

Monitoring Methods for Estuarine Benthic Systems

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MONITORING METHODS FOR ESTUARINE BENTHIC SYSTEMS

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INTRODUCTION

The purpose of this report is to present methods and procedures for monitoring estuarine benthic systems. Emphasis is given to those features of benthic systems of significant ecological importance. While the experience of the authors has been within estuarine environments, the material contained herein is relevant to a wide range of marine sediments.

Because the authors strongly feel that meaningful monitoring procedures must be based upon an understanding of the systems being monitored, a description of marine benthic systems is provided. This report is divided into two sections which describe marine benthic systems and the methods and procedures for examining and monitoring these systems.

DESCRIPTION OF ESTUARINE BENTHIC SYSTEMS

Inorganics and organics are deposited in estuarine benthic systems. Inorganics, including sands, silts and clays, are introduced into estuaries from the ocean, upstream rivers and localized runoff. Organics originate from sources outside the estuary and from primary production within the estuary. The system which results from such deposits is illustrated in Figure 1 (Bella, 1972, 1973).

The significant chemical transformations with respect to the carbon, sulfur and nitrogen cycles are primarily mediated by bacterial metabolism with carbon from the deposited organics acting as the primary hydrogen donor. However, the type of bacterial decomposition occurring at any location is determined principally by the availability of hydrogen acceptors. When available, dissolved oxygen is used as the hydrogen acceptor. In its absence and with corresponding low Eh conditions, sulfate becomes the principal hydrogen acceptor. Because nitrate concentrations are nearly always far less than sulfate concentrations within estuarine systems, nitrate reduction, which will occur before sulfate reduction, is usually not



Conceptual Diagram of Chemical Transformation in Estuarine Benthic Sediments Figure 1.

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significant in comparison to sulfate reduction.

The availability of exogenous hydrogen acceptors (DO and sulfates) depends on the mixing and advection within the deposits. Vertical mixing which transports exogenous hydrogen acceptors results from physical turnover of the deposits, and molecular diffusion. Advection through the deposits depends on the permeability of the deposits and the direction and magnitude of the hydraulic gradient.

The Sulfur Cycle

The availability of hydrogen acceptors and organics determines the nature and extent of bacterial decomposition which, in turn, largely determines the quality of the interstitial and interfacial waters. If the input of organics to deposits exceeds the transfer of DO, aerobic decomposition will not be sufficient to decompose all of the organics. Sulfate reduction will then proceed below the aerobic region. The reduction of sulfates by heterotrophic sulfate-reducing bacteria which utilize the sulfate ion as a terminal hydrogen acceptor (Baas-Becking and Wood, 1955), results in the release of hydrogen sulfide which is found in solution as part of the following pH dependent system:

$H_2S \stackrel{+}{\rightarrow} HS^- \stackrel{+}{\rightarrow} S^=$.

In the present discussion, all components of the above relationship will be defined as "free sulfide". At a pH of 6.5-7.0, the free sulfide is approximately evenly divided between H_2S and HS^- with S^- being negligible. Free sulfides are also produced during anaerobic putrefication of sulfur containing amino acids, but normally this process is of negligible importance in the marine environment (Cline and Richards, 1969; and Fenchel, 1969).

Free sulfides form insoluble compounds with heavy metals, particularly iron. Free sulfides quickly react with available iron within the deposits to form ferrous sulfide, FeS, which gives benthic deposits their characteristic black color (Berner, 1969). The input of this iron into the deposits results primarily from the deposition

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of insoluble inorganics which contain ferric oxides and other insoluble forms of iron (Berner, 1967). Not all of this iron, however, is available to react with the sulfides. Other heavy metals such as zinc, tin, cadmium, lead, copper and mercury all have solubility products significantly below that of ferrous sulfide which indicates that ionic concentrations of these metals within the interstitial waters are not likely to be significant. However, the distribution of heavy metals between the crystalline phase, the adsorbed phase, the organic phase, precipitates and free ionic species is largely unknown for high organic, sulfide-bearing sediments.

Free sulfide concentrations within benthic deposits will remain at low levels (generally below 1 mg/l) when available iron is present. If available iron is sufficiently depleted, free sulfides within the anaerobic regions of deposits will increase until their production at a given location is balanced by the advective and diffusive transport out of that location and by the loss caused by reaction with any remaining available iron. Thus, when the available iron is consumed, free sulfides may diffuse to the aerobic regions of the deposits and into the overlying waters. Physical disruption such as dredging of the deposits may also lead to the release of free sulfides.

