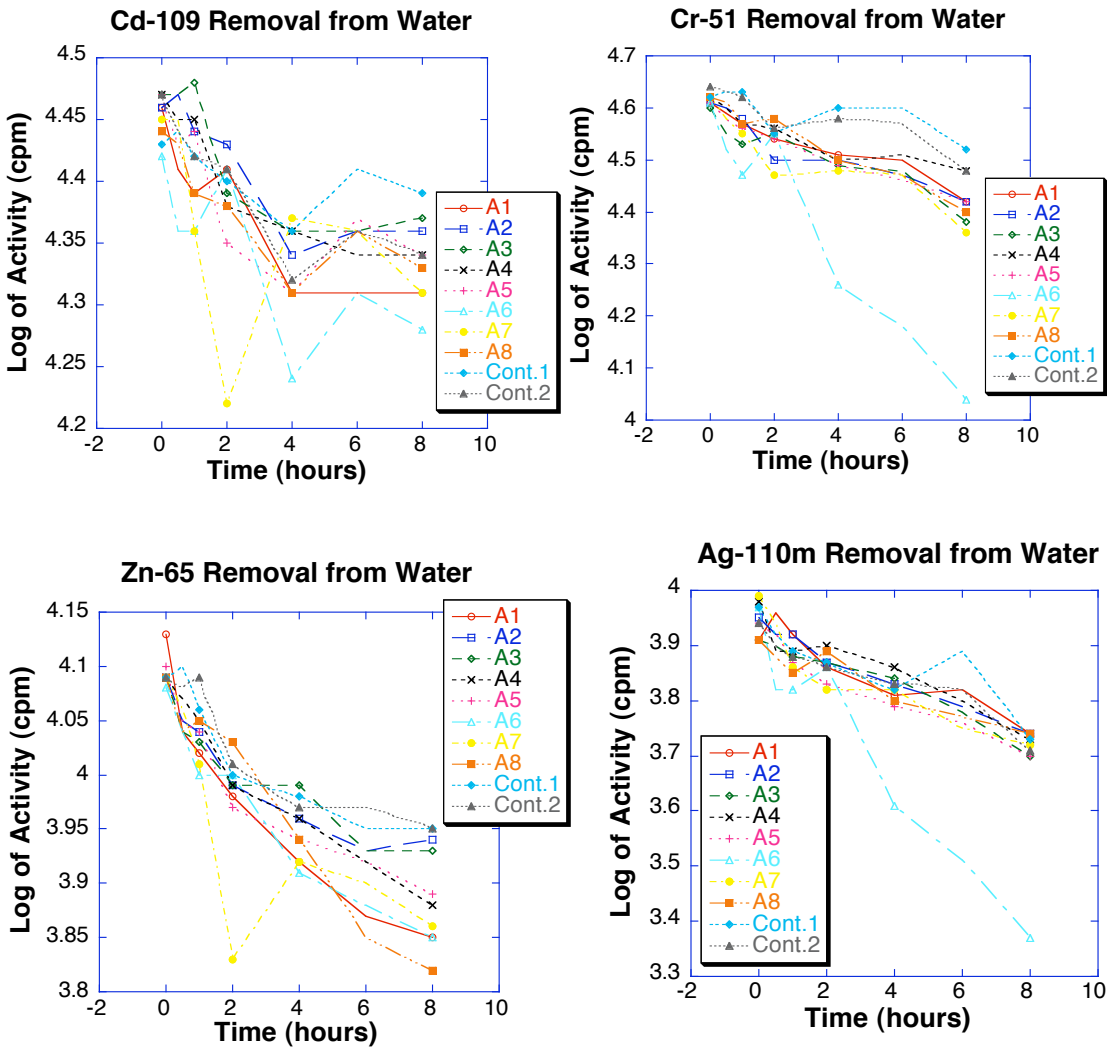
Appendix Fig. 2. Metal experiment 1 – natural colloidal organic carbon removal from the water.



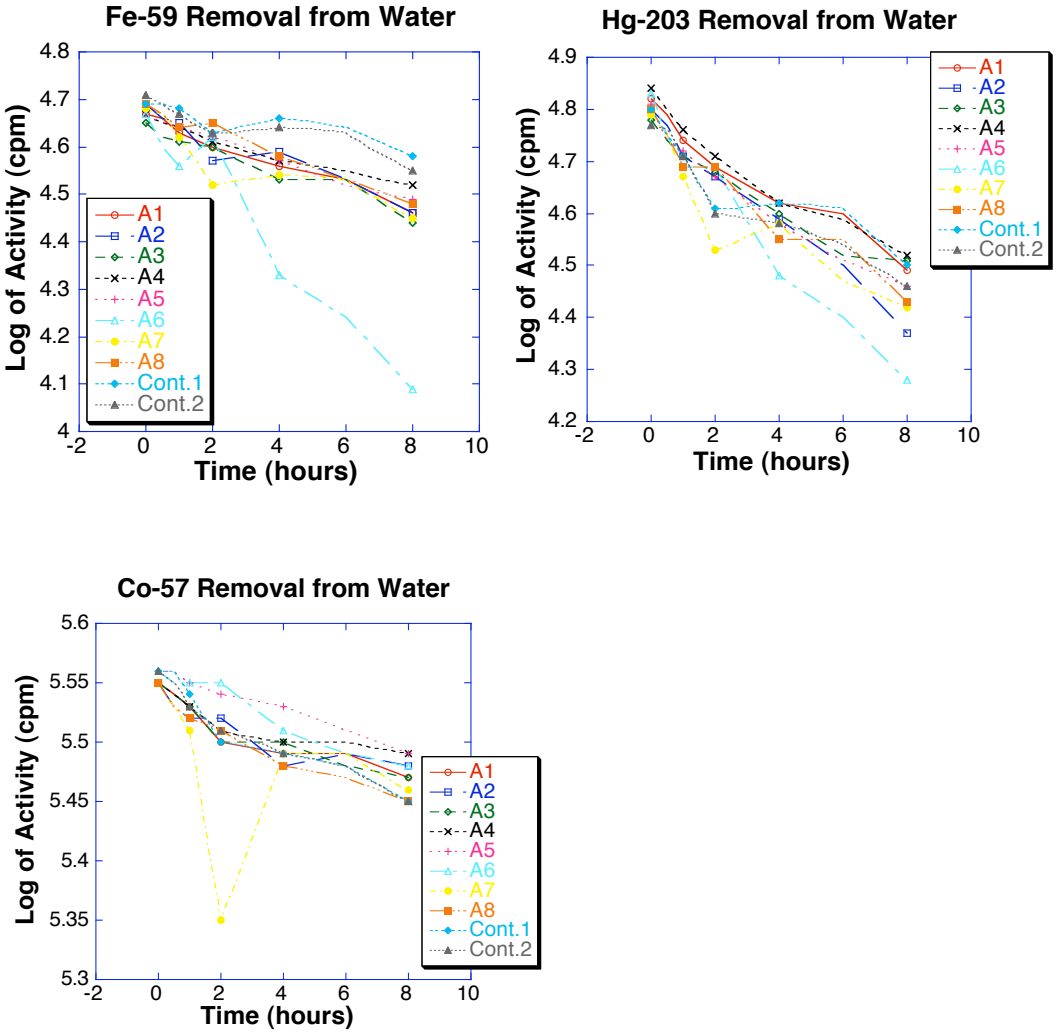
Appendix Fig. 3 - Metal experiment 2 – carrageenan colloidal matter removal from the water.



