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IDENTIFICATION OF TOTAL AND BIOLOGICALLY SENSITIVE FORMS OF TOXIC METAL INPUTS TO AN URBAN AFFECTED RIVER

by

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INTRODUCTION

Aquatic pollution biologists generally agree that knowledge of a metals chemical speciation is critical to understanding the biological impact of discharging a metal to aquatic systems. Chemical speciation may be defined as the various chemical forms of an element which together make up the total concentration of that element. The behavior of any particular element is, of course, related to its unique properties, but is also affected by the properties of the surrounding body of water or sediments. For soluble copper in the river environment, speciation primarily results from association with inorganic and organic ligands. A review of copper toxicity data suggests that toxic responses are highly correlated with the presence of the free, or ionic species (Andrew 1976). The toxicity of any particular copper complex is probably related to the stability of the complex. Copper complexes with relatively larger equilibrium constants should demonstrate less of a toxic response than a copper complex with a smaller stability constant. There is, in fact some data which suggests this is the case (Chynoweth et al. 1976), however, present water quality criteria are based upon total metal inputs and disregards chemical speciation (Environmental Protection Agency 1976).

The difficulty in determining the toxicity of single complexes of copper is due to the diversity of ligands present in natural waters and the lack of refined analytical techniques which measure specific complexes of copper. Since pollution experiments are often conducted using a natural water source as dilution or holding water, there is little that can be done to control speciation. The 96 hr LC50 values for copper, determined under standard conditions, with standard test animals vary over an order of magnitude. This variation may be due largely to different speciation of copper in different test waters.

For the regulatory agency, the bioassay is the basic tool used for making judgments on potential impact and setting water quality criteria (Environmental Protection Agency 1976). These bioassays are costly, and must be conducted using the receiving water in question to be useful. Even the most carefully conducted bioassays won't allow for natural or man-made changes in receiving water quality which may affect copper speciation. Extrapolation of these toxicity reference values to the natural setting are made with application factors, which are often chosen arbitrarily, and used to build in a margin of safety in setting site-specific restrictions. Excessive waste water treatment for metals can be costly, and these costs are ultimately passed on to consumers. The development of inexpensive, rapidly conducted analytical techniques which allow for prediction of bioavailability and toxic effects, without the difficulties and costs of laboratory bioassays is needed.

The purpose of this study was to investigate the use of differential pulse anodic stripping voltammetry (DPASV) in making predictions regarding copper speciation and biological impact which might occur as a

result of copper inputs to a river environment. Measurements were made as follows:

1. Total copper and reducible copper measurements were made on Green River water to determine the sources and forms of copper in the Green River.
2. Complexometric titrations were conducted on river water samples to determine the ability of Green River water to complex copper.
3. Reducible copper measurements were made on copper associated several organic and inorganic ligands to determine the electrochemical lability of these complexes.

Sources and Forms of Copper Inputs

Copper is a metal with broad industrial applications. Environmental Protection Agency (1974) estimates that approximately 10 tons of copper enter freshwater streams and rivers daily from human activities. Nonpoint contributions are significant in agricultural or timber management areas in which copper containing pesticides may be used. For example, copper sulfate is frequently used to control helminthiasis and infectious podermatitis in cattle and sheep (Chu et al. 1978). Copper compounds are also used in fertilizer (as a micronutrient) and in certain insecticides. Significant agricultural contributions also result from feed lots and chicken farms, where large numbers of animals are concentrated.

Point sources of copper include domestic sewage treatment plants Concentrations as high as 1.7 mg/l have been found in domestic sewage. Eight hour composite sample from these plants have resulted in a range of copper concentration from 0.2-2.6 mg/l in the final effluent (William et al. 1974). When these concentrations are combined with industrial wastes, as in the case of the Renton sewage treatment plant, treatment may remove less than 50% of the metals (William et al. 1974).

Of all major sources of copper to the environment, it is estimated that approximately 14% is discharged by major steam electric power plants. These inputs are primarily the result of corrosion of copper and brass tubing used in heat condensors, heat exchangers, and cooling towers (Environmental Protection Agency 1974). Other industrial sources include metal fabrication and processing plants, metal extractive industries, and material recovery plants. Point discharges to the Green River which are known to contain copper include waste from an industrial laboratory (Kent, Washington), a material recovery operation (Renton, Washington), and galvenizing, electroplating, and smelting operations (Seattle, Washington).

