Methods and Materials

I. Sampling Design

The sampling scheme was designed to assess the copper levels and other environmental characteristics of tidal creeks and estuaries with and without plasticulture in their drainage basin. Three watersheds with tomato plasticulture were chosen for investigation. Gargathy Creek in Accomac County, and The Gulf in Northampton County both contained an aquaculture facility located on their lower half. Gargathy Creek contained 179 acres of plasticulture in 1996, and 174 acres in 1997. The Gulf contained 167 acres in 1997, the only year for which figures are available (Hammer and Boyd, 1997). Additional sampling was conducted on Parker’s Creek, which is a non-tidal creek adjacent to tomato plasticulture.

Two additional sites were chosen as control sites because little or no agricultural activity was located in the watershed or near the sampling site. There was no plasticulture located within the basins of either of these two waterways. These sites were Queen’s Sound, an estuary with a high level of oceanic tidal flushing off the coast of Chincoteague in Accomac County, and Raccoon Creek, which is located entirely within the Eastern Shore Wildlife Refuge in Northampton County and contains no agriculture.

Systematic grab sampling for water samples was conducted at these five watersheds approximately each month over the period of thirteen months. The sampling was performed primarily at low tide, when the estuary would contain the highest volume of freshwater and the lowest volume of tidal oceanic water. This increased the chance that concentrations of contaminants from land-based runoff would be present at detectable concentrations. Sampling events preferentially followed runoff-generating rainfall, although some sampling was also performed during clear weather conditions.

Samples were taken from the middle point in the creek depth-wise, unless this was too deep to reach, in which case the sample was taken approximately one foot below the surface of the water. Samples were taken as far towards the center of the creek as was possible. Occasional samples were collected by boat; in this case, they were taken off the bow of the boat as the boat moved slowly forward through the water.
When rainfall was heavy enough to create runoff from fields, samples were taken from this runoff and from puddled runoff that had collected in roadside ditches and local low spots. Samples of runoff from a field engaged in tomato plasticulture in 1996 were taken throughout the study, as well as runoff from a non-agricultural roadside ditch located in a residential area within the same watershed. Samples of runoff from non-tomato agricultural and non-agricultural areas were collected during the heaviest rain of 1997. Besides tomato plasticulture, these samples included other plasticulture, agricultural crops grown without the use of plasticulture, natural and residential areas.

All grab water samples were collected in 500 mL high-density polyethylene containers (Fisher Scientific; Raleigh, NC). Samples to be analyzed for dissolved copper were then sub-sampled into 60 mL high-density polyethylene containers. For total organic carbon analyses, the samples were collected in 40 mL amber glass bottles with polytetrafluoroethylene (PTFE) lined caps.

Grab sediment samples were collected from five locations in four watersheds. These samples were taken in triplicate. Because the copper concentrations in sediment were not expected to fluctuate as often as water concentrations, sediment samples were taken only one time, soon after the beginning of the study. Samples were collected below the high tide mark within the creek, as close to the center of the creek as was possible at the time of sampling. Sediment samples were taken by hand in one-foot long cylindrical corers, approximately two inches in diameter. These corers were clear hard polyvinylchloride (PVC) with rubber stoppers at the two ends. (Figure 7)

Autosamplers were used to collect water from the five watersheds during the summer of 1997. This involved using autosamplers to collect approximately 24 water samples over four tidal cycles following a runoff-producing rainfall event. An initial run in May 1997 was used to assess the autosampler’s performance, and provide background with a non-rainfall sampling.

An overview of the monthly schedule of grab water sampling, water autosampling, and sediment core sampling is provided in Table 1. Multiple sites within one watershed are included together.
Figure 7. Soil Corer used in sediment grab sampling. Its dimensions are two inches in diameter, and approximately 18 inches tall.
### Table 1. An overview of the monthly schedule of water grab sampling, water autosampling, and sediment core sampling.