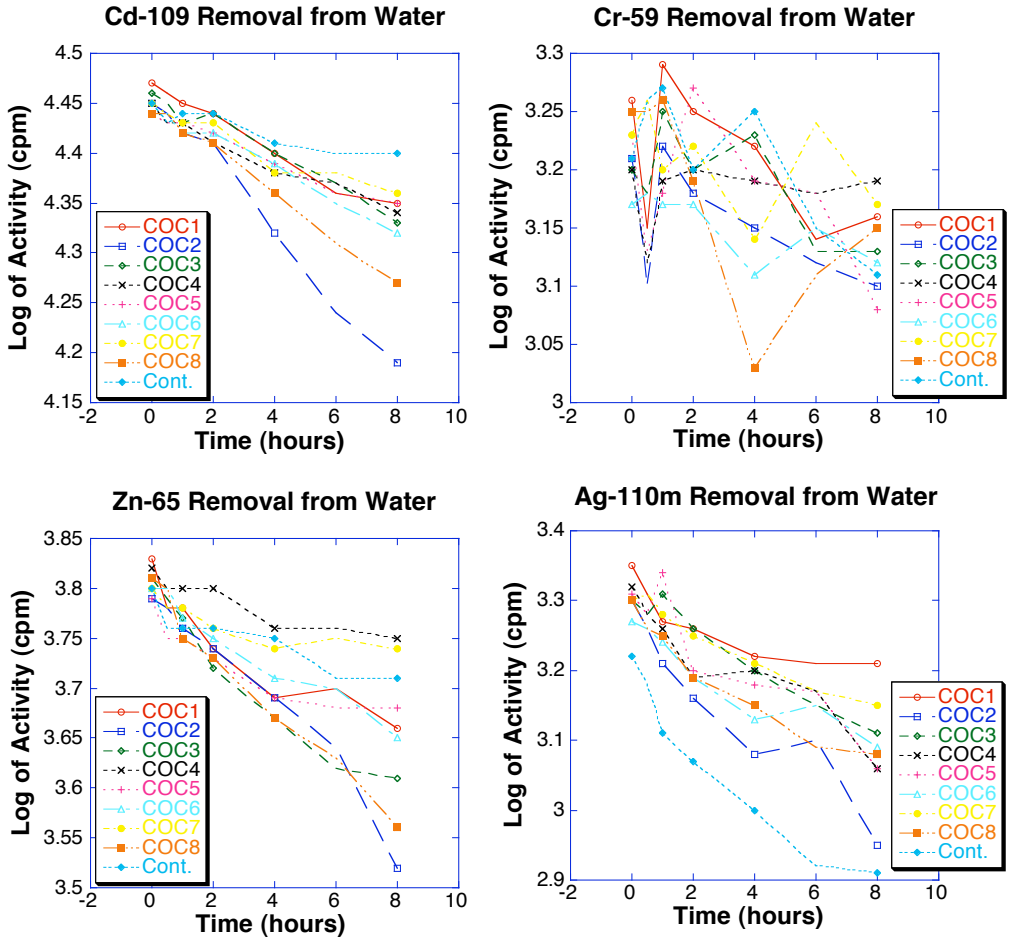
Appendix Fig. 3. Continued



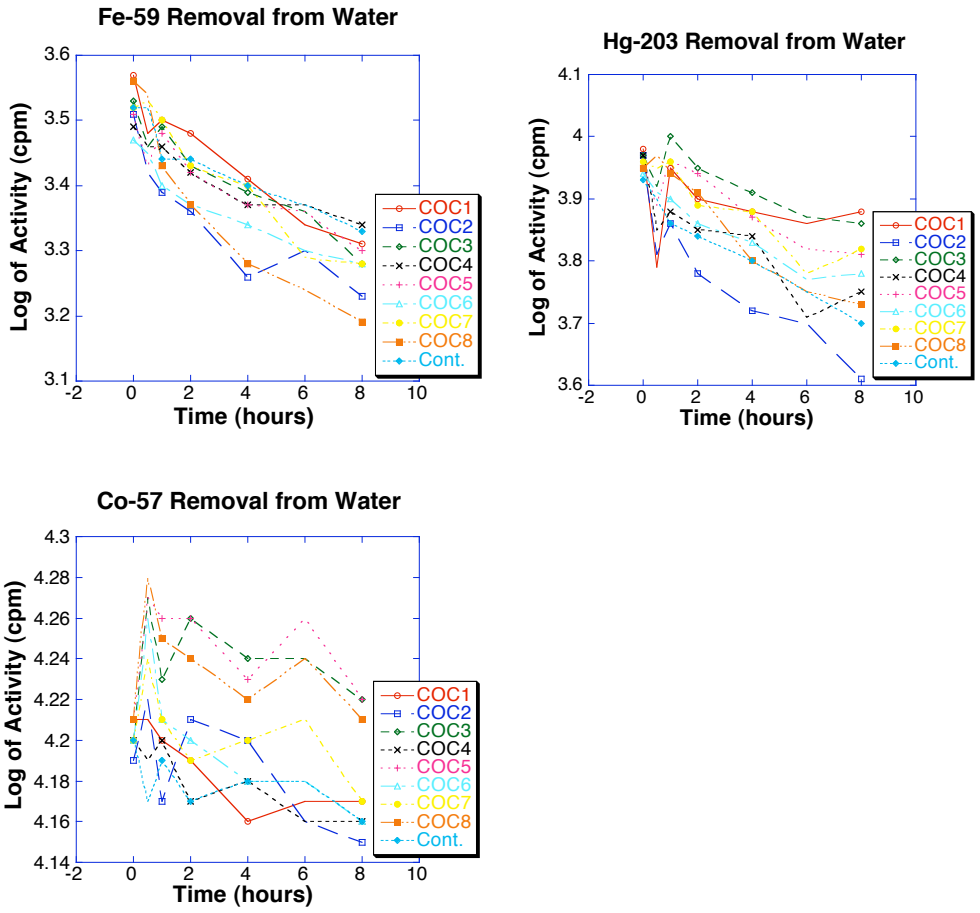
Appendix Fig. 4. Metal experiment 2 – alginic acid colloidal matter removal from the water.



Appendix Fig. 4. Continued.



Appendix Fig. 5. Metal experiment 2 – natural colloidal organic carbon removal from the water.



Appendix Fig. 5. Continued.

Appendix Table 1
Latex particle removal time series – fluorescent yellow-green CML latex particles, 0.4μm diameter

Time	**Absorbance (abs. units)**			
(hours)	Beaker-3	Beaker-4	Beaker-6	Control
0	0.12	0.18	0.15	0.14
0.5	0.094	0.095	0.12	0.11
1	0.080	0.082	0.11	0.12
2	0.054	0.050	0.10	0.12
4	0.036	0.030	0.091	0.13
16	0.015	0.0023	0.016	0.12

Appendix Table 2
Metals Removal From Water - Experiment 1. (A) Algininc acid treatment; (B) Carrageenan treatment; (C) Colloidal organic carbon

A

Cd-109	Activity (cpm)				
Time (hours)	**A1**	**A2**	**A3**	**A4**	**A5**
0	1.25E+04	1.22E+04	1.21E+04	1.19E+04	1.20E+04
1	1.11E+04	1.10E+04	1.05E+04	1.11E+04	1.13E+04
2	1.04E+04	9.98E+03	9.98E+03	1.03E+04	1.16E+04
4	9.12E+03	1.01E+04	1.00E+04	7.47E+03	1.04E+04
6	7.73E+03	9.69E+03	9.24E+03	5.43E+03	1.06E+04
8	7.48E+03	9.68E+03	9.19E+03	4.55E+03	1.02E+04
20.5	5.15E+03	5.95E+03	6.33E+03	3.28E+03	8.81E+03
Cr-51	Activity (cpm)				
Time (hours)	**A1**	**A2**	**A3**	**A4**	**A5**
0	9.30E+04	9.42E+04	9.30E+04	9.18E+04	9.24E+04
1	9.04E+04	9.15E+04	9.10E+04	9.10E+04	9.15E+04
2	8.89E+04	9.07E+04	9.01E+04	8.95E+04	9.07E+04
4	8.59E+04	8.64E+04	8.76E+04	6.01E+04	8.76E+04
6	7.95E+04	8.28E+04	8.62E+04	2.95E+04	8.23E+04
8	7.10E+04	8.82E+04	8.54E+04	1.67E+04	8.32E+04
20.5	3.12E+04	4.66E+04	5.68E+04	1.25E+04	4.29E+04
Ag-110m	Activity (cpm)				
Time (hours)	**A1**	**A2**	**A3**	**A4**	**A5**
0	8.70E+02	1.11E+03	1.25E+03	1.03E+03	1.16E+03
1	7.73E+02	9.56E+02	1.13E+03	8.85E+02	9.26E+02
2	9.28E+02	7.95E+02	9.22E+02	9.22E+02	1.11E+03
4	8.15E+02	1.10E+03	7.07E+02	7.13E+02	8.78E+02
6	1.22E+03	1.08E+03	1.10E+03	3.81E+02	1.09E+03
8	7.38E+02	7.93E+02	7.76E+02	8.26E+01	7.43E+02
20.5	1.79E+02	3.57E+02	7.20E+02	3.52E+02	2.71E+02
Zn-65	Activity (cpm)				
Time (hours)	**A1**	**A2**	**A3**	**A4**	**A5**
0	2.33E+04	2.35E+04	2.39E+04	2.29E+04	2.36E+04
1	2.07E+04	2.15E+04	2.19E+04	2.15E+04	2.12E+04
2	1.93E+04	1.97E+04	2.00E+04	2.03E+04	2.07E+04
4	1.82E+04	1.92E+04	1.88E+04	1.46E+04	1.95E+04
6	1.52E+04	1.83E+04	1.88E+04	1.05E+04	1.82E+04
8	1.48E+04	1.79E+04	1.73E+04	8.86E+03	1.81E+04
20.5	1.06E+04	1.28E+04	1.35E+04	8.01E+03	1.37E+04

Appendix Table 2. Continued.

B

Cd-109	Activity (cpm)				
Time (hours)	C1	C2	C3	C4	C5
0	9.36E+03	8.76E+03	8.34E+03	8.87E+03	9.00E+03
1	9.26E+03	8.38E+03	8.32E+03	8.38E+03	8.20E+03
2	7.42E+03	8.24E+03	6.15E+03	8.06E+03	7.83E+03
4	8.50E+03	6.27E+03	5.70E+03	5.87E+03	6.67E+03
6	7.73E+03	6.44E+03	4.40E+03	5.71E+03	5.53E+03
8	6.38E+03	5.78E+03	3.73E+03	5.29E+03	4.97E+03
20.5	4.02E+03	5.79E+03	2.43E+03	3.74E+03	3.63E+03
Cr-51	Activity (cpm)				
Time (hours)	C1	C2	C3	C4	C5
0	1.23E+05	1.04E+05	1.09E+05	1.14E+05	1.20E+05
1	1.18E+05	1.02E+05	9.69E+04	1.17E+05	1.13E+05
2	1.13E+05	9.76E+04	8.54E+04	1.06E+05	1.05E+05
4	1.09E+05	9.22E+04	7.33E+04	9.10E+04	8.93E+04
6	1.03E+05	8.90E+04	6.76E+04	8.96E+04	7.83E+04
8	9.87E+04	8.43E+04	5.99E+04	8.82E+04	7.10E+04
20.5	7.94E+04	7.39E+04	5.38E+04	6.90E+04	5.90E+04
Ag-110m	Activity (cpm)				
Time (hours)	C1	C2	C3	C4	C5
0	5.05E+03	4.24E+03	4.16E+03	4.64E+03	5.11E+03
1	4.44E+03	4.15E+03	3.72E+03	4.34E+03	4.49E+03
2	4.36E+03	4.03E+03	3.02E+03	4.15E+03	3.91E+03
4	4.42E+03	3.57E+03	2.63E+03	3.20E+03	3.20E+03
6	4.17E+03	3.36E+03	2.22E+03	3.07E+03	3.12E+03
8	3.05E+03	3.95E+03	1.57E+03	3.36E+03	2.69E+03
20.5	2.59E+03	2.40E+03	1.75E+03	2.79E+03	2.18E+03
Zn-65	Activity (cpm)				
Time (hours)	C1	C2	C3	C4	C5
0	2.76E+04	2.29E+04	2.45E+04	2.55E+04	2.71E+04
1	2.58E+04	2.24E+04	1.99E+04	2.59E+04	2.43E+04
2	2.48E+04	2.11E+04	1.74E+04	2.22E+04	2.26E+04
4	2.24E+04	1.96E+04	1.41E+04	1.97E+04	1.84E+04
6	2.19E+04	1.87E+04	1.20E+04	1.93E+04	1.69E+04
8	2.02E+04	1.73E+04	1.08E+04	1.82E+04	1.52E+04
20.5	1.65E+04	1.52E+04	9.69E+03	1.32E+04	1.21E+04

Appendix Table 2. Continued.

