

APPENDIX C. CHEMICAL METHODOLOGIES

I. SEDIMENT AND TISSUE MEASUREMENTS

A. FIELD COLLECTIONS

Sediment samples were taken with a chain-rigged Van Veen sediment sampler, and portions were removed from the top 2-cm of a single 0.1 m² grab sample for testing. The samples were analyzed for grain size, dissolved sulfides, total organic carbon, oil and grease, priority pollutant metals and priority pollutant organic compounds.

1. Grain Size.

Samples of sediment were removed from the Van Veen grab with a metal spoon, placed in plastic cups, transported to the laboratory, and refrigerated.

2. Dissolved Sulfide

Portions of the Van Veen sample were taken into an open-ended plastic syringe and placed in a pore water press. Pore water was squeezed into an attached plastic syringe containing 2-3 drops of 2 N zinc acetate solution. The mixture was placed in a polypropylene tube, capped excluding air, refrigerated, and analyzed within 16 hours.

3. Total Organic Carbon (TOC)

Test portions of Van Veen samples were removed with a metal spoon, placed in acid-rinsed amber glass bottles, covered with Teflon lined caps, and refrigerated. About 0.5 mL of H₂SO₄ was added as preservative.

4. Priority Pollutant Metals

Sample portions were removed from a Van Veen Sample with a plastic spoon, placed into acid-rinsed plastic cups, and frozen.

5. Priority Pollutant Organic Compounds

Sample portions were taken from a Van Veen sample with a metal spoon, placed into hexane- and acetone-rinsed glass containers and refrigerated. For field collection of tissue samples, see Chapter 8 (Materials and Methods).

B. LABORATORY PROCEDURES

1. Sediment Grain Size

Sediment grain size is used to normalize sediment chemistry data and to differentiate changes in benthic communities caused by the outfall from the effect of substrate type. Until 1996, sediment grain size was determined by the classical technique of particle sizing by sorting fractions through sieving and sedimentation. In 1996, manual methodology was replaced by an instrumental method using the Coulter LS 230 particle size analyzer (PSA) which measures particle size by scattering of light. Light scattering technology utilizes the relationship between particle size, wavelength and the angle of the scattered light that is transmitted through an optical system to a series of detectors. Laser diffraction particle sizing is done by analyzing the pattern of scattered light produced when particles pass through a laser beam. The angular range is from close to 0 to 160 degrees relative to the incident light beam. PSA incorporates both, laser diffraction and Polarization Intensity Differential Scattering (PIDS for the smaller particles 0.04-0.8um). The instrument calculates the results based on both the Fraunhofer and Mie theories of light scattering. It measures particle size from 0.04 um to 2000 um in a single scan using 116 size channels. A representative sample is introduced into the Fluid Module sample cell of the instrument. The sample is then dispersed in tap water and circulated through the measuring cell and the various particle sizes are determined by detection of scattered light and displayed by volume percent in differential distribution. Coarse material (gravel >2 mm) can introduce negative bias by blocking the imaging of the smaller particles. Samples with measurable particles in the higher range must be sieved with 2 mm (# 10) sieve prior to instrumental analysis. Specific particle size control sample are used for verification of the performance of particle size analyzer.

2. Dissolved Sulfide

Dissolved sulfide was determined colorimetrically using the methylene blue method from Standard Method 4500 S= D. A 7.5 mL portion of sediment pore water sample was transferred to each of two matched test tubes. Amine-sulfuric acid reagent and ferric chloride solution were added and mixed. If sulfide was present, a blue color appeared in the solution. After 3-5 minutes, diammonium hydrogen phosphate was added. Absorbance was measured after 3-15 minutes using a Shimadzu UV-Visible spectrophotometer at 664 nm. The results were compared with a standard solution of sodium sulfide, which was standardized with iodine and then diluted to the appropriate concentrations. The results were expressed in parts per million (ppm).

3. Total Organic Carbon

Total Organic Carbon was analyzed using the combustion-infrared method from Standard Method 5310 B, with modifications for solid/slurry samples. An Apollo 9000 Total Organic Carbon Analyzer with Model 183 TOC Boat Sampler for solid matrices was used.