Copper inputs to aquatic systems can be classified into forms: 1) cupric (Cu^{++}), 2) organically complexed, 3) adsorbed or exchangeable ionic, 4) soluble inorganically complexed, and 5) particulate. Each of

these forms has its own impact to aquatic systems. Cupric copper, for example, is considered the most toxic (Pagenkopf 1976). It is also very reactive, mobile, and soluble. Inorganic, insoluble copper complexes pose much less of a problem to aquatic life. The chemistry of adsorbed, or exchangeable copper is more complex, and the ultimate fate of these forms is dependent upon the material the copper is adsorbed to (often clays) and the characteristics of the surrounding water.

Environmental Chemistry of Copper

Once in solution in river water, copper can undergo numerous chemical reactions. In water of low organic carbon and moderate alkalinity, copper chemistry is dominated by its reaction with carbonates:

$$Cu^{++} + HCO_3^- \rightleftharpoons CuHCO_3^+ \quad (\log K = 12.3)$$

$$\text{or, } Cu^{++} + CO_3^{2-} \rightleftharpoons CuCO_3 \quad (\log K = 6.77)$$

$$\text{or, } Cu^{++} + 2CO_3^{2-} \rightleftharpoons Cu(CO_3)_2^{2-} \quad (\log K = 10.1).$$

For these waters, the chemical equilibrium and solubility of copper is dependent upon pH and carbonate concentrations.

The presence of soluble organic ligands, including humic and fulvic acids, polysaccharides, fatty acids, amino acids and industrial detergents may enhance copper solubility but at the same time decrease its bioavailability. The reactive groups of organic ligands are the carboxyl group, the amino group, the carbonyl group, and the sulfhydral group. Nearly all organic molecules contain one or more of these groups. It has been reported that copper forms the most stable organic complex of all the transition metals (Mahtoura et al. 1978; Irving and Williams 1953).

Florence and Batley (1980) downplay the role of organics in copper speciation in natural waters, claiming that organic ligands are not present in great enough concentrations to successfully compete for copper ions with inorganic ligands, principally carbonates. Humic and fulvic acids, and other natural organic ligands have copper stability constants in the same range or less than inorganic ligands (log K for humics and fulvics in the range of 5 to 6, Shuman and Cromer 1979) and are often present in much lower concentrations. Sylva (1976), using computer equilibrium models, determined that a theoretical organic ligand (log K equal to 4) with a 1:1 stoichiometry with copper, present at 3.15×10^{-5} M, had a negligible effect on complexation at pH values of 6 and 7. At log K values of 5, complexation with organics were significant. At pH values of 8, organic complexation was obscured by the initial inorganic complexation. Equilibrium for copper inputs to aquatic systems, especially with respect to organic complexation, may never be approached in some cases, due to the kinetics of copper at environmental concentra-

tions. A list of commonly occurring ligands is presented in Table 1 along with the equilibrium constants.

Ultimate Fate

The ultimate fate of copper in rivers and their estuaries is deposition into sediments. Deposition may occur as the result of flocculation or precipitation. Sylva (1976) reported precipitants that can occur in natural waters include CuO, $CuCO_3$, $Cu(OH)_2$, $Cu_2(OH)_2CO_3$ (malachite), and $Cu_3(OH)(CO_3)_2$ (azurite). For systems of low alkalinity, malachite may precipitate at pH = 6. Azurite and $Cu(OH)_2$ will precipitate at pH value of about 6.2 and 6.8.

The role of adsorption is probably significant in the deposition of metals to sediments. Adsorption reactions include 1) direct adsorption of copper to a solid, 2) ion exchange of copper to a solid, and 3) complexation of copper with an organic molecule containing a hydrophobic group adsorbed to a solid. The amount of adsorption may depend upon several factors. Sanchez and Lee (1973) reported a relationship between copper adsorption to sediments and increasing alkalinity and pH in sediments. The type of solids or clays present, the presence of competing ions for adsorption sites, and the amounts and types of organic material present will also affect adsorption.

