

The Analysis of Cadmium, Chromium, Copper, Iron, Lead, and Manganese in Estuarine Waters

by

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Harbor Branch Foundation Technical Report No.22

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Preface

The development of reliable, accurate analytical techniques for the analysis of selected heavy metals in a seawater matrix was the first objective of the chemistry section of the Indian River Coastal Zone Study for the past year. Although the refinement of these analytical techniques is a continuing process, the basic procedures have been developed and are not likely to change substantially.

The purpose of this technical report is to discuss the instrumentation and analytical techniques that have been developed and that will be used for the analysis of cadmium, chromium, copper, iron, lead, and manganese in saline waters. The report has been divided into two main sections, the Methods and Materials section and the Results section. Each section was further divided into several subsections, each of which deals with a specific aspect of the analysis. The Methods and Materials sections is primarily a discussion of the theory and approach that was taken toward solving the unique analytical problems of heavy metal analysis in saline waters. The Results section is a further discussion and compilation of the results of that approach.

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HARBOR BRANCH FOUNDATION, INC.

TECHNICAL REPORT

The Analysis of Cadmium, Chromium, Copper, Iron, Lead,
and Manganese in Estuarine Waters

Introduction

Trace metals have been reported in nearly all major eastern seaboard estuaries and rivers (Bopp and Biggs, 1972; Bopp, et al. 1973; Cross, et al. 1970; Rahn, 1973; and others). McKee and Wolf (1963) attribute the presence of these metals to industrial and municipal processes. In certain chemical forms and concentrations, these metals may be acutely toxic or may exert chronically toxic effects on biological organisms (Frazier, 1972; Menzel and Ryther, 1961). In many cases, synergistic effects have been reported with two metals or metals acting with other toxicants and/or stresses (Vernberg and Vernberg, 1974).

To date, there have been only a few concerted efforts to assess the levels of trace metals in the water of the State of Florida. Among these, the State of Florida initiated a project that monitored the level of selected trace elements in water and sediment in an attempt to compile an overview of the general quality of the water in the State. Flame atomic absorption techniques were used and results reported as μg metal/liter (or ppb). A wide range of values were reported for the elements analyzed. Copper, for example, ranged from 0-1200 $\mu\text{g}/\ell$.

The National Pesticide Monitoring Program (1973) established four sampling sites in the state and reported values for As, Cd, Hg, Pb, and Se in fish and starlings. Most of the metals were not detectable but at the Gainesville station, Cd was reported to range between <0.05 - 0.20 $\mu\text{g/g}$ weight in starling.

Quick and Mackin (1971) reported values for some trace metals in oysters from the Suwanee River. The highest values reported were 0.75, 1.85, 4.84, 76, 0, 1.85, and 285 ppm for Cd, Cr, Cu, Fe, Hg, Pb, and Zn, respectively.

Mathis (1973) analyzed mangrove leaf and detritus and suspended sediment from the Barron and Shark Rivers for Cd, Cu, and Fe. High values reported were 1.7, 12, and 6900 $\mu\text{g/gram}$ for Cd, Cu, and Fe, respectively.

Values for dissolved metals were reported by Windom (1975) and the Department of Environmental Regulation (DER) (1975) for the St. Johns River and Tampa Bay. Windom found Cd levels up to 1.5 $\mu\text{g/l}$, and Fe up to 90 $\mu\text{g/l}$ in the St. Johns River. The DER reported Cr values up to 200 $\mu\text{g/l}$ and Pb up to 102 $\mu\text{g/l}$ for Tampa Bay.

Studies were initiated by Harbor Branch Foundation (1974-75) as part of the Indian River Coastal Zone Study. Water samples from various stations on the Indian River were analyzed for selected heavy metals. The data acquired were part of a qualitative survey and as such give only indications of the actual concentrations of heavy metals in the estuary.

These qualitative studies and the lack of quantitative information concerning heavy metals in Floridian water

determined the present objectives of the chemistry section of the Indian River Coastal Zone Study. These are, to assess the levels of trace metals in the Indian River estuary and to determine the flux of heavy metals in the estuary.

To accomplish these objectives it was necessary to develop the techniques for the analysis of trace metals in saline waters. The purpose of this technical report is to describe the techniques that have been developed for the analysis of cadmium, chromium, copper, iron, lead, and manganese in saline water.

Methods and Materials

Analytical Methodology

The primary tool used by this and many other laboratories for the analysis of heavy metals in solution is atomic absorption spectrophotometry (AAS). Atomic absorption spectrophotometry utilizes the property that light energy is absorbed by the atoms of the metal under analysis. To take advantage of this property, the metal to be analyzed must be presented to the light source in an atomic state. To do this, the sample is either aspirated into an acetylene-air or acetylene-nitrous oxide flame which atomizes the metal or the sample may be injected into an electrically heated graphite furnace. Temperatures up to approximately 3000°C are necessary to atomize most metals.

While the metal in the sample is in an atomic state, light at the appropriate wavelength from a hollow cathode or electrodeless discharge lamp is passed through the sample. The light-energy which is absorbed is in direct relationship to the quantity of metal present in the sample.

The instrument is designed with a photodetector sensitive to the appropriate wavelength. The instrument measures the difference electronically in light intensity between the source and the photodetector and interprets that difference as an absorbance. By using standards with known concentrations of the metal, the instrument may be calibrated and samples containing unknown quantities of metals analyzed. Those requiring a more rigorous discussion of atomic absorption are referred to Burrell (1975), Perkin Elmer (Nov. 1973) and others.

There are, however, many problems associated with this type of analysis. To begin with, solutions with high dissolved solids produce severe interferences that obscure the true signal from the metal. Seawater is such a solution. Since the Indian River is a high salinity estuary, it was necessary to develop techniques to combat the interferences from the seawater matrix. The development of these techniques will comprise the text of this report.

Instrumentation

A Perkin-Elmer Model 306 Atomic Absorption Spectrophotometer with deuterium arc background correction was the basic instrument used for the analysis. Since flame atomic absorption analysis of seawater is impossible due to severe interferences and preconcentration of the seawater is subject to contamination, it was decided that an HGA 2100^R (Perkin-Elmer) graphite furnace analysis was the best analytical approach.

A Perkin-Elmer Ramp Programmer^R was added to the HGA controller which allowed the analyst to control each temperature cycle.

To improve the precision of the analysis, a Perkin-Elmer AS-1 Autosampler^R was purchased to automatically inject the samples into the furnace. Extra pumps that inject 50 and 100 μl were also purchased for the AS-1 Autosampler^R.

The Perkin-Elmer Model 56^R recorder was used to display the output signal of the Model 306. A Perkin-Elmer PRS-6 Printer Sequencer^R was used to automatically print the absorbance value of the peak.

All of the 110 volt power supplied to the instrument was first passed through an Acme Electric Corp.^R voltage stabilizer to reduce spurious signals from power fluctuations.

Instrument Operation

Since the atomic absorption spectrophotometer coupled with the HGA-2100 and its ancillary equipment is a complex system, an outline of the setup procedure for operation is necessary. This may be supplemented with Perkin-Elmer manuals #993-9312, 990-9789, and 993-9022. This will be in the form of step by step instructions and copper will be used as an example. Basic familiarity with the location of the switches is assumed.

1. Right-hand panel: DECIMAL POINT at "1", MODE switch to "ABS".
2. Left hand panel: SIGNAL at "TC1"; FUNCTION at "ABS"; RANGE at "UV"; EM CHOPPER at "OFF"; PHASE at "NORMAL"; FILTER "OFF".
3. Turn on the AAS power by depressing the switch on the left hand panel of the instrument. Rotate the HGA out of the light path.
4. Find the copper hollow cathode lamp in its mounting bracket and plug it into the receptacle in the top left hand side of the instrument. Set the amperage to 15 mA with the dial source by reading the gauge behind the lamp position.
5. Set the WAVELENGTH SELECTOR to 324.7 and the SLIT WIDTH to 4. Adjust the lamp mounting bracket adjusting screws to maximize the reading on the energy meter.

- Adjust the wavelength selector dial until the energy meter is at maximum. This operation brings the lamp beam and wavelength into proper alignment. Bring the meter to midscale by using the GAIN control.
6. Remove the windows from the HGA and clean them. Replace the windows. Zero the DVM. Rotate the HGA back into the path of the light.
 7. Push the HGA release lever to the left. Using the adjusting screws on the HGA, adjust the HGA furnace until the reading on the DVM is the lowest obtainable. This reading for copper is usually between .060 and .080 absorbance. This procedure alligns the furnace in the light path.
 8. Release the two halves of the furnace by lifting up on the button on the right lower side of the furnace. Remove the old tube. Check for ash on the cones. If present, clean with moistened Kimwipe^R paper.
 9. Allign a new tube with the tool provided and close the two halves of the furnace. There will be an audible click as the safety catch is in place.
 10. Turn on the air purge on the deuterium arc background corrector and turn on the background corrector. The energy meter will go initially to zero, then will read some value when the deuterium arc lamp lights.
 11. The PHASE switch in the "NORMAL" position now reads the energy (on the energy meter) of the deuterium background corrector. In the "REVERSE" phase the energy meter will read the energy of the copper lamp.

12. Change the PHASE to "REVERSE" and note the position of the energy meter. Change the PHASE to "NORMAL" and note the position of the meter. The two should balance. If they do not,
13. "Increase" the "REFERENCE ENERGY" by depressing that button on the deuterium power supply. If the energy still will not balance, increase the SLIT to 5.
14. Go to step 12 and repeat, bringing the energy needle back to midscale with the "GAIN" control.
15. The deuterium arc background corrector and the source lamp should now balance.
16. The instrument is now ready for the HGA controller to be turned on.
17. Turn on the cooling water, the argon purge gas supply, and the exhaust hood.
18. Lift up the purge gas valve on the controller to its "ON" position. Check the gas flow on the metering device.
19. Turn the power on using the POWER switch.
20. Depress the "HIGH TEMPERATURE" button for approximately ten seconds or until the DVM rezeroes.
21. The HGA is now ready for the temperature programs to be set. The determinations of the correct temperatures are covered in the next section.
22. To set the correct temperatures on the controller, simply depress the button above each cycle (DRY, CHAR, ATOMIZE) and set the temperature with the dial.
23. Total cycle time is set with the lower dials.