<table>
<thead>
<tr>
<th>Month</th>
<th>Gargathy Creek</th>
<th>The Gulf</th>
<th>Parker’s Creek</th>
<th>Queen’s Sound</th>
<th>Raccoon Creek</th>
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Each autosampler was an ISCO model 2700 (Isco, Inc.; Lincoln, Nebraska). These ISCO samplers were enclosed in a hard plastic weather-resistant shell. A bottom compartment contained 24 one-liter polypropylene bottles strapped into the case. Above the containers, the sampler consisted of a peristaltic pump, chemically resistant silicone pump tubing, a line to an outside power source, and the sampler’s programmable memory and clock. The pump tubing connected to external PTFE tubing, continued through the pump, and finished in a mechanical distributor arm, which rotated around the lower part of the case to position itself alternatively over each of the 24 bottles. The distributor motor was located in a sealed, waterproof box below the programmable memory and clock, along with internal desiccant for additional weather proofing. Outside of the sampler case, a 12-volt battery was contained within another waterproof case and had a connection to the sampler to provide power. When an electrical source was located close enough, a direct current powered the sampler.

Although the water level changed with the tides, the intake was suspended approximately at the center of the water column between a cement weight and a buoy. The intake consisted of a hard polyvinylchloride tube, drilled with holes to screen out solid objects that would clog the intake line. (Figure 8) One end was blocked off with a PVC cap and the other narrowed to connect to a 3/8” inch ID, 25 or 12 foot long PTFE tube that then led to the autosampler. The samplers were often located on a dock not far from the intake. (Figure 9)

At times when a dock was not located close enough to allow easy access to the sampling location, a floating platform supported the sampler and battery case. The floating platforms consisted of six inch diameter PVC piping, fitted together into two square airtight rings, approximately five feet to a side. These rings provided the floatation to support the autosampler when full of 24 one-liter samples and the battery with case. They were strapped together with duct tape and bungee cords, one on top of the other. A wooden platform, measuring approximately four feet by five feet of untreated lumber was suspended level with the top of the topmost PVC ring by bungee cords. The sampler and battery case were strapped to the surface of the wooden platform with bungee cords and metal hooks. Anchors kept the floating platforms from floating out of position with the tide and current of the creek. (Figure 10)
Figure 8. An intake screen, approximately one inch in diameter with \(\frac{1}{4}\) inch intake holes. The screen is approximately one foot tall.
Figure 9. An autosampler deployed on a dock
Figure 10. Floating autosampler with platform
II. Site Description

Below are listed all sites from which samples were collected during the course of this project. Within each watershed, if more than one sample was collected per watershed, the samples are listed by location from upstream to downstream. Land-based samples are listed by location from south to north. Figure 11 is a map showing the location of all watersheds and sampling sites.

A. The Gargathy Creek Watershed

Samples for the site entitled ‘Freshwater Creek’ were taken from a freshwater creek that feeds into the headwaters of Gargathy Creek. Sampling occurred at a site just before the creek flows beneath Metompkin Road in Modest Town, Accomac County, Va. Waters were collected one foot away from the edge of the bank, approximately midstream.

Samples for the site entitled ‘Gargathy Creek, Headwaters’ were taken upstream of Kegotank Landing in Accomac County, VA, at the point at which the creek is no longer passable by boat at mid-tide. Waters were collected off the side of the boat about midstream.

Samples for the site entitled ‘Gargathy Creek, Gully Site’ were taken upstream of Kegotank Landing, about midway alongside the large field between Gargathy Creek and Kegotank Road, in Accomac County, VA, where a gully cuts through the marsh leading from the field down to the water, about ten yards away. This site was halfway in between the midstream dock and the headwaters. Waters were collected off the side of the boat about midstream.

Samples for the site entitled ‘Gargathy Creek, Midstream Dock’ were taken from a dock upstream of Kegotank Landing, approximately midway between Kegotank Public Landing and the Headwaters of Gargathy Creek, in Accomac County, VA. Waters were collected off the end of the dock about ten feet into the stream at low tide, approximately midstream.
Figure 11. Map of the Eastern Shore of Virginia with watersheds indicated. Copyright 1996, Microsoft Corporation.
Samples for the site entitled ‘Gargathy Creek, Kegotank Landing’ were taken from Kegotank Public Landing, at the end of Kegotank Road, in Accomac County, VA. Sediments were collected about five feet into the creek at low tide, and waters were collected at the end of the dock adjacent to the landing about five feet into the creek, approximately a quarter of the way to the center of the stream. Some additional grab samples were collected from ditched runoff flowing directly into the creek through the landing.

