

Electrochemical Detection of Heavy Metals in Municipal and Ground Water Samples

JEFFREY S. ERICKSON

SCOTT A. TRAMMELL

*Laboratory for Molecular Interfaces Branch
Center for Bio/Molecular Science and Engineering Division*

LISA C. SHRIVER-LAKE

*Laboratory for Biomaterials and Systems Branch
Center for Bio/Molecular Science and Engineering Division*

SCOTT N. DEAN

*Laboratory for Bio/Nano Science and Technology
Center for Bio/Molecular Science and Engineering Division*

November 29, 2023

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

| | | | | | |
|--|-------------------------|--|--|--|--|
| 1. REPORT DATE (DD-MM-YYYY) 29-11-2023 | | 2. REPORT TYPE NRL Memorandum Report | | 3. DATES COVERED (From - To) April 2022 – September 2023 | |
| 4. TITLE AND SUBTITLE Electrochemical Detection of Heavy Metals in Municipal and Ground Water Samples | | | | 5a. CONTRACT NUMBER | |
| | | | | 5b. GRANT NUMBER | |
| | | | | 5c. PROGRAM ELEMENT NUMBER NISE | |
| 6. AUTHOR(S) Jeffrey S. Erickson, Scott A. Trammell, Lisa C. Shriver-Lake, and Scott N. Dean | | | | 5d. PROJECT NUMBER | |
| | | | | 5e. TASK NUMBER | |
| | | | | 5f. WORK UNIT NUMBER 4X53 | |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Naval Research Laboratory 4555 Overlook Avenue, SW Washington, DC 20375-5320 | | | | 8. PERFORMING ORGANIZATION REPORT NUMBER NRL/6930/MR--2023/7 | |
| 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Naval Research Laboratory 4555 Overlook Avenue, SW Washington, DC 20375-5320 | | | | 10. SPONSOR / MONITOR'S ACRONYM(S) NRL | |
| | | | | 11. SPONSOR / MONITOR'S REPORT NUMBER(S) | |
| 12. DISTRIBUTION / AVAILABILITY STATEMENT DISTRIBUTION STATEMENT A: Approved for public release; distribution is unlimited. | | | | | |
| 13. SUPPLEMENTARY NOTES | | | | | |
| 14. ABSTRACT This report describes the analysis of drinking water field samples for selected heavy metal contaminants at EPA relevant levels. First, four laboratory deionized water samples spiked with trace metal contaminants were analyzed to determine the limits of the assay when performed with the homebuilt instrument and field kit. Next, eleven tap water field samples collected from various locations in Maryland, Texas, and the Washington DC area were tested. Models for interactions between compounds are explored under the standard addition framework. Finally, recommendations for improvements to the field kit are presented. | | | | | |
| 15. SUBJECT TERMS Drinking water testing Field kit Heavy metals Field samples Electrochemistry | | | | | |
| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT U | 18. NUMBER OF PAGES 26 | 19a. NAME OF RESPONSIBLE PERSON Jeffrey Erickson |
| a. REPORT U | b. ABSTRACT U | c. THIS PAGE U | | | 19b. TELEPHONE NUMBER (include area code) (202) 767-0364 |

This page intentionally left blank.

CONTENTS

| | | |
|-----|---|----|
| 1. | INTRODUCTION | 1 |
| 1.1 | Background | 1 |
| 2. | MATERIALS AND METHODS..... | 2 |
| 3. | EXPERIMENTAL RESULTS..... | 4 |
| 3.1 | The Field Kit | 4 |
| 3.2 | Qualitative Results | 5 |
| 3.3 | Quantitative Results | 6 |
| 3.4 | Field Samples | 7 |
| 3.5 | Field Testing | 10 |
| 4. | DISCUSSION | 12 |
| 4.1 | The Method of Standard Additions for One Target | 12 |
| 4.2 | Baseline Correction Procedures | 13 |
| 4.3 | Extending the Method to Multiple Targets | 14 |
| 4.4 | Qualitative Effects of Complex Formation | 16 |
| 4.5 | Comparison to Literature | 19 |
| 4.6 | Suggested Experimental Improvements..... | 19 |
| 5. | CONCLUSIONS..... | 20 |
| 6. | REFERENCES | 20 |

FIGURES

| | | |
|-----------|--|----|
| Figure 1. | Photograph of the various instruments used for collecting data | 4 |
| Figure 2. | Screenshots of the CStat interface..... | 6 |
| Figure 3. | Timeline of experiments and sample collection..... | 7 |
| Figure 4. | Approximate location of all eleven samples collected for this study..... | 8 |
| Figure 5. | Raw data collected in the field..... | 11 |
| Figure 6. | The method of standard additions | 12 |
| Figure 7. | Standard addition curves M_{34} and M_{44} | 16 |
| Figure 8. | Simulated standard addition curves for zinc and copper..... | 17 |

TABLES

| | | |
|----------|---|---|
| Table 1. | Breakout cable connections for the CStat. | 3 |
| Table 2. | Experimental parameters used specific to each instrument..... | 4 |
| Table 3. | EPA limits | 5 |

Table 4. Quantitative test results in spiked deionized laboratory water 7

Table 5. Results from field samples tested in the laboratory..... 9

Table 6. Standard addition curve parameters and calculated target concentration..... 14

Table 7. The value of the parameters for “copper” and “zinc” signals 16

Table 8. Simulated values for slope and intercept using hypothesis one 17

Table 9. Simulated values for slope and intercept using hypothesis two 18

EXECUTIVE SUMMARY

This report describes the analysis of drinking water field samples for selected heavy metal contaminants at EPA relevant levels. The assay consists of anodic stripping voltammetry in the presence of bismuth salts using a homebuilt electrochemical instrument. Experiments were performed with a field kit based on the method of standard additions. First, four laboratory deionized water samples spiked with trace metal contaminants were analyzed to determine the limits of the assay when performed with the homebuilt instrument and field kit. Next, eleven tap water field samples collected from various locations in Maryland, Texas, and the Washington DC area were tested. Models for interactions between compounds are explored under the standard addition framework. Finally, recommendations for improvements to the field kit are presented.

This page intentionally left blank.

1. INTRODUCTION

Access to clean and safe drinking water is a global issue. Most potable water comes from one of two sources: municipal and ground water. Municipal water can have a variety of different origins but in most cases it is purified and/or treated at a local plant before being certified as clean. Once leaving the treatment facility, it is distributed through a network of water mains and service lines. These pipes can be very old. Water lines have historically been composed of lead, copper, and galvanized steel; these materials are known to leach trace amounts of heavy metals into the drinking water that they carry, especially under acidic conditions. In rural areas, ground water supplies can have significant natural mineral content and can also suffer contamination from nitrates, nitrites, and organic chemicals from farm and industrial runoff.

Small groups of military personnel working in the field for extended periods of time must rely on locally sourced drinking water. However, water quality regulations in some parts of the world are lax or completely nonexistent. Even in developed countries, the only available water may come from a questionable source. This poor water quality can directly impact the warfighter. While a large number of portable purification methods exist such as boiling, iodine, filtration membranes including activated carbon, and chlor-floc tablets, there are currently no fielded technologies to verify that the purification was successful. This is a topic of sufficient importance that it was the focus of a recent conference (February, 2022) sponsored by Army DEVCOM Soldier Center with participants representing nineteen different military and DoD groups including the Army Futures Command and the Marine Corps Systems Command. There is also significant academic interest in this topic aimed at crossover civil applications such as the evaluation of municipal and rural drinking water supplies [1], environmental monitoring [2], and the evaluation of wastewater treatment [3].

In the spring of 2022, the Naval Research Laboratory (NRL) initiated an 18 month study with two goals: to evaluate the performance of literature-reported electrochemical assays for the simultaneous detection of multiple contaminants in drinking water, and to explore the feasibility of integrating these methods into a portable field kit with a goal of sample-to-answer in one hour or less. This manuscript is the final installment in a series of four reports detailing incremental progress on the project. The first report provided details of the field kit and sample collection strategy [4]. The second report focused on the possibility of identifying organics and trace metal contaminants simultaneously [5]. The third report explored the effect of working electrode composition on detection limits [6]. The work described here focuses on the performance of the assay and field kit when testing real tap water samples collected from various locations in Maryland, Texas, and Washington, DC.

1.1 Background

Tap water analysis is typically performed in central laboratories using various forms of mass spectrometry (GC-MS, LC-MS, ICP-MS), certain optical methods (ICPOES, AAS), and cell culture. Although exquisitely sensitive, this equipment is not portable. While some simple assays for water quality have been developed for on-site use, they are usually targeted to environmental waters, wastewater streams, or analysis of other non-potable types of waters such as swimming pools. In contrast, tap water is usually very clean; typical water testing assays such as those for pH, chlorophyll content, turbidity, and salinity are not useful.

Electrochemistry is naturally suited for the detection of trace heavy metals through the use of the anodic stripping voltammetry technique. Unfortunately, the classical method for performing this analysis uses mercury, a highly toxic material that is not suitable for handling by untrained personnel. Alternative methods are necessary. One possibility is the use of gold electrodes, although they suffer from a reduced potential window compared to other electrode materials. Recently it was observed that bismuth salts can also enhance trace heavy metal detection [7]. Bismuth can be dissolved in buffer concentrates or supplied

as pre-plated film electrodes, allowing it to be used with a minimum of handling. Furthermore, when plated onto carbon it exhibits a wide potential window. These advantages, coupled with its low toxicity compared to mercury make the method attractive for use in a field kit.

There has been significant academic interest in the use of electrochemical methods that employ bismuth for the detection of trace metals, organics, and some biological substances in various media including water and beverages such as fruit juice and wine [8]. One advantage of electrochemical detection is that it can be performed with a small amount of equipment: an instrument, a set of electrodes, and in some cases, a computer. There has been a recent trend towards the development of homebuilt miniaturized potentiostats [9] which can be battery powered, handheld, and use wireless communications. Furthermore, a wide variety of inexpensive and disposable screen printed electrodes (SPEs) are now commercially available. This makes electrochemical detection an ideal candidate for incorporation into a field kit.

There is at least one commercially available product specifically designed to perform electrochemical field tests for water quality: the 946 Portable VA Analyzer from MetrOhm. This product is advertised to detect a variety of different trace metals in field samples and can be configured with one of two electrode options: either MetrOhm's proprietary scTRACE gold microwire electrode (recommended), or a general SPE attachment for user selected electrodes. Evaluation of this product suggested that it is capable of providing adequate results. However, it is only designed to analyze one contaminant at a time, and it requires a moderate amount of user expertise. Moreover, many of the published methods for this instrument require chemical modification of the working electrode specific to the particular target of interest, something not easily done in the field. A more nimble solution is desirable.