The deposition of these copper adsorbed or precipitated solids may occur anywhere along a river, but increases significantly downstream with major deposition occurring in the estuary (International Copper Research Association 1982). Blustein and Smith (1978) demonstrated decreasing copper concentrations in the water column with increasing depth and salinity in an estuary as a result of deposition. The presence of organic material promotes flocculation in the estuary. Gardiner (1974) suggests that the flocculation and precipitation of suspended and colloidal organic material results in the transfer of most dissolved metals from river water to the bottom in the freshwater/seawater mixing areas.

Use of Polarography in Pollution Studies

Definitions

In the following discussion, several different terms are used to describe the forms of copper. Total copper refers to all forms of copper in a water sample. Reducible, labile, or electrochemically labile copper refers to any form of copper which dissociates and undergoes reduction and oxidation during DPASV analysis. The difference between total copper and reducible copper is the apparent complexed copper, or those forms which are non-reducible during analysis. Calculated bound, or complexed copper, is the amount of copper which theoretically should be in a stable complex as determined by equilibrium constants.

Table 1. Commonly occurring inorganic and organic ligands and their copper complex stability constants.

Ligand	Log stability constant
PO_4	15.61[a]
P_2O_7	7.97[a]
CO_3	6.34[a]
Cl	0.32[a]
Glycine	8.42[a]
Oxalate	5.10[a]
EDTA	19.47[a]
Humic acids	5-6[b]
Fulvic acids	5-6[b]

[a]From Sillen and Martell (1964).
[b]From Shuman and Cromer (1979).

General Theory

Several approaches are currently in use to study the speciation of metals in natural waters. One approach involves the use of electrochemical properties of metals and their complexes for acquiring qualitative and quantitative information on metal speciation. These methods, collectively referred to as polarography, can be used to measure any chemical species which will undergo oxidation or reduction when applied with a controlled potential. For measuring copper speciation, a modification of classic polarographic techniques, known as differential pulse anodic stripping voltammetry (DPASV) has been proposed as a useful tool in pollution research. Florence and Batley (1980) in a review of chemical speciation in natural waters, concludes that DPASV techniques are the most useful of currently available techniques for determination of bioavailable forms, and that this approach may be closest to measuring the toxic or bioavailable forms.

DPASV is a two-step process. During the first step, copper is reduced and deposited onto a hanging mercury drop electrode by application of a negative deposition potential:

$$Cu^{++} + 2e^{-} + Hg \rightleftharpoons HgCu$$

In the second step, the deposited ions are reoxidized, and stripped from the mercury electrode. For the Princeton Applied Research Model 374 polarographic analyzer used in this study, the pulse excitation curve for this stripping step increases linearly with time, upon which are superimposed periodic fixed height potential pulses (Figure 1). Current (i) measurements are taken just prior to the pulse application, and again, just before the pulse is removed. The difference between the two current measurements is displayed on a recorder, plotted against the linear potential increase and reflects the amount of material that is oxidized at a given potential. The resulting peak current value is related to the amount of reducible copper in the sample (Figure 2). Non-reducible copper, or copper which is in a stable association with an organic or inorganic ligand should not affect the current.

Applications

Three approaches to the application of DPASV to environmental problems of copper speciation are presented here. In one approach, shifts in stripping peak potentials are interpreted to indicate the presence of a reducible copper complex in solution (Bradford 1973; Ernst et al. 1978). Due to the many poorly understood factors which may affect the potential shifts, this approach is currently of limited value for use on natural waters.