Samples for the site entitled ‘Gargathy Creek, Clam Co.’ were taken from a private dock downstream of the landing. Sediments were collected ten feet out into the creek at low tide, and waters were collected off the end of the dock, about ten feet into the creek, about a quarter of the way to the center of the stream.

B. The Gulf Watershed

Samples for the site entitled ‘The Gulf, Headwaters’ were taken at the point at which the creek is no longer passable by boat at mid-tide, in Northampton County, VA. Waters were collected off the side of the boat about midstream.

Samples for the site entitled ‘The Gulf, Dam Site’ were taken about 1000 yards upstream of the confluence of The Gulf and the Chesapeake Bay at the downstream side of a dammed up irrigation pond in Northampton County, VA. Waters were collected off the side of the boat where the waters from the dam intersect the main arm of the creek.

Samples for the site entitled ‘The Gulf, Clam Co.’ were taken from a dock on the Eastern side of the interface of The Gulf with the Chesapeake Bay, in Northampton County, VA. Sediments were collected about fifteen feet into the creek at low tide, and waters were collected at the end of the dock about fifteen feet into the water body, about a tenth of the way into the stream.

C. Parker’s Creek Watershed

Samples for the site entitled ‘Parker’s Creek’ were taken from the North Fork of Parker’s Creek before it flows under Route 661 in Accomac County, VA. Waters were
collected directly from shore about one foot into the water body, approximately
midstream.

D. Queen’s Sound Watershed

Samples for the site entitled ‘Queen’s Sound’ were taken at a public landing on
Shore Road, on the Queen Sound Channel of Chincoteague in Accomac County, VA.
Sediments were collected about three feet into the water body at low tide, and waters
were collected directly from shore one foot into the water body, very far from the center.

E. Raccoon Creek Watershed

Samples for the site entitled ‘Raccoon Creek’ were taken at the landing on
Raccoon Creek on Ramp Road in the Eastern Shore Wildlife Refuge (ESWR) of
Kiptopeke in Northampton County, VA. Sediments were collected about three feet into
the creek at low tide and waters were collected from shore about one foot into the water
body, approximately midstream.

F. Land-based Samples

Samples for the site entitled ‘ESWR, Rain Puddle’ were taken two yards away
from the side of a service road within the Eastern Shore Wildlife Refuge in Northampton
County, VA. Waters were collected from the center of the natural puddle.

Samples for the site entitled ‘Route 666, 1997 Tomato Field’ were taken at the
roadside of a field planted in tomato plasticulture in 1997 at the time of sampling on
Route 666, in Northampton County, VA. Waters were collected from the center of the
roadside puddle.

Samples for the site entitled ‘Route 666, Rain Puddle’ were taken at the roadside
of a residential yard on Route 666 in Northampton county, VA. Waters were collected
from the center of the roadside puddle.
Samples for the site entitled ‘Johnson Road, 1997 Tomato Field’ were taken at the roadside of a field engaged in tomato plasticulture in 1997 at the time of sampling on Johnson Road in Accomac County, VA. Waters were collected from the center of the roadside puddle.

Samples for the site entitled ‘Route 630, Corn Field’ were taken at the roadside of a field planted in corn at the time of sampling on Route 630 in Accomac County, VA. Waters were collected from the center of the roadside puddle.

Samples for the site entitled ‘Route 630, Forest Puddle’ were taken at the roadside of a natural forested area at the time of sampling on Route 630 in Accomac County, VA. Waters were collected from the center of the natural puddle.

Samples for the site entitled ‘Route 630, Pepper Field’ were taken at the roadside of a field planted in pepper plasticulture at the time of sampling on Route 630 in Accomac County, VA. Waters were collected from the center of the roadside puddle.

Samples for the site entitled ‘Gargatha Road, Cotton Field’ were taken at the roadside of a field planted in cotton at the time of sampling on Gargatha Road in Accomac County, VA. Waters were collected from the center of the roadside puddle.

Samples for the site entitled ‘Gargatha Road, Rain Puddle’ were taken at the roadside of a residential yard on Gargatha Road in Accomac County, VA. Waters were collected from the center of the roadside puddle.

Samples for the site entitled ‘Kegotank Road, 1996 Tomato Field’ were taken at the roadside of a field engaged in tomato plasticulture in 1996 on Kegotank Road in Accomac County, VA. Waters were collected from the center of the roadside puddle, or from runoff leaving the puddle.