Within aerobic regions, free sulfides will be oxidized (Cline and Richards, 1969; Chen and Morris, 1971; and Ostlund and Alexander, 1963). Half lives of free sulfides in aqueous solutions have been reported from 15 minutes to 70 hours. Several studies have described the oxidation of free sulfides to occur via second order kinetics (Cline and Richards, 1969; and Chen and Morris, 1971); however, in natural environments such a description is a simplification of an extremely complex chemical reaction for which temperature, pH, and initial oxygen and sulfide concentrations are all factors affecting the rate of oxidation. The oxidation of free sulfide is catalyzed by the presence of metallic ions, such as Ni, Mn, Fe, Ca, and Mg, and is accelerated by some organic substances such as formaldehyde, phenols, and urea. Thus, the oxidation of free sulfides in estuarine and marine water may

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be much more rapid than in distilled water due to the presence of catalysts. Within oxygenated sea water the half life of sulfide has been reported to vary from ten minutes to several hours (Cline and Richards, 1969; Ostlund and Alexander, 1963). Studies indicate that estuarine waters stored for a period of time after collection will display slower oxidation rates of free sulfides than freshly collected waters (Korpalski, 1973). Since HS⁻ predominates at the pH of sea water, it has been proposed that the oxidation proceeds by the following reaction (Richards, 1965):

$$2HS^{-} + 20_{2} \rightarrow S_{2}0_{3}^{=} + H_{2}0.$$

Following the above chemical oxidation, the thiosulfate ion is more slowly oxidized to sulfate, probably with the intermediate production of other oxidized forms. Sulfur oxidizing bacteria of the genus <u>Thiobacillus</u> appear to be important in this final oxidation step (Ivanov, 1968)(Sorokin, 1970).

If the deposits are physically overturned or flushed with oxygenated water, ferrous sulfide will be oxidized. The oxygenated overturned sediments will normally return rapidly to anaerobic conditions. A portion of the oxidized ferrous sulfide iron will be returned to the sediment as available iron which can further react with free sulfide to form more ferrous sulfide. Thus overturning or flushing of sediments leads to a recycling of available iron.

The oxidation of ferrous sulfide (FeS) may result in the formation of elemental sulfur which, under anaerobic conditions, will react with ferrous sulfide to form pyrite (Berner, 1970). This reaction proceeds very slowly with a time scale of months to years. The formation of pyrite, however, does mean that available iron can be relatively low. Thus the amount of ferrous sulfide may serve only as a rough indicator of the amount of available iron that has been used within a sediment and the limitations of such an indicator must be recognized. In addition, the iron that is incorporated into pyrite will not be recycled to free iron upon turnover since pyrite oxidation is very slow.

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The Nitrogen Cycle

Although nitrogenous species do not act as significant hydrogen acceptors, nitrogen is important in the chemical transformations within sediments since it acts as a nutrient. In this capacity, the nitrogen cycle can result in significant acute and chronic impacts.

Within the sediment, nitrogen primarily is released from bacterial decomposition of nitrogen-containing organics. Within the anaerobic portions, the nitrogen remains as ammonia (NH_4^+) and is probably sorbed to other organics, silts, clays and other precipitates. Within the aerobic portions the ammonia will be oxidized by nitrifying bacteria to nitrite and nitrate; the concentrations are so low that this does not probably result in a significant oxygen sink. Thus with high-nitrogen containing sediments, nitrogen will be released to the overlying water as either NH_4^+ , NO_2^- , or NO_3^- .

Significance of the Benthic System With Regard to Dredging Operations

General dredging operations can significantly influence estuarine benthic systems in a number of ways. Direct disruption of the deposits alters both the deposits and overlying waters. As an example, an investigation by the authors on a dredging operation within the Siuslaw Estuary (Oregon Coast) indicated that free sulfide concentrations were measured within the overlying waters during dredging operations.

More difficult to observe are the chronic problems resulting (either directly or indirectly) from the results of dredging operations, such as deepened channels subject to river traffic or dikes which alter circulation patterns. As an example, the construction of a dike within a region of the Isthmus Slough of Coos Bay has dramatically influenced a large tidal flat area which now releases significant amounts of free sulfides to the upper regions of the deposits and to the overlying waters (Bella, et al., 1972). The release of free sulfides from the deposits is so

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high in this area that a purple color is clearly visible on the sediment surface and immediately below algal mats due to the purple anaerobic photosynthetic sulfur bacteria.

The following subsections will briefly describe the nature of environmental impacts from dredging related to the system described in Figure 1.

Release of Free Sulfides

High concentrations of free sulfides within the deposits and the release of free sulfides to the overlying water and atmosphere as a direct or indirect result of dredging operations can be environmentally significant for a number of reasons; among these are the following. First, the release of free sulfides can increase the benthic oxygen demand rate and thus lead to a decline in the aerobic zone of the deposit and a rapid lowering of the DO concentrations within the overlying waters.

Second, free sulfides, particularly hydrogen sulfide, are toxic at low concentrations to fish, crustaceans, polychetes and a variety of benthic micro-invertebrates (Fenchel, 1969; Servizi, et al., 1969; and Ivanov, 1968). Actual toxic concentrations reported in the literature usually represent only initial sulfide concentrations and thus may be too low due to chemical oxidation throughout the test period. In tests which maintained nearly constant conditions, hydrogen sulfide concentrations below 0.075 mg/l (pH 7.6-8.0) were found to be significantly harmful to rainbow trout, sucker, and walleye, particularly to the eggs and fry of these fish (Colby and Smith, 1967).

Oxygen Depletion

Dredging operations lead to the release of organic materials and inorganic materials (such as sulfides) which create an oxygen demand within the overlying waters. Under certain conditions, significant reductions of dissolved oxygen concentrations can result during dredging operations (Brown and Clark, 1968). In addition, dredging operations may expose benthic deposits of high oxygen demand

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which previously had been covered by relatively clean materials. The deposit of organic material suspended by dredging operations on the surfaces of benthic systems can result in the decline of the aerobic region of the benthic systems and an increased benthic oxygen demand. The reverse can also be true if dredging operations lead to the removal of polluted sediments.