This report builds on an effort to combine two widely-reported technologies, anodic stripping voltammetry in the presence of bismuth salts and a homebuilt potentiostat, into a field kit through the use of the method of standard additions. In this study the field kit was evaluated against two groups of samples: deionized water spiked with known amounts of trace heavy metals, and drinking water samples taken from various sites across Maryland, Texas, and the local Washington DC area. Qualitative and quantitative results are discussed. The 946 Portable VA Analyzer was used in some cases to obtain supplementary orthogonal results; this was especially helpful for identifying contaminants in particularly dirty samples. Finally, recommendations and suggestions for improvements are discussed.

2. MATERIALS AND METHODS

Three separate instruments were used in this Report. The CStat is a custom built potentiostat designed at NRL [10-13]. In this report, it was exclusively used for testing water samples in a laboratory setting with glassy carbon working electrodes. A 3 mm diameter glassy carbon disk electrode (product #CHI104) was obtained from CH Instruments (Austin, TX). The working electrode was cleaned before use at the beginning of each day of experiments with micro polish powder, 0.05 microns, from CH Instruments. The powder was dissolved in a small amount of deionized water on a glass plate with a polishing pad. Polishing was performed for five minutes using a figure eight pattern. A platinum wire counter electrode (product #CHI115) was obtained from CH Instruments. A 6 mm diameter silver/silver chloride refillable reference electrode (product #E-Ag/AgCl_70) was obtained from Redox.me (Norrköping, Sweden). The reference electrode was stored in 1 M potassium chloride solution when not in use. Both counter and reference electrodes were cleaned by rinsing with deionized water. A USB mini-B to banana plug cable (product # RRPECBL2, Mini-USB Cell Cable) purchased from Pine Research Instrumentation (Durham, NC) was used to break out the individual lead wires from the potentiostat; the banana plugs were connected to the appropriate electrodes using alligator clips as shown in Table 1.

| RRPECBL2 Lead Color | Electrode |
|---------------------|---------------|
| White | Reference |
| Red | Working |
| Green | Counter |
| Orange | No Connection |

Table 1. Breakout cable connections for the CStat.

A C3 Cell Stand from BASi (West Lafayette, IN) was used with a magnetic stir bar to perform mixing during accumulation steps. Stirring was turned off immediately before the square wave voltammetry sweep was performed. A photograph of the CStat is shown in Figure 1.

The Series II is a homebuilt electrochemical instrument¹, also designed at NRL. Unlike the CStat, the Series II is a prototype still under active development. It incorporates a number of improvements including true bipolar design with a center tapped battery pack, potentiostat and galvanostat modes, pinouts for a daughterboard, and the ability to run without a tether to software. In this work, the Series II was exclusively used for testing water samples at the collection site with screen printed carbon electrodes (SPEs). Bismuth oxide modified SPEs (product #DRP-110BI-U50) were obtained from Metrohm DropSens (Riverview, FL). Screen printed carbon electrodes with 4x5 mm working electrode (product #RRPE1002C) were obtained from Pine Research Instrumentation (Durham, NC). A Pine Research compact voltammetry grip mount, model #AKSPEGRP1, was used to hold the SPE; it is terminated with a 5-pin card edge connector on one end and a female USB mini-B connector on the other. A standard USB mini-B to A cable was used to connect the grip mount to the potentiostat. A photograph of the Series II prototype is shown in Figure 1.

A 946 Portable VA Analyzer from MetrOhm (Riverview, FL) was used with proprietary scTRACE gold microwire electrodes and assays as orthogonal verification for the detection of single metal targets. This instrument was only used in a laboratory setting. Although a number of different assays have been published for use with this instrument, here it was only used to test for the presence of two metals: copper and zinc. A photograph of the 946 Portable VA Analyzer is shown in Figure 1.

Trace metal standard solutions of lead, copper, zinc, cadmium, and bismuth, all in nitric acid, were obtained from Agilent (Santa Clara, CA). 1 M acetate buffer at pH 4.5 was made from sodium acetate purchased from Sigma-Aldrich, and diluted to 0.1 M for final use. The chemical reagents used with the 946 Portable VA Analyzer's assays included sulfuric acid (Ultrapure, CAS 7664-93-9), hydrochloric acid (99.999%, trace metal basis, CAS 7647-01-0), nitric acid, (OPTIMA, CAS 7697-37-2) and potassium chloride, (99.999%, CAS 7447-40-7) and were purchased from Fisher Scientific Company LLC. The cleaning solution for the scTRACE gold electrodes was 0.5 M H₂SO₄ and 0.05 M KCl in deionized water. The electrolyte for the analysis of copper was a solution of 0.3 M KCl and 0.1 M HCl. The Cu standard addition was a solution of 1 mg/L in 0.065 % nitric acid. Testing was performed in glass containers and used deionized water to minimize background contamination.

¹ Technically, the prototype is named the "CStat Series II". The name has been shortened to just "Series II" in this report to avoid confusion with the original CStat instrument.

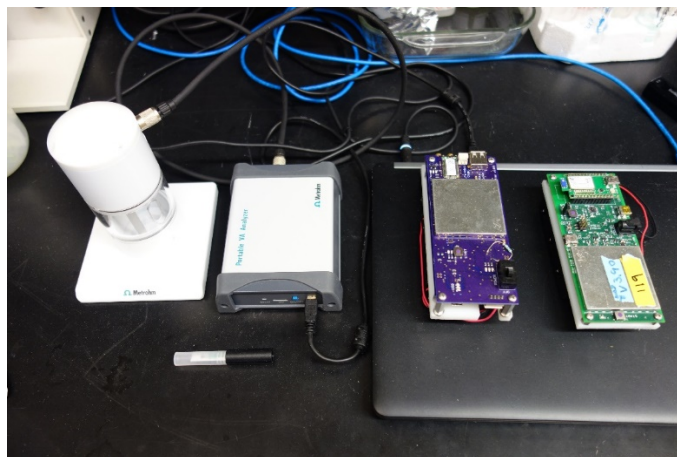


Figure 1. Photograph of the various instruments used for collecting data. From left to right: (1) the two white instruments are the 946 Portable VA Analyzer from Metrohm; the scTRACE electrode is sitting in front of them, (2) the purple circuit board is NRL's Series II prototype instrument, and (3) the green circuit board is NRL's CStat potentiostat.

All water testing experiments on the CStat and the Series II were performed in 0.1 M acetate buffer at pH 4.5. The square wave anodic stripping voltammetry technique was used for trace metals detection. Specific parameters are shown in Table 2. The 946 Portable VA Analyzer used experimental procedures specific to each particular metal tested².

| Potentiostat | Electrode | Conditioning | Accumulation | Square Wave Sweep |
|--------------|----------------------|---------------|----------------|-----------------------------------|
| CStat | Glassy Carbon | 30s at +0.3 V | 120s at -1.5 V | -1.5 V to +0.3 V, 17.5 Hz |
| Series II | BiO ₂ SPE | 5m at -1.2 V | 600s at -0.9 V | -0.9 V to -0.5 V or +0.5 V, 15 Hz |
| | Carbon SPE | N/A | 120s at -1.5 V | -1.5 V to +0.5 V, 15 Hz |

Table 2. Experimental parameters used specific to each instrument. For the CStat, stirring was performed with a stir bar on the C3 Cell Stand. It used glassy carbon electrodes and required 500 ppb bismuth added to all buffers. Series II measurements took place without stirring. Experiments used a 25 mV amplitude square wave with a 4 mV step size. All voltages in the table are vs. Ag/AgCl.

3. EXPERIMENTAL RESULTS

Two groups of samples were analyzed in this study. The first group was a blind test of four deionized water samples, each spiked with one of the four trace metals targeted by the assay. The identity and concentration of each contaminant was not known by the author in advance. The experiment provided both qualitative and quantitative verification of the electrochemical assay on NRL's homebuilt instruments and field kit, as well as an independent characterization of the 946 Portable VA analyzer used for orthogonal measurements. The second group contained eleven different samples collected from various field locations. Three of these field samples were tested at the collection site with the Series II prototype. Ten were brought back to the laboratory and tested with the CStat instrument.

3.1 The Field Kit

NRL's field kit is based on the method of standard additions, a procedure in which a sample is spiked with known amounts of target and successively measured as the spiked concentrations are increased. By

² Procedures for the analysis of various trace metals using the 946 Portable VA Analyzer are available here: https://www.metrohm.com/en_us/discover/blog/20-21/trace-metal-analysis-with-solid-state-electrodes---part-3.html

performing a linear regression on each target, the unknown amount (if any) in the original sample can be determined by extrapolating the regression line to the point where it crosses the x-axis. The field kit is designed to obtain four data points for each sample. It includes a clean blank containing only bismuth and sodium acetate, and “low”, “medium”, and “high” concentration trace metal spiked buffers. In the case of deionized water samples where all species in solution are known, there is no advantage to this technique. The power the method of standard additions becomes apparent when working with dirty field samples, where there might be unanticipated species present that interfere with the signal of each target in an unknown way. The method essentially creates calibration curves in real time.

All laboratory experiments were set up in the same manner as they would be run in the field. The kit contains four 50 mL Falcon tubes in a small plastic holder or Styrofoam block, each pre-filled with 3 mL of buffer concentrate including four spiked metals: lead, copper, cadmium, and zinc. To perform an experiment, a tap water sample is first collected in a 500 mL clean plastic bottle. Each Falcon tube is filled from this bottle to the 30 mL line, capped, and inverted 20 times to ensure good mixing. Testing is then performed. The exact volume of liquid placed in the test chamber is not important. In this work, 5 mL samples were more than sufficient to submerge the electrodes.