24. To engage the ramp programmer, press the button over each cycle on the ramp programmer unit until it lights. The ramp time for the cycle is set with the dial.
25. The Model 56^R recorder should now be engaged (see also Perkin-Elmer Manual N-1167E). Turn the POWER switch on the recorder to "SERVO" and the HGA controller RECORDER switch to "MANUAL".
26. Set the RECORDER RANGE to 10 mV, POLARITY at "+", and the CHART SPEED to the desired setting.
27. Check to see that the DVM reads ".000" and set the recorder pen to zero baseline on the chart paper using the ZERO adjust knob.
28. Switch the HGA controller recorder switch to "AUTO". The controller will automatically engage the recorder ten seconds before atomization begins.
29. To align the AS-1 Autosampler, first releast the sampler arm by loosening the knob beaneath the sampler tray platform.
30. The arm must be rotated to its sample position, then to its inject position before the tip can be alligned. Do this by moving the arm with the large black knob on the left side of the AS-1 arm movement box.
31. As the arm approaches the HGA graphite tube, position it to enter the tube by turning the red-capped knobs on the front and side of the platform. The knobs position the sampler arm in-out and left-right, respectively.
32. To position the sampler to enter the tube the proper distance, which is about two-thirds the diameter of the tube, use the knurled lever on top of the arm movement box.

It screws in and out to raise and lower the injection tip. Check its position by using the dental glass to peer inside the tube.

33. Once everything is positioned properly, lock the sampler in position by retightening the knob beneath the sample tray platform.

34. There are three pumps and two positions on the AS-1 controller in which these pumps may be located. The position labeled "20 μl " will inject the rated volume of the pump (i.e., it will inject 100 μl from a 100 μl pump). If the pump is placed in the "10 μl " position, it will inject one-half of the rated volume of the pump (i.e., a 100 μl pump in the 10 μl position will inject 50 μl). Position the desired pump in position. For copper, the 50 μl pump is placed in the 20 μl position.

35. Make certain that the wash reservoir is filled with highly pure water. Rotate the sample tray to position 1. Set the "MULTI" dial on the AS-1 controller to the desired number of replicates to be taken from each sample cup.

36. To start the AS-1 and the HGA which is controlled by the AS-1, first press the red button on the AS-1 controller. The white button below it will flash while the pump is rinsing.

37. When it stops flashing, push the white button in to its'lock position to start sampling. It will automatically continue to sample position 30, but periodic checks are recommended.

Optimization of Temperature Parameters

The methods described in Perkin-Elmer manual 990-9972, "Analytical Methods for Atomic Absorption Spectroscopy Using the HGA-Graphite Furnace", were used initially to determine the optimum temperature settings for the determination of Cd, Cr, Cu, Fe, Pb, and Mn in aqueous solutions. Those who require greater detail may refer to this manual. This method may be summarized as:

1. Prepare a sample of the metal to be analyzed that will yield 0.25-0.40 absorbance units (the concentration needed may be calculated from the formula given in the manual). For example, a 10 $\mu\text{g}/\ell$ solution of manganese will produce an absorbance of approximately 0.370 using a 20 $\mu\ell$ injection volume.
2. Set the dry cycle to 100°C for 40 sec. for a 20 $\mu\ell$ sample and the char cycle at 300°C for 40 sec.
3. Atomize at 1000°C for 15 sec. Record the absorbance.
4. Increase the atomization temperature by 100 degree increments until no further increase in absorbance is noted. The optimum atomization temperature is the lowest temperature that will produce maximum absorbance. Atomization time should be long enough to allow the signal to return to baseline, otherwise a "memory" effect may occur.
5. To optimize the char cycle, set the atomization temperature and time to the optimum settings determined in #4. Increase the char cycle temperature incrementally until a decrease in absorbance signal is noted. The

optimum charring temperature will be the highest temperature that produces maximum absorbance. The charring time will depend upon the samples' volume. Large volumes require longer drying and charring times and increased dissolved solids often require longer drying and charring time as well as temperature ramping.

Although instrument settings are given in the Perkin-Elmer^R manual, they should be taken as general indicators only since temperatures with the HGA-2100 are dependent upon voltage supplied to the controller, which may vary from one laboratory to another.

Initially, aqueous standard solutions of the trace metals were analyzed to determine the optimum instrument parameters. Next, seawater samples were analyzed at absorbing and at non-absorbing wavelengths. The latter is necessary to assess the magnitude of the non-atomic signal resulting from the volatilization of the bulk salts which comprise a major portion of the seawater matrix. Non-absorbing wavelengths may be obtained from the Handbook for Chemistry and Physics (1975-76). The non-atomic signal is reduced as much as possible by adjusting the instrument parameters without reducing the absorbance signal due to the metal under investigation. The non-atomic signal should be reduced to a level that can be compensated for by the deuterium arc background corrector. This may not be possible, however, without chemical manipulation of the sample (e.g., matrix modification) as will be discussed in a following section.

Ramp Programmer

The temperature ramp accessory allows the operator to program the dry, char, and atomize cycles to reach their maximum temperatures gradually. This is especially useful when samples that contain large amounts of dissolved salts, such as seawater, are being analyzed and/or volumes of sample greater than about 20 μl are injected into the furnace. These samples have a tendency to "spatter" which results in poor reproducibility and may even contaminate the quartz windows of the furnace.

To eliminate this problem, the total drying cycle time is increased and the cycle is allowed to reach temperature slowly. Further, drying temperatures lower than 100°C are used. The total charring time is also increased and ramped slowly to maximum temperature. By careful adjustment of drying and charring total and ramp time, settings that dry and char the sample without spattering may be obtained. It should be noted, however, that the ramp setting is for duration of ramp only, the rate of change in temperature will be determined by the temperature to be reached. If the temperature is increased, for example from 500°C to 1000°C which occurs when changing the analysis from one metal to another, less volatile metal, the rate of change increases. This may produce spatter. Therefore, it is best to set the ramp using the least volatile metal to be analyzed. Changing to a lower charring temperature will only decrease the rate of change which will cause no problems with spattering. If the injection volume is not changed, then the only adjustments that

need to be made when analyzing different metals are the maximum charring and atomization temperatures and possibly the atomization time.

The atomization ramp was found to be useful for lead, but not particularly useful for the other metals. It is thought that the metals do not volatilize at discrete temperatures; rather, they will atomize at low temperatures (actually any temperature greater than the maximum charring temperature) but the atomization will be slower. By ramping the atomize cycle, the metal atomizes throughout the ramp cycle, resulting in a broad, poorly defined peak.

Contamination and Blanks

The contamination of samples is one of the greatest problems that can plague any analytical procedure. There have been many discussions of sources of contamination (Their, 1957; Patterson and Settle, 1976; and others) from which a general consideration of contamination may be obtained. Essentially, the sources of contamination may be divided into 4 sections. These are,

1. sample and reagent containers
2. reagents
3. airborne particles, and
4. instrument components

Each component of contamination was tested for its contribution to the total blank. An effort was then made to eliminate or minimize the contamination from the contributing component.

Sample and Reagent Containers

Sample and reagent containers are a source of contamination and may be conveniently divided into five components:

1) sample bottles, 2) volumetric flasks necessary for the method of additions, dilution when necessary, etc. 3) the autosampler cups in which the sample is held prior to analysis, 4) the teflon autosampler tip, line and rinse bottle, and 5) disposable Eppendorf pipette tips.

All containers including field sample bottles and AS-1 sample cups were washed using a modification of the procedure described by Patterson and Settle (1976). The cleaning procedure involves first a soap (Triton-x^R, 10%) and deionized water soak for three days at 70°C. Second, the articles are soaked in a 3:1 solution of nitric/hydrochloric acid (reagent grade) for 3-8 hours. Finally, the containers are filled with a 0.1 N HNO₃ solution made from redistilled Ultrex^R nitric acid and high purity deionized water. Between each step, the containers are rinsed with copious quantities of high quality water (approximately 18 megohm/cm resistance).

To evaluate the contamination contribution from the containers, Milli-Q water and Milli-Q water acidified to the same pH as the samples (to check for desorption of metals from the walls of the containers) were held for several hours in each type of container used, then analyzed.

Teflon Autosampler cups were washed using the procedure outlined above, and are stored in 0.1 N HNO₃. Before using the cups, they are rinsed with copious quantities of deionized water followed by a rinse with the sample just prior to use.

To evaluate the contamination resulting from the cups, a tray of cups containing blanks has been analyzed.

To evaluate the effect of the disposable pipette tips, water was injected from a teflon beaker that had been subjected to the cleaning procedure and rinsed with quantities of deionized water using the Eppendorf^R pipettes. Further, each pipette tip is rinsed with 0.1N HNO₃ followed by several rinses with the sample before using.

Reagents

Reagents are perhaps the largest source of contamination to the analysis. There are four reagents used consistently: 1) water, 2) nitric acid, HNO₃, 3) ammonium nitrate, NH₄NO₃, and 4) trace metal standards. The procedure used to control contamination for each of these reagents will be discussed separately.