C

Cd-109	Activity (cpm)				
Time (hours)	COC1	COC2	COC3	COC4	COC5
0	1.46E+04	1.40E+04	1.47E+04	1.42E+04	1.52E+04
1	1.38E+04	1.33E+04	1.48E+04	1.42E+04	1.50E+04
2	1.35E+04	1.23E+04	1.02E+04	1.27E+04	1.15E+04
4	1.07E+04	9.92E+03	8.72E+03	1.24E+04	9.98E+03
6	1.01E+04	9.30E+03	8.07E+03	1.08E+04	9.86E+03
8	9.13E+03	8.09E+03	7.26E+03	1.04E+04	9.30E+03
19	7.46E+03	4.83E+03	4.97E+03	8.11E+03	6.27E+03
Cr-51	Activity (cpm)				
Time (hours)	COC1	COC2	COC3	COC4	COC5
0	1.15E+05	1.16E+05	1.18E+05	1.23E+05	1.19E+05
1	1.06E+05	1.07E+05	1.10E+05	1.12E+05	1.07E+05
2	9.76E+04	9.94E+04	9.24E+04	1.02E+05	9.76E+04
4	8.53E+04	9.04E+04	6.30E+04	9.62E+04	9.22E+04
6	7.83E+04	8.17E+04	5.51E+04	8.85E+04	8.45E+04
8	7.21E+04	7.65E+04	4.18E+04	8.21E+04	8.10E+04
19	4.70E+04	5.17E+04	2.90E+04	5.62E+04	5.41E+04
Ag-110m	Activity (cpm)				
Time (hours)	COC1	COC2	COC3	COC4	COC5
0	2.75E+04	2.92E+04	2.93E+04	2.94E+04	2.74E+04
1	2.58E+04	2.56E+04	2.58E+04	2.68E+04	2.63E+04
2	2.34E+04	2.48E+04	2.18E+04	2.44E+04	2.48E+04
4	2.08E+04	2.22E+04	1.71E+04	2.34E+04	2.41E+04
6	1.92E+04	1.97E+04	1.54E+04	2.19E+04	2.20E+04
8	1.74E+04	1.83E+04	1.28E+04	1.99E+04	2.03E+04
19	1.21E+04	1.27E+04	9.69E+03	1.42E+04	1.47E+04
Zn-65	Activity (cpm)				
Time (hours)	COC1	COC2	COC3	COC4	COC5
0	7.14E+04	7.08E+04	7.20E+04	7.50E+04	7.26E+04
1	6.31E+04	6.55E+04	6.43E+04	6.43E+04	6.55E+04
2	5.69E+04	5.55E+04	4.77E+04	5.65E+04	5.80E+04
4	4.79E+04	4.90E+04	3.74E+04	5.21E+04	5.66E+04
6	4.39E+04	4.26E+04	3.57E+04	4.78E+04	5.21E+04
8	3.95E+04	4.16E+04	3.11E+04	4.59E+04	4.97E+04
19	2.72E+04	2.96E+04	2.16E+04	3.26E+04	3.82E+04

Appendix Table 3
Metals Removal From Water - Experiment 2. (A) Alginic acid treatment; (B) Carrageenan treatment; (C) Colloidal organic carbon treatment

A

Cd-109	Activity (cpm)									
Time (hr)	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A Cont.1**	**A Cont.2**
0	2.86E+04	2.87E+04	2.95E+04	2.92E+04	2.98E+04	2.62E+04	2.82E+04	2.75E+04	2.68E+04	2.94E+04
0.5	2.58E+04	2.95E+04	2.97E+04	2.83E+04	2.72E+04	2.31E+04	2.80E+04	2.72E+04	2.73E+04	2.77E+04
1	2.48E+04	2.77E+04	3.02E+04	2.83E+04	2.76E+04	2.28E+04	2.31E+04	2.44E+04	2.65E+04	2.62E+04
2	2.55E+04	2.70E+04	2.43E+04	2.42E+04	2.23E+04	2.55E+04	1.67E+04	2.39E+04	2.50E+04	2.56E+04
4	2.04E+04	2.21E+04	2.27E+04	2.31E+04	2.04E+04	1.74E+04	2.32E+04	2.05E+04	2.31E+04	2.07E+04
6	2.03E+04	2.30E+04	2.28E+04	2.18E+04	2.34E+04	2.05E+04	2.29E+04	2.28E+04	2.60E+04	2.28E+04
8	2.04E+04	2.31E+04	2.37E+04	2.18E+04	2.20E+04	1.90E+04	2.05E+04	2.13E+04	2.45E+04	2.21E+04
Co-57	Activity (cpm)									
Time (hr)	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A Cont.1**	**A Cont.2**
0	3.54E+05	3.54E+05	3.57E+05	3.56E+05	3.59E+05	3.59E+05	3.57E+05	3.54E+05	3.59E+05	3.62E+05
0.5	3.45E+05	3.39E+05	3.47E+05	3.45E+05	3.59E+05	3.58E+05	3.39E+05	3.38E+05	3.59E+05	3.58E+05
1	3.38E+05	3.30E+05	3.38E+05	3.36E+05	3.57E+05	3.53E+05	3.26E+05	3.30E+05	3.46E+05	3.38E+05
2	3.19E+05	3.28E+05	3.18E+05	3.27E+05	3.48E+05	3.51E+05	2.25E+05	3.21E+05	3.17E+05	3.20E+05
4	3.10E+05	3.05E+05	3.18E+05	3.18E+05	3.37E+05	3.21E+05	3.12E+05	3.02E+05	3.10E+05	3.11E+05
6	3.08E+05	3.06E+05	2.99E+05	3.17E+05	3.23E+05	3.09E+05	3.07E+05	2.92E+05	3.04E+05	3.01E+05
8	2.95E+05	3.01E+05	2.93E+05	3.07E+05	3.10E+05	3.02E+05	2.90E+05	2.80E+05	2.81E+05	2.82E+05
Hg-203	Activity (cpm)									
Time (hr)	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A Cont.1**	**A Cont.2**
0	6.59E+04	6.24E+04	6.05E+04	6.84E+04	6.52E+04	6.71E+04	6.10E+04	6.25E+04	6.24E+04	5.93E+04
0.5	6.19E+04	5.84E+04	5.49E+04	6.26E+04	5.54E+04	5.66E+04	5.65E+04	5.62E+04	5.61E+04	5.69E+04
1	5.45E+04	5.17E+04	5.15E+04	5.70E+04	5.21E+04	4.88E+04	4.67E+04	4.93E+04	5.14E+04	5.07E+04
2	4.86E+04	4.72E+04	4.76E+04	5.17E+04	4.72E+04	4.94E+04	3.37E+04	4.87E+04	4.03E+04	3.99E+04
4	4.16E+04	3.92E+04	4.02E+04	4.19E+04	3.78E+04	3.00E+04	3.81E+04	3.52E+04	4.19E+04	3.78E+04
6	3.95E+04	3.16E+04	3.30E+04	3.89E+04	3.24E+04	2.52E+04	2.94E+04	3.52E+04	4.05E+04	3.49E+04
8	3.11E+04	2.33E+04	3.20E+04	3.32E+04	2.86E+04	1.90E+04	2.62E+04	2.70E+04	3.16E+04	2.89E+04
Cr-51	Activity (cpm)									
Time (hr)	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A Cont.1**	**A Cont.2**
0	4.03E+04	4.11E+04	3.94E+04	4.18E+04	4.11E+04	4.11E+04	4.15E+04	4.20E+04	4.18E+04	4.37E+04
0.5	3.87E+04	4.00E+04	3.58E+04	3.99E+04	3.78E+04	3.30E+04	3.84E+04	4.04E+04	4.26E+04	4.23E+04
1	3.74E+04	3.78E+04	3.42E+04	3.69E+04	3.66E+04	2.97E+04	3.58E+04	3.74E+04	4.22E+04	4.19E+04
2	3.50E+04	3.17E+04	3.53E+04	3.63E+04	3.48E+04	3.56E+04	2.93E+04	3.82E+04	3.52E+04	3.63E+04
4	3.22E+04	3.19E+04	3.10E+04	3.14E+04	3.10E+04	1.83E+04	3.00E+04	3.19E+04	3.95E+04	3.76E+04
6	3.14E+04	2.98E+04	2.99E+04	3.22E+04	2.89E+04	1.52E+04	2.94E+04	2.95E+04	3.99E+04	3.71E+04
8	2.62E+04	2.63E+04	2.39E+04	2.99E+04	2.62E+04	1.10E+04	2.31E+04	2.49E+04	3.33E+04	3.02E+04

Appendix Table 3. Continued.