In the second approach, peak current variations with pH, organic chelating material, or carbonate alkalinity are observed and related to the formation or dissolution of nonreducible complexes, solid phases, or colloidal species (Chau et al. 1976). This approach has been used to

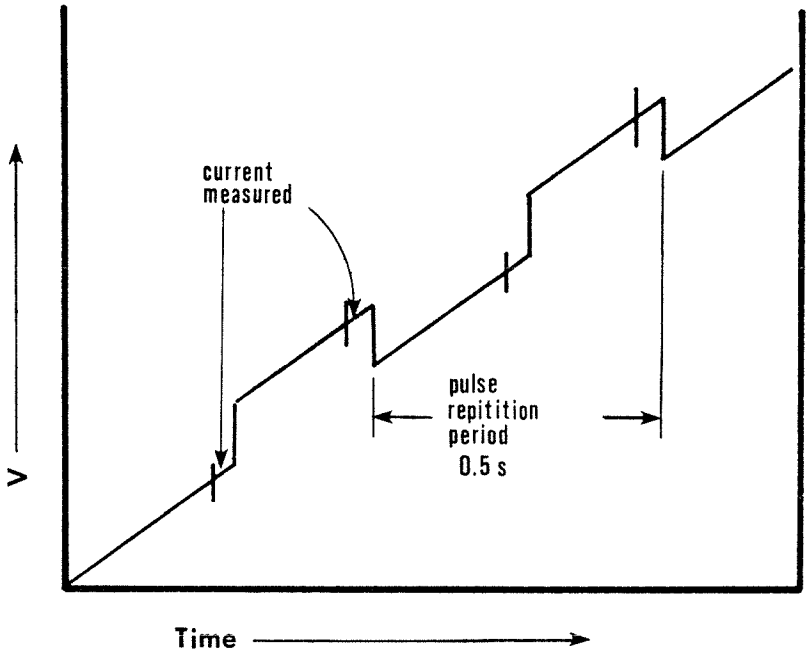

Fig. 1. Pulse excitation curve for DPASV.

