Video Article

Clean Sampling and Analysis of River and Estuarine Waters for Trace Metal Studies

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Abstract

Most of the trace metal concentrations in ambient waters obtained a few decades ago have been considered unreliable owing to the lack of contamination control. Developments of some techniques aiming to reduce trace metal contamination in the last couple of decades have resulted in concentrations reported now being orders of magnitude lower than those in the past. These low concentrations often necessitate preconcentration of water samples prior to instrumental analysis of samples. Since contamination can appear in all phases of trace metal analyses, including sample collection (and during preparation of sampling containers), storage and handling, pretreatments, and instrumental analysis, specific care needs to be taken in order to reduce contamination levels at all steps. The effort to develop and utilize "clean techniques" in trace metal studies allows scientists to investigate trace metal distributions and chemical and biological behavior in greater details. This advancement also provides the required accuracy and precision of trace metal data allowing for environmental conditions to be related to trace metal concentrations in aquatic environments.

This protocol that is presented here details needed materials for sample preparation, sample collection, sample pretreatment including preconcentration, and instrumental analysis. By reducing contamination throughout all phases mentioned above for trace metal analysis, much lower detection limits and thus accuracy can be achieved. The effectiveness of "clean techniques" is further demonstrated using low field blanks and good recoveries for standard reference material. The data quality that can be obtained thus enables the assessment of trace metal distributions and their relationships to environmental parameters.

Video Link

The video component of this article can be found at https://www.jove.com/video/54073/

Introduction

It has been commonly recognized that some trace metal results obtained for natural waters may be inaccurate owing to artifacts arising from inadequate techniques applied during sample collection, treatments and determination[1,2]. The true concentrations (in sub-nM to nM range in surface waters [3]) of dissolved trace metals are now up to two orders of magnitude lower than previously published values. The same situation has been found in marine chemistry where the accepted dissolved trace metal concentrations in oceanic waters have decreased by orders of magnitude over the last 40 years or so as improved sampling and analytical methods have been introduced. Efforts have been made to improve data quality with the developments of "clean techniques" aiming at the reduction or elimination of trace metal contamination throughout all phases of trace metal analysis [4-8]. For the determination of trace metal concentrations at ambient levels, preconcentration is often required. Ion-exchange techniques [8-12] have been commonly applied for efficient preconcentration.

Contamination can arise from the walls of containers, the cleaning of the containers, the sampler, sample handling and storage, and sample preservation and analysis [7,13]. All studies using clean methods conducted more recently indicate that trace metal concentrations in natural waters are typically well below the detection limits of routine methods [7]. Since the recognition of suspect trace metal data in the early 1990s, clean methods have been incorporated into US EPA (Environmental Protection Agency) Guidelines for trace metal determination [14] and the US Geological Survey has adopted clean methods for their water quality monitoring projects [15]. Clean methods for trace metal studies need to be employed in all projects in order to create a firm and accurate data base.

In principle, water samples used for trace metal determination should be collected with appropriate sampling gears of a particular material and composition, stored and treated properly using appropriate containers and apparatus, before proceeding with instrumental analysis. Since suspended particulate matter (SPM) can undergo changes during the sample storage period and alter water composition, rapid separation of SPM from water samples is a common practice for trace metal studies in aquatic environments. For the determination of dissolved trace metal concentrations in natural waters, filtration is necessary and in-line filtration techniques are suitable and efficient.

Distribution and behavior of trace metals in aquatic environments such as surface and ground waters can be affected by natural (*e.g.*, weathering) and anthropogenic (*e.g.*, wastewater effluents) factors, as well as other environmental conditions, such as regional geology, morphology, land use and vegetation, and climate [16-19]. This can then lead to differences in physicochemical parameters such as concentrations of suspended particulate matter (SPM), dissolved organic carbon (DOC), anthropogenic ligands (*e.g.*, ethylenediaminetetraacetic acid, EDTA), salt, redox potential and pH [17-20]. Therefore, accurate and relevant trace metal studies require appropriate collection of samples for trace metal analysis as well as for the determination of related factors and parameters.