Water

Water may be treated in a variety of ways to reduce contamination. The water used in this laboratory goes through three separate treatments before it is used for dilution, preparation of standards, etc. Water is first passed through a large industrial reverse osmosis unit before it enters our laboratory. In the laboratory, it is processed through a Continental^R reverse osmosis unit followed by mixed bed ion exchange columns, then through three mixed bed ion exchange columns (Milli-Q^R, Millipore^R Corp.). The Milli-Q^R unit is equipped with a resistance meter. The water was processed through the unit until the meter reached 18 megohms/cm.

Contamination from water was assessed by analyzing de-ionized water. Since we could detect no signal or at best a very small signal from the normal injection volume, the injection volume was increased to 100 μl , the maximum that can be injected into the furnace.

Reagents

Reagent acids used were Ultrex^R acids which are high purity acids. These acids were processed through a Quartz Products^R PB2 Highly Pure Water Generator^R, a quartz subboiling still. Redistilled acid is stored in the original quartz glass bottles in which the Ultrex^R acid was shipped. To determine the contribution from the acids, a water blank was prepared which contained ten to one hundred times as much acid as would be used in the samples. This method was used to produce a signal that could be detected using the graphite furnace.

To reduce the trace metal contaminants, the ammonium nitrate was cleaned by recrystallizing in a cleaned teflon jar with highly pure water. The contamination from ammonium nitrate was evaluated by analyzing a water blank that contained ten times as much ammonium nitrate as the sample.

Trace Metal Standards

Trace metal standards are ordered new each year through Fisher Scientific Company and are kept in the plastic bottles in which they are shipped. For daily use, small aliquots of each standard are poured into 25 ml teflon jars.

Airborne Particles

Airborne particles such as dust, particles from ceiling tiles, etc. also contribute to contamination of the analysis by settling in unsealed glassware, exposed samples and onto the teflon tip of the Autosampler and/or pipette tips. The contribution of contamination resulting from airborne particles is difficult to evaluate, but may be minimized with certain techniques. These include 1) filtration of air delivered to the laboratory and/or directly over the bench where samples are prepared, and 2) covering samples at all times from collection to analysis.

Our sample collection units (described in a separate paper) are washed using the technique already described. Sample collection units are kept in plastic bags until they are attached to the pump, and are returned to the bag after the sample is collected. In the laboratory, the sample bottles are kept in bags until they are analyzed. The AS-1 sample tray is kept covered at all times with the plexiglass cover provided.

Further, a plexiglass housing has been built to protect the samples in the AS-1 Autosampler. This housing encloses the Autosampler except for the space needed for the sampler arm to inject samples into the furnace. Filtered air is blown gently into the housing, creating a positive pressure that prevents vagrant air currents from blowing particles into the housing. The only opportunity that exists for airborne contamination occurs 1) when the sample bottle is emptied of the 0.1N HNO₃ solution stored in it prior to collection of

a sample and 2) during transfer of the covered sample tray from the lab bench to the plexiglass housing.

Air in our laboratory is 90% filtered, and we have purchased laminar air flow hood with HEPA^R filters which are mounted over the lab bench and provide a 99% dust-free environment in which to prepare samples. With these precautions there is little opportunity for contamination from airborne particles.

Instrument Components

The final source of contamination may be the instrument itself. The graphite furnace is essentially a graphite tube held between two graphite cones. Contamination from the instrument may be the result of 1) an initially contaminated lot of cones, tubes, or both (which may be checked by running a program with no sample), or 2) contamination from one sample to the next; that is, the "memory effect". The latter may be eliminated by a combination of several methods: a) a distilled water blank may be run with replicates between samples, b) a number of replicates of each sample may be run, in which case the first one or two replicates are discarded, or c) the auto high temp option on the ramp programmer may be engaged. The latter automatically cycles the program to 2500°C between replicates. This will eliminate any carry-over in the graphite tube from one sample injection to the next, but will not eliminate carry-over that may results from contamination of the Autosampler tip; however, we have not found this to be a problem. Instrument contamination is best evaluated using a

very sensitive metal such as zinc or cadmium. Generally, any carry-over from the tip or contamination of the tubes or cones will show up if the standards are placed in the sample try randomly and an ANOVA produced for the linear regression. This has been done and no significant difference between random and non-random samples found.

Analytical Considerations

There are five factors relative to the analysis of saline water samples that must be examined before a definitive assessment of trace metal levels can be made. These are 1) the effect of salinity, 2) the effect of matrix modification, 3) the effect of volume, 4) the effect of the purge gas flow rate on the absorbance signal, and 5) the effect of the graphite tube on the absorbance signal.

Salinity and Matrix Modification

The effect of salinity on the absorbance signal is of prime consideration in the analysis of estuarine waters. This is especially true since tidal fluctuations, rainfall, etc., all affect the salinity of a station which, in turn, not only affects the chemical form of the metal at that station, but also affects the quality of the analysis. That is, as the salinity increases, the quantity of bulk salts also increases. If these salts are not removed during the charring cycle, considerable non-atomic signals are produced which may overwhelm the background corrector.

Matrix modification functions by reacting either with the major bulk salts in the sample or with the metal under analysis to produce a more volatile species, or a more thermally stable

metal, respectively. The quantity of matrix modifiers added will, in the case of bulk salt reactions, also be a function of the quantity of bulk salts present in the sample; i.e., salinity of that sample. Generally, however, an excess of matrix modifier is added to the sample.

Volume

The absorbance signal is also a function of the volume injected into the furnace. Since some metals are very sensitive (e.g. cadmium, zinc) and others are relatively insensitive (e.g., copper, lead) it is advantageous to the analysis to vary the injection volume to accommodate the sensitivities involved; i.e., to maximize the slope of the analytical curve.

Analysis of saline water samples compounds the problems since the quantity of bulk salts injected also increases. Therefore, the effect of an increased injection volume, with and without matrix modification was checked to assess the non-atomic signal produced by the volatilized bulk salts.

Purge Gas

Argon gas was used to purge the graphite furnace. It is possible to change the purge gas flow rate which will change the rate at which the sample is swept from the path of the light source. This phenomenon can be used advantageously by removing volatilized bulk salts more efficiently and/or by increasing the residence time of the metal atoms under analysis. The purge gas flow rate may also be stopped entirely by analyzing the sample in the "Interrupt" mode. Some authors (Segar and Cantillo, 1975) also noted an increase in precision with increased purge gas flow rate.

Tube Life

Since the entire analytical procedure is ultimately dependent upon the graphite tube, the effect of the tube on the absorbance signal over its lifetime is especially important.

There are two types of graphite tubes that may be considered: 1) commercially available (Perkin-Elmer part no. 2901766) pyrolytically coated graphite tubes, and 2) non-pyrolytically coated graphite tubes (part no. 2901633). Each of these tubes was evaluated.

Accuracy

There are presently no standard solutions of seawater that contain known amounts of trace metals. Consequently, the determination of the accuracy of an analysis is difficult. However, there are two methods that may be used to assess the accuracy of the analysis:

1. EPA aqueous standards may be analyzed.
2. An alternate method of analysis may be used and results compared.

Both of these methods were used to assess the accuracy of the trace element analysis. The standards were analyzed using atomic absorption spectrophotometry and anodic stripping voltametry and the results compared.

Statistics and Quality Control

Several statistical parameters are used to manipulate the data produced by the analytical procedure. Each will be discussed as the data produced by the analytical procedure is described.

The analytical procedure has been refined so that a fairly standard set of samples and standards are run each time. Manganese will be described as an example; however, each metal is treated similarly.

A typical AS-1 sample tray contains a set of three blanks followed by five standards: 0.5, 1.0, 2.0, 5.0, and 10.0 $\mu\text{g}/\ell$, the concentrations of which have been found to bracket the concentrations found in the unknown samples. Both blanks and standards are, of course, prepared in the same matrix as the unknown samples. Replicates are analyzed from each cup for a total of 16 points.

To begin with, the blanks are analyzed and the mean and standard deviation recorded. Next, the mean blank is subtracted from the standards. The corrected absorbances are used to calculate a linear regression for the standards using a "least squares" fit. The linear regression produces a slope and intercept, which are recorded. Next, an ANOVA table is calculated for the data and the "F statistic" recorded. The slope is compared with slopes from previous runs using a V-mask quality control chart (Montgomery, 1976), which utilizes a cumulative sum procedure, to determine if the slope is within acceptable limits. If the slope is acceptable, the slope is pooled and a mean slope calculated. The blanks are compared with blanks from previous runs using the non-paired "T" test to determine if they are acceptable. If there is no significant difference between blanks, the blanks are pooled and a mean and standard deviation calculated. If both slope and blanks are acceptable, the minimum detectable concentration

(M.D.C.) is calculated using the standard deviation of the blanks, the students "t" value at the 95% confidence level and the mean slope. The M.D.C. is also recorded.

Once it has been determined that an acceptable standard curve has been produced, the calculations proceed to unknown samples.

Since the samples have been diluted 1:1 with high purity water, the absorbances are multiplied by two to obtain the absorbance of the original sample. Blanks are subtracted to produce the corrected absorbance. Next, the concentrations present in each replicate is determined by solving for "x" in the equation: $y = a + bx$; where, y = absorbance; a = intercept; b = slope of the linear regression, and x = concentration.

Each unknown was replicated and two absorbance values determined for each replicate, for a total of four values for each sample. Next, the mean concentration, standard deviation and coefficient of variation is determined for each sample. If there is any significant difference between replicates, another sample is prepared and analyzed and the contaminated sample discarded.

Blanks with standard deviations, the slope and intercept of the analytical curves, the unknown concentrations' mean, standard deviation and coefficient of variation are filed on "floppy discs" for retrieval and manipulation by a DEC, PDP 11/34 minicomputer.