Samples for the site entitled ‘Kegotank Road, 1997 Tomato Field’ were taken at the roadside of a field engaged in tomato plasticulture in 1997 at the time of sampling on Kegotank Road in Accomac County, VA. Waters were collected from the center of the roadside puddle.

Samples for the site entitled ‘Metompkin Road, 1997 Tomato Field’ were taken at the roadside of a field engaged in tomato plasticulture in 1997 at the time of sampling on Metompkin Road in Accomac County, VA. Waters were collected from the center of the roadside puddle.
Samples for the site entitled ‘Metomkin Road, Soybean/Wheat Field’ were taken at the roadside of a field planted in soybeans growing through a cover crop of wheat at the time of sampling on Metomkin Road in Accomac County, VA. Waters were collected from the center of the roadside puddle.

III. Preparation of Samples and Sampling Containers, Transportation and Preservation of Samples

A. Water Sampling

A flow chart describing the sampling and analysis of water samples is contained in Figure 12. Samples for total copper were collected and stored according to Standard Method 3010 (Eaton, et al., 1995). For total copper, samples were collected in 500 mL high-density polyethylene containers by hand, or in 1 L polypropylene bottles by autosampler. All sample bottles and their caps were soaked into 10% concentrated nitric acid (Fisher Scientific; Raleigh, NC) for 12 hours, rinsed with Type II water (reagent grade water from a Milli Q purification system) three times, and allowed to air dry prior to sampling. After dissolved copper sub-samples were removed, and measurement of total suspended solids, salinity and pH were completed, the samples were acidified to pH <2 by the addition of 50% trace metal grade nitric acid (Fisher Scientific; Raleigh, NC) in Type II water. The trace metal grade nitric acid was used only for addition to samples and contained 0.005 ppm Cu.

All samples for total metals were transported to the laboratory on ice to maintain a low temperature and avoid unnecessary water loss, and were stored in a refrigerator at eight degrees Celsius until analyzed. Samples were not held longer than six months before analysis.
A 500 mL sample is taken from the waterway in a high-density polyethylene container.

50 mL is subsampled into a high-density polyethylene container, acidified and analyzed for dissolved metals.

50 mL is subsampled into a high-density polyethylene container, acidified and analyzed for dissolved metals.

pH is determined directly in the sample by a pH meter.

250 mL is transferred to a graduated cylinder and salinity is determined by floating a hydrometer in the sample.

200 mL is acidified and transferred to digestion containers and analyzed for total metals.

250 mL is filtered and dried for total suspended solids analysis.

A 40 mL sample is taken from the waterway in an amber glass bottle.

Saline samples are filtered with a Dionex Onguard-Ag™ filter to remove chloride.

Saline samples are analyzed for total organic carbon.

Freshwater samples are analyzed for total organic carbon.

Figure 12. Flow chart for water sampling, storage and analysis of total copper, dissolved copper, salinity, total suspended solids, and total organic carbon.
Samples for dissolved copper were collected and stored according to Standard Method 3010 (Eaton, et al., 1995). For dissolved copper, samples were stored in 60 mL high-density polyethylene containers. These sample bottles and their caps were soaked in 10% concentrated nitric acid for 12 hours, rinsed with Type II water three times, and allowed to air dry prior to sampling. Samples for dissolved copper were sub-sampled from total copper samples and filtered. The filter holders and syringes used in filtering were always washed thoroughly with detergent (Fisher Scientific; Raleigh, NC), rinsed with tap water three times, rinsed with Type II water three times, and allowed to air dry prior to filtration. After drying, 0.45 um filters (Gelman Sciences) were placed in the filter holders in the laboratory, coming into contact only with clean laboratory gloves. Filter holders and syringes were transported to the site in an airtight container, to avoid contamination (Benoit, et al., 1997). Filtering was completed while wearing clean laboratory gloves. The 500 mL samples were shaken thoroughly before the start of the filtering. The filters were then rinsed with 50 ml of Type II water, and 10 mL of sample, the filtrate of which was discarded. Following this, 40 mL of sample was filtered into the sample container. The samples were acidified to pH <2 by the addition of 50% trace metal grade nitric acid in Type II water. All sub-sampling for dissolved metals was completed within three hours of being collected or within three hours of the completion of the autosampling run, depending on the sample. All samples for dissolved metals were transported to the laboratory in ice to maintain a low temperature and avoid unnecessary water loss, and were stored in a refrigerator at eight degrees Celsius until analyzed. Samples were not held longer than six months before analysis.