Protocol

1. Sampler Preparation

1. Sampler
   1. Assemblage of sampler
      1. Connect a 4 m long fluorinated ethylene propylene (FEP) tubing (I.D. 0.635 cm, O.D. 0.95 cm or similar) to a 1.5-m chemically resistant silicone pumping tube (O.D. 0.635 cm).
      2. Insert a polypropylene Y-connector into the pumping tube, and connect a 50-cm pumping tube to one outlet, and a 0.45 µm capsule filter (by a 20-cm pumping tube) to the other.
      3. Assemble the tubings in a clean room (bench) after they are cleaned (see below), and store the assemblage in two layers of polyethylene bags.
   2. Sampler cleaning
      1. Fill (by attaching the 1.5-m pumping tube onto a peristaltic pump) the tubing set with lab detergent solution and soak it for 24 hr. Flush the tubing set with de-ionized water, then fill it with 10% (v/v) HCl (Reagent Grade) and soak for 48 hr.
      2. Thoroughly flush the tubing set with de-ionized water several times, and store the assemblage in plastic bags. Clean the Y-connectors and short pumping tubes by soaking them in a 50% (v/v) HNO_3 (Reagent Grade) solution for 24 hr.
   3. Capsule filters
      1. Clean the capsule filters by first flushing them with de-ionized water, then 10% (v/v) HCl solution for a 48-hr soaking step.
      2. After acid soaking, flush the filters with de-ionized water and add 1 ml of 21% (weight) NH_4OH (sub-boiled) solution into each filter to neutralize the acid.
      3. Seal individual filter with a loop of 30-cm cleaned-pumping tube connecting the inlet and outlet, and store in polyethylene zippered bags.
2. Sample containers
   1. Use polyethylene (PE, 1,000-ml) and FEP (500 ml and 1,000 ml) bottles for containers for trace metal determination.
      1. Clean the bottles by soaking them first in detergent (1%), then in 50% (v/v) HNO_3 (Reagent Grade) and 10% (v/v) HCl (Reagent Grade) solutions for 24, 48 and 24 hr, respectively, and rinse the bottles with de-ionized water between the two soaking steps.
      2. After a final HCl soaking, rinse the bottles thoroughly with de-ionized water (DIW) and dry the bottles (with cap sealed) in a clean room or class-100 clean bench.
      3. Seal cleaned bottles in polyethylene zippered bags and double-bag them in polyethylene bags for transport.
   2. Clean glass bottles for dissolved organic carbon (DOC) determination
      1. Soak amber borosilicate glass bottles (40-ml), for analysis of dissolved organic carbon (DOC), in 10% HCl for 48 hr. Rinse cleaned glass bottles with de-ionized water, and combust them at 480 °C for 2 hr before use. Seal the bottles individually in zippered polyethylene bags for transport.

2. Sampling

1. Water sample collection
   1. Upon arrival at the sampling site, mark sample identification number on the outer bag, and keep the bottles in their original bags.
   2. At the river bank or on a boat, have one person open the bag with the sampler and attach the 4-m FEP tubing to a 3-m polypropylene pole (cleaned). Extend the pole as far from the bank as possible and keep the inlet of the FEP tubing approximately 30 cm below the surface of running river water before the pump is turned on.
   3. Have another person attach the pumping tube to the pump head of a peristaltic pump (sampling pump with internal batteries). Start the pump and drain the water (downstream side) at least 3 times of the total sampler volume. Put on powder-free gloves and open the bag of bottles and caps to start filling the sample bottles.
      1. Alternatively, if another person is available, have the third person be in charge of opening the inner bags and sample caps, and holding the sampling tube that drains the sample into the bottle.
   4. Collect an unfiltered sample in a 125-ml plastic bottle for measurements of conductivity, temperature and pH in the field.