Ag-110m	Activity (cpm)									
Time (hours)	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A Cont. 1**	**A Cont. 2**
0	8.17E+03	8.83E+03	8.17E+03	9.62E+03	8.77E+03	9.26E+03	9.74E+03	8.17E+03	9.26E+03	8.77E+03
0.5	9.13E+03	8.29E+03	7.87E+03	7.75E+03	8.23E+03	6.62E+03	8.71E+03	7.52E+03	8.35E+03	7.93E+03
1	8.29E+03	8.23E+03	7.64E+03	7.82E+03	7.47E+03	6.64E+03	7.23E+03	7.11E+03	7.82E+03	7.59E+03
2	7.19E+03	7.42E+03	7.47E+03	7.88E+03	6.78E+03	7.19E+03	6.66E+03	7.76E+03	7.36E+03	7.30E+03
4	6.52E+03	6.81E+03	6.98E+03	7.21E+03	6.18E+03	4.09E+03	6.65E+03	6.30E+03	6.68E+03	6.72E+03
6	6.63E+03	6.19E+03	5.98E+03	6.34E+03	5.79E+03	3.25E+03	5.60E+03	5.95E+03	7.79E+03	6.58E+03
8	5.53E+03	5.47E+03	5.01E+03	5.43E+03	4.96E+03	2.37E+03	5.28E+03	5.50E+03	5.36E+03	5.11E+03
Fe-59	Activity (cpm)									
Time (hours)	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A Cont. 1**	**A Cont. 2**
0	4.70E+04	4.85E+04	4.51E+04	4.63E+04	4.69E+04	4.70E+04	4.79E+04	4.88E+04	4.91E+04	5.10E+04
0.5	4.57E+04	4.57E+04	4.19E+04	4.45E+04	4.50E+04	3.97E+04	4.66E+04	4.65E+04	4.88E+04	4.92E+04
1	4.30E+04	4.44E+04	4.09E+04	4.35E+04	4.41E+04	3.59E+04	4.16E+04	4.40E+04	4.80E+04	4.71E+04
2	3.96E+04	3.75E+04	3.98E+04	4.09E+04	4.15E+04	4.13E+04	3.30E+04	4.44E+04	4.29E+04	4.25E+04
4	3.64E+04	3.85E+04	3.36E+04	3.73E+04	3.71E+04	2.16E+04	3.47E+04	3.78E+04	4.54E+04	4.35E+04
6	3.38E+04	3.40E+04	3.39E+04	3.57E+04	3.31E+04	1.73E+04	3.41E+04	3.36E+04	4.34E+04	4.23E+04
8	2.88E+04	2.90E+04	2.76E+04	3.34E+04	3.07E+04	1.23E+04	2.79E+04	3.02E+04	3.76E+04	3.57E+04
Zn-65	Activity (cpm)									
Time (hours)	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A Cont. 1**	**A Cont. 2**
0	1.35E+04	1.23E+04	1.22E+04	1.22E+04	1.25E+04	1.20E+04	1.22E+04	1.24E+04	1.22E+04	1.23E+04
0.5	1.09E+04	1.13E+04	1.10E+04	1.18E+04	1.13E+04	1.09E+04	1.15E+04	1.10E+04	1.25E+04	1.21E+04
1	1.05E+04	1.10E+04	1.08E+04	1.11E+04	1.09E+04	9.94E+03	1.02E+04	1.11E+04	1.14E+04	1.23E+04
2	9.62E+03	9.86E+03	9.80E+03	9.80E+03	9.33E+03	9.91E+03	6.78E+03	1.06E+04	9.97E+03	1.03E+04
4	8.26E+03	9.13E+03	9.82E+03	9.13E+03	8.78E+03	8.08E+03	8.32E+03	8.74E+03	9.60E+03	9.29E+03
6	7.36E+03	8.53E+03	8.53E+03	8.36E+03	8.25E+03	7.63E+03	8.00E+03	7.05E+03	8.89E+03	9.24E+03
8	7.04E+03	8.62E+03	8.53E+03	7.59E+03	7.69E+03	7.13E+03	7.32E+03	6.66E+03	8.94E+03	8.86E+03

Appendix Table 3. Continued.

B

Cd-109	Activity (cpm)								
Time (hours)	C1	C2	C3	C4	C5	C6	C7	C8	Car. Cont.
0	3.85E+03	4.01E+03	4.02E+03	3.92E+03	3.84E+03	3.89E+03	3.92E+03	3.85E+03	1.00E+00
0.5	3.58E+03	3.91E+03	4.18E+03	3.45E+03	3.60E+03	3.40E+03	1.01E+04	3.35E+03	-7.09E+00
1	1.56E+04	3.54E+03	3.68E+03	2.76E+03	3.33E+03	3.66E+03	3.59E+03	3.45E+03	1.05E+03
2	8.55E+03	3.55E+03	3.23E+03	2.12E+03	3.07E+03	3.33E+03	3.33E+03	3.37E+03	3.01E+01
4	2.42E+03	3.05E+03	3.09E+03	1.43E+03	2.88E+03	2.94E+03	3.29E+03	3.55E+03	1.29E+01
6	1.73E+03	2.72E+03	2.82E+03	8.18E+02	2.51E+03	2.55E+03	2.87E+03	3.45E+03	3.86E+01
8	1.82E+03	2.48E+03	2.72E+03	6.35E+02	2.50E+03	2.50E+03	2.62E+03	3.12E+03	1.06E+01
Co-57	Activity (cpm)								
Time (hours)	C1	C2	C3	C4	C5	C6	C7	C8	Car. Cont.
0	2.80E+03	2.63E+03	2.71E+03	2.57E+03	2.52E+03	2.81E+03	2.90E+03	2.49E+03	2.60E+00
0.5	2.98E+03	3.10E+03	3.00E+03	2.32E+03	2.65E+03	2.83E+03	8.32E+03	2.58E+03	1.76E+01
1	9.34E+03	2.60E+03	3.06E+03	2.23E+03	2.29E+03	2.63E+03	2.84E+03	2.59E+03	1.75E+00
2	7.21E+03	2.43E+03	2.86E+03	2.33E+03	2.68E+03	2.85E+03	2.91E+03	2.69E+03	4.58E-01
4	2.37E+03	2.48E+03	2.27E+03	2.17E+03	2.03E+03	2.66E+03	2.98E+03	2.77E+03	1.36E+01
6	2.13E+03	2.52E+03	2.24E+03	2.14E+03	1.92E+03	2.48E+03	2.65E+03	2.75E+03	6.29E+00
8	2.06E+03	2.27E+03	2.24E+03	2.03E+03	1.83E+03	2.52E+03	2.44E+03	2.90E+03	3.01E+01
Hg-203	Activity (cpm)								
Time (hours)	C1	C2	C3	C4	C5	C6	C7	C8	Car. Cont.
0	7.95E+02	8.04E+02	7.20E+02	6.73E+02	7.01E+02	3.96E+02	7.37E+02	7.33E+02	3.91E+01
0.5	6.81E+02	6.37E+02	8.45E+02	4.29E+02	4.36E+02	5.96E+02	1.73E+03	5.92E+02	6.40E+01
1	4.08E+03	7.07E+02	7.79E+02	5.15E+02	5.08E+02	6.49E+02	6.00E+02	6.12E+02	2.78E+01
2	1.04E+03	5.36E+02	6.28E+02	4.07E+02	3.40E+02	4.68E+02	4.76E+02	5.64E+02	3.23E+01
4	3.91E+02	4.75E+02	4.86E+02	2.61E+02	3.45E+02	5.82E+02	4.78E+02	5.89E+02	4.77E+01
6	2.53E+02	3.43E+02	2.48E+02	3.70E+02	2.25E+02	3.07E+02	4.31E+02	4.88E+02	2.44E+01
8	2.83E+02	3.19E+02	3.55E+02	2.57E+02	2.38E+02	3.84E+02	4.18E+02	4.29E+02	3.21E+01
Cr-51	Activity (cpm)								
Time (hours)	C1	C2	C3	C4	C5	C6	C7	C8	Car. Cont.
0	4.71E+02	5.58E+02	4.60E+02	5.40E+02	5.36E+02	4.85E+02	5.55E+02	5.03E+02	0.00E+00
0.5	4.82E+02	5.07E+02	5.05E+02	4.21E+02	4.85E+02	4.86E+02	1.45E+03	4.86E+02	4.85E+02
1	1.14E+03	5.71E+02	4.87E+02	3.87E+02	3.18E+02	5.15E+02	5.15E+02	4.48E+02	2.75E+00
2	7.20E+02	4.43E+02	4.50E+02	4.34E+02	2.22E+02	5.12E+02	4.57E+02	4.60E+02	2.61E+01
4	3.01E+02	3.15E+02	3.43E+02	3.27E+02	2.18E+02	4.55E+02	4.35E+02	3.60E+02	4.04E+00
6	3.22E+02	3.50E+02	1.78E+02	2.75E+02	1.78E+02	1.19E+02	4.35E+02	4.10E+02	3.69E-01
8	2.38E+02	2.23E+02	2.47E+02	2.48E+02	1.63E+02	2.65E+02	3.12E+02	3.66E+02	1.10E+01

Appendix Table 3. Continued.