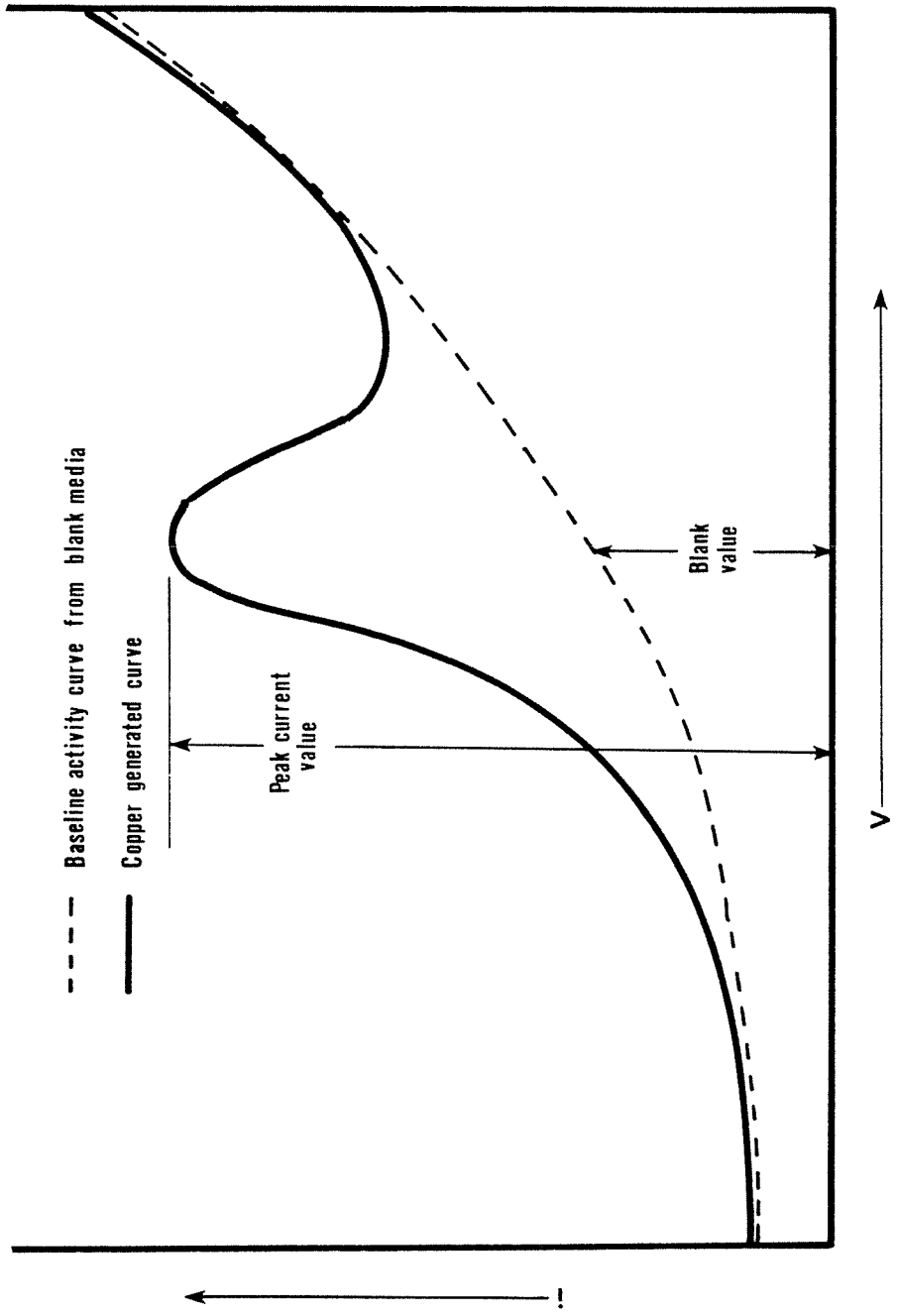

Fig. 2. Typical DPASV curves generated for blank media and copper standard at pH=7.

determine an 'apparent complexing capacity value' to characterize natural waters in their ability to complex copper additions. The method is based upon the measurement of DPASV reducible copper after a number of copper spikes have been made and allowed to equilibrate with the complexing agents in a water sample. The apparent complexing capacity of a sample is given by the intercept on the abscissa of a plot of the peak current values versus the concentration of copper added. While ligands remain available for complexation, any spiked copper would be complexed, and therefore not measurable by the instrument. When all ligands are saturated, any spiked copper would remain in the ionic form and analysis should result in a linear relationship between copper added and instrument response. A theoretical representation is presented in Figure 3.

The third approach involves measurement of reducible copper during a complexometric titration. Serial additions of copper are made to a single solution containing ligands which form nonreducible complexes. Shuman and Woodward (1977) and Srna et al. (1980) have used this approach to estimate complexing capacities in fresh and seawater. As in Chau's (1974) method the results are a plot of total copper added versus instrument response. The endpoint, or complexing capacity, is considered to be the point where the slope of the curve changes dramatically (Fig. 4). Shuman and Woodward (1977) reported generating curves with two distinct endpoints, suggesting the presence of 2 or more ligands in the sample.

Several investigators have used this third approach to study copper complexation with fulvic and humic acids, two naturally occurring metal chelators. Wilson et al. (1980) studied binding by fulvic acids. At constant copper concentrations, additions of fulvic acid caused 1) a decrease in peak current values, 2) the appearance of a subsidiary peak approximately 12-15 mV anodic of the assumed Cu^{++}/Cu potential, and 3) a cathodic shift in the baseline wave. Wilson et al. (1980) concluded that copper-fulvic acid complexes may be partially reducible during analysis, and that fulvic acids may be adsorbing to the mercury electrode, causing a local pH change, thereby affecting the reduction potential for copper. Shuman and Cromer (1979) investigated binding of humics and fulvics and concluded, as Wilson et al. (1980), that some dissociation of fulvic-copper complexes was occurring during the deposition step of the analysis and recommended the use of correction factors to compensate. In another paper, Shuman and Michael (1978) determined that in a sample of low carbonate alkalinity, Cu^{++} itself may be adsorbing to the mercury electrode prior to reduction and deposition; conclusions warned against the use of shifts in reduction potentials (first approach) and of comparing peak heights from water samples with gross differences in solution composition.