Results

Optimizations of Parameters

DRY, CHAR, AND ATOMIZE CYCLES

The determination of the maximum charring temperature and optimum atomization temperature for aqueous solutions of manganese is illustrated in Figures 1 and 2, respectively. For These determinations, a 10 $\mu\text{g/l}$ aqueous sample of manganese was prepared. Twenty microliters of the sample were injected using the AS-1 Autosampler^R into a pyrolytically coated graphite tube. Four replicates were determined and the mean and standard deviation calculated. The "confidence intervals" shown are two times the standard deviation of the mean. It can be seen that the absorbance signal becomes not only smaller but also more variable as the maximum charring temperature is exceeded. The maximum charring temperature was found to be 800°C. The optimum atomization temperature was 2700°C.

Similar tests were performed on each metal to be analyzed. The results are summarized in Table 1. For all of the metals it was found that the dry and char cycles should be ramped to improve the reproducibility of the analysis.

Lead shows improved results if the atomize cycle is ramped for the seawater analysis. These results are also summarized in Table 1. When the atomize cycle is ramped, the metal is volatilized at a discrete time in the cycle and can be more readily recognized. The analysis is improved because the deviation of the zero spike (used to calculate the minimum detectable concentration) is reduced.

Table 1. Conditions for the Analysis of Selected Trace Metals in Aqueous and Seawater Matrices

METAL	CADMIUM		CHROMIUM		COPPER		IRON		LEAD		MANGANESE	
CONDITION												
Lamp	E.D.L.		E.D.L.		H.C.		H.C.		E.D.L.		H.C.	
Wavelength	228.8		357.9		324.7		248.3		217.0		279.5	
Slit (nm)	0.7		0.7-2.0		0.7-2.0		0.7-2.0		0.7		0.2-2.0	
Injection Volume (μl)	20		50		50		50		50		50	
Mode	Interrupt		Interrupt		Interrupt		Interrupt		Interrupt		Interrupt	
Tube Type	Coated		Coated		Coated		Coated		Coated		Coated	
ml/min. Gas Flow	100		100		100		100		100		100	
Deuterium	yes		yes		yes		yes		yes		yes	
°C Dry Temp.	100		100		100		100		100		100	
Dry Time (sec)	40		80		80		80		80		80	
Dry Ramp (sec)	30		70		70		70		70		70	
°C Char Temp.	350		950		800		950		600		800	
Char Time (sec)	40		80		80		80		80		80	
Char Ramp (sec)	30		70		70		70		70		70	
°C Atomize Temp.	2100		2600		2600		2600		2300		2700	
Atomize Time (sec)	10		10		10		10		17		12	
Atomize Ramp (sec)	0		0		0		0		15		0	
Matrix	Aqueous	Seawater	Aqueous	Seawater	Aqueous	Seawater	Aqueous	Seawater	Aqueous	Seawater	Aqueous	Seawater
Dilution of Matrix	-	1:1	-	1:1	-	1:1	-	1:1	-	1:1	-	1:1
Matrix Modifier	-	0.5	-	0.5	-	0.5	-	0.5	-	0.5	-	0.5
Slope (abs/μg metal)	.140	.102	.020	.010	.024	.024	.024	.018	.035	.010	.0995	.030
S.D. Slope	.017	.032	.002	.002	.007	.006	.004	.007	.007	.003	.010	.007
Blank (abs.)	.003	.019	.002	.003	.009	.009	.025	.031	.022	.008	.007	.030
S.D. Blank	.004	.013	.002	.002	.010	.010	.013	.016	.009	.003	.004	.008
N	20	37	11	11	20	39	12	31	15	11	6	6
"t"	2.093	2.021	2.228	2.228	2.093	2.021	2.201	2.042	2.131	2.201	2.571	2.571
M.D.C. (μg/l)	.06	0.26	0.22	0.4	0.87	0.9	1.2	1.8	0.55	0.66	0.40	0.70
mm/min. Chart Speed	20	80	20	40	20	40	20	40	20	80	20	40
Peak to Read	one only	First	one only	one only	one only	one only	one only	one only	one only	First	one only	one only