The pH of the sample was determined directly from the 500 mL or one liter total copper sample using an Acumet Portable pH meter, Model 156 (Fisher Scientific; Raleigh, NC). All pH measurements were completed within three hours of being collected or within three hours of completion of the autosampling run, depending on the sample. The meter was calibrated with pH seven and ten solutions previously brought to the temperature of the samples. A check on the calibration of the pH meter was performed every three samples, and corrected immediately. The pH probe was rinsed thoroughly with Type II water in between each sample.
Salinity and total suspended solids were measured within a week from a 250 mL sub-sample of the total copper sample. A 250 mL graduated cylinder was rinsed thoroughly with Type II water. Then 250 mL was poured from the 500 mL or one liter sample bottle for total copper into the graduated cylinder. A hydrometer was rinsed thoroughly with Type II water and then allowed to float in the cylinder. After the hydrometer came into hydrostatic equilibrium with the sample, the salinity was read off the side of the hydrometer at the water level. Total suspended solids were determined immediately afterwards using the 250 mL used in the salinity analysis.

Total organic carbon was sampled by hand in 40 mL amber glass bottles with PTFE lined lids. The glass bottles were washed thoroughly with detergent, rinsed with tap water, soaked in 10% concentrated nitric acid for twelve hours, rinsed three times with Type II water, and allowed to air dry. The lids were washed in detergent, rinsed three times with tap water, rinsed three times with Type II water, and allowed to air dry. The samples were kept in ice during transport to discourage biological activity, and stored in a refrigerator at eight degrees Celsius until analysis.

B. Sediment Sampling

A flow chart describing the sediment sampling and analysis is contained in Figure 13. The corers used in sediment sampling were washed thoroughly with detergent, rinsed with tap water, allowed to soak in 10% nitric acid for twelve hours, rinsed three times with Type II water, and allowed to air dry. The rubber stoppers at the ends of the sediment corers were washed thoroughly with detergent, rinsed three times with tap water, rinsed three times with Type II water, and allowed to air dry. Cores were kept upright in a core case, and kept in a cooler in ice during transport to the laboratory. All cores were stored in a refrigerator at four degrees Celsius until each core was ready to be analyzed.

First, the core was taken from the refrigerator to the freezer and left overnight to freeze at –16° C. The cores were then removed and laid sideways to begin to thaw. A hard PVC pole was washed with detergent and rinsed five times with Type II water, and was used to push the core out of its plastic case and onto clean aluminum foil when the
Sediment core is collected from the waterway, and transported back to the laboratory and frozen.

Sediment core is sub-sampled into five sections according to depth.

Section 0
Section A
Section B
Section C
Section D

2 g of each section are transferred to digestion containers and tested for total copper.

2 g of each section are transferred to a weighing pan and tested for total, volatile, and fixed solids.

All remaining solids are thoroughly mixed and stored.

A 40 g sample is submitted to the soils laboratory for analysis of % silt, % clay, and % sand.

Figure 13. Flow chart for sediment sampling, storage, and analysis
sides of the core had thawed enough to permit transference, approximately 20 minutes later. When the core had thawed enough to be cut, a hard plastic spatula, soaked in 10% concentrated nitric acid for 12 hours and rinsed three times with Type II water, made each cut and transferred sections to 500 mL high-density polyethylene containers. These containers had been soaked in 10% concentrated nitric acid for 12 hours, rinsed three times with Type II water, and allowed to air dry. The spatula was rinsed with Type II water five times in between each section. Each of the 500 mL containers were then stored at eight degrees Celsius until analyzed.

The cores were cut into two-inch sections. The top section was ‘A,’ the next ‘B,’ and so on down to ‘D’. If any section had less than a complete two inches then that part was discarded. For the last two cores analyzed from each location, section ‘A’ was cut down the center vertically into two sub-sections. One of these sub-sections comprised ‘A,’ and the top one-inch of the other one comprised ‘0’.