3.2 Qualitative Results

The method of standard additions is most effective when the unknown sample concentration falls somewhere near the spiked concentrations, so that the extrapolation of the regression line is not extensive³. For drinking water testing, relevant concentrations should be near the EPA limits for each of the targets. However, the four trace metals in the assay have very different allowable levels, as shown in Table 3.

| Metal or Buffer | EPA Limit (MCL) [ppb] | Spiked Concentration [ppb] | | | |
|-----------------|-----------------------|----------------------------|----------|----------|----------|
| | | Buffer 0 | Buffer 1 | Buffer 2 | Buffer 3 |
| Zinc | 5000 | 0 | 10 | 20 | 30 |
| Cadmium | 5 | 0 | 5 | 10 | 15 |
| Lead | 15* | 0 | 5 | 10 | 15 |
| Copper | 1300 | 0 | 5 | 10 | 15 |

Table 3. EPA limits (listed as “maximum contaminant levels”; aka MCL) and spiked buffer concentrations used with the Method of Standard Additions for the four targets in this study. The concentrations listed are the values each tube will have when diluted with sample to the 30 mL mark. In addition, every tube will contain 500 ppb bismuth and 0.1 M acetate buffer after sample is added. The lead concentration listed here is the “EPA Action Level” set by the Lead and Copper Rule, most recently revised in December, 2021; the MCL for lead is zero. Zinc is not considered a hazardous substance; the limits here are published as a secondary standard. Parts per billion (ppb) and $\mu\text{g/L}$ are equivalent terms.

The initial hypothesis was to use different spiked target concentrations, each appropriate to the EPA level for the particular target. However, the resulting very broad copper signal quickly swamped all other signals in the voltammogram and made quantification impossible (data not shown). Since it is highly unlikely to find field samples with copper and zinc at levels this high, an alternative hypothesis of using equal concentrations of all four spiked targets near the EPA action level for lead was implemented. Experiment showed that this worked well for all selected contaminants except for zinc, for which peaks were often completely absent. A review of the scientific literature suggests that zinc and copper interact

³ One rule of thumb suggests the most useful range of standard additions should increase the analytical signal to between 1.5 and 3 times the original value. Since NRL’s test kit buffers are pre-made in the laboratory, the effective range of the field kit is fixed and is designed to be approximately 2.5 ppb – 50 ppb for all trace metals except zinc, which is doubled. A standard addition regression line slope smaller than $1 \mu\text{A/ppb}$ will stretch this range.

with each other to form complexes. These intermetallic compounds mask the signal for zinc, but not necessarily for copper. Following Wang [14], the amount of spiked zinc was doubled, although in samples where natural copper content is very high, all zinc signal including the spiked amounts may still be masked. Final concentrations used in each standard addition buffer are presented in Table 3.

Typical voltammograms from spiked deionized laboratory water measured with NRL's CStat are presented in Figure 2. The presence of trace metals at 15 ppb is visually apparent in the data when observed at full scale. This illustrates the sensitivity of the method. Also of interest is the presence of copper in NRL's deionized water (Figure 2a). This was calculated from the CStat with a glassy carbon electrode and verified by the 946 Portable VA Analyzer with an scTRACE gold microwire electrode to be present at roughly 2 ppb in most circumstances, although it can be higher at the beginning of the day when the water is first turned on. It is likely that the deionized water is purified in the basement and transported to the third floor laboratory where testing was performed with copper pipes.

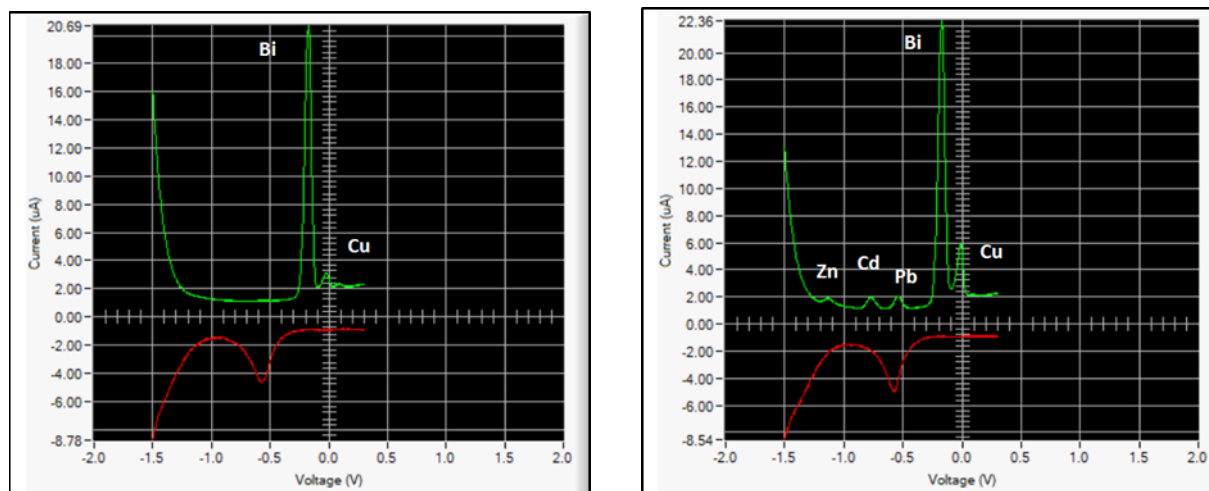


Figure 2. Screenshots of the CStat interface. The red curve is the working electrode cleaning sweep and can be ignored. The green curve is the anodic stripping voltammetry trace. (a) “Clean” acetate buffer made from laboratory deionized water with 500 ppb bismuth; no spiked analytes added. Interestingly, NRL’s “deionized” water has some copper in it. This was verified by the 946 Portable VA Analyzer (data not shown). (b) Acetate buffer with 500 ppb bismuth and all four spiked trace metals at 15 ppb (30 ppb for zinc). Peaks for each of the four trace metals can be qualitatively identified by eye.

3.3 Quantitative Results

Obtaining accurate quantitative measurements of the detected metals was challenging. The first issue was obtaining clean water with no measurable trace metal signal, something that has proven difficult to achieve due to the sensitivity of the assay. For this report, it is assumed that laboratory deionized water used to make buffers will have trace copper levels on the order of 2-10 ppb. In practice, the contribution of this metal in the buffer concentrate to the real sample is vanishingly low because it is diluted 10:1 by the field kit test procedure. That is, the signal on the CStat for a 3 mL buffer concentrate diluted to the 30 mL line of a falcon tube with field sample is

$$\text{Signal} = 0.9(\text{sample}) + 0.1(\text{trace metal in buffer})$$

The EPA action level for copper is 1300 ppb and the deionized water copper levels add 10% of approximately 2-10 ppb. Furthermore, performing dilutions in the field without the use of analytical glassware also contributes to the uncertainty of the final result. It is unlikely that the assay will be sensitive to the estimated 1 ppb maximum of copper added due to NRL’s deionized water.

The second challenge to obtaining quantitative results is comparison to ground truth. In the case of spiked samples, the amount of contaminant present is known a priori. However, field samples are true

unknowns and need to be characterized by an orthogonal method in order to draw any conclusions about the accuracy of the field kit. In this study, we had intended to use the 946 Portable VA Analyzer as a gold standard for two reasons. First, it uses discrete assays for each target in the study, optimized by chemically modifying the scTRACE gold microwire working electrode in a specific way for each species. Second, because it is a commercial product developed by professionals it is expected to be reasonably accurate. However, the assay for lead required a reagent that did not arrive in time to complete the study, and there is no published assay for cadmium. As a result, the 946 Portable VA Analyzer was only used to obtain orthogonal measurements for copper and zinc.

Table 4 presents the results from the CStat and the 946 Portable VA Analyzer on the four blind deionized water samples. Each was spiked with one trace metal. Neither the identity of the metal nor the concentration was known to the author at the time the testing was performed.

| Sample | Contaminant | Portable VA Analyzer | CStat | CStat Rel. Error |
|--------|-----------------|----------------------|------------------|------------------|
| 1 | Cadmium, 7 ppb | N/A | 6.07 ± 0.27 ppb | 13.3% |
| 2 | Lead, 11 ppb | N/A | 6.05 ± 0.82 ppb | 45% |
| 3 | Zinc, 15 ppb | 11.29 ± 0.572 ppb | 12.05 ± 2.57 ppb | 19.7% |
| 4 | Copper, 10 ppb* | 9.6 ± 0.51 ppb | 7.99 ± 1.23 ppb | 20.1% |

Table 4. Quantitative test results in spiked deionized laboratory water. Relative error for the CStat is calculated against the known (spiked) sample concentration, not based on the error bars reported from the measurement. Note that the value for copper is the amount that was added to NRL's water. The true concentration is difficult to gauge. For the copper relative error calculation, a "true" value of 10 ppb is used. The large relative error for lead may be due to interactions between lead and copper [15], which can change the value of the observed signal.

Given the limited size of the data set, it is hard to draw firm conclusions about the performance of the CStat with glassy carbon working electrodes using NRL's field kit. These spiked samples suggest the method has a relative quantitative accuracy between about 20% and 50%. Visual observations of the raw data suggest that concentrations below 5 ppb can be detected. Although NRL's field kit cannot compete with the accuracy and precision of standard laboratory assays such as AAS, the small size weight and power (SWaP) characteristics of the CStat and the short sample-to-answer time of the assay make the method a potential candidate for field work.

3.4 Field Samples

The progression of tasks in this 18 month study is shown in Figure 3. Field work and assay development overlapped, and the characterization of the working electrodes was not performed until the last few months of the project. For this reason, data obtained in the laboratory and data obtained at the collection site are difficult to compare: these data sets were obtained with different instruments and different working electrodes. They are presented separately.

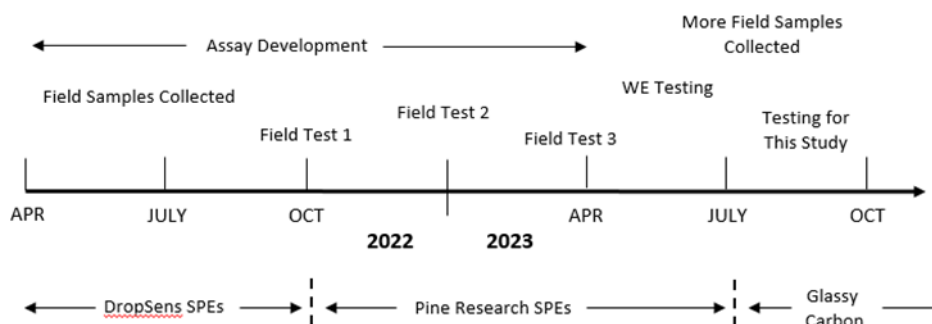


Figure 3. Timeline of experiments and sample collection associated with the 18-month study. The working electrode used with the assay is indicated on the bottom of the image.