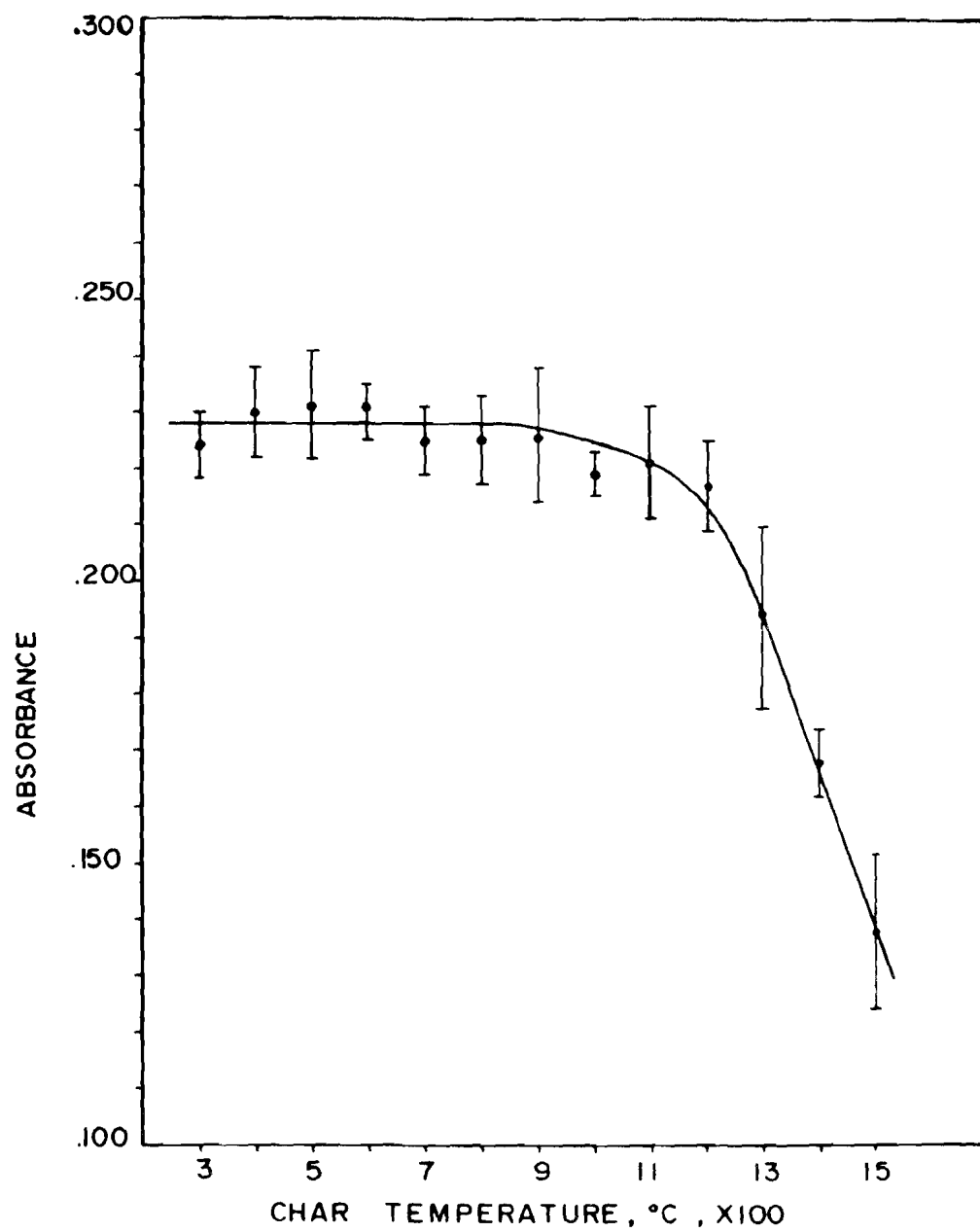


FIG. 1: Determination of Manganese char temperature

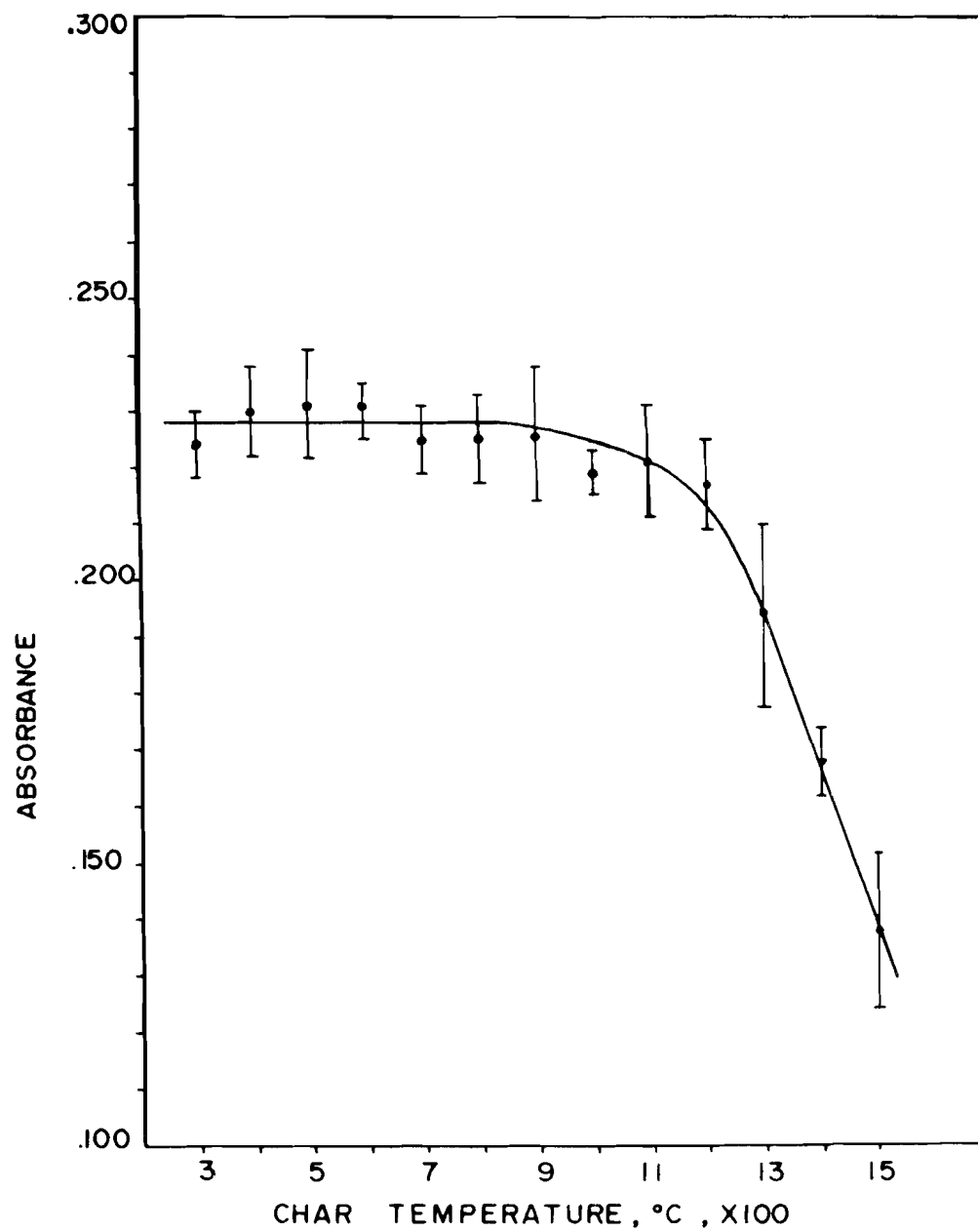


FIG.1: Determination of Manganese char temperature

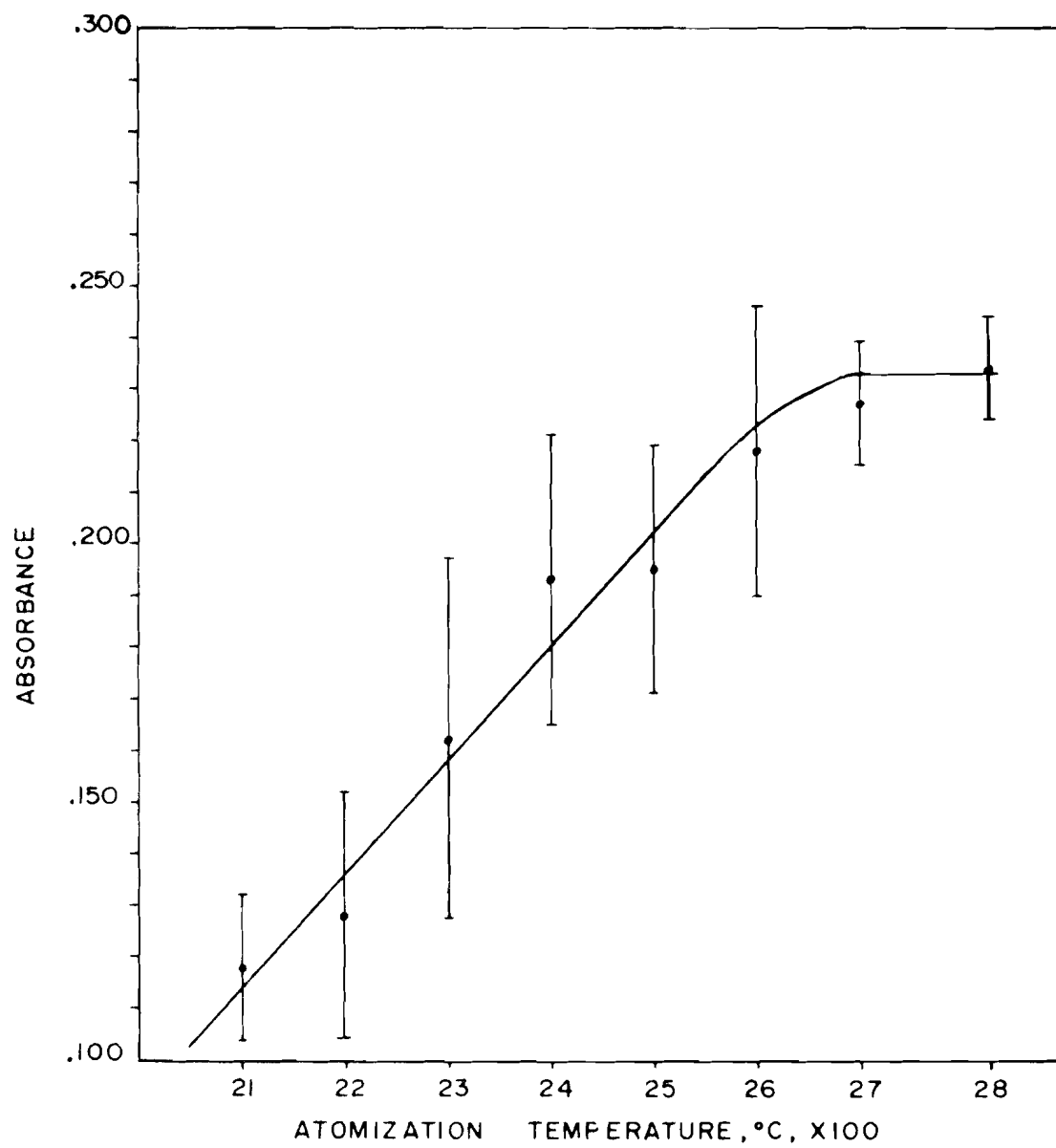


FIG. 2: Determination of optimum atomization temperature for Manganese

Mode

Of the two modes available for operation of the HGA Controller, the interrupt mode was found to be the more sensitive. The interrupt mode shuts off the purge gas during the atomization cycle thereby allowing the metal atoms a longer residence time in the light path.

A comparison of the normal versus the interrupt mode for manganese was compiled in Table 2. It can be seen that with the aqueous matrix, the slope is greater and the minimum detectable concentration (M.D.C.) is lower using the interrupt mode. In a seawater matrix, the slope is greatly improved using the interrupt mode. The standard deviation of the blank was greater, however, which resulted in about the same M.D.C. for manganese.

Volume Injected

There are three pumps available with the AS-1 Autosampler which enable the analyst to inject volumes of 10, 20, 25, 50 or 100 μl into the graphite tube. Each metal has an optimum injection volume dependent upon the sensitivity of the metal, the matrix under analysis, and the concentration of the metal in that matrix.

In aqueous solutions, as the injection volume increases, the slope of the linear regression also increases. Since a high slope is desirable, a large injection volume may be used if the trace metal concentration is low and the solution is aqueous. As an example, Table 3 illustrates the effect of different injection volumes in aqueous solution for manganese. The slope increases from 0.021 abs/ μg Mn with 10 μl injection to 0.207 abs/ μg Mn with a 100 μl injection.

In saline solutions, the optimum injection volume is not

Table 2. A Comparison of the "Normal" and "Interrupt" Modes
Using Manganese in Aqueous and Seawater Matrices.

Inject Volume	50 $\mu\ell$		50 $\mu\ell$	
Mode	Normal		Interrupt	
Matrix	Aqueous	1:1 Seawater	Aqueous	1:1 Seawater
Slope(abs/ μg metal)	.026	.020	.0995	.058
Intercept	.001	-.013	.003	-.007
Mean Blank (abs.)	.007	.003	.008	.073
S.D. Blank	.004	.003	.002	.014
M.D.C. ($\mu\text{g}/\ell$)	0.40	0.40	0.052	0.62

Table 3. The Effect of Injection Volume on Manganese Determinations in an Aqueous Matrix.

Parameter	Condition			
Wavelength, nm	279.5			
Mode/Tube	Interrupt/Pyrolytically Coated			
Matrix	Aqueous Solution			
Inject Volume (μl)	10	20	50	100
Dry Temp($^{\circ}\text{C}$)	95	95	95	95
Dry Time(sec)	20	40	80	110
Dry Ramp Time(sec)	10	30	70	90
Char Temp($^{\circ}\text{C}$)	800	800	800	800
Char Time(sec)	20	40	80	110
Char Ramp Time(sec)	10	30	70	90
Atomize Temp($^{\circ}\text{C}$)	2700	2700	2700	2700
Atomize Time(sec)	10	10	12	15
Atomize Ramp Time(sec)	0	0	0	0
Mean Blank(abs.)	.004	.006	.008	.027
S.D. Blank	.001	.002	.002	.008
"t"	2.571	2.571	2.571	2.571
Slope(abs/ μg metal)	.021	.037	.0995	.207
Intercept	-.006	.037	.003	-.00008
M.D.C. ($\mu\text{g}/\text{l}$)	0.12	0.14	0.052	0.099

always the maximum available. The limiting factor is the variability of the blank, which increases with increasing injection volume. It appears that by increasing the injection volume, the quantity of bulk salts is also increased, apparently to a point where the deuterium background corrector cannot compensate for the non-atomic signal resulting from the volatilization of the bulk salts. Table 4 shows the effect of increasing the injection volume for the analysis of manganese in a seawater matrix. The optimum injection volume for manganese was found to be 50 μ l. At this volume, the slope is greatest (0.058 abs/ μ g Mn as compared with .0056, μ g/l as compared with 0.92, 1.07, and 2.57 μ g/l) of all the injection volumes examined.

Graphite Tubes/Sample Cups

There are two types of graphite tubes available for analysis: the pyrolytically coated and the non-pyrolytically coated tubes. Each of these was tested for effectiveness in the analysis of the trace metals.