The exact causes of oxygen depletion resulting from dredging operations are unknown even though a number of studies have been completed on the oxygen demand of resuspended bottom sediment (Seattle University, 1970; and Touhey, 1972). The reported insensitivity of the oxygen uptake rates to both organism concentrations and salinity strongly suggests that the majority of the demand is chemical in nature, not biochemical. The most probable species involved are various iron sulfides which are rapidly oxidized. Preliminary studies have shown that the oxidation of $10^{-3}M$ of FeS to Fe(OH)₃ and oxidized sulfur compounds occurs within several minutes in an aerobic environment.

The adverse impacts of low dissolved oxygen concentrations on a variety of pelagic and benthic organisms is well documented. The recycling of iron, sulfur and heavy metals that results from the oxidation of various chemical species, however, may be of equal or greater importance within estuarine ecosystems.

Release of Nitrogen Species

From anaerobic sediments, the primary soluble release will be ammonium ions which can have several effects. First, ammonium ions are toxic to a number of organisms at levels of only approximately 1 mg/l (McKee and Wolf, 1963). Values in the overlying waters in excess of 8 mg/l have been reported by Windom (1973) from his studies of resuspended sediments. Values several orders of magnitude larger than toxic levels have been reported for interstitial waters (Konrad, et. al., 1970; and Gahler, 1969). When this ammonia is released to the overlying waters, problems of algal growth can result.

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Sulfide-Iron-Heavy Metals Interactions

The release of heavy metals from polluted sediments as a result of dredging has been postulated by many authors and has resulted in specific criteria being developed by the EPA (O'Neil and Sceva, 1971; EPA, 1973). However, in sediments where sulfides are being produced, the possible chemical transformations from resuspension become quite complex. Presently it is unknown whether heavy metals will be released from sulfide bearing sediments.

Ferrous sulfides are common minerals in anaerobic sediments and are probably responsible for the characteristic black colors. Although many reactions have been postulated for their formations (Berner, 1971), the exact end products depend on a number of environmental factors and will be termed FeS_x for simplicity.

Preliminary studies have shown that heavy metals adsorb to both Fe(III) oxides and Fe(II) sulfides. In addition, the heavy metals are readily co-precipitated in both Fe(III) oxides and Fe(II) sulfides. From these results, it is hypothesized that heavy metals will not be released to the water column upon resuspension and either will be adsorbed, co-precipitated or precipitated and incorporated within the sediments; a similar hypothesis has been proposed by Windom (1973).

METHODS AND PROCEDURES FOR MONITORING MARINE BENTHIC SYSTEMS

Scope

Most of the following methods represent an attempt to observe certain chemical characteristics and their vertical concentration gradients within the estuarine benthic sediments. Particular emphasis has been placed on observing the sulfur cycle and its associated variables within the upper, more biologically active, layers. Typically, the gradients are large as illustrated in the three example core profiles shown in Figure 2. As a result, the following methods have been chosen to enable relatively undisturbed sampling and analysis with small samples.

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Only in this manner can representative, detailed core profiles be obtained. In general, these procedures represent techniques developed and refined at Oregon State University's Civil Engineering Department for monitoring the chemical state of the estuarine environment.

Siting of Sampling Stations

The importance of obtaining good representative samples cannot be overstressed; however, this is usually easier said than done. Special care should be taken when trying to obtain benthic samples from an estuary. Estuaries are difficult to sample because of the constantly changing currents and water elevation. Weather and commercial activity, such as log storage and transport, compound the problem.

Currently the effort required to obtain and analyze benchic core samples limits the amount of data that can be gained. It is, therefore, important to take steps to permit the relation of the detailed information from the specific site of the sample to the surrounding area. Knowledge of the bottom contour and the sample site location is necessary to interpret the results and to extrapolate the results to the surrounding area.

Contour of the Bottom

Experience has shown that the contour of the bottom should be known before taking samples. Without such data, sampling can be attempted on submerged, steeply sloping surfaces. Data from such sites are difficult to interpret because the bottom contour, current velocity and type of sediment are all interrelated. Consequently, it is important to know if the sample site is in an area of abrupt transition or an area of reasonably uniform slope. A small electronic depth finder with a chart recorder is normally adequate to provide contour information when detailed soundings are not necessary.

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Location

The ideal sample location from an operational point of view is next to a stationary object like a piling. Securing the sampling boat to a stationary object minimizes the problem of initial location and inadvertent changes in location from currents and wind during sampling. In addition, return to a site at a later time is much easier.

Frequently, no suitable stationary object is available adjacent to the selected site. In this situation the best that can be done is to take bearings on landmarks. Landmarks should be prominent and above high tide. Often bearings can be determined in the boat with adequate accuracy using a sighting compass.

Staying in position during sampling is always difficult. Two anchors, one at each end of the boat, are a minimum, and three are better. The anchor resisting the greatest force, either wind or current, must be the furthest away from the boat in order to hold.

For underwater sampling with SCUBA equipment, stations can be marked with "deadman" anchors which are screwed into the sediments. These anchors are easily located again if marked with colored flagging.

Sampling

Core sampling procedures have been developed for both surface and underwater sampling. When possible, underwater sampling is preferred because of greater reliability and visual examination of the sampling site. However, underwater sampling requires more time and equipment and is sometimes restricted in high currents.