Several two-gram sub-samples were taken from each container for total metals analysis. Two additional two-gram sub-samples were extracted for total, volatile and fixed solids analysis. All of these sub-samples were transferred to weighing dishes with the help of a glass stirring rod that had been washed thoroughly with detergent, rinsed with tap water, soaked for twelve hours in 10% nitric acid, rinsed three times with Type II water and allowed to air dry. The two-gram aliquots were measured by a Mettler H10 balance.

After the total copper and solids analyses were completed, the samples were amalgamated by site for % clay, silt, and sand analysis. The five remaining samples were well mixed and stored in 500 mL high-density polyethylene bottles that were soaked in 10% nitric acid for twelve hours, rinsed three times with Type II water, and allowed to air dry. These sediments were then sub-sampled for 100 grams of well-mixed sample, and dried at 103 C for twelve hours in aluminum pans, which had been washed with detergent, rinsed with Type II water three times, and allowed to air dry. Then the samples were sieved through a #10 sieve and stored in soil boxes designed for submission to a soil testing lab for analysis.

IV. Pre-concentration and Analysis Procedures
A. Water Samples

A complete set of quality control samples were completed along with the analysis, including travel blanks, analysis blanks, and analysis spikes. The results are listed in Appendix B.

Total copper analysis was performed according to SW-846 Method 3020A, entitled “Acid Digestion of Aqueous Samples and Extracts for Total Metals Analysis by GFAA Spectroscopy” (USEPA, 1997). Although copper was not specifically listed as an analyte routinely determined by this method, an official at the Environmental Protection Agency reported it to be applicable as long as appropriate quality control samples read acceptably (Fordham, 1997). All digestion containers, lids, funnels, volumetric flasks, and pipettes were soaked in 10% concentrated nitric acid for 12 hours, rinsed with Type II water three times, and allowed to air dry.

Blanks and sample spikes were digested for quality control purposes. Blanks were made of Type II water. Spikes were diluted in Type II water from a Fisher Scientific Copper Reference Solution, 1000 ppm ± 1% in 2% nitric acid, expiration date April 1999. All pipettes and volumetric flasks used in creating sample spikes, and the 60 mL high-density polyethylene sample bottles in which the spikes were stored were soaked in 10% nitric acid for twelve hours, rinsed three times with Type II water and allowed to air dry. Spikes were created with the use of a five mL autopipette designated solely for Type II water dilutions, and a one mL autopipette for the concentrated copper solution. Spikes were stored in a refrigerator at four degrees Celsius to prevent metal losses, and were discarded after three months.

In the first digestion step, the sample was shaken thoroughly, and 100 mL was pipetted by hand into a digestion vessel, either a 250 mL Erlenmeyer flask, or a 250 mL beaker with ribbed watch glass. Three mL of acid were added, and the sample was allowed to gently reflux with the acid until it reached a low volume. Another three mL aliquot of acid was added and again refluxed, adding more acid until the digestion was complete and evaporated to a volume of approximately three mL. The sample was then warmed with ten mL of Type II water for ten more minutes, and allowed to cool.
completely. The sample was transferred to 100 mL volumetric flask by funnel, carefully washing the sides of the digestion container twice with Type II water. The sample was brought up to 100 mL and shaken for two minutes to assure thorough mixing. The sample was then ready for analysis by graphite furnace atomic adsorption spectroscopy (GFAAS).

All dissolved copper samples were analyzed directly by GFAAS without digestion.

The graphite furnace was a Perkin-Elmer 5100, and was operated according to manufacturer’s specifications. All pipettes and volumetric flasks used for creating the standards, check samples, and blanks were soaked in 10% concentrated nitric acid for twelve hours, rinsed three times with Type II water, and allowed to air dry. They were diluted with Type II water from a Fisher Scientific Copper Reference Solution, 1000 ppm ± 1% in 2% nitric acid, expiration date April 1999 for the standards, and SPEX Certiprep, Alternate Metals I, ten ppm in 2% nitric acid, expiration date Nov. 1998 for the check sample. All checks, standards, and blanks contained 5% concentrated trace metal grade nitric acid to closely duplicate the matrix of the samples. Standards for 20 ug/L and 40 ug/L were used in conjunction with a Type II water blank to create a three-point curve. A check sample of 10 ug/L was then analyzed. If it did not register within 1 ug/L of its expected value, then the three-point curve was regenerated. The detection limit of the GFAAS for copper was 0.5 ug/L. Any measurement below 0.5 ug/L was reported as 0, or not detected, and all measurements above 0.5 ug/L were rounded to the closest integer.