A comparison of coated versus non-coated tubes was produced. Table 5 shows the results of the comparison. For manganese, the pyrolytically coated tube produced the greater slope and a similar M.D.C. in an aqueous matrix, and a lower M.D.C. in a seawater matrix. Similar tests were run for each metal.

There are also two types of sample cups available for use with the AS-1 Autosampler. One type is a clear, polystyrene and the other is opaque teflon. An analysis of blanks for each showed that the teflon cups, which can be rigorously washed with the cleaning method described previously, and reused, had a lower blank (.005 absorbance as compared with .024 absorbance

Table 4. The Effect of Injection Volume on Manganese Determination in a Seawater Matrix.

Parameter	Condition			
Wavelength	279.5			
Mode/Tube	Interrupt/Pyrolytically Coated			
Matrix	Seawater 1:1			
Inject Volume (μl)	10	20	50	100
Dry Temp($^{\circ}\text{C}$)	95	95	95	95
Dry Time(sec)	20	40	80	110
Dry Ramp Time(sec)	10	30	70	90
Char Temp($^{\circ}\text{C}$)	800	800	800	800
Char Time(sec)	20	40	80	110
Char Ramp Time(sec)	10	30	70	90
Atomize Time($^{\circ}\text{C}$)	2700	2700	2700	2700
Atomize Time(sec)	10	10	12	15
Atomize Ramp Time(sec)	0	0	0	0
Mean Blank(abs.)	.007	.013	.073	.112
S.D. Blank	.002	.005	.014	.032
"t"	2.571	2.571	2.571	2.571
Slope(abs/ μg metal)	.0056	.012	.058	.032
Intercept	.0002	-.0008	-.007	-.019
M.D.C. ($\mu\text{g}/\text{l}$)	0.92	1.07	0.62	2.57

Table 5. A Comparison of Pyrolytically Coated with Non-Coated Graphite Tubes in Aqueous and Seawater Matrices for Manganese Determinations.

Inject Volume	50 $\mu\ell$		50 $\mu\ell$	
Mode	Interrupt		Interrupt	
Matrix	Aqueous		Seawater	
Tube Type	Non-Coated	Coated	Non-Coated	Coated
Slope(abs/ μg metal)	.059	.0995	.021	.058
Intercept	.005	.003	-.011	-.007
Mean Blank(abs.)	.008	.008	.147	.073
S.D. Blank	.0009	.002	.015	.014
"t"	2.571	2.571	2.571	2.571
M.D.C. ($\mu\text{g}/\ell$)	0.040	0.052	1.8	0.62

for manganese) and lower variance (.002 absorbance as compared with .013 absorbance for manganese) than the polystyrene cups. This comparison is illustrated in Table 6. Consequently, the teflon cups are used exclusively for analysis.

Contamination and Blanks

Spurious contamination of the sample under analysis may result from three major sources: from sample containers, reagents, and from airborne particles. Each of these potential sources must be controlled in order to reduce the blank and lower the M.D.C.

Sample containers are washed using the procedure described earlier.

Water is purified as described in the Methods and Materials section. The contamination from water is examined in Table 6. This shows that water from the Continental^R system (called DHOH in the table) is not adequately purified. However, if this water is passed through the Millipore^R Milli-Q^R system, very high quality water is produced. This cleaning is shown to be especially critical for copper, iron, lead and manganese. Cadmium and chromium do not show significant levels in the Continental^R water.

Acid reagents are redistilled to reduce trace metal contamination. Table 7 shows the results of progressive cleaning of concentrated HNO₃. It is obvious that the redistilled Ultrex acid shows the lowest blank and standard deviation.

Airborne particles also contribute trace metal contamination. As stated previously, the samples are kept sealed in plastic bags until they are used. Further, the samples are

Table 6. A comparison of the blanks resulting from the type of water and the type of AS-1 sample cups used.

Type Cup		Teflon			Polystyrene	
Metal	Matrix Parameter	DHOH	Milli-Q	Redistilled Acid/MilliQ	Milli-Q	Redistilled Acid/MilliQ
CADMIUM	Mean Blank(abs)	.000	.000	.003	.000	.056
	S.D. Blank	0	0	.004	0	.030
	Conc. Blank	-	-	.0002	-	.0040
	(µg/100 µl acid)					
CHROMIUM	Mean Blank(abs)	.000	.000	.004	.000	.006
	S.D. Blank	0	0	.003	0	.005
	Conc. Blank	-	-	.0013	-	.0018
	(µg/100 µl acid)					
COPPER	Mean Blank(abs)	.014	.000	.006	.000	.014
	S.D. Blank	.001	0	.003	0	.005
	Conc. Blank	0.68	-	.0026	-	.0050
	(µg/100 µl acid)					
IRON	Mean Blank(abs)	.014	.000	.025	.000	.084
	S.D. Blank	.008	0	.013	0	.075
	Conc. Blank	0.5	-	1.2	-	8.03
	(µg/100 µl acid)					
LEAD	Mean Blank(abs)	.009	.000	.022	.000	.029
	S.D. Blank	.004	0	.010	0	.028
	Conc. Blank	0.25	-	.0088	-	.011
	(µg/100 µl acid)					
MANGANESE	Mean Blank(abs)	.028	.000	.005	.000	.024
	S.D. Blank	.018	0	.002	0	.013
	Conc. Blank	0.25	-	.00035	-	.0021
	(µg/100 µl acid)					

DHOH is water that passed through the Continental^R system only. MilliQ^R water had a further cleaning through the Millipore^R system. The concentrations of metals in these categories are given as µg/l.

Table 7. Comparison of reagent grade nitric acid with Ultrex^R and redistilled Ultrex^R nitric acid blanks in an aqueous matrix for selected trace metals.

MATRIX	AQUEOUS SOLUTION																	
MODE/TUBE TYPE	INTERRUPT/PYROLYTICALLY COATED																	
ACID SOURCE	REAGENT GRADE HNO ₃						ULTREX ^R HNO ₃						REDISTILLED ULTREX ^R HNO ₃					
METAL ANALYZED	Cd	Cu	Cr	Fe	Pb	Mn	Cd	Cr	Cu	Fe	Pb	Mn	Cd	Cr	Cu	Fe	Pb	Mn
SLOPE(abs/μg metal)	.140	.022	.026	.024	.021	.0995	.140	.026	.022	.024	.021	.0995	.140	.026	.022	.024	.021	.0995
MEAN BLANK(abs)	.117	.015	.070	.084	.072	.016	.016	.008	.015	.097	.027	.018	.003	.004	.006	.025	.022	.005
S.D. BLANK	.009	.004	.008	.013	.028	.008	.004	.002	.003	.004	.019	.004	.004	.003	.003	.013	.010	.002
M.D.C.(μg/l)	.16	0.5	0.80	1.4	3.43	0.21	.07	.20	0.4	0.4	2.3	0.10	.06	.26	0.3	.12	1.1	.05
CONCENTRATION OF METAL IN ACID AS μg METAL/100 μl ACID	.0084	.0056	.026	.034	.033	.0014	.0012	.0021	.0056	.039	.01	.0015	.0002	.0013	.0026	.011	.008	.00035

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Table 6. A comparison of the blanks resulting from the type of water and the type of AS-1 sample cups used.

Type Cup		Teflon			Polystyrene	
Metal	Matrix Parameter	DHOH	Milli-Q	Redistilled Acid/MilliQ	Milli-Q	Redistilled Acid/MilliQ
CADMIUM	Mean Blank(abs)	.000	.000	.003	.000	.056
	S.D. Blank	0	0	.004	0	.030
	Conc. Blank	-	-	.0002	-	.0040
	($\mu\text{g}/100 \mu\text{l}$ acid)					
CHROMIUM	Mean Blank(abs)	.000	.000	.004	.000	.006
	S.D. Blank	0	0	.003	0	.005
	Conc. Blank	-	-	.0013	-	.0018
	($\mu\text{g}/100 \mu\text{l}$ acid)					
COPPER	Mean Blank(abs)	.014	.000	.006	.000	.014
	S.D. Blank	.001	0	.003	0	.005
	Conc. Blank	0.68	-	.0026	-	.0050
	($\mu\text{g}/100 \mu\text{l}$ acid)					
IRON	Mean Blank(abs)	.014	.000	.025	.000	.084
	S.D. Blank	.008	0	.013	0	.075
	Conc. Blank	0.5	-	1.2	-	8.03
	($\mu\text{g}/100 \mu\text{l}$ acid)					
LEAD	Mean Blank(abs)	.009	.000	.022	.000	.029
	S.D. Blank	.004	0	.010	0	.028
	Conc. Blank	0.25	-	.0088	-	.011
	($\mu\text{g}/100 \mu\text{l}$ acid)					
MANGANESE	Mean Blank(abs)	.028	.000	.005	.000	.024
	S.D. Blank	.018	0	.002	0	.013
	Conc. Blank	0.25	-	.00035	-	.0021
	($\mu\text{g}/100 \mu\text{l}$ acid)					

DHOH is water that passed through the Continental^R system only. MilliQ^R water had a further cleaning through the Millipore^R system. The concentrations of metals in these categories are given as $\mu\text{g}/\text{l}$.

opened under a laminar flow hood, the air of which passes through a HEPA filter.

The effectiveness of the filter was demonstrated graphically to us quite accidentally. A set of samples was left on the counter but not under the filter. Some of the sample vials opened accidentally and were left standing over a weekend. The laboratory was unoccupied during this time and the opened samples were discovered and analyzed the following Monday. Table 8 is a compilation of the results of the test.

This illustrates the need for a laminar flow filtration system in an analytical laboratory. Although such dramatic contamination would not be expected, some contamination is inevitable unless filtered air is used.