   5. Collect unfiltered samples (500-ml or 1,000 ml for particulate sample collection) first (through the outlet without a filter) and then close the outlet with a plastic clamp to force water to go through the capsule filter to collect a filtered water sample (1-L for dissolved trace metal determination).
   6. Collect filtered samples (through the outlet with the filter) in 40-ml amber glass bottles for DOC and EDTA measurements.
   7. Collect water samples at a flow rate of approximate 500 ml/min to 1,000 ml/min. Replace capsule filters when the pressure starts to build up (according to the filters' specification). For each type of sample, collect extra samples, as well as field blanks, at selected locations to serve as quality control aliquots.
   8. Place the 40-ml glass bottles on dry ice and stored in an ice chest, and polyethylene bottles in ice chests.

2. Collection of suspended particulate matter (SPM)
   1. Collect SPM on 0.4 µm polycarbonate (PC) membrane filters (acid washed and pre-weighed) by vacuum filtration, using a plastic filtration funnel and vessel.
   2. Freeze dry membrane filters in the lab to yield SPM concentrations and provide particle samples for particulate trace metal determination.

3. Sample Pretreatment (Dissolved Trace Metals)

1. For dissolved trace metal determination, add 2-ml concentrated sub-boiled HNO_3 (per 1-L sample) into sample bottles. Transfer dissolved trace metal samples (acidified) into FEP bottles. Alternately, samples can be collected directly into FEP bottles. UV-irradiate the samples in FEP bottles for 24 hr (8 15-watt UV lamps).

4. Preconcentration and Treatments for Trace Metal Analysis

1. Preconcentration resin
   1. Weigh 2 g of cation exchange resin into a small (10-30 ml capacity) plastic cup and add a small amount of 2 N HNO_3 solution into the cup. Pour the resin into a 10-ml capacity chromatography column. Clean the resin by washing the column with 5-ml 2 N HNO_3 (sub-boiled) twice, and rinse with high purity water (HPW) 3 times.
   2. Add 10 ml of 1 M NH_4OH (sub-boiled) into the column to convert the resin to NH_4^+-form.

2. Buffer solution (1 M ammonium acetate)
   1. Add 57 ml of glacial acetic acid (sub-boiled) in approximately 800 ml of HPW. Add ~60 ml of ammonium hydroxide (21%, sub-boiled) and mix with acetic acid. Adjust the final pH to 5.5 and the final volume to 1,000 ml.

3. Preconcentration procedure (dissolved trace metals)
   1. Adjust the pH of the acidified, UV-irradiated samples to 5.5 ± 0.3 by adding 30 ml of 1 M ammonium acetate buffer solution and some (~2.8 ml) NH_4OH (sub-boiled) into the samples.
   2. Place the sample bottle ~30 cm above the column packed with cationic exchange resin (Section 4.1) and connect the sample bottle and preconcentration column by a ~60 cm FEP tube and chromatography cap and connector (female Luer).
   3. Control the flow rate at 3-5 ml/min using a 2-way stopcock, connected above the column. Allow the samples to pass through the preconcentration columns. After the samples have passed the column, disconnect the tubes and caps from the columns.
   4. Treat the columns with 2 x 5-ml HPW and 4 x 5-ml of 1 M ammonium acetate (pH 5.5), and 2 x 5-ml of HPW to separate major cations from other trace metals.
   5. Place a 30-ml acid-washed PE bottle just below the column and wash the column with 7 x 1-ml 2 N HNO_3 (sub-boiled) into the PE bottle.
   6. Determine the volume of 2 N HNO_3 effluent (~8.0 ml) by obtaining the weight and specific gravity of the 2 N HNO_3 effluent.

4. Digestion of suspended particulate matter
   1. Freeze dry PC filters with SPM samples and weigh them after drying. Place SPM samples, with filters, into pre-weighed perfluoroalkoxy alkanes (PFA) vessels (60-ml capacity), and add 3 ml of concentrated HNO_3 into the vessels.
   2. Tighten the vessels to a constant torque of 2.5 kg-meters, and digested in a conventional oven at 130 °C for 12 hr. After cooling, open the vessels and add 2 ml of HF into the vessels.
   3. Tighten the vessels to a constant torque of 2.5 kg-meters, and digested in a conventional oven at 130 °C for 12 hr. After cooling, open the vessels and add 16 ml of 4.5% boric acid solution into the vessels.
   4. Tighten the vessels to a constant torque of 2.5 kg-meters, and digest in a conventional oven at 130 °C for 12 hr. Weigh each vessel and determine specific gravity of each digest solution to yield final digest volume.
   5. Calculate a dilution factor for each digest from the final volume and weight of SPM on filter (final digest volume divided by sample weight on filter).