Ag-110m	Activity (cpm)								
Time (hours)	C1	C2	C3	C4	C5	C6	C7	C8	Car. Cont.
0	8.05E+01	1.62E+02	9.81E+01	9.67E+01	1.21E+02	1.29E+02	1.21E+02	1.14E+02	7.94E-01
0.5	5.21E+01	7.32E+01	1.25E+02	1.02E+02	1.10E+02	9.77E+01	2.68E+02	9.98E+01	1.54E+01
1	7.22E+02	1.13E+02	1.27E+02	9.89E+01	1.09E+02	1.26E+02	1.04E+02	7.31E+01	4.40E-01
2	3.03E+02	1.39E+02	7.28E+01	7.68E+01	4.01E+01	1.20E+02	9.00E+01	8.89E+01	1.19E+00
4	5.45E+01	4.94E+01	5.79E+01	7.07E+01	3.17E+01	9.97E+01	4.36E+01	8.22E+01	5.95E-02
6	7.22E+01	9.48E+01	8.44E+01	3.49E+01	4.04E+01	8.92E+01	5.53E+01	9.02E+01	2.97E+00
8	5.19E+01	6.22E+01	3.84E+01	1.02E+02	4.63E+01	6.03E+01	7.00E+01	8.10E+01	1.38E+00
Fe-59	Activity (cpm)								
Time (hours)	C1	C2	C3	C4	C5	C6	C7	C8	Car. Cont.
0	9.53E+02	9.86E+02	9.17E+02	9.08E+02	9.69E+02	1.02E+03	9.38E+02	8.49E+02	0.00E+00
0.5	8.79E+02	1.03E+03	9.19E+02	9.08E+02	8.43E+02	9.07E+02	2.64E+03	8.90E+02	4.63E+00
1	1.84E+03	8.87E+02	8.05E+02	7.39E+02	6.64E+02	9.38E+02	9.79E+02	8.55E+02	6.81E-01
2	1.44E+03	7.67E+02	7.80E+02	7.66E+02	4.67E+02	8.39E+02	8.78E+02	9.13E+02	1.32E+01
4	5.91E+02	5.85E+02	5.87E+02	5.90E+02	3.69E+02	7.24E+02	8.14E+02	6.83E+02	7.18E-02
6	5.38E+02	5.02E+02	4.44E+02	5.15E+02	3.26E+02	4.34E+02	6.26E+02	7.26E+02	1.36E+01
8	4.35E+02	4.32E+02	4.79E+02	5.79E+02	3.45E+02	4.80E+02	5.87E+02	7.82E+02	8.19E+00
Zn-65	Activity (cpm)								
Time (hours)	C1	C2	C3	C4	C5	C6	C7	C8	Car. Cont.
0	1.96E+03	2.15E+03	2.00E+03	2.00E+03	2.07E+03	2.10E+03	2.05E+03	1.91E+03	1.33E+00
0.5	1.89E+03	1.91E+03	1.94E+03	1.91E+03	1.81E+03	1.92E+03	5.76E+03	1.81E+03	3.64E+00
1	3.78E+03	1.99E+03	1.95E+03	1.70E+03	7.99E+02	4.48E+03	2.00E+03	4.76E+03	4.40E+00
2	4.06E+03	1.63E+03	1.54E+03	1.48E+03	1.11E+03	1.77E+03	1.90E+03	1.81E+03	1.56E+01
4	1.29E+03	1.44E+03	1.22E+03	1.24E+03	9.87E+02	1.56E+03	1.80E+03	1.65E+03	5.54E+00
6	1.04E+03	1.19E+03	1.08E+03	1.15E+03	8.74E+02	1.32E+03	1.41E+03	1.75E+03	6.15E+00
8	9.20E+02	9.94E+02	8.98E+02	1.10E+03	7.75E+02	1.13E+03	1.20E+03	1.64E+03	3.45E-01

Appendix Table 3. Continued.

C

Cd-109	Activity (cpm)								
Time (hours)	COC1	COC2	COC3	COC4	COC5	COC6	COC7	COC8	COC Cont.
0	2.93E+04	2.83E+04	2.89E+04	2.82E+04	2.77E+04	2.76E+04	2.80E+04	2.76E+04	2.82E+04
0.5	2.87E+04	2.73E+04	2.80E+04	2.71E+04	2.72E+04	2.77E+04	2.77E+04	2.77E+04	2.71E+04
1	2.79E+04	2.61E+04	2.68E+04	2.70E+04	2.68E+04	2.66E+04	2.68E+04	2.61E+04	2.73E+04
2	2.75E+04	2.56E+04	2.76E+04	2.59E+04	2.62E+04	2.65E+04	2.71E+04	2.57E+04	2.78E+04
4	2.54E+04	2.08E+04	2.52E+04	2.42E+04	2.43E+04	2.44E+04	2.42E+04	2.29E+04	2.60E+04
6	2.31E+04	1.74E+04	2.34E+04	2.32E+04	2.30E+04	2.22E+04	2.39E+04	2.05E+04	2.52E+04
8	2.22E+04	1.56E+04	2.15E+04	2.18E+04	2.26E+04	2.10E+04	2.30E+04	1.86E+04	2.53E+04
Co-57	Activity (cpm)								
Time (hours)	COC1	COC2	COC3	COC4	COC5	COC6	COC7	COC8	COC Cont.
0	1.63E+04	1.55E+04	1.59E+04	1.60E+04	1.61E+04	1.59E+04	1.59E+04	1.62E+04	1.59E+04
0.5	1.64E+04	1.65E+04	1.88E+04	1.55E+04	1.85E+04	1.81E+04	1.72E+04	1.90E+04	1.48E+04
1	1.59E+04	1.49E+04	1.70E+04	1.58E+04	1.80E+04	1.63E+04	1.62E+04	1.76E+04	1.55E+04
2	1.55E+04	1.62E+04	1.81E+04	1.48E+04	1.83E+04	1.58E+04	1.56E+04	1.72E+04	1.49E+04
4	1.46E+04	1.60E+04	1.74E+04	1.52E+04	1.69E+04	1.53E+04	1.57E+04	1.67E+04	1.51E+04
6	1.48E+04	1.46E+04	1.73E+04	1.45E+04	1.83E+04	1.52E+04	1.64E+04	1.75E+04	1.50E+04
8	1.47E+04	1.41E+04	1.67E+04	1.43E+04	1.66E+04	1.46E+04	1.49E+04	1.62E+04	1.43E+04
Hg-203	Activity (cpm)								
Time (hours)	COC1	COC2	COC3	COC4	COC5	COC6	COC7	COC8	COC Cont.
0	9.57E+03	9.26E+03	9.37E+03	9.23E+03	9.20E+03	8.70E+03	9.07E+03	8.82E+03	8.60E+03
0.5	6.20E+03	6.41E+03	8.29E+03	7.03E+03	7.76E+03	8.10E+03	8.87E+03	9.27E+03	7.97E+03
1	9.01E+03	7.28E+03	9.95E+03	7.62E+03	9.17E+03	7.95E+03	9.21E+03	8.65E+03	7.31E+03
2	7.96E+03	6.04E+03	8.93E+03	7.13E+03	8.80E+03	7.25E+03	7.82E+03	8.17E+03	6.98E+03
4	7.54E+03	5.19E+03	8.09E+03	6.95E+03	7.34E+03	6.72E+03	7.58E+03	6.35E+03	6.37E+03
6	7.24E+03	5.06E+03	7.45E+03	5.16E+03	6.66E+03	5.90E+03	5.96E+03	5.58E+03	5.66E+03
8	7.63E+03	4.05E+03	7.17E+03	5.61E+03	6.53E+03	6.07E+03	6.67E+03	5.39E+03	5.06E+03
Cr-51	Activity (cpm)								
Time (hours)	COC1	COC2	COC3	COC4	COC5	COC6	COC7	COC8	COC Cont.
0	1.82E+03	1.63E+03	1.60E+03	1.58E+03	1.69E+03	1.48E+03	1.69E+03	1.78E+03	1.62E+03
0.5	1.40E+03	1.27E+03	1.50E+03	1.33E+03	1.35E+03	1.53E+03	1.84E+03	1.78E+03	1.83E+03
1	1.93E+03	1.65E+03	1.78E+03	1.56E+03	1.53E+03	1.49E+03	1.59E+03	1.81E+03	1.85E+03
2	1.76E+03	1.51E+03	1.60E+03	1.57E+03	1.85E+03	1.47E+03	1.67E+03	1.56E+03	1.60E+03
4	1.65E+03	1.41E+03	1.68E+03	1.54E+03	1.56E+03	1.28E+03	1.37E+03	1.08E+03	1.77E+03
6	1.39E+03	1.33E+03	1.34E+03	1.50E+03	1.50E+03	1.41E+03	1.73E+03	1.28E+03	1.41E+03
8	1.46E+03	1.25E+03	1.35E+03	1.54E+03	1.19E+03	1.33E+03	1.48E+03	1.41E+03	1.30E+03

Appendix Table 3. Continued.

