UNITED STATES DEPARTMENT OF THE INTERIOR

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TRACE ELEMENT ANALYSIS OF MARINE ORGANISMS

BY ANODIC STRIPPING VOLTAMMETRY (ASV)

By

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INTRODUCTION

Trace element analysis of marine organisms has generally been conducted by (1) sophisticated instrumentation (e. g., atomic absorption), (2) complicated cookbook laboratory procedures utilizing a host of chemicals and glassware, or (3) a combination of the above. All these procudures present problems to the investigator. For example, analysis by methods other than instrumentation are unable to detect minute amounts of various trace elements and in general are not extremely accurate. Problems common to all procedures are (1) relatively large amounts of sample (2 grams or more) are required for analyses of even the more common trace elements and (2) contamination of samples is almost a certainty due to complicated sample preparation procedures which include large amounts of glassware, chemicals and excessive handling of the sample.

The development of new anodic stripping voltammetry (ASV methods (Matson, 1968) has provided investigators with an instrument which (1) is capable of accurately detecting trace elements in minute quantities, (2) utilizes very small amounts of sample (0.01 mg), (3) works on a simple operating principle which simplifies sample preparation thereby reducing the possibility of contamination, (4) can be used aboard small boats, and (5) is relatively inexpensive (compared to other instruments used for trace element analysis). Recent ASV literature (Matson, 1968, Fitzgerald, 1970; Bradford, 1972) has dealt exclusively with the analysis of salt water and sediments and does not provide an adequate method for analysis of marine organic matter. This paper reports on an investigation designed to develop the methodology necessary for ASV analysis of organic material from the marine environment.

REGIONAL AND LOCAL SETTING

Marine organisms used in this study were collected from Corpus Christi Bay, Texas; a bay located on the south-central Texas gulf coast. Corpus Christi Bay is approximately 4 m deep and nearly equidimensional, covering an area of approximately 300 km^2. In the saucer-like central part of the bay, the sediments consist of fine silt and clays; sands composed mainly of shell debris rim the bay on shallow shelves. A dredged ship channel 15 m deep and 123 m wide nearly bisects the bay and terminates in a narrow, 13 km long, landlocked harbor, along which petrochemical and other industries are located.

Average tidal fluctuations in Corpus Christi Bay is .5 m. Wind tides, however, are common to 1-1.5 m and during hurricane conditions, tides of greater than 3 m have been recorded.

Water temperatures in Corpus Christi Bay vary seasonally from 10 to 28° C. Salinity ranges from 25-30 ppt during an average year.

MATERIALS AND METHODS

Marine polychaetes and crabs were collected for analysis. Polychaetes were collected using a grab sampler and a five gallon homemade bucket dredge. A minnow seine was used in collecting crabs.

Sediment samples were washed through a 1 mm mesh sieve. Calgon[1] served as a dispersing agent in preventing floculation of clay minerals. Mechanical picking and flotation techniques were used to separate polychaetes from shell hash (a residue of sieving sediment samples). Organisms were preserved in a 10 percent solution of formalin, or 40 percent solution isopropyl alcohol, or by freezing.

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[1]Trade names used in this report are given as examples of suitable equipment and are not endorsed by the Geological Survey.

All glassware was "conditioned" prior to usage. Conditioning of glassware consisted of refluxing each container with nitric acid for two hours. The glassware was kept covered with Saran Wrap to prevent contamination.

In the laboratory, extreme care was necessary to prevent contamination of samples by (1) keeping all glassware (test tubes and ASV cells) covered with Saran Wrap, (2) handling samples only when absolutely necessary and (3) cleaning any equipment to be used on the samples with dilute HNO_3 followed by deionized water. Samples were weighed on an Ainsworth (type 28N) electric balance accurate to .0001 g. All sample weights are wet weights.

A Quigley-Rochester sonic dismembrator (Model 121000) was utilized in breaking samples into small enough units as to assure complete acid digestion. Subsamples were treated with nitric-sulfuric-perchloric acid and refluxed on hot plates (Cole-Parkel, Mark I). NaAc-NaCl solution was used to buffer the subsample.