Surface Core Sampler

<u>Description</u>. The sampler consists of the following major components: (a) frame, (b) sliding weight, (c) valves, (d) core tubes, and (e) core catchers. Total weight of the assembly is 46 pounds (Figures 3, 4, and 5).

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Figure 3. Core Sampler



Figure 4. Check Valve and Core Catcher Retaining Lanyard



The frame and sliding weight are low carbon steel weldments. Weighted Lavelle Rubber Company "korky" flapper tank balls are used as valves. Core tubes are fabricated from extruded acrylic tubing. The core catchers consist of two metal rings with a waterproof nylon fabric sleeve between them. A twisting action is imparted to the sleeve by stretched latex tubing attached to the sides of the rings.

<u>Operation</u>. A core tube is inserted down through the hole at the end of an arm of the frame. A wetted core catcher is twisted open and slid over the bottom end of the core tube. Loops of the core catcher retaining lanyards are slipped over the bolt lugs on the frame. The valve and core tube retaining plate are bolted in place. A metal bar is tightened in place over the valve. The above operation is repeated for the other two core tubes.

The sampler is lowered slowly to the bottom. At this time the depth of overlying water is measured by observing marks on the taut hoist line. Keeping the hoist line taut has been found to be good practice to minimize tipping over of the sampler due to current or sloping bottom. The sliding weight of the sampler is raised by pulling on the hoist line until the weight hits the upper limit of its travel. Often it is possible to hear the sliding weight strike both its upper and lower limits. Quick release of the line sends the weight sliding back down. Resultant impact between the weight and the sampler frame drives the core tubes into the sediment. Generally between 40 and 50 blows are delivered.

Extraction of the cores from the sediment is achieved by cranking the hoist winch until the line tightens, often causing the boat to list slightly until the sediment releases its grip on the core tubes. Occasionally it is necessary to rock the listing boat for several minutes until release occurs.

When the core is extracted from the sediment, the surrounding sediment drags the sleeve below the core tube end. The lanyards keep the upper ring above the bottom end of the core tube. Once the sleeve is below the core tube end, it

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twists closed and keeps the core in place.

After bringing the sampler on board, the core tubes are released from the sampler, core catchers are slipped out of the way and replaced with firmly inserted rubber stoppers.

Underwater Sampling

Underwater sampling is accomplished with two SCUBA divers. Twenty-inch long, two-inch diameter Plexiglas tubes are forced into the sediments and capped with rubber stoppers. The tubes are pulled from the sediments and the bottom of the tubes capped with rubber stoppers.

Sample Preservation

The shorter the interval between sampling and analysis, the better the results are likely to be. A mobile laboratory is being used to enable analysis of all parameters that are likely to be altered during storage.

Consideration must always be given to the effect time, cooling, freezing or vehicular vibrations may have on subesequent analysis. Freezing is not recommended for cores from which interstitial water is to be extracted for analysis. Extraction of interstitial water in the field as soon as possible after cores are collected is recommended. Core samples that are to be frozen are placed in a freezer in the mobile laboratory.

Sample Preparation

Sample preparation depends on the state of the core sample and the type of **analysis** that is to be performed. With the exception of cores used for "total **core**" analysis, cores are cut into disk=shaped sections. Cores assigned to intersti**tial** water analysis are further processed by squeezing the sections in a press. core Sectioning

Core sectioning is done in one of two ways. Unfrozen cores are sectioned in a core cutting fixture (usually in the field) and frozen cores are sectioned by sawing.

Sectioning Unfrozen Cores. The core cutting fixture consists of a stack of acrylic plastic rings held in alignment by an open sided 26 guage stainless steel trough. Dimensions of the rings are 2-inch (5 cm) inside diameter by 3 inch thick wall by various standard heights ranging from 1 cm to 8 cm. Rings are held in the trough with a few medium weight rubber bands. Cutter plates are fabrica ted from 2-3/4 inch wide 26 guage stainless steel sheet. One edge is sharpened and the opposite edge has a small 90° bend flange that allows pushing the cutter plate with the palm of the hand.

Rings are selected and assembled to yield the desired arrangement and section thicknesses. With a cutter plate held in position over the top of the stack, the core is pushed up into the stack from the core tube using a pushrod with a 5 cm diameter head. The cutter plate at the top of the stack provides a means of exerting a downward force on the stack and also positions the top of the core with the top of the stack of rings. A cutter plate inserted next to the bottom of the vertical stack retains the core after the pushrod is removed. A pair of cutter plates is inserted between rings at the top and bottom of each desired section. The section of core, contained laterally by the ring and sandwiched between two cutter plates, is withdrawn. If the section removed is not at the top of the stack, the overlying portion of core will slip down and neatly fill the vacancy.

The method is ideally suited to fine particle sediments. Cores containing pieces of fairly friable wood or bark particles have been successfully sectioned. The method does not, however, work well for sandy cores often because too much interstitial water is lost.

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<u>Sectioning Frozen Cores</u>. Frozen cores are removed from the core tubes by running hot water over the exterior of the tube until the core can be pushed out of the tube. The core is wrapped in Saran Wrap and placed in a sawing fixture. With the core in a horizontal position, sawing is done easily with a hacksaw. A large quantity of cores may justify the use of a power saw. Depending on the analysis to be performed, the core section can be wrapped in plastic and kept frozen or can be allowed to thaw in a closed container. This method is particularly suited to coarse-grained, sandy sediments and cores that are mostly wood chips.