Ag-110m	Activity (cpm)								
Time (hours)	COC1	COC2	COC3	COC4	COC5	COC6	COC7	COC8	COC Cont.
0	2.23E+03	2.00E+03	1.99E+03	2.07E+03	2.02E+03	1.87E+03	2.00E+03	2.00E+03	1.67E+03
0.5	2.04E+03	1.82E+03	1.91E+03	1.92E+03	1.89E+03	1.82E+03	2.02E+03	1.84E+03	1.53E+03
1	1.88E+03	1.62E+03	2.06E+03	1.82E+03	2.20E+03	1.75E+03	1.89E+03	1.77E+03	1.30E+03
2	1.82E+03	1.46E+03	1.84E+03	1.56E+03	1.57E+03	1.54E+03	1.77E+03	1.55E+03	1.17E+03
4	1.67E+03	1.19E+03	1.60E+03	1.59E+03	1.50E+03	1.36E+03	1.63E+03	1.41E+03	1.01E+03
6	1.64E+03	1.26E+03	1.40E+03	1.47E+03	1.49E+03	1.41E+03	1.49E+03	1.24E+03	8.23E+02
8	1.64E+03	8.95E+02	1.29E+03	1.16E+03	1.16E+03	1.24E+03	1.40E+03	1.19E+03	8.06E+02
Fe-59	Activity (cpm)								
Time (hours)	COC1	COC2	COC3	COC4	COC5	COC6	COC7	COC8	COC Cont.
0	3.69E+03	3.20E+03	3.42E+03	3.08E+03	3.22E+03	2.93E+03	3.32E+03	3.66E+03	3.31E+03
0.5	2.99E+03	2.63E+03	2.87E+03	2.87E+03	2.72E+03	2.85E+03	3.40E+03	3.47E+03	3.34E+03
1	3.19E+03	2.46E+03	3.06E+03	2.87E+03	3.01E+03	2.54E+03	3.19E+03	2.70E+03	2.78E+03
2	3.05E+03	2.29E+03	2.67E+03	2.66E+03	2.64E+03	2.35E+03	2.70E+03	2.35E+03	2.75E+03
4	2.60E+03	1.84E+03	2.47E+03	2.37E+03	2.33E+03	2.17E+03	2.49E+03	1.92E+03	2.52E+03
6	2.19E+03	2.00E+03	2.30E+03	2.32E+03	2.27E+03	2.01E+03	1.94E+03	1.73E+03	2.33E+03
8	2.05E+03	1.69E+03	1.91E+03	2.21E+03	1.99E+03	1.89E+03	1.89E+03	1.55E+03	2.13E+03
Zn-65	Activity (cpm)								
Time (hours)	COC1	COC2	COC3	COC4	COC5	COC6	COC7	COC8	COC Cont.
0	6.73E+03	6.10E+03	6.53E+03	6.57E+03	6.16E+03	6.37E+03	6.38E+03	6.46E+03	6.38E+03
0.5	6.06E+03	6.05E+03	6.13E+03	6.29E+03	5.62E+03	6.25E+03	6.00E+03	6.28E+03	5.75E+03
1	5.98E+03	5.71E+03	5.95E+03	6.35E+03	5.58E+03	5.88E+03	6.08E+03	5.59E+03	5.77E+03
2	5.51E+03	5.48E+03	5.28E+03	6.27E+03	5.36E+03	5.60E+03	5.72E+03	5.33E+03	5.69E+03
4	4.86E+03	4.92E+03	4.72E+03	5.69E+03	4.90E+03	5.18E+03	5.50E+03	4.65E+03	5.63E+03
6	4.99E+03	4.40E+03	4.16E+03	5.73E+03	4.81E+03	4.98E+03	5.65E+03	4.23E+03	5.17E+03
8	4.56E+03	3.31E+03	4.04E+03	5.58E+03	4.75E+03	4.46E+03	5.49E+03	3.67E+03	5.08E+03

Appendix Table 4
Mass Balance for Metal Radioisotpes - Experiment 1. (A) Alginic acid treatment; (B) Carrageenan treatment; (C) Colloidal organic carbon treatment

A

Alginic Acid Mass Balance		**% Activity in Matrices**				
Cd-109	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**
T=20.5h	water	41.2	48.8	52.3	27.6	73.4
	meat	0.21	0.04	0.24	7.98	0.26
	shell	10	11.6	13.5	14.3	2.08
	wall	48.6	39.6	34.0	50.2	24.
	Act.Tot.	100	100	99.9	100	99.9
Ag-110m	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**
T=20.5h	water	20.6	32.2	57.6	34.2	23.4
	meat	0.71	0.12	-0.03	4.38	0.21
	shell	5.86	7.14	5.18	5.84	6.07
	wall	72.9	60.6	34.3	55.6	70.4
	Act.Tot.	100	100	97.0	100	100
Zn-65	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**
T=20.5h	water	45.5	54.5	56.5	35	58.1
	meat	0.24	0.05	0.26	14.24	0.5
	shell	20.0	20.6	19.1	21.0	11.1
	wall	34.2	24.8	24.2	29.8	30.4
	Act.Tot.	100	100	100	100	100
Cr-51	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**
T=20.5h	water	33.5	49.5	61.1	13.6	46.4
	meat	0.41	0.05	0.2	1.05	0.46
	shell	1.69	2.94	2.2	1.08	1.94
	wall	64.4	47.5	36.5	84.3	51.2
	Act.Tot.	99.9	100	100	99.9	99.9

Appendix Table 4. Continued.

B

Carrageenan Mass Balance		**% Activity in Matrices**				
Cd-109	**Location**	**C1**	**C2**	**C3**	**C4**	**C5**
T=20.5h	water	42.9	66.1	29.1	no data	40.3
	meat	0.11	2.29	20.1	no data	2.23
	shell	10.1	13.4	21.1	no data	19.7
	wall	46.8	18.3	29.6	no data	37.8
	Act.Tot.	100	100	100	0	100
Ag-110m	**Location**	**C1**	**C2**	**C3**	**C4**	**C5**
T=20.5h	water	51.3	56.6	42.1	no data	42.7
	meat	0.02	2.57	3.61	no data	2.13
	shell	2.77	4.27	2.84	no data	4.56
	wall	45.9	36.6	51.5	no data	50.7
	Act.Tot.	100	100	100	0	100
Zn-65	**Location**	**C1**	**C2**	**C3**	**C4**	**C5**
T=20.5h	water	59.8	66.4	39.6	no data	44.6
	meat	0.13	2.51	16.7	no data	2.12
	shell	11.5	14.6	17.9	no data	19.08
	wall	28.6	16.5	25.9	no data	34.2
	Act.Tot.	100	100	100	0	100
Cr-51	**Location**	**C1**	**C2**	**C3**	**C4**	**C5**
T=20.5h	water	64.6	71.1	49.4	no data	49.2
	meat	0.04	1.13	0.41	no data	0.98
	shell	0.69	0.95	0.83	no data	0.99
	wall	34.7	26.9	49.4	no data	48.9
	Act.Tot.	100	100	100	0	100

Appendix Table 4. Continued.

C

COC Mass Balance		% Activity in Matrices				
Cd-109	**Location**	**COC1**	**COC2**	**COC3**	**COC4**	**COC5**
T=20.5h	water	27.1	16.5	17.0	27.6	22.9
	meat	0.4	0.2	8.5	0.1	0.8
	shell	9.5	8.3	9.7	3.7	8.5
	wall	63.1	75.0	64.9	68.6	67.8
	Act.Tot.	100.0	100.0	100.0	100.0	100.0
Ag-110m	**Location**	**COC1**	**COC2**	**COC3**	**COC4**	**COC5**
T=20.5h	water	44.0	43.5	33.1	48.3	53.6
	meat	0.85	0.14	5.46	0.15	3.18
	shell	5.56	4.69	5.46	3.98	4.96
	wall	49.6	51.7	56.0	47.6	38.2
	Act.Tot.	100	100	100	100	100
Zn-65	**Location**	**COC1**	**COC2**	**COC3**	**COC4**	**COC5**
T=20.5h	water	16.9	17.9	13.5	18.9	20.2
	meat	0.75	0.33	18.33	0.23	2.45
	shell	23.5	20.3	25.6	1.48	19.2
	wall	58.8	61.4	42.7	79.4	58.2
	Act.Tot.	100	100	100	100	100
Cr-51	**Location**	**COC1**	**COC2**	**COC3**	**COC4**	**COC5**
T=20.5h	water	23.7	25.5	18.3	26.5	32.1
	meat	0.67	0.21	1.81	0.13	1.88
	shell	2.42	2.05	2.05	1.58	1.9
	wall	73.3	72.2	77.8	71.8	64.1
	Act.Tot.	100	100	100	100	100

Appendix Table 5
Mass Balance for Metal Radioisotpes - Experiment 2. (A) Alginic acid treatment; (B) Carrageenan treatment; (C) Colloidal organic carbon treatment