Trace element concentrations were determined for each sample by anodic stripping voltammetry (ASV) using the ESA Model SA 2011. Basically, the technique of ASV relies on concentrating electroactive metal ions onto a mercury film coated electrode by application of a negative potential (plating voltage) for a period of from 5 to 30 minutes. Following plating, the potential is varied linearly in an anodic direction and the metal ions are stripped out of the electrode. As each metal is returned to solution, a sharp current peak occurs, the peak height of which is directly proportional to the concentration of the metal ion in the mercury film. By plating standards under identical conditions as those used for the unknown, one can obtain accurate concentration values for the unknowns by peak height comparison. During plating all the metal ions which have formal potentials sufficiently below the plating potential are reduced into the electrode following the general reaction

$$M^{++} + 2e^{-} \rightarrow M^{o}.$$

The ASV technique is essentially reversible. There is less than 1 percent electrode memory and the analytical sample is not destroyed - it can be stored and rerun. The sensitivity and precision of the technique lends itself to the analysis of micro samples, and the simultaneous analysis of more than one metal in a sample.

Micro-pipetting was accomplished by using precision pipettes (Oxford Laboratories). Chemicals were purchased from G. Frederick Smith Chemical Co. and were double vacuum distilled to reduce trace element concentrations. All stock chemical solutions were analyzed ASV) to determine trace element concentrations.

RESULTS AND DISCUSSION

Presampling

Problem: Contamination of samples

Solution: Considerable thought, centered around the problem of sample contamination, needs to be given by the investigator before any sampling program is undertaken. It must be remembered that contamination could occur at any time during the investigation. Detailed solutions to this problem will be given throughout the rest of this section as they pertain to various methods, storage problems, etc. The investigator must be "contamination conscious" throughout the investigation.

Problem: Ionic exchange capacity of glass

A large amount of glassware is normally employed in any investigation of this type. Glassware is involved in sample storage, preparation, and analysis. Glass has an ion exchange capacity which presents two immediate problems to the investigator; (1) trace elements could "leach" out of the glassware into the sample, thereby contaminating the sample and/or (2) the glassware could "rob" the sample of certain trace elements by absorption.

Solution: Conditioning of glassware and reduction in amount of glassware used.

Conditioning of glassware to reduce its ion exchange capacity and remove trace metals is accomplished by refluxing each glass container with boiling nitric acid for two hours. Refluxing should be followed by the rinsing of each container with deionized water at least four times. Glassware treated in this manner is then stored upside down and covered with Saran Wrap.

Initially during this investigation, samples were transferred into many different glass containers (beakers) during sample collection, preservation, preparation and analysis. It became essential to reduce the amount of glassware used. This reduction served two purposes: (1) the less glassware used, the less chance of sample alteration and (2) the amount of laboratory time necessary for sample preparation was reduced.

For the organisms chosen to be used in this study, it was found that disposable test tubes served nicely as sample storage, preservation, and preparation containers. Twelve dozen test tubes were conditioned as described above, numbered, weighed, and wrapped in Saran Wrap. ASV cells were also conditioned and stored in a warm laboratory oven.

Sample Collection and Preservation

Problem: Collection of blue crabs (*Callinectes sapidus* Rathbun)

Solution: Use of minnow seine

Pulling a 3 m minnow seine (1.28 cm stretch mesh) in water depths from 0.3-1.0 m around the margin of Corpus Christi Bay provided all the blue crabs necessary for the study. Any number of seines, trawls or dredges would be effective in collecting blue crabs from any water depth or local environment due to the abundance of blue crabs.

Problem: Collection of marine polychaetes

Solution: Dredging, sieving, hand sorting or floatation techniques

A grab sampler capable of obtaining a sediment sample of 1/50 m^3 was first employed. Due to the scarcity of polychaetes in such a small sediment sample, a five gallon bucket dredge was constructed for the purpose of obtaining larger sediment samples.