Samples were analyzed in its original slurry state. Inorganic carbon was removed by reaction with acid prior to analysis. Portions of each sample were dried at 105°C to determine the percentage dry weight. Total organic carbon was then calculated based on dry sediment weight

and reported as mg/Kg.

4. Cyanide

Cyanide was analyzed using the semi-automatic colorimetric measurement method from EPA Method 335.4. Cyanide, as molecular hydrogen cyanide (HCN), is released from samples containing cyanide by means of a reflux-distillation operation under acidic conditions and absorbed in a scrubber containing sodium hydroxide solution. The cyanide in the absorbing solution is then determined using the Lachat Flow Injection Analyzer (FIA).

5. Ammonia Ammonia values were determined following EPA protocol 350.1 for NH₃, utilizing the Lachat Flow Injection Analyzer (FIA)

6. Acute toxicity Receiving water samples were evaluated for acute toxicity utilizing the Mysid *Mysidopsis bahia* following the EPA Protocol “Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms”, (EPA-821-R-02-012, 2002). Percent survival was the reported endpoint.

7. Chronic toxicity Chronic toxicity was evaluated using the Red Abalone *Haliotis rufescens*, following the EPA method described in “Short Term Methods For Estimating The Chronic Toxicity of Effluents and Receiving Waters To West Coast Marine And Estuarine Organisms”, (First Edition, August 1995, EPA-600-R-95_136). Chapman, Denton, and Lazorchak. Endpoints were reported as Chronic Toxicity Units (TUc).

8. Priority Pollutant Metals

a. Sediments

All priority pollutant metals in sediment samples were analyzed by EPA method 200.7 using a Varian Vita Pro inductively coupled plasma spectrophotometer (ICP). Prior to analysis of all metals, except mercury, the sediment samples were digested according to EPA method 3050. This digestion procedure was used to prepare sediment, sludge, and soil samples for analysis.

The sediments were pre-dried in 60°C before sample preparation. Approximately 2 grams of well-mixed sample was digested according to EPA method 3050 and diluted to 100 mL in volumetric flasks with distilled deionized water.

Mercury was analyzed by cold vapor technique in Standard Method 3112 with a Cetac M6000 mercury analyzer. Approximately 1.0 gram of pre-dried sample was digested with acids in the presence of potassium permanganate. The digested sample was diluted to 50 mL with deionized distilled water.

Reagent blanks were run throughout the preparation and analytical procedures. Necessary corrections of the data were made using reagent blank results. The sediment results were expressed as mg/kg of dry sediment. Approximate detection limits of various metals analyzed according to the above conditions, are presented in Table C-1. To check the precision and accuracy of analytical data,

a duplicate, a spiked sample, a QC sample, a NIST standard, and a reference sample were run with each batch or every ten samples analyzed, whichever was more frequent.

All reagents were analytical reagent grade. The water was distilled and deionized. The glassware and plastic containers were cleaned by soaking in 30 percent nitric acid solution overnight, rinsed with distilled water, and rinsed again with deionized water.

b. Tissue

Metals in tissues were analyzed essentially by the same procedures as described above for sediment metal analysis, except for a few modifications. The modifications are described below.

Frozen tissue samples were thawed and, immediately before sample preparation, the tissue samples were homogenized thoroughly using mortar and pestle. Approximately 2 grams of homogenized tissue was used for digestion by EPA Method 3050. The digested samples were diluted to 50 mL with distilled deionized water.

For mercury analysis, approximately 1 gram of tissue sample was used for digestion. The rest of the digestion and analytical procedures were the same as those described for sediment analysis.

All results were expressed as $\mu\text{g}/\text{kg}$ (wet weight). The approximate detection limits of metals in various tissues analyzed by the above conditions are presented in Table C-1.

9. Priority Pollutant Organic Compounds in Sediment and Tissue

Sediment and tissue samples were thawed, extracted with solvents and the extracts further processed to remove interfering substances and to concentrate analytes. Base-Neutral and Acid extractable organic compounds (BNA) were analyzed with a gas chromatograph/mass spectrometer (GC/MS). Organochlorine pesticides and polychlorinated biphenyls (PCB) were analyzed using a GC equipped with electron capture detectors (ECD).