A

Alginic Acid Mass Balance		**% Activity in Matrices**							
Cd-109	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
T=8.0h	water	82.8	88.0	89.6	86.8	84.9	85.8	89.8	85.1
	fecal	0.24	0.08	0.20	0.39	0.21	0.14	0.23	0.14
	shell	8.79	6.56	2.89	9.67	8.39	8.02	3.72	10.6
	leach	7.84	3.76	7.13	2.82	5.30	3.14	5.83	3.97
	meat total	0.31	1.66	0.17	0.33	1.19	2.89	0.43	0.21
	Act. Tot.	100	100	100	100	100	100	100	100
Ag-110m	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
T=8.0h	water	93.6	94.6	90.51	93.3	90.7	74.2	939	94.2
	fecal	0.26	0.09	0.24	0.46	0.29	0.41	0.30	0.16
	shell	3.07	2.90	5.05	3.65	3.62	15.08	2.66	4.18
	leach	2.82	2.01	3.94	2.41	4.77	7.94	2.47	1.23
	meat total	0.24	0.36	0.25	0.23	0.67	2.35	0.71	0.23
	Act. Tot.	100	100	100	100	100	100	100	100
Zn-65	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
T=8.0h	water	75.1	87.9	82.9	79.2	80.4	80.0	83.0	73.1
	fecal	0.24	0.08	0.21	0.39	0.22	0.16	0.23	0.15
	shell	13.8	6.97	10.5	15.9	12.0	15.9	8.35	17.5
	leach	10.7	4.46	6.16	4.36	6.48	3.08	8.00	9.01
	meat total	0.28	0.62	0.19	0.23	0.87	0.83	0.42	0.25
	Act. Tot.	100	100	100	100	100	100	100	100
Cr-51	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
T=8.0h	water	75.5	78.5	66.1	80.5	72.9	40.3	73.7	75.1
	fecal	0.22	0.09	0.16	0.30	0.20	0.20	0.21	0.12
	shell	12.3	10.1	16.7	11.0	13.1	41.9	10.4	14.9
	leach	11.9	10.9	16.9	8.04	13.5	16.4	15.3	9.76
	meat total	0.16	0.31	0.11	0.11	0.32	1.22	0.37	0.10
	Act. Tot.	100	100	100	100	100	100	100	100
Co-57	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
T=8.0h	water	94.8	95.6	94.7	95.4	94.6	93.3	95.8	94.3
	fecal	0.21	0.07	0.18	0.33	0.18	0.16	0.20	0.14
	shell	2.62	2.57	3.08	3.00	3.16	4.88	1.88	3.78
	leach	2.16	1.51	1.91	1.17	1.75	1.39	1.85	1.61
	meat total	0.18	0.28	0.15	0.13	0.29	0.26	0.23	0.19
	Act. Tot.	100	100	100	100	100	100	100	100
Hg-203	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
T=8.0h	water	88.9	87.5	87.8	87.0	86.4	74.4	87.3	82.1
	fecal	0.24	0.08	0.26	0.44	0.27	0.27	0.22	0.18
	shell	7.30	8.15	8.52	8.50	9.20	18.9	6.70	9.63
	leach	2.99	2.76	3.03	3.54	3.14	4.77	3.22	2.56
	meat total	0.55	1.51	0.35	0.50	1.04	1.61	2.60	5.50
	Act. Tot.	100	100	100	100	100	100	100	100
Fe-65	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
T=8.0h	water	85.6	87.9	83.3	90.0	86.5	69.5	84.6	87.6
	fecal	0.25	0.10	0.20	0.35	0.25	0.35	0.22	0.13
	shell	1.97	1.75	2.85	2.08	1.93	9.53	1.53	2.38
	leach	11.8	9.86	13.5	7.39	10.9	18.4	13.2	9.70
	meat total	0.20	0.33	0.14	0.13	0.33	2.14	0.44	0.13
	Act. Tot.	100	100	100	100	100	100	100	100

Appendix Table 5. Continued.

B

Alginic Acid Mass Balance		% Activity in Matrices							
Cd-109	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
T=8.0h	water	82.8	88.0	89.6	86.8	84.9	85.8	89.8	85.1
	fecal	0.24	0.08	0.20	0.39	0.21	0.14	0.23	0.14
	shell	8.79	6.56	2.89	9.67	8.39	8.02	3.72	10.6
	leach	7.84	3.76	7.13	2.82	5.30	3.14	5.83	3.97
	meat total	0.31	1.66	0.17	0.33	1.19	2.89	0.43	0.21
	Act. Tot.	100	100	100	100	100	100	100	100
Ag-110m	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
T=8.0h	water	93.6	94.6	90.51	93.3	90.7	74.2	939	94.2
	fecal	0.26	0.09	0.24	0.46	0.29	0.41	0.30	0.16
	shell	3.07	2.90	5.05	3.65	3.62	15.08	2.66	4.18
	leach	2.82	2.01	3.94	2.41	4.77	7.94	2.47	1.23
	meat total	0.24	0.36	0.25	0.23	0.67	2.35	0.71	0.23
	Act. Tot.	100	100	100	100	100	100	100	100
Zn-65	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
T=8.0h	water	75.1	87.9	82.9	79.2	80.4	80.0	83.0	73.1
	fecal	0.24	0.08	0.21	0.39	0.22	0.16	0.23	0.15
	shell	13.8	6.97	10.5	15.9	12.0	15.9	8.35	17.5
	leach	10.7	4.46	6.16	4.36	6.48	3.08	8.00	9.01
	meat total	0.28	0.62	0.19	0.23	0.87	0.83	0.42	0.25
	Act. Tot.	100	100	100	100	100	100	100	100
Cr-51	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
T=8.0h	water	75.5	78.5	66.1	80.5	72.9	40.3	73.7	75.1
	fecal	0.22	0.09	0.16	0.30	0.20	0.20	0.21	0.12
	shell	12.3	10.1	16.7	11.0	13.1	41.9	10.4	14.9
	leach	11.9	10.9	16.9	8.04	13.5	16.4	15.3	9.76
	meat total	0.16	0.31	0.11	0.11	0.32	1.22	0.37	0.10
	Act. Tot.	100	100	100	100	100	100	100	100
Co-57	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
T=8.0h	water	94.8	95.6	94.7	95.4	94.6	93.3	95.8	94.3
	fecal	0.21	0.07	0.18	0.33	0.18	0.16	0.20	0.14
	shell	2.62	2.57	3.08	3.00	3.16	4.88	1.88	3.78
	leach	2.16	1.51	1.91	1.17	1.75	1.39	1.85	1.61
	meat total	0.18	0.28	0.15	0.13	0.29	0.26	0.23	0.19
	Act. Tot.	100	100	100	100	100	100	100	100
Hg-203	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
T=8.0h	water	88.9	87.5	87.8	87.0	86.4	74.4	87.3	82.1
	fecal	0.24	0.08	0.26	0.44	0.27	0.27	0.22	0.18
	shell	7.30	8.15	8.52	8.50	9.20	18.9	6.70	9.63
	leach	2.99	2.76	3.03	3.54	3.14	4.77	3.22	2.56
	meat total	0.55	1.51	0.35	0.50	1.04	1.61	2.60	5.50
	Act. Tot.	100	100	100	100	100	100	100	100
Fe-65	**Location**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
T=8.0h	water	85.6	87.9	83.3	90.0	86.5	69.5	84.6	87.6
	fecal	0.25	0.10	0.20	0.35	0.25	0.35	0.22	0.13
	shell	1.97	1.75	2.85	2.08	1.93	9.53	1.53	2.38
	leach	11.8	9.86	13.5	7.39	10.9	18.4	13.2	9.70
	meat total	0.20	0.33	0.14	0.13	0.33	2.14	0.44	0.13
	Act. Tot.	100	100	100	100	100	100	100	100

Appendix Table 5. Continued.

C

COC Mass Balance		% Activity in Matrices							
Cd-109	**Location**	**COC1**	**COC2**	**COC3**	**COC4**	**COC5**	**COC6**	**COC7**	**COC8**
T=8.0h	water	87.4	58.1	80.7	88.5	88.1	82.3	90.7	64.7
fecal	fecal	0.30	0.31	0.59	0.37	0.24	0.35	0.10	0.31
	shell	11.4	11.8	6.64	8.47	6.31	7.30	6.10	5.83
	leach	0.35	0.05	0.21	0.28	0.30	0.21	0.39	0.15
	meat total	0.45	29.6	11.7	2.33	5.03	9.81	2.64	28.0
	Act. Total	100	100	100	100	100	100	100	100
Ag-110m	**Location**	**COC1**	**COC2**	**COC3**	**COC4**	**COC5**	**COC6**	**COC7**	**COC8**
T=8.0h	water	96.8	77.8	88.4	88.8	87.1	81.1	92.4	87.75
	fecal	0.29	12.7	4.61	2.98	2.66	3.98	0.09	5.41
	shell	1.61	3.38	2.96	4.18	4.49	4.78	3.67	3.97
	leach	1.18	1.58	1.00	1.52	1.75	1.31	0.92	0.98
	meat total	0.11	4.43	3.03	2.46	3.98	8.82	2.90	1.90
	Act. Total	100	100	100	100	100	100	100	100
Zn-65	**Location**	**COC1**	**COC2**	**COC3**	**COC4**	**COC5**	**COC6**	**COC7**	**COC8**
T=8.0h	water	82.42	67.7	68.7	92.5	82.6	77.1	91.1	59.7
	fecal	0.32	0.10	1.13	0.37	0.48	0.47	0.10	0.76
	shell	15.82	16.21	15.40	5.04	12.2	12.4	5.71	15.85
	leach	0.96	0.58	0.66	1.01	0.53	1.00	0.92	1.43
	meat total	0.48	15.3	14.0	0.99	4.09	8.94	2.10	22.1
	Act. Total	100	100	100	100	100	100	100	100
Cr-51	**Location**	**COC1**	**COC2**	**COC3**	**COC4**	**COC5**	**COC6**	**COC7**	**COC8**
T=8.0h	water	87.76	88.3	87.1	89.6	89.6	90.0	90.1	90.3
	fecal	0.36	2.17	2.04	0.78	0.87	1.75	0.09	2.03
	shell	7.57	7.24	6.66	6.70	4.71	6.87	5.48	6.64
	leach	4.09	1.83	3.42	2.61	3.97	0.65	3.53	0.00
	meat total	0.23	0.43	0.74	0.31	0.79	0.66	0.77	0.98
	Act. Total	100	100	100	100	100	100	100	100
Co-57	**Location**	**COC1**	**COC2**	**COC3**	**COC4**	**COC5**	**COC6**	**COC7**	**COC8**
T=8.0h	water	96.66	95.0	95.8	95.4	97.0	95.5	96.5	95.2
	fecal	0.33	0.48	0.63	0.37	0.22	0.39	0.10	0.48
	shell	2.74	3.34	2.59	2.91	1.90	2.39	2.31	3.19
	leach	0.13	0.22	0.23	0.38	0.18	0.20	0.27	0.33
	meat total	0.14	0.91	0.71	0.86	0.62	1.47	0.83	0.79
	Act. Total	100	100	100	100	100	100	100	100
Hg-203	**Location**	**COC1**	**COC2**	**COC3**	**COC4**	**COC5**	**COC6**	**COC7**	**COC8**
T=8.0h	water	96.0	74.1	88.9	86.9	87.9	82.4	91.7	83.1
	fecal	0.23	14.3	4.92	3.30	2.81	4.84	0.12	6.83
	shell	2.53	4.49	2.86	5.10	4.58	5.26	3.92	4.83
	leach	0.89	0.98	0.92	1.81	1.15	0.82	1.12	1.85
	meat total	0.27	6.05	2.32	2.84	3.55	6.63	3.14	3.29
	Act. Total	100	100	100	100	100	100	100	100
Fe-65	**Location**	**COC1**	**COC2**	**COC3**	**COC4**	**COC5**	**COC6**	**COC7**	**COC8**
T=8.0h	water	91.0	83.8	87.0	90.6	88.4	88.2	88.4	81.8
	fecal	0.49	7.16	5.96	1.59	2.45	4.19	0.16	8.56
	shell	6.93	5.85	4.11	5.18	5.19	4.47	6.45	6.81
	leach	1.20	1.20	1.63	1.32	1.54	1.93	2.54	1.76
	meat total	0.30	1.95	1.23	1.24	2.42	1.21	2.36	1.05
	Act. Total	100	100	100	100	100	100	100	100