The bottom of the bucket dredge was covered with 1 mm mesh wire screen. Because of the consistency of the mud bottom in Corpus Christi Bay, sediment would not wash through the wire screen, thereby filling the dredge. The sediment collected by the dredge was washed through a 1044 cm^2 sieve (1 mm mesh) aboard the boat. This reduced the five gallon sample down to mixture of shell hash and some mud. This sediment "residue" averaged about 900 ml in volume for every five gallons of mud sieved.

The polychaetes were sorted from the shell hash in two ways. First, hand picking the polychaetes was tried but proved difficult. Secondly, the shell hash was mixed with a 10 percent solution of Calgon, which was then mixed with water that had been saturated with sugar. The Calgon served to separate the clay minerals and prevent clay floculation. The sugar water was used as a flotation medium. When the shell hash plus Calgon was dropped into the sugar water, the shell dropped to the bottom while the polychaetes tended to float and could easily be revoved. (Note: flotation techniques work on the principal that most marine organisms have a density of 2.54 and shell hash, sediment, etc. have greater densities. A medium (denisty greater than 2.54) is provided such that the "light" marine organisms float while the "heavier" sediment sinks.)

Problem: Collection of tissue in blue crabs

Blue crabs, immediately after being collected, were frozen in the initial

stages of this project. It appeared, however, that some "drying out" of tissue occurred.

Solution: Immediate dissection of blue crabs in the field

Blue crabs were dissected in the field as soon after capture as possible. Instruments used for dissection were stainless steel and had been washed in deionized water and wrapped in Saran Wrap. A container of deionized water was transported to the field for rinsing of instruments during dissection.

The dissecting procedure was as follows: (1) remove all legs and place in a clean dissecting tray, (2) remove the carapace, exposing the internal cavity, (3) extract tissue samples from the liver, hypodermis, fluke meat, claw meat, eyes, gills, testes, heart, and carapace and place each tissue in individual test tube, (4) record wet weight of all tissue samples (Note: In sampling the carapace, algae must be removed from outer surfaces and the hypodermis from the inner surfaces).

Problem: Preservation of samples

Preservation of samples in the initial phases of this study was with 10 percent formalin or 40 percent isopropyl alcohol solutions. Efforts to accurately determine trace element concentrations (Pb, Cu) in these samples were fruitless due to nonreproductability of the results (Table 1).

Solution: Freezing of samples

After polychaetes and blue crab tissue samples were collected and weighed, it was convenient to freeze the samples in their test tubes thereby eliminating sample alteration by formalin or alcohol (Table 1).

Sample Preparation and Analysis

Problem; Leaching of trace elements from samples

Initially, simple acid digestion was thought to be the method by which free and organically bound trace elements could be put into suspension so their concentration could be determined. Two problems developed in this method; (1) the ASV unit works only when the sample has a pH in the range of 5-7, thereby restricting of the amount of acid that could be used for the digestion of the sample and (2) this reduced amount of acid (1 ml) was not enough to completely digest the sample, leaving a residue. (Note: An acid solution with a pH of 2-3 is necessary to free all organically bound trace elements). Sample preparation resulting in low pH values or incomplete sample digestion yielded nonreproductable results (Table 2) - results erratically high in value due to the effect of the acid on the ASV electrode.

Solution: Sonication, acid digestion, buffer solution

Each sample is removed from the freezer and allowed to thaw out after which 3 ml of deionized water is added (Note: all sample preparation is carried out in the original test tube used for sample storage). The sample is then sonicated (sonic dismembration) until no precipatation occurs within 3 hours after after being removed from the sonic dismembration. The time interval for a sample to be reduced to this state varied with amount of sample and, in the case of blue crab tissues, the toughness of the sample (e.g., carapace samples take several hours).

From the sonicated sample, a .1 ml subsample was removed and placed directly into a conditioned ASV analytical cell. In the ASV cell, the subsample is refluxed in 1 ml of boiling nitric-sulfuric-perchloric acid to dryness. A buffer solution of 1 M NaAc - 0.2 M NaCl (5 ml) is added to the ASV cell and the sample

is sonicated for 2 minutes. The sample is now ready to be run on the ASV. (Note: in the final calculations, the trace element concentrations in both the 1 ml of nitric-sulfuric-perchloric acid and the 5 ml of NaAc-NaCl solution must be accounted for before any sample concentrations can be determined). Samples prepared in the above manner give reproducible results (Table 2).