No studies have been conducted which attempt to demonstrate a direct relationship between DPASV reducible copper concentrations and

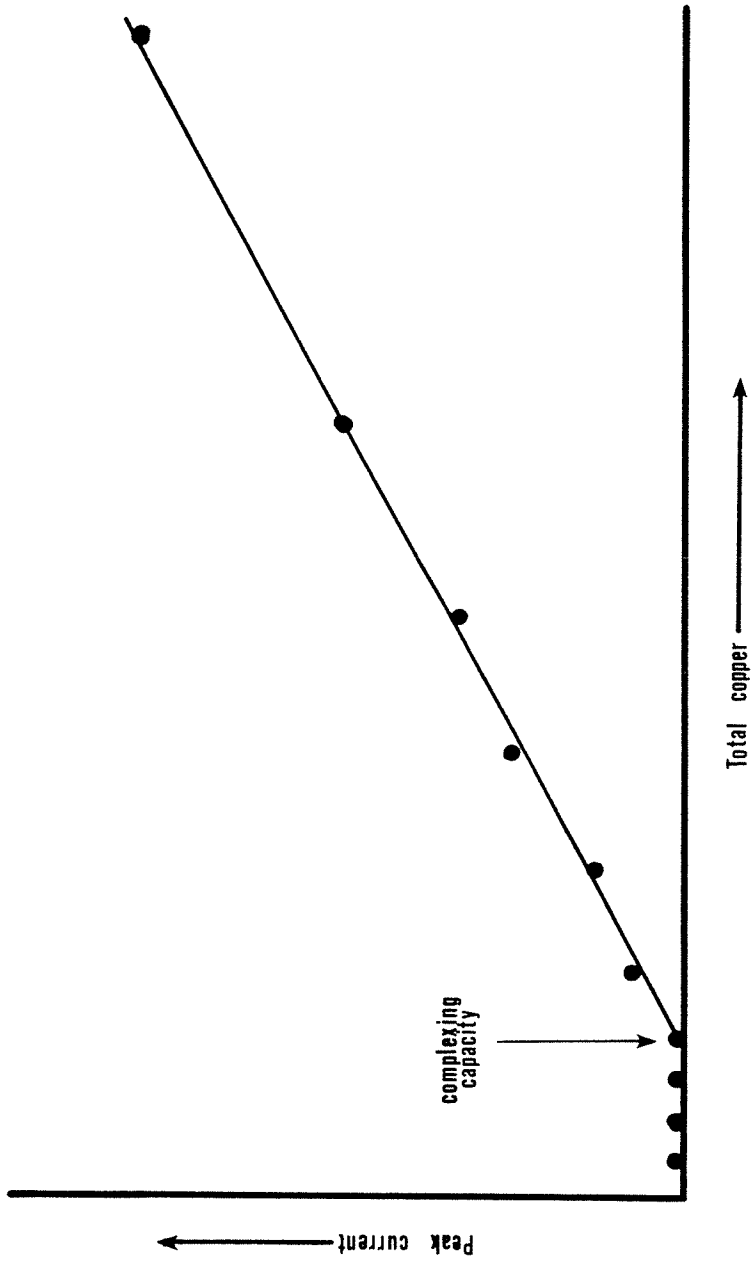

Fig. 3. A theoretical representation of apparent complexing capacity as described by Chau et al. (1974).