Appendix Table 6
Percent metal activity removed from water. (A) Experiment 1; (B) Experiment 2

A

	% Activity Removed from Water			
COM Type	**Cd-109**	**Cr-51**	**Ag-110m**	**Zn-65**
AA	51.3 +/- 16.8	59.1 +/- 18.1	66.4 +/- 14.7	50.1 +/- 9.7
C	55.8 +/- 13.5	41.0 +/- 9.7	49.4 +/- 8.2	47.5 +/- 10.9
COC	56.4 +/- 10.4	59.8 +/- 8.9	55.4 +/- 7.7	58.8 +/- 8.2

B

	% Activity Removed from Water						
COM Type	**Hg-203**	**Ag-110m**	**Zn-65**	**Cd-109**	**Co-57**	**Cr-51**	**Fe-59**
AA	56.9 +/- 3.6	43.6 +/- 2.5	38.8 +/- 7.5	24.5 +/- 13.6	16.5 +/- 13.4	41.6 +/- 13.7	41.6 +/- 6.5
C	48.8 +/- 19.5	42.2 +/- 13.7	46.4 +/- 20.8	41.2 +/- 12.5	14.5 +/- 23.1	49.5 +/- 17.6	44.6 +/- 14.6
COC	32.9 +/- 8.8	38.4 +/- 6.2	30.1 +/- 11.5	26.3 +/- 8.5	4.45 +/- 9.2	16.8 +/- 8.6	42.3 +/- 12.1

Appendix Table 7
^{14}C Activity Removed from Water. (A) Experiment 1; (B) Experiment 2

A

C-14 AA	**Activity (cpm) ($x10^5$)**				
Time (hours)	**A1**	**A2**	**A3**	**A4**	**A5**
0	1.18	1.18	1.22	1.30	1.38
1	1.15	1.18	1.21	1.24	1.25
2	1.14	1.21	1.16	1.13	1.17
4	0.938	1.10	1.11	0.986	1.11
6	1.07	1.12	1.08	1.19	1.26
8	1.11	1.21	1.14	1.15	1.23
19	0.960	1.17	0.970	1.21	1.15
C-14 COC	**Activity (cpm) ($x10^5$)**				
Time (hours)	**C1**	**C2**	**C3**	**C4**	**C5**
0	4.13	4.06	4.15	3.98	4.39
1	3.53	3.90	4.17	4.29	4.68
2	4.08	4.10	3.80	4.09	4.58
4	4.02	3.96	4.00	3.62	3.94
6	3.82	3.80	3.94	3.85	4.10
8	3.68	4.00	4.08	3.87	4.27
19	3.99	3.94	4.03	3.78	4.30

Appendix Table 7. Continued.

B

C-14 AA	Activity (cpm) ($x10^4$)								
Time(hours)	A1	A2	A3	A4	A5	A6	A7	Cont.A	Cont.B
0	7.44	8.15	7.29	8.05	8.14	8.19	7.67	7.58	7.78
0.5	7.18	7.22	7.18	7.58	7.67	7.51	6.99	7.44	7.57
1	6.81	6.14	6.94	7.07	6.6	7.01	6.85	7.23	7.35
2	6.45	6.12	6.73	7.84	6.56	6.79	6.58	6.96	7.2
4	5.34	5.56	6.3	6.44	6.65	6.18	5.85	6.77	6.97
6	5.05	5.43	6.08	6.05	5.97	5.47	5.46	6.38	6.48
8	4.99	5.04	5.69	5.82	5.77	5.36	5.4	6.22	6.2
C-14 COC	**Activity (cpm) ($x10^5$)**								
Time	COC1	COC2	COC3	COC4	COC5	Cont.A			
0	3.51	2.25	2.64	2.43	2.19	0.941			
0.5	2.30	2.41	2.53	2.33	2.14	1.00			
1	2.35	2.30	2.33	2.21	2.01	0.986			
2	2.26	2.19	2.29	2.12	1.94	0.944			
4	2.20	2.12	2.20	1.96	1.89	0.923			
6	2.11	2.04	2.12	1.89	1.83	0.893			
8	2.04	2.03	2.10	1.82	1.79	0.848			
C-14 Car	**Activity (cpm) ($x10^5$)**								
Time	C1	C2	C3	C4	C5	C6	C7	Cont.A	Cont.B
0	0.769	1.97	1.46	0.847	2.58	1.37	1.38	1.83	3.56
0.5	1.17	1.50	1.53	1.14	1.26	1.26	2.12	1.68	1.63
1	1.15	1.09	1.55	1.11	1.15	1.21	1.51	1.59	1.57
2	1.15	1.05	1.16	1.11	1.14		1.44	1.58	1.53
4	1.09	1.10	1.12	1.05	1.08	1.11	1.41	1.51	1.51
6	1.15	1.02	1.05	0.991	1.00	1.08	1.33	1.47	1.43
8	1.04	1.00	1.03	1.01	1.00	1.05	1.30	1.43	1.42

Appendix Table 8
Comparison of COM for ^{14}C. (A) Experiment 1; (B) Experiment 2

A

	AA	COC
meat	20.0	5.71
shell	1.45	1.31
water	78.4	87.0
% Total	99.9	94.1

B

	AA	Car	COC
water	69.4	74.7	82.5
leach	1.97	1.04	1.62
fecal mat.	4.00	2.39	3.09
meat	15.5	1.56	1.03
shell	3.84	1.29	1.97
% total	94.8	81.1	90.2

Appendix Table 9
^{14}C Uptake Data. (A) Experiment 1; (B) Experiment 2

A

	Activity	**(cpm)**	
Oyster ID	meat	% Activity	Total t=0
A1	27878	23.63	118002
A2	29179	24.57	118758
A3	16533	13.54	122130
A4	25575	19.68	129942
A5	25628	18.62	137670
C1	37796	9.13	413826
C2	21506	5.29	406590
C3	21458	5.17	415308
C4	18705	4.70	398136
C5	18672	4.25	439416

B

	Activity (cpm)		
ID	**meat**	**meat %**	**total**
A2	9195	**11.27**	81570
A4	3320	**4.12**	80538
A5	2598	**3.19**	81468
A6	3757	**4.58**	81960
A7	6256	**8.15**	76782
C1	5431	**3.81**	142686
C2	1931	**1.35**	142686
C3	1828	**1.28**	142686
C4	2020	**1.42**	142686
C5	1104	**0.77**	142686
C6	1665	**1.17**	142686
C7	1640	**1.15**	142686
COC1	2356	**0.99**	238062
COC2	2681	**1.19**	225030
COC3	2774	**1.05**	264110
COC4	2223	**0.91**	243525
COC5	2232	**1.02**	219585

Appendix Table 10
^{14}C Activity Removed from Water. (A) Experiment 1; (B) Experiment 2

A

	Activity (cpm)			
ID	Initial	Final	% Remaining	% Removed
AA	2088 +/- 139	1820 +/- 196	87.2 +/- 12.7	12.8 +/- 2.0
COC	6910 +/- 257	6689 +/- 316	96.8 +/- 6.0	3.2 +/- 0.3

B

COM Type	Activity (cpm) Initial	Final	% Activity Remaining	% Activity Removed
Alg.	148538 +/- 3763	106683 +/- 3373	71.8 +/- 4.1	28.2 +/- 0.2
Car.	148538 +/- 63164	106683 +/- 10633	71.8+/-43.7	28.2 +/- 19.1
COC	260701 +/- 53548	196012 +/- 14019	75.2 +/- 21.7	24.8 +/- 4.7

Appendix Table 11
Dry Weights of Oysters for C-14 Experiment 2

Oyster	**Dry Weight (g)**	**Oyster**	**Dry Weight (g)**	**Oyster**	**Dry Weight (g)**
A1	0.323	Car1	0.735	COC1	0.262
A2	0.252	Car2	0.563	COC2	0.409
A3	0.439	Car3	0.312	COC3	0.345
A4	0.485	Car4	0.386	COC4	0.216
A5	0.424	Car5	0.200	COC5	0.297
A6	0.366	Car6	0.336	COC6	0.151
A7	0.587	Car7	0.497	COC7	0.267
A8	0.495	Car8	0.375	COC8	0.363

VITA

Jennifer Marcelle Haye

3102 69th Street, #95

Galveston, Texas 77551

e-mail: hayej@tamug.edu

Education:

M.S. Oceanography, Texas A&M University, May 2005.

B.S. Marine Science, Texas A&M University, Galveston, December 2000.

Publications:

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