Problem: Trace element concentrations of the 1 ml of nitric-sulfuric- perchloric acid and 5 ml of NaAc-NaCl solution

Solution: Pipette 1 ml of nitric-sulfuric-perchloric acid into a clean conditioned ASV cell and boil on a hot plate to dryness. To the ASV cell, add 5 ml of NaAc-NaCl solution and sonicate for two minutes. This solution can now be run on the ASV unit and the concentration of trace elements determined. These values are then subtracted out of the gross sample values to determine true trace element concentrations in the organic samples. Determination of trace elements in these two solutions needs to be done daily (contamination of stock supplies may have occurred) and/or when new stock supplies of either solution are prepared. Table 3 illustrates the variation in trace element concentrations for four different stock solutions of the buffer solution (NaAc-NaCl).

Problem: Contamination of standards

Solution: Samples of the standard solutions should be run daily to check for contamination. If contamination is suspected, a new standard should be prepared (see Anonymous, 1972 for instructions on preparations of standards). Table 4 illustrates the effect of contaminated pipette inserted into a 20 ppm standard solution for lead and copper.

Problem: Sample contamination during sonication

Solution: After each sample has been sonicated, a test tube of deionized water should be sonicated for one minute. The sonic probe is stainless steel and cleaning by the above manner is sufficient. Change the water used for cleaning after each sample. Table 5 illustrates how 5 ml of deionized water become contaminated first by not cleaning the probe and secondly by not changing the cleaning water.

Problem: ASV analysis of trace element concentration in each sample

Solution: The procedure and instructions for operating an ESA Anodic Stripping Analyzer Model 2011 can be found in the instruction manual (Anonymous, 1972).

Problem: Accuracy of results

Solution: In evaluating this new method, there was a need to show not only reproducibility but accuracy. This was accomplished by comparing the quantities of trace metals in subsamples analyzed first by the ASV method and then by atomic absorption. The AA units were callibrated against EPA standard materials designed specifically for trace metal analysis. Table 6 illustrates the two methods were comparable in the accuracy of results.

SUMMARY

A procedure was developed for determining trace element concentrations in marine organisms by anodic stripping voltammetry. A general description of the procedure and precautions are outlined.

1. The investigator must become and remain "contamination conscious" in the initial planning of the project.
2. All glassware to be used in sample storage, preservation, preparation and analysis should be conditioned by refluxing with boiling nitric acid for two hours, rinsed with deionized water at least four times

and then covered with a plastic wrap. The amount of glassware used should be kept at a minimum.

3. All dissecting instruments or other equipment that comes in contact with the samples should be of stainless steel and continually washed down with fresh deionized water.

4. Samples taken in the field should be frozen as a means of preservation. The sample to be processed should be weighed while the material is fresh, preferably before freezing. (Note: In some cases such as the blue crab, dissection was necessary in the field to obtain fresh tissue samples.) Samples should remain frozen until the day they are to be analyzed. Only thaw out the number of samples that can be processed that day.

5. Preparation of a sample for analyses is outliend:

   a. Thaw out sample and add 3 ml of deionized water

   b. Sonicate sample for at least one minute before removing subsamples.

   c. Remove 1 ml (subsample) of sample and place in a conditioned ASV analytical cell.

   d. Reflux the subsample in 1 ml of boiling nitric-sulfuric-perchloric acid. Boil sample to dryness.

   e. Add 5 ml of NaAc-NaCl solution to ASV cell and sonicate for two minutes.

6. Run sample on ASV unit to determine trace element concentrations following procedures outlined in the instruction manual for the anodic stripping analyzer.

The procedure described in this report yields reproducible accurate trace element concentrations found in marine organisms (e.g., Table 7).