After four successful sample analyses, the check sample and the blank were measured again. If the results did not register within 1 ug/L of their expected value, then the four previous data points were discarded and the three-point curve was regenerated. A sample registering within each 2 ug/L range was reanalyzed and analyzed with the addition of 10 ug/L to check the accuracy of the three-point curve. Approximately every 50 samples, a sample was measured four or more times to check reproducibility.

All dilutions were made with the use of a five mL autopipette designated solely for Type II water dilutions, and a one mL autopipette with a new pipette tip for each sample. Dilutions were made in centrifuge tubes that had been soaked in 10% concentrated nitric acid for twelve hours, rinsed three times with Type II water, and
allowed to air dry. These dilute samples were mixed thoroughly with the help of a touch mixer before analysis.

The pH of the solution was read directly from the readout of a calibrated pH meter, and salinity was read directly from the hydrometer. After the salinity measurement, total suspended solids were determined according to Standard Method 2540D (Eaton, *et al.*, 1995). An unused glass-fiber filter disk was inserted by tweezers into a filtering apparatus and secured over a suction flask. Three successive 20 mL aliquots of Type II water were then applied and pulled through the filter by suction pump. The filter was moved to an aluminum weighing dish and the whole placed in an oven to dry at 103 C. After two hours, the dish with filter were allowed to cool, and were weighed on a Mettler H10 scale to 0.1 mg. Repeating the drying and weighing process, the two weights agreed to within 0.5 mg or the process was repeated a third time. The filter was then replaced on the filtering apparatus and seated with the addition of a small amount of Type II water. Then the 250 mL sample was slowly pulled through the filter by suction pump. Afterwards, the filter was washed with three aliquots of ten mL of Type II water; following the last one, the suction was continued for three minutes. The double drying and weighing was carried out as before, with additional weightings if the two did not meet within 0.5 mg.

Total organic carbon was determined by Standard Method 5310B (Eaton, *et al.*, 1995). Chloride was a major interference in determining TOC by this method, so a pre-treatment to remove chloride was utilized in those samples from saline and estuarine environments. A Dionex ONGUARD-Ag™ filter was used to filter out the chloride. Due to the low loading rates of the filters and the high concentration of chloride in the samples, the saline and estuarine samples were diluted by a factor of five with Type II water for the analysis. The dilutions were made with the use of a five mL autopipette designated solely for Type II water dilutions, and a one mL autopipette with a new pipette tip for each sample. The five mL syringe used in filtering the samples was rinsed five times with Type II water. First, two mL of Type II water were flushed through the filter, and then three mL of sample, all of which were discarded according to manufacturer’s specifications. The following two mL were filtered and recovered for the analysis.
All samples at this point were treated similarly, except that values for the saline and estuarine samples were multiplied by five at the end to create their accurate value for TOC. A Type II water blank was run for quality control purposes. The TOC analyzer was a Dohrmann Carbon Analyzer Horiba PIR-2000, and was operated according to manufacturer’s specifications. A ten mg/L TOC standard was created for the analysis. The 500 mL amber glass container used for storing the standard was soaked in 10% nitric acid for twelve hours, rinsed three times with Type II water, and allowed to air dry. The standard was stored in a refrigerator at four degrees Celsius, and was discarded at the end of a week. The dilution was made with the use of a five mL autopipette designated solely for Type II water dilutions, and a one mL autopipette for the concentrated solution.

Samples were placed in 10 mL glass containers that were soaked in 10% nitric acid for twelve hours, rinsed three times with Type II water, and allowed to air dry. Two drops of phosphoric acid were added to reduce the pH to less than two, and the samples were purged for five minutes to remove all dissolved carbon dioxide, another interference. A one uL needle was rinsed three times with the sample, discarding the rinsate. Then the sample was measured to exactly one uL and inserted into the TOC analyzer. The process was run in duplicate and the two results agreed within 10% or the analysis was repeated. The results of this analysis are included in Appendix C.

All statistical analyses were performed with NCSS 97: Statistical System for Windows, Kayesville Utah, 1997.

B. Sediment Samples

A complete set of quality control samples were completed along with the analysis, including analysis blanks, and analysis spikes. The results are listed in Appendix B.