Total organic halides (TOX) were analyzed by Mitsubishi TOX Analyzer using SW 846 Method 9020.

a. Sample Preparation and Extraction

Sediment and tissue sample preparation and extraction were done using SW-846 EPA Method 3545, Pressurized Fluid Extraction (PFE) procedure. A portion of sample was weighed and dried with anhydrous sodium sulfate to form a homogenized fine powder. Extraction was performed using a Dionex Accelerated Solvent Extraction (ASE) 200 model extractor. The powder was loaded into an extraction cell and extracted three times with 1:1 methylene chloride/acetone mixture for 5 minutes at 100 degrees Celsius and 1500 psi. The extract from the ASE was ready for BNA, Pesticides, and PCBs cleanup and analyses.

b. Base-Neutral and Acid Extractable Organics

The method for BNA in SW 846 EPA Method 8270 was followed. Prior to quantitative analysis, the ASE extract was cleaned by acid-base partition. Sample extract was adjusted to a pH > 12 to “salt” out the acids. The Base/Neutral components were extracted 3 times with methylene chloride. The organic phases were combined and concentrated to an appropriate volume. This was followed with a florisil cleanup used to further reduce matrix interferences. The aqueous phase, after the extraction at pH 12, was adjusted to pH 2 to convert salts back to acids, and then extracted with three portions of methylene chloride. The acid portion extracts were combined with the B/N portion before concentrating to 1 mL for GC/MS analysis.

A Hewlett Packard 5890 GC equipped with a 5972 MSD, an auto sampler model 7673 and a 30 m x 0.25 mm i.d. DB5-ms fused silica capillary column was used for identification and quantification by means of internal standards. It was operated in the electron-impact-ionization mode and checked with decafluorotriphenylphospine.

c. Organochlorine Pesticides and Polychlorinated Biphenyls (PCB)

The procedure given in SW 846 EPA Methods 8081 and 8082 was followed to determine pesticides and PCB. The methylene chloride/acetone extract of tissue or sediment was concentrated and solvent exchanged to hexane. This extract was placed in a separatory funnel and extracted several times with hexane saturated acetonitrile. The acetonitrile extracts were dissolved in water and extracted twice with hexane. The combined hexane extracts were washed twice with water and dried by passing through anhydrous sodium sulfate. The extract was reduced in volume with a K/D concentrator, cleaned through a Florisil column, and separated into two fractions by eluting the column with 6% ether/hexane and 50% ether/hexane. The volume of each fraction was further reduced in a K/D and placed in a vial for pesticides and PCB determination using GC/ECD.

Analysis was performed on a Hewlett Packard Model 5890 gas chromatograph equipped with dual ECD detectors and a HP Chem Station. Two fused silica capillary columns (DB5 and DB1701) were used for identification and confirmation. Quantification was relative to external standards.

Table C-1. Method detection limits of metals in sediments and tissues.

METALS	SEDIMENTS	FISH MUSCLE	FISH LIVER
	mg/dry kg	µg/wet kg	µg/wet kg
As	0.13	100	500
Cd	0.03	30	100
Cr	0.10	40	100
Cu	0.27	90	500
Hg	0.7*	219	100
Pb	0.25	110	500
Ni	0.32	70	500
Ag	0.04	20	50
Zn	0.59	1140	1000

* Mercury unit is in µg/dry kg.

II. LITERATURE CITED

American Public Health Association. 1998. Standard Methods for the Examination of Water and Wastewater, 20th Ed. American Public Health Association, Washington, D.C., unpagued.

USEPA 1986. Test Methods for evaluating Solid Waste: Physical/Chemical Methods. 3rd Ed. Revision Dec 4, 1996. Publication No. SW-846, Office of Solid Waste & Emergency Response, Washington, D.C.).

APHA. See American Public Health Association.

Plumb, R. H. Jr. 1981. Procedure for handling and chemical analysis of sediment and water samples. Technical Report EPA/CE-81-1. Prepared by Great Lakes Laboratory, Buffalo, NY. For US EPA/ Corps of Engineers Technical Committee on Criteria for Dredge and Fill Material. Published by the US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi: 3-28 - 3-47.

Royse, C. F., Jr. 1970. An Introduction to Sediment Analysis. Arizona State University. Units 1 - 6. 169 pp.