5. Sample Analysis

1. Trace metals
   1. Determine trace metals (Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn) concentrations in pre-concentrated dissolved sample and solution from particulates using flame atomic absorption spectrometry, graphite furnace atomic absorption spectrometry, and/or inductively coupled plasma mass spectrometer.
   2. Determine major ions concentrations and some trace metals, in sub-samples drawn prior to preconcentration and digests of suspended particulate matter, by an inductively coupled plasma-atomic emission spectrometry.

2. Ancillary parameters
    1. Determine sample temperature, pH, salinity and conductivity in the field, using portable devices.
    2. Determine dissolved organic carbon (DOC) concentrations by a Total Organic Carbon Analyzer, based on wet oxidation with carbon dioxide detection by infrared spectrometry [21]. Determine total EDTA concentration by a high performance liquid chromatography (with a SPD-M10AV Diodearray detector) following the established procedures [22,23].

Representative Results

With the development and use of "clean techniques", it is now well recognized that in order to obtain accurate trace metal concentrations in ambient waters, preconcentration of trace metals in waters samples is a common practice. While most water quality criteria for trace metals in natural waters are in the low µg/L range, lower detection limits are needed to investigate geochemical and biological effects on trace metals at ambient concentrations in aquatic environments.

With improved detection limits following the use of "clean techniques", it was clearly shown from a data set of 141 randomly collected river water samples from Texas rivers, that preconcentration is needed (**Figure 1**). The histogram of trace metal data for Cd, Cu, Ni, and Pb shows that, while some concentrations of specific elements may be relatively readily determined (*e.g.*, Cu), some elements (*e.g.*, Cd and Pb) have concentrations in ambient waters well below detection limits of routine analytical methods. The comparison was made with the Texas river data set against USEPA Methods 1637, 1638, 1639, and 1640. Methods 1637 and 1640 are techniques for trace metal determination with preconcentration and detected by graphite furnace atomic absorption spectrometry or inductively coupled plasma-mass spectrometry, respectively. Methods 1639 and 1638 are for the determination of trace metals in ambient waters for the above-mentioned instruments, without preconcentration.