LITERATURE CITED

Anonymous. 1972. Instruction Manual for ESA Anodic Stripping Analyzer Model 2011. Environmental Sciences Associates, Inc. 28 p.

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Table 1. Trace element (Cu, Pb) concentrations in three samples of marine polychaete species A where each sample was preserved in solutions of 10 percent formalin (A), 40 percent isopropyl alcohol (B), or by freezing (C)

Sample	A					B					C				
Subsample	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Cu (ppm)	6	16	1	40	13	9	2	64	10	22	11	14	11	13	10
Pb (ppm)	74	19	26	31	52	15	34	92	70	83	20	22	22	19	21

Table 2. Trace element (Cu, Pb) concentrations in three samples of blue crab fluke meat where each sample was prepared for analysis by complete acid digestion (A), partial acid digestion (B), or by sonication, acid digestion (1 ml) and butter solution (C).

Sample	A					B					C				
Subsample	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Cu (ppm)	68	101	74	122	91	111	43	90	150	161	13	16	15	15	13
Pb (ppm)	116	150	94	202	171	83	124	93	38	107	19	19	20	18	17

Table 3. Trace element (Cu, Pb) concentration in four stock solutions of NaAc-NaCl buffer solution

Stock Solution	A			B			C			D		
Subsample	1	2	3	1	2	3	1	2	3	1	2	3
Cu (ppm)	2	2	1	1	1	1	2	4	4	1	1	2
Pb (ppm)	9	8	9	11	10	12	15	15	15	10	9	9

Table 4. Trace element (Cu, Pb) concentrations in 20 ppm standard solution for copper and lead before (A) and after (B) a contaminated pipette was inserted into the standard solution

Standard	A					B				
Subsample	1	2	3	4	5	1	2	3	4	5
Cu (ppm)	20	20	19	21	20	26	24	24	28	30
Pb (ppm)	20	19	20	20	20	31	33	33	28	30

Table 5. Trace element (Cu, Pb) concentrations in 5 ml of deionized water before contamination (A), after sonication with an unwashed probe (B), after sonication with a probe that had been cleaned in the same 50 ml of deionized water used to rinse off the probe after each of five samples (C), and after sonication with a probe cleaned in "fresh" deionized water after each sample (D).

Sample	A		B				C				D		
Subsample	1	2	1	2	3	4	1	2	3	4	1	2	3
Cu (ppm)	0	0	6	5	5	7	2	1	1	0	0	0	0
Pb (ppm)	0	0	9	9	8	9	7	6	7	5	0	0	0

Table 6. Trace element (cu, Pb) concentrations in various tissue samples from a female blue crab as determined by ASV and atomic absorption (AA) methods.

Sample	Liver			Eyes			Carapace		
Subsample	1	2	3	1	2	3	1	2	3
AA method									
Cu (ppm)	12	12	10	74	75	73	111	113	110
Pb (ppm)	3	3	3	27	27	29	166	165	168
ASV method									
Cu (ppm)	11	12	11	75	75	74	110	114	109
Pb (ppm)	4	3	3	28	27	29	167	165	170

Table 7. Trace element (Cu, Pb) concentrations in various tissue samples from a mature male blue crab seined from Corpus Christi Bay one mile west of the Naval Air Station.

Sample	Liver				Hypodermis				Fluke Meat			
Subsample	1	2	3	4	1	2	3	4	1	2	3	4
Cu (ppm)	8	8	7	8	122	121	122	123	30	29	29	31
Pb (ppm)	2	3	3	2	30	30	30	29	34	33	30	33

Sample	Claw Meat				Eyes				Gills			
Subsample	1	2	3	4	1	2	3	4	1	2	3	4
Cu (ppm)	19	18	18	18	85	86	85	84	10	12	11	12
Pp (ppm)	20	20	19	19	30	30	28	30	4	4	4	4

Sample	Testes				Heart				Carapace			
Subsample	1	2	3	4	1	2	3	4	1	2	3	4
Cu (ppm)	13	11	11	11	97	97	95	98	134	134	132	135
Pb (ppm)	4	3	4	3	13	13	12	13	182	181	185	183