Sediment Squeezing

Aqueous samples for interstitial water analysis are squeezed from the sediments using a specially fabricated "mud press". The press is essentially the same as that described by Reeburgh (1967). The inside diameter has been made 5 cm to provide compatibility with the core sample diameter (Figures 6 and 7).

A piston made from a modified laboratory rubber stopper has been incorporated between the diaphragm and the sediment to reduce diaphragm breakage. Breakage was caused by shell fragments and jagged woody particles. The discharge port has been equipped with a Luer-Lok fitting that allows syringes to be quickly and easily connected. The extract is collected directly in a syringe.

Total Core Analysis

A core sample may be divided into portions that are subsequently analyzed or it may be analyzed as an entity in itself. The latter case is herein designated "total core" analysis and includes determinations such as redox and pH.

Redox and pH

The following procedure makes it possible to measure the redox and pH of a relatively undisturbed whole core. In addition, the method of measurement allows

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Figure 6. Mud Press



Figure 7. Press-Cylinder Assembly

the observation of variations of these two characteristics with distance from the mud-water interface.

<u>Redox-pH Fixture Description</u>. The fixture is composed of a 5 cm inside diameter acrylic tube with three vertical sets of ports (Figure 8). Each set has twelve ports spaced on one inch centers. The inside diameter of the ports is 1/2inch. Rubber ended stoppers are used to plug each port.

<u>Redox-pH Operation</u>. The redox measuring system is checked by immersing the platinum electrodes and saturated calomel reference electrode in a fresh pH 7 buffer solution saturated with quinhydrone. The meter should read within 5 mv of the following values (Jones, 1966):

+47 mv at 20°C +41 mv at 25°C +34 mv at 30°C

Calibration of the pH meter is made with the same solution. Cores are temperature equilibrated by leaving them at room temperature overnight.

The upper end of a core tube is inserted into the recess in the bottom of the fixture. By applying a force at the bottom of the core, the core is pushed upward and into the fixture until an alignment mark is reached. Core temperature is measured with a mercury thermometer.

A saturated calomel reference electrode is inserted through the hole in the top stopper so that it makes good contact with the core.

Redox measurements are made first using the millivolt scale of an Orion Model 407 Specific Ion Meter. One platinum wire electrode is used in one set of vertical ports. The other platinum wire electrode is used in the other set of ports. Measurements are made alternately from one set of ports to the other set moving downward from the top. A combination of light force and gentle jiggling is often necessary to get maximum response and minimize the measurement difference between the two electrodes at the same elevation. Port plugs are removed and replaced before and after each reading. Between measurements electrodes are rinsed clean in distilled water and wiped with a tissue. -22-



Figure 8. Redox-pH Fixture

<u>Redox Calculation</u>. Redox (Eh) is calculated algebraically using the following formula (Society of American Bacteriologist, 1957): Eh = $E_{obs} + E_{sce}$.

where: Eh is the potential in my referred to the standard normal hydrogen half-cell.

E_{obs} is the average of the two platinum electrode readings in mv.
E_{sce} is the value of mv of the saturated calomel reference electrode corrected for temperature according to the following table (Ives and Janz, 1961):

Temperature in °C	E _{sce} in my
15	+250.9
20	+247.7
25	+244.4
30	+241.1

Interstitial Water Analysis

The following methods are used in analyzing the aqueous samples squeezed from the sediments. They have been selected or modified so that small samples, 10 ml or less, can be analyzed.

Chloride

<u>Apparatus</u>. The procedure is based on the use of the following pieces of Orion Research equipment: a chloride ion electrode, model 94-17; a single junction reference electrode, model 90-01; and a specific ion meter, model 407.

Procedure

- 1) Select a location that is free from drafts, temperature changes and vibrations.
- Set up specific ion probe assembly.
- 3) Place sample (1.0 ml maximum) in tall test tube. Add 0.5 ml of 30 per cent hydrogen peroxide and 1 drop 1 + 1 sulfuric acid. Cover the top of the test tube with aluminum foil.

- 4) Autoclave the sample for 30 minutes at 121°C and 15 psi.
- 5) Dilute (q.s.) the sample in a 100 ml volumetric flask and pour into a 150-ml beaker.
- 6) Stir.
- 7) Lower electrodes into sample.
- 8) Fill a thoroughly cleaned 2.0 ml pipette graduated in tenths of a ml with 50,000 mg/l chloride solution.
- 9) Adjust indicator needle to mid-scale with slope set at 100 per cent and temperature setting at room temperature.
- 10) Turn stirrer to medium speed.
- 11) Position tip of pipette within beaker such that it does not touch anything, and its discharge falls directly into the sample solution.
- 12) Add NaCl solution dropwise until desired reading is obtained on the "known addition" scale.
- 13) Record the volume of NaCl used and meter reading.
- Calculate concentration of unknown, applying a previously determined correction factor for salinity matrix effects.
- 15) Rinse and blot dry the electrodes before using again.