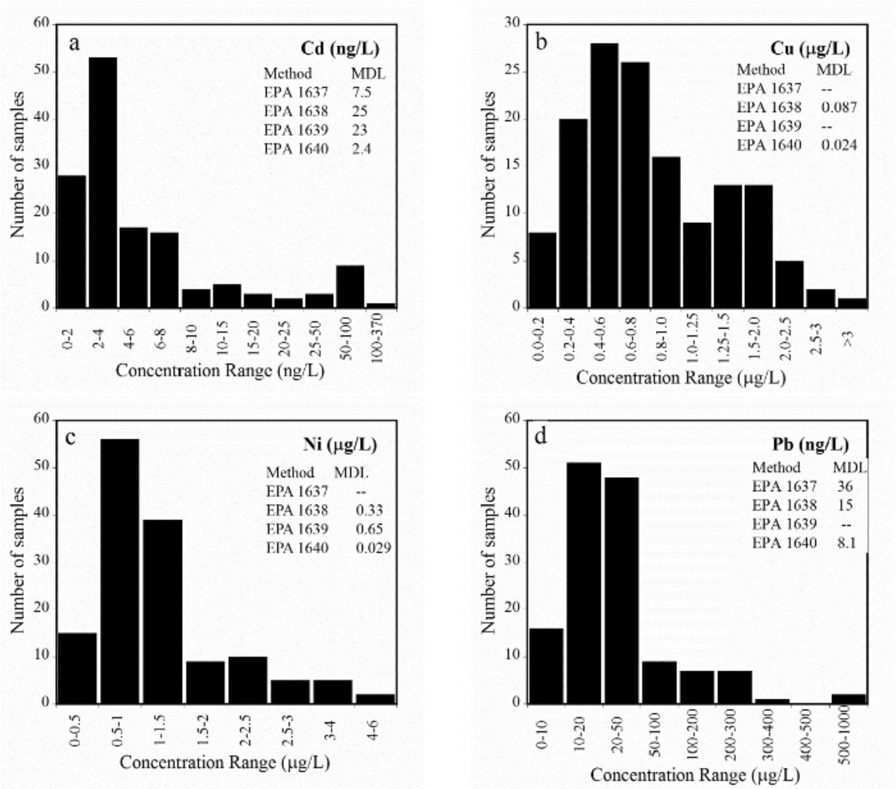

Figure 1. Histogram of data collected from Texas, USA rivers, and comparison with USEPA method detection limits for Cd (a), Cu (b), Ni (c), and Pb (d). A total of 141 samples, randomly collected from different locations, were processed using the sampling and analysis protocol described here. Comparing the distributions of data and the EPA detection limits shows that a large fraction of samples from Texas rivers will need to be preconcentrated in order to obtain best data quality. The detection limits (ML) shown in the graphs are those listed in USEPA Methods 1637, 1638, 1639, and 1640 (method details see text). Please click here to view a larger version of this figure.

Results of detection limits, recoveries for a reference material (SRM), and blank concentrations of field blanks, high purity water brought to the field and treated and processed as samples, show the effectiveness of "clean techniques" (**Table 1**). Reducing contamination results in lower detection limits, good SRM recoveries, and very low field blank concentrations. The results shown in **Table 1** were obtained based on processing 1 L of blank samples. The low detection limits imply that trace metal concentrations can be obtained with good data resolution, which is critical when trace metal distributions are to be compared to environmental conditions (parameters) that influence trace metal geochemistry [20]. Comparing our results (method detection limits) with those of EPA Methods mentioned above, which, however, do not include field sampling, comparable or better (lower) detection limits were obtained using the protocol provided here. Good precision was also achieved, as evidenced by similar results of field blanks. Replicate samples were collected and processed separately for some field samples and the differences of trace metal concentrations were generally less than 10%.

	Cd	Co	Cu	Fe	Mn	Ni	Pb	Zn
	ng/L	mg/L	mg/L	mg/L	mg/L	mg/L	ng/L	mg/L
MDL	1	0.01	0.06	0.3	0.1	0.1	10	0.1
SLRS-3 (N=11)								
Certified	13	0.027	1.35	100	3.9	0.83	68	1.04
Measured (avg)	12.6	0.026	1.29	97.2	3.86	0.77	71	1.13
Standard deviation	0.9	0.008	0.09	4.2	0.20	0.06	9	0.12
Recovery(%)	97	97	96	97	99	93	105	109
Field blank 1	0.2	0.000	0.003	0.03	0.00	0.01	2.5	0.02
Field blank 2	0.2	0.000	0.011	0.02	0.01	0.00	2.9	0.03
Field blank 3	0.1	0.000	0.011	0.03	0.00	0.00	2.5	0.01

Table 1. Results of data quality achieved using the sampling and analysis protocol described here. Detection limits, statistical results for a trace metal standard reference material (SLRS-3) and illustration of trace metal concentrations in 3 filed blanks (high purity water treated as samples in the field) indicate the effectiveness of the described protocol. Concentration units are ng/L for Cd and Pb, and µg/L for the other elements. Low detection limits, good recoveries for a standard reference material, and low field blank concentrations were achieved.

Results obtained using the sampling and analysis protocol described here demonstrates that it not only produces trace metal data with low detection limits, it also provides a large range for application. As shown in **Figure 2** with the comparison of trace metal concentrations determined by two independent methods, good correlation (R^2>0.994) with a linear regression slope near 1 indicate that these techniques can be applied in aquatic environments. Since an ion exchange preconcentration technique was used to generate those results, the linear range indicate that the described protocol can be applied in distinctively different aquatic environments where trace metal concentrations vary over a large range.