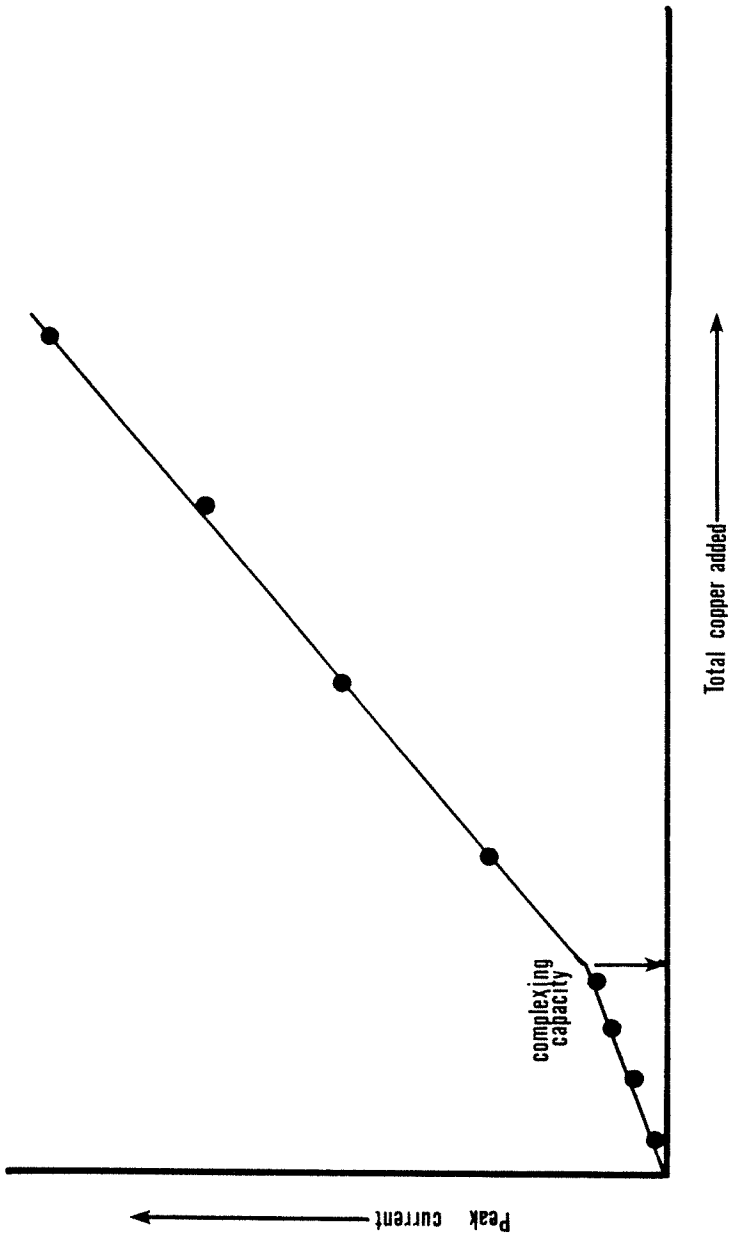

Fig. 4. A theoretical representation of complexing capacity as described by Shuman and Woodward (1977).

toxic response in fresh waters. Young et al. (1979) investigated mortality of larval coonstripe shrimp (Pandalus danae) and determined a general correlation between DPASV reducible copper in seawater and toxic response. A causal relationship was not established. Delayed moulting of larvae occurred at concentrations (5 µg/l) less than the calculated apparent complexing capacity (19 µg/l) which suggests that Chau's approach for characterizing natural waters may be misleading. Harrison et al. (1980) concluded a strong relationship exists between DPASV reducible copper and toxicity to oyster larvae (Crassostrea gigas), with toxic effect is observed at 6 µg/l Cu. The seawater in this experiment was determined to have an apparent complexing capacity of 3 µg/l copper. Srna et al. (1980) compared toxic response in marine algae and the measurement of copper ligands with DPASV, and concluded that measured concentrations of ligands were lower than those which could account for observed reduction of toxicity to phytoplankton. All chemical measurements from Srna et al. (1980), however, were determined at pH 4.8, rather than at ambient pH, which may account for apparent lower binding.

MATERIALS AND METHODS

Objectives and Assumptions

The primary objective of this study was to investigate the use of DPASV in making predictions regarding copper speciation and biological impact which might occur as a result of copper inputs to a river system. The assumptions of using DPASV to measure copper complexation of natural waters are, for any complex:

$$Cu + L \rightleftharpoons CuL,$$

1) The complex, CuL, does not contribute to the stripping current, i.
2) $i = R(Cu^{++} + CuL_1 + CuL_2 + \ldots + CuL_n)$, where R is the instrument response to all soluble forms of metal not bound to the complex under study, and L_n are the ligands which form electrochemically labile, or reducible, complexes.
3) CuL does not dissociate appreciably during the period of metal deposition.
4) The concentration of L must be uniform throughout the test cell.

Study Area

Description

The Green River, approximately 89 miles long, is an ideal river to study becaue of diversity in the drainage topography and adjacent land utilization, ranging from high mountainous headwaters to meandering low-

land areas, from limited access areas to intense industrialization. The origin of the river is about 30 miles northeast of Mt. Rainier. The first 25 miles are characterized by dense forests, steep slopes, and narrow valleys. The Howard Hansen Dam and Reservoir are located at river mile no. 65 (RM 65). Three miles below this dam, the City of Tacoma maintains a municipal water supply diversion facility. Due to the physical barrier, this is the upper limit for all anadromous fish migration. For the next 25 miles, rugged terrain persists, until the river emerges from the Green River Gorge at RM 46.5. Downstream, the river acquires a smaller gradient, passing through open farmland, mixed with patches of conifers and deciduous growth. This stretch includes two of the most significant tributaries, the Newaukum and Soos creeks. Below RM 26, the river meanders through farmland and eventually into heavily urbanized areas. The final 11 miles of the system, known as the Duwamish Waterway, flow through areas of intense industrialization, and are characterized by diking and channelization.