Pressed Sediment Analysis

Sulfates

Sulfates are determined turbidimetrically in accordance with Method 156C of the 13th edition of Standard Methods with the following additions or exceptions. Modifications have been made to make the procedure applicable to estuarine waters.

<u>Procedure</u>. A filtered field sample of nominal 10 ml size is preserved by adding two drops of concentrated HC1.

An aliquot of the field sample (normally 2 ml to 7 ml, depending on the expected sulfate concentration) is diluted to 100 ml with distilled water. The entire 100 ml diluted sample is used as described in the Standard Methods procedure. Generally the field sample sizes are too small to allow running blanks for sample color and turbidity correction. It is assumed that the error is minimized by filtration and subsequent dilution.

Turbidity is determined on a Beckman Model DB spectrophotometer with a light path of 1 cm.

Free Sulfide

Free sulfides (H_2S , HS^- and S^-) have proven very difficult to measure when the concentrations are low and the sample volumes small. Two methods have been successfully used. They are the "known subtraction" technique, and the "titration" technique. At this time, it is felt the titration technique is superior because it allows measurement of lower concentrations and minimizes the need to make an initial estimate of the concentration. Both methods are presented.

Known Subtraction Method. This method is fairly fast, but accuracy drops rapidly for field samples below 10 mg/1. At 10 mg/1, results are 9 per cent low and at 2.5 mg/1, they are 30 per cent low. A lower limit, using an appropriate correction factor for this method appears to be 1.0 mg/1. Failing to estimate the initial concentration range can result in the sample being wasted. The following procedure represents a supplement to Orion Research's procedures (Instruction Manual, 1969)(Application Bulletin, 1969).

Precautions must be taken to minimize the loss of sulfides. Both sediment sample extract must be protected from the atmosphere. A syringe containing 10 ml of 50% sulfide antioxidant buffer (SAOB) collects the extract during squeezing until about 10 ml are delivered. The exact volume must be recorded for later computation. The buffered sample is stored in a screw top test tube.

The procedure is based on the use of the following pieces of Orion equipment: a sulfide specific ion electrode, model 94-16; a double junction sleeve-type

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electrode, model 90-02; and a specific ion meter, model 407.

The following procedure is utilized:

- 1) Select a location that is free from drafts, temperature changes and vibrations.
- 2) Set up specific ion probe assembly with a nitrogen sparge tube made of flexible tubing. Pass tubing through third hole of electrode holder. Adjust sparge tube so that its discharge end is about 2 cm above the sensing end of the electrode.
- 3) Slip the electrodes and sparge tube through a piece of thin rubber sheeting with separate tight fitting holes for each item. The piece of sheeting should be large enough to cover the top and drape down the sides of a 50 ml beaker.
- 4) Adjust nitrogen to a slow bubbling rate using a beaker of water.
- 5) Place buffered sample (at room temperature) and magnetic stirring rod in 50 ml beaker. Volume of actual sample must be recorded.
- 6) Lower electrodes into sample leaving the sparge tube above the surface of the sample. Slide rubber sheet down to top of beaker. Position beaker so that the pour lip is toward you.
- 7) Stir briefly.
- Read potential using mv scale.
- Select concentration of complexing solution, lead perchlorate, Pb(Cl04), based on the mv reading obtained. See table below.

The following table is approximate and based on a combined volume of about 10 ml

of unknown sample of 50 per cent "sulfide anti-oxidant buffer" (SAOB).

Potential Reading (mv, negative)	Appropriate Concentration of Pb(C104) ₂ (molarity)	Equivalent Sulfide Con- centration (mg/l)
780	0.1	3200
750-780	0.01	320
730-750	0.001	32

- 10) Fill a thoroughly cleansed 1.0 ml pipette, graduated in hundredths and equipped with a discharge control device, with the appropriate concentration of Pb(Cl0₄)₂. Make final filling adjustment with tip of pipette touching inside of reagent bottle neck.
- 11) Place filled pipette in a stand that holds it vertically until needed.
- 12) Turn meter selector switch to S⁻⁻ position.
- 13) Adjust indicator needle to mid-scale (stirrer turned off) with slope at 100 per cent and temperature setting at room temperature.
- 14) Turn stirrer on to medium speed and fold rubber cover back a small amount.

- 15) Carefully position tip of pipette within beaker at the pour lip such that it doesn't touch anything and its discharge will fall directly into the sample solution. Reposition rubber cover.
- 16) Add Pb(Cl04)₂ dropwise until desired reading is obtained on "known increment" scale. Turn mixer off.
- 17) Fold back sheet and carefully withdraw pipette.
- 18) Record volume of Pb(Cl04)2 used and meter reading. Stable meter readings for low concentrations are slow to develop and are of short duration.
- 19) Calculate concentration of unknown.
- 20) Rinse and blot dry the electrodes before using again.

<u>Titration Method</u>. The titration method utilizes the same procedure as in the known subtraction method except for the step involving the addition of Pb^{++} . In that step, the Pb^{++} solution is added in 0.01 ml increments with a 2 microliter Gilmont burette and the electrode potential recorded after each addition. The equivalent sulfide is calculated from the breakpoint on the titration curve where the slope reaches a maximum negative value. With this method, free sulfide within estuarine waters can be measured accurately down to approximately 0.3 mg S⁼/1.

Soluble Organic Carbon

Soluble organic carbon as determined by the following method as the filterable total organic carbon passing through a Whatman No. 40 filter. The interstitial water passes through this filter during squeezing.