Drinking water was collected from various locations and brought back to NRL for laboratory testing. These samples are referred to as “field samples”. There were eleven total field samples, ten of which were analyzed in the laboratory. Six of these field samples were collected from the Washington DC metropolitan area. One field sample was collected in Texas. The remaining four were collected from sites in Maryland. Figure 4 illustrates the general area of collection for all eleven samples. Precise locations are not specified in order to protect the identities of the providers.

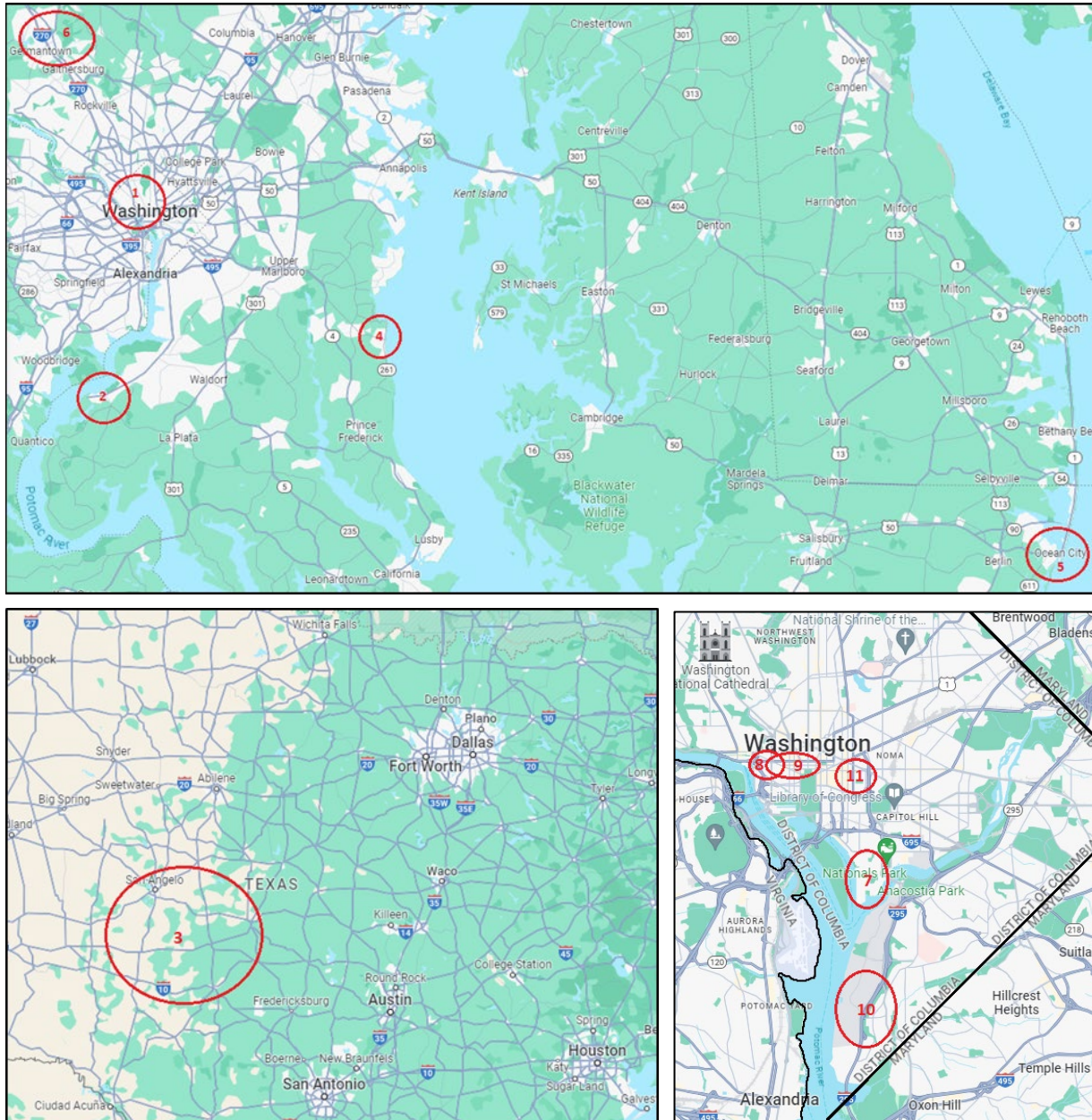


Figure 4. Approximate location of all eleven samples collected for this study. Samples 1-3 were tested on site with the Series II prototype using SPEs. All samples except for number 2 were also brought back into the laboratory and tested with the CStat instrument using a glassy carbon electrode. Google Maps was used to generate this figure.

Data from laboratory testing with the CStat are presented in Table 5. There was some noise in the data which sometimes resulted in very small negative concentrations. These were interpreted as 0 ppb. It was observed that copper strongly interferes with the zinc assay. As copper concentration increases, the slope (sensitivity) of the zinc assay gets smaller and eventually goes to zero. This is problematic because

the calculated concentration from the standard addition method is proportional to $1/\text{slope}$, which will diverge in the limit of zero slope. In cases where the measured standard addition regression line was less than roughly $0.005 \mu\text{A/ppb}$, the test was ruled inconclusive and marked with "N/A". Signals were not normalized to bismuth except for copper in sample six. Comparison data from the 946 Portable VA Analyzer was available for some of the samples. A blank entry indicates no data was collected.

| Field Sample | NRL CStat [ppb] | | | | Portable VA [ppb] | | Notes |
|--------------|-----------------|-----|------|-------|-------------------|------|--|
| | Zn | Cd | Pb | Cu | Zn | Cu | |
| 1 | N/A | 0 | 0 | 26.24 | 44.58 | 68.5 | |
| 2 | | | | | | | Tested on site. No laboratory measurement |
| 3 | 132.8 | 0 | 1.19 | 52.27 | 117 | 85.0 | Used alternate zinc analysis. Sample was very dirty |
| 4 | 78.35 | 0 | 0.97 | 16.75 | 113 | 9.77 | Used alternate zinc analysis |
| 5 | 22.73 | 0 | 2.26 | 14.23 | 35.1 | 29.2 | |
| 6 | N/A | 0 | 6.65 | 80.21 | 32.12 | 147 | Copper normalization required for CStat |
| 7 | N/A | 0 | 0 | 53.18 | | | Large difference between copper & normalized result |
| 8 | N/A | N/A | 0.19 | 60.57 | | | Very large error bars on cadmium, slope below 0.005 |
| 9 | N/A | N/A | 0 | 56.13 | | | Very large error bars on cadmium, slope below 0.005. Large difference between copper & normalized result |
| 10 | N/A | 0 | 0.85 | N/A | 24.27 | 11.6 | No visible copper peak in data. Sample was very dirty |
| 11 | N/A | 0 | 0 | 26.88 | | | Large difference between copper & normalized result |

Table 5. Results from field samples tested in the laboratory. Negative concentrations were interpreted as zero. Slope smaller than roughly $0.005 \mu\text{A/ppb}$ was recorded as inconclusive or "N/A". Sample 2 was only tested on site and will be discussed in the next section. Blank entries indicate no data.

Every sample collected from the Washington, DC metropolitan area was observed to have significant copper concentration (samples one, and seven through eleven); this masked the detection of zinc by the CStat due to the formation of intermetallic compounds. Given the age of the infrastructure in Washington, it is highly likely that these samples passed through copper interior pipes. Field samples were collected from Washington during the day, without allowing water to sit in the pipes overnight to accumulate trace metals. These data suggest that copper from pipes quickly and easily dissolves in tap water. It may be the case that lead dissolves much more slowly.

Field samples three, four, and six were collected from ground water sources (wells). Two of these samples had very high zinc concentrations. In all cases where copper was present in concentrations significantly larger than zinc, the zinc results from NRL's assay were either low or zero. In the opposite case (samples three and four), the zinc peaks were so prominent that an alternate baseline estimation procedure had to be used. Specific baseline estimation procedures are described in Section 4.

Also of interest was field sample ten, which was taken from the bathroom sink of a military facility in the Washington DC area. The sample was so dirty that both the CStat and the 946 Portable VA Analyzer performed poorly, as indicated by the noise levels in the signal and the size of the error bars. It is suspected that an unknown substance(s) in the sample is fouling the electrodes and interfering with the assay, possibly a surfactant.

In samples with the largest copper concentrations, it was observed that the bismuth peak height decreased significantly. In these cases, the difference between the bismuth-normalized and non-normalized copper results differed. Normalized copper values were found to be far too low to be realistic, although in the case of sample six (which had the largest amount of copper present), the non-normalized regression fit had a zero slope and the normalized value had to be used.

Finally, all of NRL's copper measurements seem to be low, in some cases by a factor of two. Some of the errors may be related to the much larger than expected concentrations in the samples: the standard

addition used 5, 10, and 15 ppb, but samples with as much as 147 ppb copper were observed. The largest error contribution probably comes from copper forming intermetallic compounds with other trace metals in solution, especially copper-zinc complexes. These intermetallic compounds are likely to have a different signal contribution and/or peak voltage than copper alone. Zinc concentrations were also found to be much higher than anticipated when formulating the field kit buffers.

3.5 Field Testing

In addition to laboratory testing, field samples 1-3 were also tested at the collection site with NRL's Series II prototype instrument. As shown in Figure 3, on-site testing took place with preliminary versions of the assay; the full suite of four trace metals was not tested. It should also be noted that these tests were performed with screen printed electrodes instead of glassy carbon.

The first field test sampled municipal water from a private residence in Washington, DC that did not belong to any of the authors of this study. The owner had recently purchased a condominium and was in the process of moving out of the current location (an apartment building). The location had been unoccupied for about one week, although it was visited daily to move boxes and for cleaning. Water was allowed to sit in the pipes undisturbed overnight before sampling. The assay used bismuth oxide screen printed electrodes from DropSens and was designed to detect lead only. Given the number of other units in the building, it is unlikely that stagnant water from the service pipe was sampled, only the interior pipes in the apartment unit itself. Therefore, a positive result was not expected. Prior to use, the electrodes were activated for 5 minutes in 0.1 M KOH at -1.2 V. A standard addition curve was constructed from 0, 10, 50, and 100 ppb spiked lead in 0.1 M acetate buffer, pH 4.5. The raw data and resulting standard addition curve is shown in Figure 5a. No lead was detected in the sample.