Ammonium nitrate is used as a matrix modifier and also contains trace metal contamination. It is recrystallized to eliminate or reduce the contamination. An analysis of recrystallized and reagent grade ammonium nitrate, the results of which are summarized in Table 9, shows that there is approximately a four fold improvement in the contamination for chromium, a two fold improvement in the contamination for copper, a six fold improvement for iron, and a two fold improvement for manganese. There was no improvement found for lead, but cadmium showed a lower standard deviation, consequently a lower M.D.C., when recrystallized ammonium nitrate was used.

Finally, the minor components of contamination are the graphite tubes and cones. Although no contamination of these has been found, each tube is routinely checked by analyzing

Table 8. Comparison of Contamination from Airborn Particles
in Sample Vials for Selected Trace Metals.

Metal	Conc. Closed (µg/l)	Conc. Opened (µg/l)	µg/l Contamination	% Contamination
Cadmium	0.050	0.086	.036	72
Chromium	0.52	1.540	1.02	196
Copper	0.570	0.940	.37	65
Lead	0.576	0.964	.388	67
Iron	0.813	1.445	.632	78

Table 9. A comparison of contamination between recrystallized and reagent grade ammonium nitrate in a dilute seawater matrix.

METAL	AMMONIUM NITRATE	(abs/μg) SLOPE	(μg/l) M.D.C.	(abs.) BLANK	Mean BLANK	Concentration of Metal as μg/0.5 μl NH ₄ NO ₃
Cd	Recrystallized	.238	.01	.020	.001	.002
	Reagent Grade		.08	.022	.007	.002
Cr	Recrystallized	.009	0.6	.003	.002	.005
	Reagent Grade		1.14	.0175	.004	.022
Cu	Recrystallized	.015	0.17	.009	.001	.007
	Reagent Grade		1.03	.023	.006	.017
Fe	Recrystallized	.012	0.43	.0098	.002	.017
	Reagent Grade		1.93	.109	.009	.100
Pb	Recrystallized	.012	0.64	.008	.003	.0078
	Reagent Grade		0.64	.008	.003	.0078
Mn	Recrystallized	.039	0.33	.064	.005	.015
	Reagent Grade		0.33	.132	.005	.032

The concentration of the ammonium nitrate stock is 500 g/l, of which 0.5 ml is mixed into a total volume of 10 ml of 1:1 diluted seawater. This produces an addition of 125 mg ammonium nitrates per 50 μl injection.

a set with nothing injected into the tube. If any peaks are found and persist, the tube is discarded.

Seawater Analysis and Matrix Modification

Various matrix modifiers have been tried in an attempt to improve the analysis of the trace metals in seawater, especially lead. Among the modifications tried were:

1. Dilution of the sample to reduce the quantity of bulk salts to a level that the deuterium background corrector could compensate.
2. Addition of phosphoric acid
3. Addition of peroxide
4. Addition of ammonium molybdate
5. Addition of ammonium bifluoride.

These were attempts to react the lead in the sample with a compound that would produce a less volatile lead species. All of these experiments met with good results in aqueous solutions, but were failures in seawater solutions.

6. Addition of ammonium nitrate, as suggested by Ediger (1975) in which the major bulk salt, NaCl, is reacted with the ammonium nitrate to produce ammonium chloride and sodium nitrate, both of which are volatile at temperatures less than 500°C.

Table 10 shows the effect of sample dilution and matrix modification on the analysis of seawater samples. As is shown, an improvement in the analysis was found when seawater samples were diluted and a further improvement noted when 0.5 ml of a 500 g/l stock of ammonium nitrate added to the diluted sample.

Table 10. A comparison of the ammonium nitrate with diluted and undiluted seawater samples.

METAL	CONDITION	(abs/ μ g metal) SLOPE	INT.	F	R	(μ g/l) M.D.C.
Cd	1	.069	-.007	400.85	.9925	.40
	2	.088	.011	70.20	.9598	.20
	3	.237	-.020	294.62	.98669	.11
Cr	1	.026	.020	56.84	.9511	1.3
	2	.028	-.005	28.42	.9037	1.1
	3	.010	-.003	167.0	.9768	0.6
Cu	1	.008	.010	132.67	.97813	3.6
	2	.008	.001	15.17	.8465	2.6
	3	.015	-.002	149.28	.9742	0.2
Fe	1	.0053	-.011	14.357	.8398	6.8
	2	.0039	.12	0.4634	.1928	-
	3	.012	-.011	123.57	.96912	0.4
Pb	1	.0067	.008	253.74	.98838	6.6
	2	.017	.062	15.08	.8458	1.1
	3	.014	.004	688.87	.99424	0.6
Mn	1	.028	-.010	78.7546	.96395	0.6
	2	.015	.002	12.728	.82439	0.7
	3	.039	.007	240.88	.98379	0.6

Seawater used was collected in the Gulf Stream, approximately 25 miles off Ft. Pierce inlet. Dilutant water was processed through the Milli-Q^R reverse osmosis unit. Diluted seawater was diluted 1:1 with Milli-Q^R water.

CONDITION 1: Undiluted seawater, with 1 ml NH_4NO_3

CONDITION 2: Seawater diluted 1:1, no NH_4NO_3

CONDITION 3: Seawater diluted 1:1, with 0.5 ml NH_4NO_3

The addition of this quantity of ammonium nitrate resulted in an addition of 1.25 mg NH_4NO_3 per 50 μl injection (or 0.5 mg/l 20 μl injection) which has been found to be adequate for these purposes. Addition of more ammonium nitrate was found to reduce the slope of the linear regression.

It can be seen by examining Table 10 for manganese, for example, that the slope and F-value are greatest and the M.D.C. lowest for the diluted, matrix modified samples.

Copper, iron, lead and manganese show very low f-values for diluted seawater samples with no matrix modification. However, even the undiluted samples show significant f-values if ammonium nitrate matrix modifier is added. This shows that matrix modification is necessary for the determination of these metals in a seawater matrix.

Accuracy

Accuracy was first assessed by analyzing EPA standards solutions in aqueous solution. There are three concentration ranges available from the Environmental Protection Agency, all of which were analyzed. Table 11 shows the results of that analysis. It can be seen that the percent error was quite low, except for cadmium at the lowest concentration.

Statistics and Quality Control

Several programs for use with the PDP 1134 computer have resulted from the statistics and quality control program that was initiated. As an example, the process from raw data collected from the atomic absorption spectrophotometer to final form ready for manipulation will be illustrated using manganese

Table 11. Analysis of EPA standards in aqueous solution. Standard solutions are available through the Environmental Protection Agency, Cincinnati, Ohio.

ACCURACY

METAL	SAMPLE	EPA CONC	HBF CONC	% CV	% ERROR
Cadmium MDC = 0.2 (µg/ℓ)	EPA 1	5.2	3.75	8.5	28.8
	EPA 2	23	24.4	1.3	6.1
	EPA 3	73	83.4	3.0	14.2
Chromium MDC = 0.7 (µg/ℓ)	EPA 1	16.0	15.11	1.5	5.6
	EPA 2	154.0	155.5	2.5	1.0
	EPA 3	209.0	208.4	2.9	0.3
Copper MDC = 0.3 (µg/ℓ)	EPA 1	16.0	15.3	2.6	4.4
	EPA 2	72.0	71.2	5.6	1.1
	EPA 3	102.0	95.1	7.5	6.8
Lead MDC = 1.8 (µg/ℓ)	EPA 1	22.0	22.9	6.1	4.1
	EPA 2	298.0	280.4	4.5	5.9
	EPA 3	352.0	341.6	4.2	2.9

as an example. The description of the programs may be followed by using the attached computer printout provided for this purpose.

Access is gained to the initial data entry file by calling up "CD FILE" (refer to Figure 3, page 1). Next, the type of instrument used for the analysis is coded by the program and the analyst enters the appropriate code. In this case, the appropriate code is AAS. The data may now be entered, but must be entered in the appropriate order. This order was established by analyzing many sets of standards and unknowns and has been found to be applicable to almost all the cases normally encountered. If a different set of standards are run then the program must be modified to accept the new data form.

The program next asks for the total number of data points to be entered. In this case, a total of 30 points will be entered. These are the uncorrected absorbance values from the spectrophotometer. The correct entry form is

DATA 1-6 are blanks

DATA 7-16 are five standards with two replicates per standard. For manganese, these standards are 0.5, 1.0, 2.0, 5.0. and 10.0 $\mu\text{g}/\ell$.

DATA 17-N are unknowns, where N = the number of total data points to be entered. There are two samples per unknown and two replicates per sample. This is a total of four numbers per unknown concentration. In this example, 30 points were entered so that DATA 17-30 are unknown values.

DATA 17-18 is replicate 1, sample 1

DATA 19-20 is replicate 2, sample 1

DATA 21-22 is replicate 1, sample 2

DATA 23-24 is replicate 2, sample 2, etc.

Once all of the data has been entered, the program asks if there are any corrections to be made (Figure 3, page 2). If not, a tabular printout of the data that was entered can be obtained by typing "YES" after the question "WOULD YOU LIKE A LISTING OF THE DATA?" The program again asks if there are any corrections. If not then the program is ended.

Once the data has been entered on the "CD FILE" program, another program must be called up to manipulate the data. This program is called "PAAS1" (Figure 4, page 1). These programs were designed specifically for the weekly sampling effort; consequently, the program first asks for the week of sampling. However, if the particular run under analysis is not part of the weekly cruises, the calculation may continue by entering "99999" for the week number. The calculation will proceed but the data will not be entered on the master file since "99999" is a "default" code. Next, the number of samples entered in program "CD FILE" must be entered. This program assumes that the analyst entered two replicates of each sample; therefore, enter the number of replicates - in this example there are 8 samples. The final step is to enter the metal analyzed. In this case, manganese, Mn, was entered.

The program proceeds automatically from this point. First, the statistics for the blank are calculated, and the mean, standard deviation, and coefficient of variation printed.