Organic carbon is determined by the method described by Menzel and Vaccaro (1964) which allows measurement down to 0.5 mg/l. The aspirated CO₂ from the glass ampoules are analyzed by the carbon analyzer marketed by Oceanography International Corporation.

Pressed Sediment Analysis

The following methods are used in analyzing samples of solid material taken from the sediments. They have been selected or modified so that small samples can be analyzed.

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Volatile Solids

Volatile solids are determined in accordance with Method 224G of the 13th edition of Standard Methods.

Total Nitrogen

Total nitrogen is run using a Coleman Model 29 Nitrogen Analyzer based on the Dumas Method. The sample is combusted in a CO_2 atmosphere in the presence of catalysts. The gas is passed through an alkali absorbing the CO_2 carrier leaving the nitrogen which is collected volumetrically in a micro-syringe.

Total Organic Carbon

Total carbon is run using Coleman Model 33 Carbon Analyzer. The sample is combusted in an O_2 atmosphere in the presence of catalysts. The gas is passed through various absorption tubes where water and other interfering gases are absorbed and the remaining CO_2 is absorbed in a tube containing ascarite. The ascarite tube is weighed before and after the analysis, the weight difference indicating the amount of carbon in the sample.

Total Sulfides

The method for determining total sulfides in solid samples is based on methods described by Jeffery (1970) and Standard Methods (1971) with the following exceptions:

- a. 0.100 N I2 solutions were used.
- b. 3-holed rubber stoppers were used on the reaction flasks, the extra hole being used for addition of reagents.
- c. 100 ml distilled water was initially added to the reaction flask, and the 3-flask sample train purged with N₂ for 15 minutes. A frozen 10-25 g sample was quickly added and the system repurged with N₂ before addition of H₂SO₄.

Total Sulfide Capacity

A 250 ml wide-mouthed Erlenmeyer sample flask (containing 100 ml of distilled water) and two 250 ml collecting flasks (each containing 5 ml of 2 N Zn(Ac)₂ and 95 ml of distilled water) were prepared for each sample. The sample flask had a rubber stopper fitted with a glass inlet tube extending below the surface of the liquid and two short glass tubes which extend just below the stopper. The latter tubes served as gas outlet and reagent injection ports. All three tubes were fitted with serum stoppers. Each of the collecting flasks had a gas inlet tube extending below the liquid's surface, a short gas exit tube, and a reagent addition tube fitted with a serum stopper. The flasks were connected with Tygon tubing; in the case of the sample flasks, gas entry and exit tubing were fitted with syringe needles in order to disconnect the flask from the train. A simple water-filled beaker placed after the last collecting flasks sealed the system.

In a typical run, the system was swept with pre-purified nitrogen for ten minutes. The sample flask was disconnected, tared, a frozen sample (10 to 20 g) added quickly, and the flask reweighed. Gaseous H_2S [Matheson] was introduced through the inlet tube and the exit tube was connected with Tygon tubing to an empty syringe. Metering was accomplished by observing the syringe filling to 150 ml. The sample flask was shaken during the addition of H_2S .

The sample flask was placed on a mechanical shaker for one hour, then purged with nitrogen which was introduced through the entry tube and vented through an exit tube fitted with a syringe needle. The progress of the purging was followed with moistened $Pb(Ac)_2$ paper until a black coloration was absent. This required a period of 2 to 3 hours. The flask was reconnected to the collecting flasks, nitrogen again swept through the system, and 10 ml of concentrated H_2SO_4 added with a syringe. The flasks then were shaken occasionally during a subsequent 1.5 hour purge time. A white precipitate of ZnS, somewhat foamy at first, accumulated in

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the collecting flasks. The nitrogen was shut off, and enough excess standardized iodine solution (0.100M) was added to the collecting flasks to impart a yellowbrown color to the solution. Shaking was required to hasten the reaction, and a yellow precipitate of sulfur was noted. The flasks then were acidified with 2-3 ml of concentrated HCl, combined into a 500 ml Erlenmeyer flask, and titrated with standardized 0.025 N thiosulfate using a starch indicator. Details of solution preparation may be found in Standard Methods, Section 228. A blank was carried through the same procedure.

Pyrite Iron

Wet samples are dried overnight at 100-110° C. Any lumps are crushed into a fairly homogeneous sample to facilitate extraction. A 0.50 to 1.00 g sample was weighed to the nearest .01 g, then placed in a 250 ml flat-bottomed flask. Addition of 25 ml of 50% HCl solution and two boiling beads was followed by refluxing for two hours. Bumping was common for sandy samples; fine samples boiled gently without bumping. Following reflux, the inside of the condenser was washed into the flask with distilled water; any material on the joint was also rinsed into the flask. Often some material clung to the cold finger of the condenser; this was washed into the flask.

The slurry was then filtered through 0.8μ Millipore filter paper, and the residue rinsed thoroughly with distilled water. The clear filtrate was analyzed for iron by diluting to 250 ml with distilled water, then diluting again by a factor of ten. More dilution was required to obtain the proper concentration region for atomic absorption analysis.