The second field test was performed in Indian Head, MD. The water at this site was also municipal. Unlike all other testing, a sample was not retained at this location to bring back to the laboratory at the request of the host. Tests were run on site for both lead and copper. It was not possible to guarantee that the water had sat undisturbed in the pipes overnight. It was likely that the particular tap where water was collected from had been unused for at least 12 hours, but similar to the prior field test, occupants in the building were active and water had been running from other taps all morning. A standard addition curve was constructed from 0, 10, 50, and 100 ppb spiked lead; and 0, 500, 1000, and 1500 ppb spiked copper. Results are shown in Figure 5b. No lead was detected in the tap water, but the method of standard additions indicated roughly 86 ppb copper. There is no orthogonal measurement to which this result can be compared, but it is not an unrealistic number for a building with copper interior pipes, and falls well below the EPA action level.

The third field test was performed near Eldorado, TX. Ground water was sourced directly from a well. The sample seemed to be significantly dirtier than any of the previous field tests. Unlike all other data from this study, the voltage (vs. Ag/AgCl) for the peaks of both lead and zinc were not steady, but floated around slightly. The best standard addition curve was obtained when a potential was simply assigned that matched the peak in the highest concentration, with all other concentrations measured at that assigned voltage rather than their maxima. Results are shown in Figure 5c.

There were other problems with field test three. It appears that there was no bismuth in the blank buffer, which resulted in that particular point being excluded. Moreover, the copper standard curve did not show up. Interestingly no copper data was observed in field sample ten either, which was also known to be "dirty". However, copper did show up in the retained sample tested in the laboratory with glassy carbon. It is likely that there are additional compounds in field sample that interfered with the copper measurement to the point that the SPE could not detect it. No lead was observed in the sample, but testing indicated about 92 ppb zinc. Orthogonal results from the 946 Portable VA Analyzer put this number at 117 ppb, which gives the Series II zinc measurement a relative error of about 21.37%.

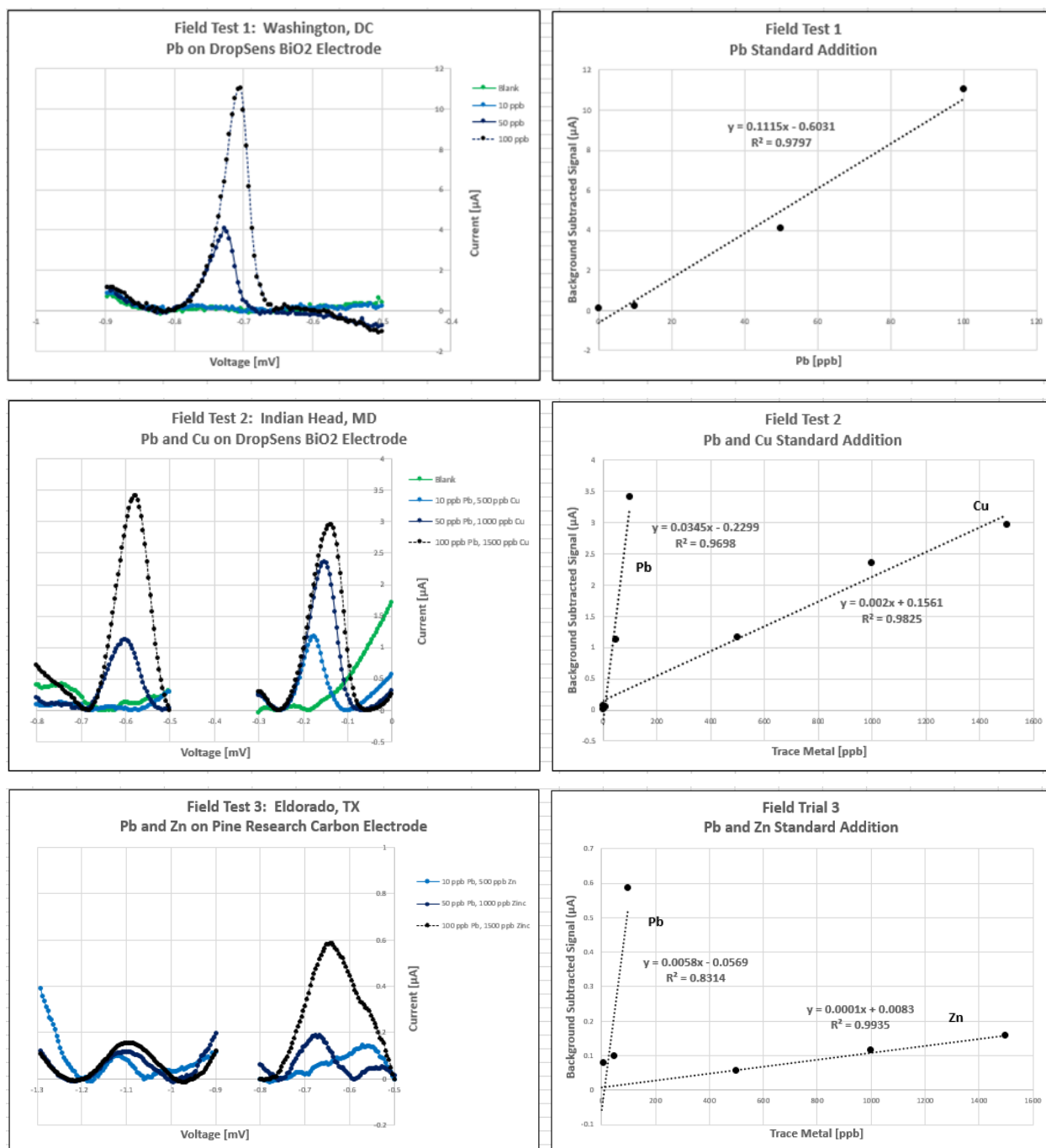


Figure 5. Raw data collected in the field using the method of Standard Additions and carbon SPEs. These studies were performed while assay development was ongoing. (a) First field trial, performed in Washington, DC. The only target is lead. (b) Second field trial, performed at Indian Head, MD. Lead and copper are targets. (c) Third field trial, performed in Eldorado, TX. There were supposed to be three metal standards, but copper did not show up in the test.

Perhaps the most important lesson learned from testing field samples was that most publicly accessible tap water in the United States is already in compliance with EPA limits and is therefore “uninteresting”. To obtain a true measure of the effectiveness of the field kit, samples need to be collected from sites that are known a-priori to produce contaminated tap water. These types of samples

are very difficult to find randomly. A partner who can provide them, such as a water utility company, would be highly useful.

4. DISCUSSION

Experimental data obtained in this study as well as from many other literature reports suggest that the formation of intermetallic compounds can impact the signals produced by anodic stripping voltammetry. In this section, the method of standard additions is examined in greater depth. Two simple interaction models are introduced into these equations in an attempt to explain the general trends observed in this study.

4.1 The Method of Standard Additions for One Target

In most anodic stripping voltammetry studies with bismuth salts, quantitative evaluation takes place through the use of a calibration curve. A series of dose/response experiments are performed by spiking target into solutions with a similar composition to the unknowns that will be measured. These data are fit to a regression line that can be extrapolated to find a limit of detection at approximately 3x the signal to noise ratio. The signal from each unknown is then compared to this calibration fit to determine the amount of target present. While this approach can be successfully used in well-designed experiments, it is not likely to produce good results in the field where unknown amounts and types of interfering species may be present. Under these conditions, the method of standard additions is a preferred alternative.

With a calibration curve approach, a true blank sample is usually included that can be used to tack down the instrument signal at zero concentration. Then, the only important variable is the slope (sensitivity) of the resulting calibration regression line fit. In contrast, the method of standard additions does not have a measured zero. Both the slope and the y-intercept of the regression line fit are important.

The method of standard addition relies on the assumption that when the target is at zero concentration, the instrument signal is also zero. Then, if the standard addition data points are fit to a regression line, the negative of the value at which it crosses the x-axis is the concentration of target in the original sample. This is illustrated in Figure 6.

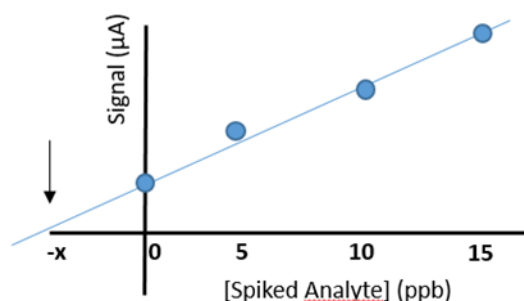


Figure 6. The method of standard additions. Here, the sample is divided into four parts, each of which is spiked with a known amount of target: zero, low, medium, and high concentration. A least squares regression line is fit to the data. The point at which the line crosses the x-axis is the negative of the concentration of target in the original sample. The experimentally determined target concentration is labeled in the Figure as (-x). Note that these are imaginary data points for illustrative purposes only and the x-axis concentrations shown here may differ from actual assay results.

That is, if the standard addition signal is fit to a regression line of the form

$$y = mx + b$$

then the concentration of target in the original sample is found by solving for $-x$:

$$C \text{ [ppb]} = \frac{b}{m}$$

This equation shows that for standard addition regression line fits with identical slope, the calculated amount of target is proportional to the value of the y-intercept. Interactions between the target and other substances in solution can change the value of the slope; the method of standard additions automatically takes this into account. However, it is also possible that some unknown compound in solution will create a background signal at the same voltage as the target is measured. In this case, the instrument signal at zero target concentration is not zero. These are known as matrix effects, and unless the value of the zero concentration signal is somehow known in advance, the method of standard additions cannot intrinsically correct for it. Matrix effects change the value of the y-intercept. Manual intervention is required to estimate the shape of the baseline in the absence of interferences, and subtract it from the signal of the target peak. This is referred to “background subtraction” in this study. The procedures used to perform it have a very large effect on the accuracy of the final result.

4.2 Background Subtraction Procedures

Background subtraction procedures create an artificial “zero” against which peak height can be measured. This is important, because in order for the method of standard additions to succeed, it is necessary to pin down one point on the regression line with an absolute concentration value. The accuracy of this artificial zero will strongly influence the results of the calculation. To do this, we need to estimate the shape of the signal in the absence of a peak. We make the following assumptions:

- (1) The endpoints of each peak can be accurately estimated by a set of minima, and
- (2) The background signal between these minima can be estimated by a polynomial fit

The goal is to introduce a single set of procedures that is applied in the same way to every voltammogram in this study. A series of macros were created in an Excel spreadsheet. Then by copying and pasting the various data sets into this spreadsheet, standard addition plots were created the same way each time. The procedure is as follows. First, the incoming data is passed through a 5-point moving average filter to remove high frequency noise.

$$\bar{y}_n = \frac{y_{n-2} + y_{n-1} + y_n + y_{n+1} + y_{n+2}}{5}$$

Next, it was observed that measurements could sit on a variable background voltage. This was eliminated by selecting a point on the voltammogram (here $V = -0.9$ V vs. Ag/AgCl) and subtracting it from every other point in the scan. At this point, each of the individual peaks were treated separately.