Figure 3. Computer Printout of the "CDFILE" program. This program files absorbance data for manipulation using subsequent computer programs. (page 1 of 2).

RUN CDFILE

ENTER THE INSTRUMENT-PROCESS CODE NAME

VALID CODES AND NAMES ARE AS FOLLOWS:

ASV = ANODIC STRIPPING VOLTOMETRY

DOC = DISSOLVED ORGANIC CARBON

AAN = AUTO-ANALYZER

AAS = ATOMIC ABSORPTION SPECTROPHOTOMETER

? AAS

YOUR DATA WILL BE STORED IN VIRTUAL FILE DX1:AAS.DCM

ENTER THE NUMBER OF DATA POINTS TO BE STORED

? 30

ENTER THE DATA

1 -? .01

2 -? .005

3 -? .005

4 -? .005

5 -? .005

6 -? .005

7 -? .01

8 -? .01

9 -? .025

10 -? .027

11 -? .06

12 -? .057

13 -? .14

14 -? .14

15 -? .265

16 -? .265

17 -? .265

18 -? .27

19 -? .287

20 -? .29

21 -? .25

22 -? .247

23 -? .26

24 -? .265

25 -? .245

26 -? .23

27 -? .23

28 -? .235

29 -? .200

30 -? .195

Figure 3. "CDFILE" program printout. (page 2 of 2).

ARE ANY CORRECTIONS NECESSARY				
? NO				
WOULD YOU LIKE A LISTING OF THE DATA				
? YES				
1	2	3	4	5
.01	5.00000E-03	5.00000E-03	5.00000E-03	5.00000E-03
6	7	8	9	10
5.00000E-03	.01	.01	.025	.027
11	12	13	14	15
.06	.057	.14	.14	.265
16	17	18	19	20
.265	.265	.27	.287	.29
21	22	23	24	25
.25	.247	.26	.265	.245
26	27	28	29	30
.23	.23	.235	.2	.195
WOULD YOU LIKE TO MAKE ADDITIONAL CORRECTIONS ?				
? NO				
READY				

Next, a "non-paired 't' test" is calculated which compares the present set of blanks with the pooled blanks for all of the previously analyzed sets. The calculated "t", and table "t" at the appropriate degrees of freedom are printed. If there is a significant difference the program will exit. If there is no significant difference between the blanks, the program pools the blanks with the previous blanks and calculates a mean and standard deviation.

The ANOVA table is produced for the linear regression as well as the slope and intercept. The analyst checks that the F-statistic for the linear regression is significant.

Next, the cumulative sum is calculated. The analyst must first enter the desired probability level; i.e., 0 = 95%, 1 = 90%, 2 = 80%. When this is done, the program calculates the cumulative sum and checks that against the critical height, if the cumulative sum is less than the critical height, then the slope is within the control range. In this case, the slope is pooled with all of the previously determined slopes and a mean slope calculated. If the slope is not in control, that slope is "flagged" with a negative sign and the calculation proceeds.

Next, the M.D.C. is calculated using the standard deviation of the blanks, the "t" value for the blanks, and the mean slope.

The concentrations of the unknowns is calculated next. The mean concentration, standard deviation, and coefficient of variation are calculated for each replicate and filed. If any concentrations are less than the M.D.C., they are "flagged" with a negative value and the concentration filed as one-half

the M.D.C.

Those desiring a detailed listing of the program are referred to the Data Management Department, Harbor Branch Foundation, Inc.

Figure 4. Computer printout of "PAAS1" program. This program manipulates data that was filed using the "CDFILE" program. (page 1 of 2).

RUN PAAS1

ENTER THE WEEK NUMBER? 99999

ENTER 1 FOR DISSOLVED OR 2 FOR PARTICULATE? 1

ENTER THE NUMBER OF SAMPLES? 7

ENTER THE METAL (CD,CU,FE,PB,MN,ZN, OR CR)? MN

STATISTICS FOR BLANKS

MEAN= 5.83333E-03

S.D.= 2.04124E-03

C.O.V.= 34.9927

T-TEST:OLD-NEW

T CAL= .0993237

T TABLE= 1.981 D.O.F.= 113

NEW AND OLD BLANKS DO NOT DIFFER

RESULTS FOR STD CURVE

LINEAR REGRESSION

ANOVA RESULTS

VAR	SOURCE	DF	SS	MS	F
AMOUNG	GRPS	4	.0892464	.0220616	16978.5
LIN	REG	1	.0880908	.0880908	1698.11
DEV	FROM REG	3	1.55628E-04	5.18759E-05	39.9236
WITHIN	GRPS	5	6.49691E-06	1.29938E-06	
TOTAL		9	.0882529		

SLOPE = .0266966

INTERCEPT = -4.71075E-03

CUM. SUM RESULTS FOR DISSOLVED MN

ENTER THE PROBABILITY LEVEL NUMBER FROM THE FOLLOWING LIST:

0=95% 1=90% 2=80% PROB. LEVEL= ? 0

T= 2.16 FOR 13 DEG OF FREEDOM (MAX D.O.F.=120)

DELTA EQUALS 2.89526E-03

DIFF BETWEEN MEAN AND EXP. SLOPES 3.44046E-03

CUM. SUM 1.53263E-03

DISTANCE 19.9699

CRITICAL HT. .028909

CUM. SUM IS WITHIN CONTROL RANGE

MINIMUM DETECTABLE CONCENTRATION IS 2.10071

Figure 4. "PAAS1" computer printout. (page 2 of 2).

WEEK 99999 RESULTS

RESULTS FOR STATION 1 SAMPLER 1 REF 1

MEAN = 20.2165

STANDARD DEVIATION = .26482

COEFFICIENT OF VARIATION = 1.30992

RESULTS FOR STATION 1 SAMPLER 1 REF 2

MEAN = 21.7897

STANDARD DEVIATION = .158769

COEFFICIENT OF VARIATION = .728642

RESULTS FOR STATION 1 SAMPLER 2 REF 1

MEAN = 18.7931

STANDARD DEVIATION = .158961

COEFFICIENT OF VARIATION = .845849

RESULTS FOR STATION 1 SAMPLER 2 REF 2

MEAN = 19.8419

STANDARD DEVIATION = .264935

COEFFICIENT OF VARIATION = 1.33523

RESULTS FOR STATION 1 SAMPLER 3 REF 1

MEAN = 17.969

STANDARD DEVIATION = .794574

COEFFICIENT OF VARIATION = 4.42192

RESULTS FOR STATION 1 SAMPLER 3 REF 2

MEAN = 17.5944

STANDARD DEVIATION = .264935

COEFFICIENT OF VARIATION = 1.50579

RESULTS FOR STATION 1 SAMPLER 4 REF 1

MEAN = 14.9723

STANDARD DEVIATION = .264877

COEFFICIENT OF VARIATION = 1.76911

?ENL AT LINE 400

READY

Summary and Conclusions

The collection and analysis of samples for trace metals is an exacting, often tedious procedure. This is a result of three factors: first, the concentrations of trace metals in estuarine waters are quite low (in the low parts per billion range); second, contamination of the sample by fortuitous contact with boats, fingers, gear, etc. is difficult to avoid (it is apparent that even a small amount of contamination could easily double the concentration detected in a sample); and third, the analysis of low trace metal levels is difficult, especially in estuarine waters which have high dissolved solid contents. However, all of these factors may be overcome by following the procedures discussed in this report.

To summarize the important points, the discussion will be divided into three sections: collection of samples, preparation of samples for analysis and analytical factors.

Collection

It is absolutely imperative that samples be collected so that the concentration of the metal in the sample is representative of the concentration of metal in the area from which the sample was collected; indeed, any analysis is only as good as the sample collected. This is accomplished by:

1. using only teflon or polyethylene collecting gear,
(these are non-contaminating);
2. cleaning all sampling gear rigorously and maintaining the cleanliness by storing the gear in plastic bags;
3. allowing only the sample liquid to contact the gear;

4. acidifying the sample immediately with high quality acid; and,
5. transporting and storing the sample so that nothing contacts the sample.

Analysis

We have found that estuarine water samples may be analyzed with acceptable accuracy and precision if the following treatments are observed:

1. Samples are opened only under a "class 100" filtered air box.
2. Teflon sample cups are used throughout, where it is possible.
3. The samples are diluted 1:1 with high purity water. We have found that this reduces the quantity of bulk salts and improves the slope and M.D.C. for our analysis. However, if the concentrations are lower than those found in estuarine waters, this procedure may not be viable (e.g., if it were necessary to analyze deep ocean water).
4. A matrix modifier is used. We use a 500 g/l solution of recrystallized ammonium nitrate. In a 10 ml volumetric, 0.5 ml of this solution is added and the volumetric brought to volume.

Analytical considerations

Relative to the analysis of the samples using the HGA-^R 2100, we have found the following:

1. A pyrolytically coated graphite tube improves the sensitivity of the analysis.
2. It is necessary to ramp both the dry and char cycles to eliminate spattering of the sample.
3. A 50 μl injection volume is usually necessary to enable the analyst to detect some of the metals.
4. A close watch must be kept on the blanks and on the slopes of the standard curves for each metal and each analytical run. We have accomplished this with computer programs; however, these are not necessary. Manual methods are available and have been used by us successfully.

It has been shown that these techniques may be used to successfully analyze cadmium, chromium, copper, iron, lead, and manganese in estuarine waters. Furthermore, a system has been developed that statistically monitors each analysis so that changes in sensitivity may be observed, documented, and corrected.

Although these techniques are acceptable for estuarine waters which have relatively high trace metal concentrations, we are continually attempting to improve the sensitivity of the analysis so that extremely low trace metal levels (less than 1 $\mu\text{g}/\text{l}$), such as are found in Gulfstream waters, can be successfully analyzed.

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