The residue, including the filter paper, was returned to its original flask using about 10 ml of distilled water to wash material down the sides; 25 ml of concentrated HNO₃ and two boiling beads were added, and the mass again refluxed for two hours. After cooling, the condenser, joint, and cold finger were rinsed and the contents poured into a 100 ml graduated cylinder. This was allowed to

settle overnight (it may also be filtered) to clarify. This solution was checked by atomic absorption to see whether further dilution was required.

The analysis was done with a Perkin-Elmer 290 atomic absorption spectrophometer. The wave-length desired was 2483 Å, which corresponds to a Select Element setting of 143.8. Standards (0, 5, 10, and 20 mg/l Fe) were used in calibration Samples were diluted to give readings in the 0 to 10 mg/l region which followed Beer's Law. All pyrite values were expressed as FeS-S equivalent (0.5 times the actual FeS₂ sulfur concentration).

Ferric Hydroxide

Ferric trihydroxide $(Fe(OH)_3)$ is formed in the air oxidation of FeS in sediments. Iron is oxidized from the <u>+2</u> to the <u>+3</u> state during the reaction. Iron of either oxidation state is readily soluble in sodium citrate solutions, but the <u>+3</u> state is preferentially complexed.

Citric acid is a tricarboxylic acid which is converted to its salt by reaction with base:

COOH
 COO Na
 COOT

$$CH_2$$
 CH_2
 CH_2
 CH_2

 HO-C-COOH
 BASE
 HO-C-COO Na
 F_e^+3
 CH_2
 $COOH$
 $COO Na$
 $COO^-/$

Complexing occurs due to the strong interaction of the trivalent cation with the citrate anion. Since the Fe^{+3} - complex is water soluble, citrate is readily _sable in solubilizing rather insoluble ferric salts. This is also useful since this allows iron analysis by atomic absorption.

<u>General Procedure</u>. FeS is produced by the reaction of ferrous ammonimum sulfate with sodium sulfide. This precipitate is washed to exclude excess reactants. FeS or sediment samples are added as a slurry to centrifuge tubes. Addition of buffered sodium citrate solution followed by heating dissolves the $Fe(OH)_3$ Centrifuging the sample and filtering the supernatant liquid gives a sample ready for analysis by atomic absorption.

<u>Solution</u>. All solutions are made by dissolving the salts in distilled water. They should be purged with nitrogen (using an air stone) for 15 minutes as soon after preparation as possible.

- S⁻ Dissolve 320 g. of Na₂S · 9H₂O in 750 ml water, and dilute to 1 liter. This gives a solution that is 1.3 M in S⁻.
- Fe⁺² Dissolve 196 g. of Fe(NH₄SO₄)₂ · 6H₂O in 750 ml water, add l ml of concentrated sulfuric acid and dilute to l liter. This gives a soluthat is 0.50 M in Fe⁺².
- Citrate Dissolve 147 g. of Sodium Citrate 2H₂O, 0.54 g. NH₄Cl, and 12 g. Ascorbic Acid in 750 ml water and dilute to 1 liter. Adjust the pH to 8.5 using NaOH. The NH₄Cl is used for buffering; the ascorbic acid is used as an antioxidant to prevent FeS oxidation.

NaCl - Dissolve 50 grams of NaCl in 300 ml water. Dilute to 500 ml. Distilled Water - Purge with N_2 .

<u>Standards</u>. In N₂ purged glove box, mix 10 ml of Fe⁺² solution with 5 ml S⁼ solution in a 50 ml centrifuge tube. Add ~ 30 ml distilled water; cap tube and shake well. Centrifuge the solid FeS to the bottom and pour off supernatant liquid. To solid, add 10 ml NaCl solution and 30 ml water and shake well to dislodge solid from bottom; centrifuge, pour off liquid and repeat washing at least five times. Check for residual ions by adding supernatant liquid to separate solutions of Fe⁺² and S⁼.

Wash precipitate into 100 ml volumetric flask, add 15 ml citrate solution and fill to mark. Shake well to suspend FeS and pour 40 ml into a clean centrifuge tube. Cap tube and heat at 85-87°C for 45 minutes, shaking the tube every 5 to 10 minutes. Remove from heat and cool to room temperature. Centrifuge and filter the supernatant liquid through a 0.45 μ Millipore filter. Analyze for iron by atomic absorption, making dilutions if necessary to keep sample in 0-10 mg/l range. Three FeS blanks should be run.

<u>Sediment Samples</u>. In glove box, add 20 ml water to weighed 10 gram frozen sediment sample in beaker; add sediment slurry to 100 ml volumetric flask and repeat procedure used for standards. Very high Fe(OH)₃ levels may require additional citrate; standards will have to be analyzed at this higher citrate level.

<u>Discussion</u>. Analysis of $Fe(OH)_3$ using known samples gives values that are within 10% of real values. FeS blanks without $Fe(OH)_3$ give values of ~10-15 mg/l Fe; these are subtracted from the known $Fe(OH)_3$ or sediment samples to give resultant values.

To determine mg/g Fe in sediment:

mg Fe(OH)₃-Fe/g-sediment =
$$\frac{(Fe_s - Fe_b)(0.10)}{wt. sample}$$

 $Fe_s = mg/l$ Fe in sample $Fe_b = mg/l$ Fe in blank

Particle Size

Sediment particle size is determined in accordance with "Standard Method for Particle-Size Analysis of Soils," ASTM Designation D422-63.

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