Total copper analysis was performed according to SW-846 method 3050, entitled “Acid Digestion of Sediments, Sludges, and Soils” (USEPA, 1997). Although copper was not specifically listed as an analyte routinely determined by this method, an official at the Environmental Protection Agency reported it to be applicable as long as appropriate quality control samples read acceptably (Fordham, 1997). All digestion containers, lids, ribbed watch glasses, funnels, volumetric flasks, centrifuge tubes, and
pipettes for sample transference were soaked in concentrated nitric acid for 12 hours, rinsed with Type II water three times, and allowed to air dry.

Blanks and sample spikes were digested for quality control purposes. Blanks were made of Type II water. Spikes were diluted in Type II water from a Fisher Scientific Copper reference Solution, 1000 ppm ± 1% in 2% nitric acid, expiration date April 1999. All pipettes and volumetric flasks used in creating sample spikes, and the 60 mL high-density polyethylene sample bottles in which the spikes were stored were soaked in 10% nitric acid for twelve hours, rinsed three times with Type II water and allowed to air dry. Spikes were created with the use of a 5 mL autopipette designated solely for Type II water dilutions, and a one mL autopipette for the concentrated copper solution. Spikes were stored in a refrigerator at four degrees Celsius to prevent metal losses, and were discarded after three months. Samples were spiked in the digestion containers, by the addition of one mL of liquid spike by autopipette. The spiked liquid was applied to the sediment and allowed to soak into the soil before digestion.

First step in the digestion, two grams of sediment were carefully weighed within the digestion vessel, either a 250 mL Erlenmeyer flask or a 250 mL beaker with ribbed watch glass, to within 0.01 grams on a Mettler PM 1000 balance. Ten mL of acid were added and mixed into the sample, and allowed to reflux with the sample for fifteen minutes. The sample was cooled, and twice received five more mL of acid and refluxed for 30 minutes. The second time, evaporation reduced the volume down to five mL. Three mL of hydrogen peroxide were then added and allowed to react with the solution. The hydrogen peroxide contained 0.03 ppm Cu. After the reaction had subsided, the solution was cooled. More hydrogen peroxide was added until the reaction was complete, usually indicated by a lack of vigorous oxidation with the hydrogen peroxide addition. No more than ten mL of hydrogen peroxide was added to the solution.

Samples were then transferred to 50 ml centrifuge tubes by way of funnel, carefully washing the sides of the digestion container twice with Type II water. The samples were centrifuged in a Beckman Model J-21C centrifuge for 20 minutes at 4000 rpm with an average force of 1200 G. The supernatant was poured off, the sample and sides of centrifuge tube were rinsed with Type II water, and the centrifuging was
repeated. After pouring off the supernatant the second time, the sediments were discarded.

The supernatant was poured into 100 mL volumetric flasks by funnel, and brought up to volume with Type II water. The samples were shaken for two minutes to assure thorough mixing. The sample was then ready for analysis by graphite furnace atomic adsorption spectroscopy. The procedure for analyzing samples by GFAAS was outlined in an earlier section.

The sediments were tested for total, volatile, and fixed solids according to Standard Methods 2540G. All evaporation dishes were washed with detergent, rinsed with tap water, and allowed to air dry before analysis, and were handled with tongs throughout the process. All weighing was done to 0.0001 g on a Mettler H10 scale, which was tared prior to each use. The drying oven was a Thelco model 28, and the Furnace was a Fisher Scientific Standard Furnace. The desiccant material was desiccated one day prior to the first use, and all analyses were completed within three months.

Aluminum pans were prepared by placing them in the furnace at 450 C for 45 minutes, and desiccating them for two minutes. They were then weighed before and after the addition of approximately two grams of wet sediment. The pans with sediment were dried for twelve hours at 103 C, desiccated and weighed again to determine total solids. Afterwards, the pans with sediment were ignited in the furnace for 45 minutes at 450 C, desiccated and weighed again to determine fixed solids. Volatile solids were determined from subtracting the fixed solids from the total solids.

The Soil Testing Lab of the Crop and Soil Science Department of Virginia Tech University determined the particle size distribution of the sediments. The analysis was for % clay, % sand, and % silt fractions.

All statistical analyses were performed with NCSS 97: Statistical System for Windows, Kayesville Utah, 1997.