Lead and cadmium were the easiest two peaks to correct; they occur in a portion of the voltammogram with a reasonably flat background. The two minima for each peak were identified, and the background was estimated by constructing a straight line between them. By subtracting the signal value of this line from every point in the peak, it obtains a zero baseline. After looking at the full data sets, modeling cadmium with a cubic (or higher) fit may be a better choice because after a linear fit the original sample often had a very small peak in the negative direction. While unimportant in this study because no field samples picked up any cadmium and there is no reason to believe that any of them should have, it may impact future studies.

Copper is slightly more complex. As long as the concentration of copper is not too high, it occurs at slightly more positive voltages than the bismuth peak and also seems to have a flat baseline, although it is elevated above zero because it appears on the bismuth shoulder. The same procedures described above were used to correct copper peaks. Copper data can have significant scatter in it, probably because of its interaction with zinc and because it may compete with bismuth for active sites on the electrode. Most of

the calculated copper results showed little change when normalized to bismuth signal, but a few of the field samples (six, seven, nine, and eleven) had significant differences. In these cases, more realistic numbers were obtained without normalization.

The most difficult peaks to correct in this study come from zinc. In cases where zinc is not highly concentrated it occurs as a shoulder on a steeply rising background. A fifth order polynomial fit was required for these samples. Unlike the other metals, this fit required manual adjustment of the parameters to make the fitted region as narrow as possible. When deviation appeared between the fit and the data on the edges of the peak, adjustment was considered complete. Maxima were calculated for zinc by subtracting the fitted baseline from the actual data and looking for the largest value in the set. When zinc concentrations in solution were smaller than copper, the zinc signals tended to be very small. This is problematic because the standard addition calculation is proportional to $1/\text{slope}$; as slope becomes small this calculation will diverge. In cases where the slope of the zinc standard addition line were less than about $0.005 \mu\text{V/ppb}$, the concentration was assumed to be too small to measure. When zinc concentrations were significantly larger than copper, the zinc peak obtained a more traditional Gaussian-like shape. In these cases, the alternative baseline correction procedure used for lead and cadmium was employed.

In all cases, after performing a background subtraction on the peaks for each target, the maxima were found and used to calculate signal.

4.3 Extending the Method to Multiple Targets

Using the background subtraction procedures outlined above and a set of four spiked laboratory samples, quantitative results were determined for instrument validation. Table 6 contains a more complete set of data from this experiment, including the calculated slope and intercept from the standard addition curve fit. Focusing on the calculated slopes, it is interesting to note that the values were mostly identical for each species of interest throughout the experiments, with exceptions shown in red.

| | Cadmium | | | Lead | | | Zinc | | | Copper | | |
|-----------|---------|---------------|---------|-------|--------|---------|-------|---------------|---------|--------|---------------|--------|
| | C | m | b | C | m | b | C | m | b | C | m | b |
| Sample #1 | 6.07 | 0.0691 | 0.3779 | 0.67 | 0.0626 | 0.0377 | 7.95 | 0.0115 | 0.0822 | 2.75 | 0.1881 | 0.4657 |
| Sample #2 | -1.01 | 0.0624 | -0.0565 | 6.05 | 0.0795 | 0.4327 | 4.24 | 0.0076 | 0.0291 | 5.77 | 0.1645 | 0.8547 |
| Sample #3 | -0.89 | 0.0629 | -0.0506 | 0.15 | 0.0561 | 0.0077 | 12.05 | 0.0122 | 0.1327 | 2.33 | 0.1067 | 0.2234 |
| Sample #4 | 0.49 | 0.0915 | 0.0400 | -0.11 | 0.0711 | -0.0071 | -0.60 | 0.0224 | -0.0121 | 7.99* | 0.0903 | 0.6494 |

Table 6. Standard addition curve parameters and calculated target concentration from the four spiked laboratory samples. The slope of the standard addition curve fit for each of the four targets remains relatively constant across the four samples except where highlighted in red. Note that the sample 4 copper standard addition label order has been swapped as described in the text.

In order to draw conclusions from these data, it is important to understand where the standard addition fit parameters originate. In general, the following observations can be made:

- (1) Scalar multiplication of every scan on the voltammogram by the same constant (eg, changes to the instrument gain) has no effect on either the slope or the intercept of the standard addition curve fit.
- (2) Adding or subtracting a constant value to every point of each scan on the voltammogram (eg, shifting the y-axis) changes only the value of the y-intercept of the standard addition curve fit. The slope is not affected.
- (3) To change the slope of the standard addition curve fit without affecting the y-intercept, the rise over run value needs to change. This is equivalent to scalar multiplication, but the value of the scalar is different for each scan in the voltammogram: it is proportional to the concentration of target. Then, for example, the value of the scalar multiplier for a 20 ppb scan is twice the value of the scalar multiplier for a 10 ppb scan.

(4) Any manipulation to the scans on the voltammogram that does not fit these rules will change both the slope and the intercept of the standard addition curve fit. However, there is one special case: if the ratio of the y-intercept to slope does not change, then the shape of the standard addition curve fit will be altered but the calculated result remain unchanged.

The method of standard additions is normally applied in the case of one unknown target. In this work, the method is extended from one target to four. This introduces a new possibility: in addition to interacting with unknown constituents in solution, the four spiked targets could also interact with each other. To see this more clearly, the value of the slope from each standard addition fit in Table 6 is extracted and placed into a matrix. Following the format of Table 6, the values in each row come from a single experiment. Each column represents the standard addition curve for one particular metal. Then M_{xy} is the slope of the standard addition regression line fit for component y from the sample spiked with extra component x

$$M = \begin{pmatrix} M_{11} & M_{12} & M_{13} & M_{14} \\ M_{21} & M_{22} & M_{23} & M_{24} \\ M_{31} & M_{32} & M_{33} & M_{34} \\ M_{41} & M_{42} & M_{43} & M_{44} \end{pmatrix} = \begin{pmatrix} 0.0691 & 0.0626 & 0.0115 & 0.1881 \\ 0.0624 & 0.0795 & 0.0076 & 0.1645 \\ 0.0629 & 0.0561 & 0.0122 & \mathbf{0.1067} \\ \mathbf{0.0915} & 0.0711 & \mathbf{0.0224} & \mathbf{0.0903} \end{pmatrix}$$

where

1 = cadmium (signal measured at about -0.776 V vs. Ag/AgCl)

2 = lead (signal measured at about -0.552 V vs. Ag/AgCl)

3 = zinc (signal measured at about -1.14 V vs. Ag/AgCl)

4 = copper (signal measured at about -0.016 V vs. Ag/AgCl)

From the third row slope M_{34} , it is observed that the addition of zinc affects the copper slope, and from the fourth row slope M_{43} the addition of copper affects zinc slope. These data suggest that copper and zinc probably form a complex. However, the addition of copper in the fourth row also causes a change in copper slope M_{44} , which is highly unusual. Values along the main diagonal represent self-interactions, and none of them should be anomalous unless the solution concentration becomes so high that they form dimers. It is unlikely that dimer formation is occurring here.

The standard addition curves that produced matrix entries M_{34} and M_{44} are shown in Figure 7. The un-spiked buffer 0 has a very high signal in M_{44} that quickly drops as the standard addition proceeds. The “anomalous slope” in M_{44} clearly results from a very poor curve fit to a group of data with a large amount of scatter. It is possible that this particular experiment was bad, but these results were the average of three samples run sequentially. That is, one full standard addition curve was completed before the next one was started. The trend exhibits itself in all three of them. Therefore, in order for the data to be bad, either the stock solutions would have had to be made incorrectly or the order of the labels on the Falcon tubes were accidentally switched. Given that the same unknown was tested with the 946 VA analyzer and the recovered value (Table 4) was very close to the spiked amount, it is unlikely that there was an error in the way the solution was made. If the data are left in their labeled order (as shown in Figure 7b) the slope of the data points is 0.0261, which is significantly smaller than any of the other copper slopes. Re-arrangement of the data points in Excel into monotonically increasing order (as if the labels were switched) produces a slope of 0.0903, which is still more than 50% low compared to the average of M_{14} and M_{24} . Both of these cases need to be considered.

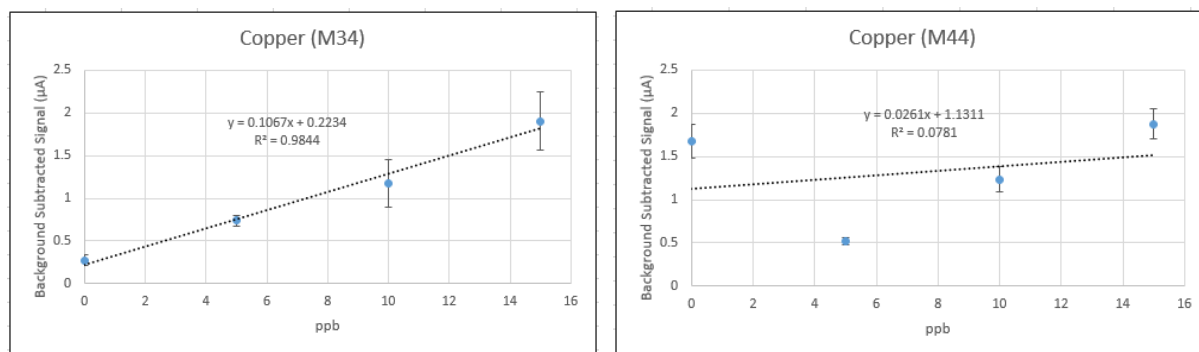


Figure 7. Standard addition curves that produced entries M₃₄ (spiked sample 3) and M₄₄ (spiked sample 4). The data points used to construct M₄₄ are in their original order. If they are re-arranged (as if the labels were accidentally switched), the M₄₄ slope becomes 0.0903 as reported in Table 6.