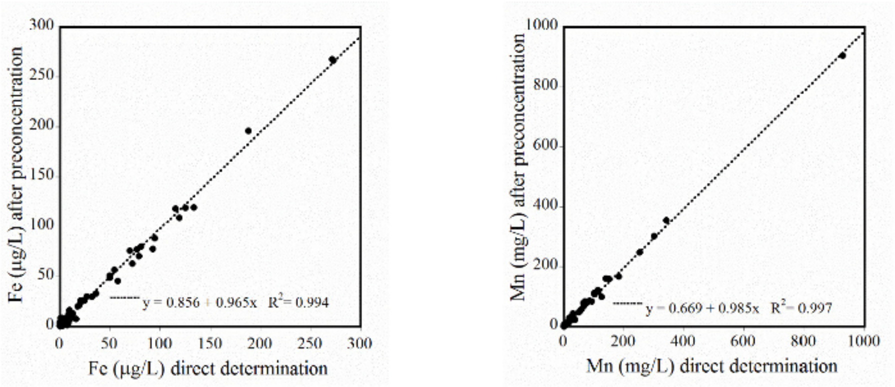

Figure 2. Comparison of trace metal concentrations obtained from two independent methods. Very good agreement was found between trace metal concentrations determined using two independent methods in separate aliquots of the same samples. One sample was analyzed directly by inductively coupled plasma atomic emission spectrometry and the other was preconcentrated by ion exchange techniques following determination by inductively couple plasma mass spectrometry. Large concentration range implies that the techniques are suitable for trace metal studies in distinctively different environments where trace metal concentrations show significant differences. Please click here to view a larger version of this figure.

Discussion

Obtaining reliable trace metal data in natural waters requires great care as emphasized during sample collection, processing, pretreatments, and analysis that aim to reduce contamination. Trace metal concentrations in natural waters obtained using "clean techniques" in the last two decades found that the concentrations can be orders of magnitude lower than previously reported. Water quality criteria for trace metals in waters are now more readily assessed when trace metal levels are accurately measured resulting in better assessment of harmful effects to humans and higher organisms. Bioavailability and toxicity of trace metals in aquatic environments require more rigorous investigations in the lower concentration ranges. This is not an easy task, as distribution and behavior of trace metals are controlled by many other physicochemical parameters in the environment, and because of that, reliable assessments of bioavailability and toxicity of trace metals are challenging. Sampling and analysis of ancillary parameters related to trace metals need to be included in such assessments.

Eliminating contamination and ultimately lowering detection limits for trace metal concentrations in ambient waters, therefore, require scientific parties to pay special attention to all phases of trace metal analysis. From lab ware, sampling gears, sample containers, apparatus and materials used during sample pretreatments, chemicals and reagents, to instrumental setting, contamination becomes additive and all those factors mentioned above need to be specified when reporting results from trace metal analysis. Therefore, the procedures to prepare sampling gear, sample containers, and materials used to process and analyze samples are all critical steps toward obtaining high data quality for trace metals in aquatic environments. When compared with existing methods (*e.g.*, USEPA Methods), data obtained using the protocol outlined above result in better or comparable detection limits and very low blanks. A large suite of trace elements (8, **Table 1**) can be assessed.

The protocol described here can be easily applied to collection of different types of aquatic samples, in addition to river and estuarine waters, *e.g.*, oceans, lakes and groundwaters. Since sample preconcentration can be time consuming, the protocol provided here can be modified according to specific characteristics in different aquatic environments. In highly impacted waters, samples should still be collected cleanly, but they can be determined directly by suitable instruments if no matrix interference is encountered. Sample volume can be adjusted if greater or smaller preconcentration factors are required. In cases where the ion exchange capacity needs to be increased, larger amounts of resin can be used.

This work demonstrates that, determination of trace metals using "clean techniques" that include sampling and pre-concentration, in conjunction with determining related environmental parameters, the distribution of trace metals in aquatic environments can be better assessed, taking into account specific environmental conditions and the extent of natural and anthropogenic influences [20]. The large concentration ranges that this protocol is capable of applying to imply that investigations of trace metal distributions and behavior can also be conducted in environments that change over time and space.

Disclosures

The authors have no conflict of interests to disclose.

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