4.4 Qualitative Effects of Complex Formation

A more in-depth analysis of the anomalous slope of M₄₄ considers that column 4 represents the signal from both copper and a copper-zinc complex. To maintain symmetry, it is assumed that column 3 also represents signal from both zinc and the copper-zinc complex. Then

$$\text{"copper" signal} = k_1[\text{Cu}] + k_2[\text{Cu}_a\text{Zn}_b]$$

and

$$\text{"zinc" signal} = k_3[\text{Cu}_a\text{Zn}_b] + k_4[\text{Zn}]$$

An Excel spreadsheet was used to explore different interaction ratios and coefficient values along with different starting values for zinc and copper. Although quantitative fits are impossible with such a small data set, possibilities can be eliminated by considering the known behavior of the system at certain limits (eg, boundary conditions). Two different hypotheses were considered, as shown in Table 7. Although any copper to zinc ratios could be considered for these hypotheses, we were guided by the structure of the (solid) brass alloy, which has three phases [16]: a face-centered cubic α -phase $\text{Cu}_{0.64}\text{Zn}_{0.36}$, a body-centered cubic β -phase CuZn , and a complex cubic γ -phase Cu_5Zn_8 . This seems plausible given that each ionic species in solution (eg, Cu^{2+}) is reduced at the electrode during the accumulation step and deposited as a solid. The fused alloy being formed with bismuth may be composed of small grains of crystalline brass with bismuth at the grain boundaries.

| Copper-Zinc complex | Hypothesis | | |
|---------------------|------------|-----|-----|
| | Null | One | Two |
| Ratio (a:b) | N/A | 3:2 | 1:1 |
| k_1 | 1 | 1 | 1 |
| k_2 | 0 | 1 | 2 |
| k_3 | 0 | 0 | 0 |
| k_4 | 1 | 1 | 1 |

Table 7. The value of the parameters for “copper” and “zinc” signals as shown in the equations above for the three hypotheses tested. The null hypothesis assumes there is no complex formation. The composition of the complex in hypotheses one and two is based on part from observations of the structure of brass.

The first hypothesis was designed to match the shape of the standard addition curve M₄₄ shown in Figure 7b. A 3:2 copper-zinc complex is very close to the composition ratio of the α -phase of brass. When $k_1 = k_2 = k_4 = 1$ and $k_3 = 0$, this model produces simulated standard addition plots with a buffer 1 signal smaller than a buffer 0 signal when solutions have copper in the unknown, as shown in Figure 8. If

this model is correct, the “copper” signal does not depend on the concentration of total copper in solution, rather it depends on the overall concentration of copper-containing species, where the formation of a 3:2 complex greatly reduces the signal because it removes three contributing copper atoms and replaces it with one contributing copper-zinc complex.

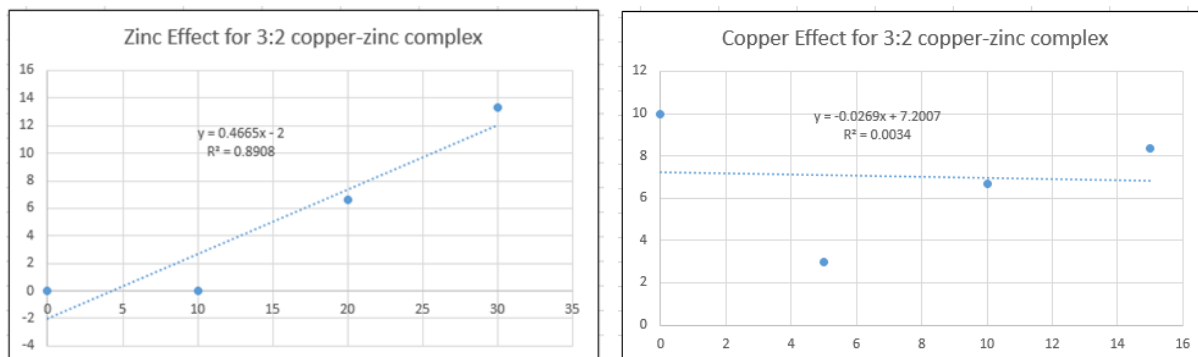


Figure 8. Simulated standard addition curves for (a) zinc and (b) copper with 10 ppb spiked copper in the sample and accounting for the formation of a 3:2 copper-zinc complex. In this case, the total copper signal is proportional to $[Cu] + [CuZn]$, while the total zinc signal is proportional only to $[Zn]$.

When there is copper in the unknown, hypothesis one makes two predictions. First, there is a dip in the copper standard addition curve as a complex starts to form (Figure 8b; compare to Figure 7b). Second, the calculated value from the zinc standard addition curve becomes smaller (eg, the x-intercept becomes more positive because the signal of the smaller standard additions is pushed to zero; Figure 8a). When there is free zinc in solution, both the zinc and copper standard addition curves appear linear. Quantitative predictions from the copper standard addition curve will be accurate, but the zinc standard addition curve will produce quantitatively incorrect calculations without a correction.

Simulation shows that both the M_{44} and M_{34} slope and y-intercept values change as the amount of free copper in solution changes. But since the zinc standard addition curve is only affected by the concentration of zinc in solution, only the y-intercept associated with M_{34} will change when there is free zinc in solution; M_{44} and its y-intercept are unaffected. Some sample data is presented in Table 8.

| Hypothesis One | | Standard Addition Curve Fit | | | |
|----------------------|----------|-----------------------------|--------|-----------------------|--------|
| Sample Concentration | | Copper | | Zinc | |
| Cu [ppb] | Zn [ppb] | m (=M ₄₄) | b | m (=M ₃₄) | b |
| 20 | 0 | -0.6004 | 18.668 | 0.1998 | -1.332 |
| 15 | 0 | -0.3334 | 13.333 | 0.3333 | -1.667 |
| 10 | 0 | -0.0269 | 7.2007 | 0.4665 | -2 |
| 5 | 0 | 0.1336 | 3.998 | 0.5665 | -1 |
| 0 | 0 | 0.3334 | -0.003 | 0.6668 | -.002 |
| 0 | 5 | 0.3334 | -0.003 | 0.6668 | 4.998 |
| 0 | 10 | 0.3334 | -0.003 | 0.6668 | 9.998 |
| 0 | 20 | 0.3334 | -0.003 | 0.6668 | 19.998 |

Table 8. Simulated values for slope and intercept of standard addition curve using hypothesis one. In this case, the addition of copper causes the slope of both the copper and zinc curve to change. Addition of zinc does not affect the slope of either curve. The red numbers indicate conditions that would produce anomalous slope values. Interestingly, as the copper in the sample concentration gets large enough, the zinc standard addition plot fits become poor and eventually the slope of the signal M_{34} shrinks to zero, which is experimentally observed. However, slope M_{44} becomes negative, which has not been observed.

Interestingly, this first hypothesis (a 3:2 copper zinc-complex) qualitatively explains some of the data in Table 6. The addition of zinc such as sample 3 results in steady slope values, which would suggest that the experimentally observed M_{34} isn't anomalous, there is just a lot of scatter in the data. The addition of copper in solution will change the slope (and intercept) values of both copper and zinc. The biggest problem with the first hypothesis is that as the amount of copper in the original sample becomes large, slope M_{44} becomes negative. This has not been observed in any of the field samples. The origin of this behavior in the model comes from the fact that the "copper" signal is based on the number of molecules present, not the total number of atoms. That is, the copper-zinc complex is assumed to contribute the same value to the signal as free copper. Since the standard addition procedure will always bind some of the initially present copper in solution, it will always produce a negative slope for sufficiently high copper concentrations in the original sample and therefore this assumption cannot be correct.

Hypothesis one was designed to match the data shown in Figure 7, which assumes that the labels in spiked sample #4 were ordered correctly. Suppose the labels on the samples were wrong. A second hypothesis can be produced where copper-zinc forms a 1:1 complex; this has the same concentration ratio as β -brass. The selected parameters for hypothesis two are $k_1 = k_4 = 1$, $k_2 = 2$, and $k_3 = 0$. In this case, copper binds zinc and removes signal from the zinc peak location, but adds both the zinc and copper signal to the copper peak location instead. For this model, the zinc equation has not changed and therefore all of its trends are identical to hypothesis one. Simulation data for hypothesis two is presented in Table 9.

| Hypothesis Two | | Standard Addition Curve Fit | | | |
|----------------------|----------|-----------------------------|-----|-----------------------|------|
| Sample Concentration | | Copper | | Zinc | |
| Cu [ppb] | Zn [ppb] | m (=M ₄₄) | b | m (=M ₃₄) | b |
| 20 | 0 | 3 | 20 | 0 | 0 |
| 15 | 0 | 3 | 15 | 0 | 0 |
| 10 | 0 | 2.7 | 11 | 0.15 | -1 |
| 5 | 0 | 2.3 | 6.5 | 0.35 | -1.5 |
| 0 | 0 | 2 | 0 | 0.5 | 0 |
| 0 | 5 | 2 | 0 | 0.5 | 5 |
| 0 | 10 | 2 | 0 | 0.5 | 10 |
| 0 | 20 | 2 | 0 | 0.5 | 20 |

Table 9. Simulated values for slope and intercept of standard addition curve using hypothesis two.

Similar to the previous model, slopes M_{34} and M_{44} are stable when zinc is in the present in the sample. When copper is present in the original sample, slope M_{34} gets smaller and eventually goes to zero, which is experimentally observed. The difference between the two models is the behavior of slope M_{44} , which goes through an intermediate region of signal enhancement and eventually stabilizes as the copper in the original sample increases to a sufficiently high value. This behavior seems much more realistic; as a result the swapped label order was adopted as correct and used in Table 4.

Although the ratios in this analysis were chosen to somewhat match those observed in a solid copper-zinc alloy (brass), the actual ratios that are selected for these two hypotheses are not extremely important in the analysis because the trends we are looking for are qualitative. The important part is the four interaction coefficients; these dictate how and where the copper-zinc complex contributes to the signal in a voltammogram which in turn produce changes in the standard addition curve fits that can be observed.

As a final comment, it should be noted that these models are simply working hypotheses that qualitatively explains the small existing data set. Even though the experiments used to construct the table were performed in triplicate, each spiked metal is represented at just one concentration. It is possible that

errors may be present, especially because the experiment was designed to be a blind study, which means the actual solutions were prepared by someone other than the author. Further studies will be required to lend additional evidence to these models.

4.5 Comparison to Literature

The biggest issue with the equations presented above is that they require knowledge of the concentration of the hypothesized copper-zinc complex. This presents a system of five unknowns with only four experimental measurements, which is underspecified and has no unique solution. Little insight to this problem exists in the scientific literature for anodic stripping voltammetry with bismuth solutions. However, a search of older literature regarding the use of mercury films reveals a number of related studies that discuss the formation of “intermetallic compounds”, especially related to the presence of copper. Several observations can be extracted that lend support to the data collected in this work and the arguments made in the previous section.

First, Tibbets et al studied the linear stripping behavior of lead in the presence of copper on glassy carbon electrodes [15]. In this work, it was shown that the lead peak at -0.52 V vs. Ag/AgCl on glassy carbon (no bismuth) decreased with the addition of copper, while simultaneously a new Cu-Pb peak appeared at around -0.3 V vs. Ag/AgCl. This provides direct evidence for the formation of a copper-lead complex, although if the new peak exists in the data from this study, it is masked by the tail of the bismuth peak. Assuming the complex does form, it may explain the large error in the calculated lead concentration in the spiked deionized water samples.

Second, there is also conflicting evidence of a copper-cadmium effect. Some authors claim it exists, while others do not [17]. If it does exist, the effect would probably be small and may explain the slightly anomalous slope M_{41} .

Finally, copper-zinc intermetallic compounds have also been studied. Gerlach et al [18] present an analysis very similar to that developed in the previous section, but without any dependence on the copper-zinc complex in the equations developed in this study. Gerlach’s equations use only copper and zinc. The framework can be extended to all species in solution and has been named the “Generalized Standard Addition” approach. The advantage of Gerlach’s formulation is that it does not require knowledge of the structure or concentration of any intermetallic compounds. Therefore, its application would result in a series of four equations with four unknowns, which should have a unique solution. Gerlach does make predictions for copper and zinc coefficients in his experiments with mercury. This approach is not further pursued here due to the small size of our data sets.

4.6 Suggested Experimental Improvements

Clearly, the inclusion of copper in the standard addition sets complicates the analysis of other trace metals present. However, given the prevalence of copper pipes in construction, it is likely that many field samples will contain trace copper. A number of approaches have been utilized in the literature to address this difficulty. The simplest approach seems to be the most promising: bind all of the free copper in the system with another species such as gallium [14, 19]. Prior et al found this procedure restored the bismuth signal almost to its unperturbed value, and while the copper-gallium complex stripped off at approximately the same potential as bare copper, the shoulder between the bismuth peak and the copper peak disappeared. The result was much higher resolution. However, it was noted that in the presence of excess gallium a new peak appeared at -0.85 V vs. Ag/AgCl, which may overlap with cadmium (-0.776 V in this work). Given that cadmium is unlikely to be found in any tap water samples, eliminating it from the list of trace metals in the assay and potentially replacing it with another may be a reasonable choice.

An additional issue observed in this study was the difficulty obtaining good results in field samples three and ten. It is hypothesized that these samples were especially “dirty” and some unknown substance(s) were fouling the both the CStat glassy carbon working electrode and the 946 VA Portable

Analyzer scTRACE gold electrode, resulting in noisy measurements and large error bars. One possible solution that has been explored in the literature is the use of nafion films [20], which not only protect against surfactants but can also enhance the electrode signal in some situations. Modification of the working electrode on site may be difficult or impossible to implement in our field kit. The incorporation of these two solutions will require further study. In addition, it would be worthwhile to include a much larger test of spiked deionized water samples for additional instrument characterization, and to add lead measurements to the orthogonal test performed by the 946 Portable VA Analyzer.

5. CONCLUSIONS

This manuscript reports a study with a homebuilt potentiostat and field kit for the quantitative detection of four separate trace metals in tap water samples collected in the field. Qualitative observations suggest that trace metal concentrations at or below 15 ppb can be visually identified from the voltammograms. However, characterization with spiked laboratory samples suggested that the quantitative accuracy of the method was no better than 20-50%. Field samples performed even worse, with relative errors as high as 100% or slightly more compared to an orthogonal detection method.

Quantitative errors were attributed to high copper concentrations in many of the samples, probably due to the formation of copper-zinc complexes. In addition, two of the field samples gave poor results that were attributed to interferents such as surfactants in solution fouling the working electrode. It is hypothesized that if these two sources of error can be removed, it may be possible to obtain a much higher accuracy.

6. REFERENCES

- [1] Z. Shi, C. W. K. Chow, R. Fabris, J. Liu, and B. Jin, "Applications of Online UV-Vis Spectrophotometer for Drinking Water Quality Monitoring and Process Control: A Review", *Sensors*, 2022, 22(8), 2987. DOI: 10.3390/s22082987.
- [2] M. Karydis and D. Kitsiou, "Marine water quality monitoring: A review", *Marine Pollution Bulletin*, 2013, 77(1-2), 23. DOI: 10.1016/j.marpolbul.2013.09.012.
- [3] S. S. Chong, A. R. Aziz, and S. W. Harun, "Fibre optic sensors for selected wastewater characteristics", *Sensors*, 2013, 13(7), 8640. DOI: 10.3390/s130708640.
- [4] J. S. Erickson, M. D. Pruessner, S. A. Trammell, and L. C. Shriver-Lake, "Assay and Test Kit for the Electrochemical Detection of Lead in Municipal Tap Water", *NRL Memorandum Report*, 2022, NRL/6930/MR--2022/4. (Distribution A: Approved for Public Release)
- [5] J. S. Erickson, S. A. Trammell, B. P. London, and L. C. Shriver-Lake, "Simultaneous Detection of Organics and Trace Heavy Metals in Tap Water", *NRL Memorandum Report*, 2023, NRL/6930/MR--2023/1. (Distribution A: Approved for Public Release)
- [6] J. S. Erickson, S. A. Trammell, C. P. Misener, and L. C. Shriver-Lake, "A Comparative Study of Electrode Materials for the Analysis of Tap Water", *NRL Memorandum Report*, 2023, NRL/6930/MR--2023/2. (Distribution A: Approved for Public Release)
- [7] J. Wang, J. Lu, S. B. Hocevar, and P. A. M. Farias, "Bismuth-Coated Carbon Electrodes for Anodic Stripping Voltammetry", *Analytical Chemistry*, 2000, 72, 3218. DOI: 10.1021/ac000108x.
- [8] I. Švancara, C. Prior, S. Hocevar, and J. Wang, "A Decade with Bismuth-Based Electrodes in Electroanalysis", *Electroanalysis* 2010, 22, 1405. DOI: 10.1002/elan.200970017.
- [9] A. A. Rowe, A. J. Bonham, R. J. White, M. P. Zimmer, R. J. Yadgar, T. M. Hobza, J. W. Honea, I. Ben-Yaacov, and K. W. Plaxco, "CheapStat: An Open-Source, "Do-It-Yourself" Potentiostat for

- Analytical and Educational Applications”, PLoS ONE, 2011, 6(9), e23783. DOI: 10.1371/journal.pone.0023783.
- [10] J. S. Erickson, L. C. Shriver-Lake, D. Zabetakis, D. A. Stenger, and S. A. Trammell, “A Simple and Inexpensive Electrochemical Assay for the Identification of Nitrogen Containing Explosives in the Field”, *Sensors*, 2017, 17, 1769-1780. DOI: 10.3390/s17081769.
- [11] S. N. Dean, L. C. Shriver-Lake, D. A. Stenger, J. S. Erickson, J. P. Golden, and S. A. Trammell, “Machine Learning Techniques for Chemical Identification using Cyclic Square Wave Voltammetry”, *Sensors*, 2019, 19, 2392. DOI: 10.3390/s19102392.
- [12] L. C. Shriver-Lake, R. L. Myers-Ward, S. N. Dean, J. S. Erickson, D. A. Stenger, and S. A. Trammell, “Multilayer Epitaxial Graphene on Silicon Carbide: A Stable Working Electrode for Seawater Samples Spiked with Environmental Contaminants”, *Sensors*, 2020, 20, 4006. DOI: 10.3390/s20144006.
- [13] J. R. Hajzus, L. C. Shriver-Lake, S. N. Dean, J. S. Erickson, D. Zabetakis, J. Golden, D. J. Pennachio, R. L. Myers-Ward, and S. A. Trammell, “Modifications of Epitaxial Graphene on SiC for the Electrochemical Detection and Identification of Heavy Metals in Seawater”, *Sensors*, 2022, 5367. DOI: 10.3390/s22145367
- [14] J. Wang, J. Lu, U. A. Kirgoz, S. B. Hocevar, and B. Ogorevc, “Insights into the anodic stripping voltammetric behavior of bismuth film electrodes”, *Analytica Chimica Acta*, 2001, 434, 29. DOI: 10.1016/S0003-2670(01)00818-2.
- [15] D. Tibbetts, J. Davis, and R. Compton, “Sono-electroanalytical detection of lead at a bare copper electrode”, *Fresenius Journal of Analytical Chemistry* 2000, 368, 412. DOI: 10.1007/s002160000523.
- [16] C. Yang, Z. Ding, Q. C. Tao, L. Liang, Y. F. Ding, W. W. Zhang, and Q. L. Zhu, “High-strength and free-cutting silicon brasses designed via the zinc equivalent rule”, *Materials Science & Engineering A*, 2018, 723, 296. DOI: 10.1016/j.msea.2018.03.055.
- [17] D. A. Roston, E. E. Brooks, and W. R. Heineman, “Elimination of intermetallic compound interferences in twin-electrode thin-layer anodic stripping voltammetry”, *Analytical Chemistry* 1979, 51 (11), 1728. DOI: 10.1021/ac50047a033.
- [18] R. W. Gerlach and B. R. Kowalski, “The generalized standard addition method: intermetallic interferences in anodic stripping voltammetry”, *Analytica Chimica Acta* 1982, 134(1), 119. DOI: 10.1016/S0003-2670(01)84183-0.
- [19] C. Prior, C. Lenehan, and G. Walker, “Enhanced Resolution of Copper and Bismuth by Addition of Gallium in Anodic Stripping Voltammetry with the Bismuth Film Electrode”, *Electroanalysis* 2006, 18, 2486. DOI: 10.1002/elan.200603705.
- [20] G. Kefala, A. Economou, and A. Voulgaropoulos, “A study of Nafion-coated bismuth-film electrodes for the determination of trace metals by anodic stripping voltammetry”, *Analyst* 2004, 129, 1082. DOI: 10.1039/B404978K.