Three species of Pacific salmon utilize the Green River system for migration, spawning, or rearing: Chinook, coho, and chum. Pink salmon, once abundant in the Green, have not been recorded since 1935. Other anadromous game fish found in the basin include steelhead, sea-run cutthroat, and Dolly Varden. Poor water quality conditions in the Duwamish portion of the basin have been cited as a limiting factor for anadromous fish populations.

Sampling Scheme

Samples were collected 3 times during the study period, on April 19, June 14, and August 2, 1982. Duplicate samples for all measurements were taken in 250 ml polyethelene bottles, from each of 5 (June 14, August 2) or 6 (April 19) stations.

Sampling stations were chosen based upon 1) accessibility, 2) isolation of past and present watershed use patterns (farming, industrialization, logging, urbanization) and 3) isolation of known metal-containing point discharges. Sampling stations were labelled 1 through 6, and are described in Table 2.

Chemistry

Total Copper

Total copper in river water and bioassay water was determined by atomic absorption spectrophotometry (AAS) with a graphite furnace atomizer.

Standard Preparation. Standard stock solutions of 1.0 and 10.0 mg/l were made by diluting a 1000 mg/l commercial AAS standard with 0.5%

Table 2. A description of sampling stations on the Green River.

Station No.	River mile index	Descriptive
1	68.5	Limited access area; sample taken just below railroad bridge; logging only human activity; above Howard Hansen Reservoir; no anadromous fish.
2	46.5	Bottom of Green River Gorge at Flaming Geyser Park; upstream is heavily forested with second growth conifers and deciduous trees.
3	34.0	Above confluence with Big Soos Creek, upstream crop and dairy farming.
4	24.0	Below Highway 167 overpass, near Kent, WA; upstream highly urbanized, Auburn.
5	10.0	Under 50th Pl. south bridge, 1 mile downstream from Renton sewage treatment plant.
6	0.5	East channel at Spokane Street bridge; brackish water; upstream heavily industrialized.

Ultrex HNO_3 in deionized water. Daily instrument standards were made up by pipetting small amounts of the standard stock solutions (10-100 1) into disposable centrifuge tubes containing 10 mls 0.5% Ultrex HNO_3. The preparation of daily instrument standards was checked by running a 0.0185 mg/l quality control standard obtained from the Environmental Protection Agency, Monitoring and Support Laboratory, Cincinnati, Ohio, along with daily samples. For obtaining blank values, 0.5% Ultrex HNO_3 in deionized water was used.

Absorbance values for individual standards and samples were obtained by taking a mean of at least two injections. A set of instrument standards was run every 10 samples, with a single standard and blank run every 5 samples during a day's analysis.

Precision and Accuracy. Precision refers to the reproducibility of a method when it is repeated under controlled conditions; precision can be expressed by the standard deviation. Accuracy refers to the agreement between the amount of copper measured, and the amount actually present. Recovery procedures allow the investigator to determine a qualitative estimate of the presence or absence of interfering substances in a sample. Estimates of precision, accuracy, and recovery for total copper analysis were made by running a series of tests on:

1. A 0.020 mg/l Cu standard,
2. river water samples spiked with 0.020 mg/l Cu,
3. 50% dilution of the 0.020 mg/l Cu spiked river water sample,
4. 0.0185 mg/l Cu EPA quality control standard, each in 5 replicates. These 20 samples were run with a standard curve. Detection limits were determined as the lowest standard run which exceeded twice the range of blank values observed. Mean instrument response, standard deviations, and coefficient of variation were determined for each standard.

DPASV Reducible Copper

DPASV reducible copper was determined on a Princeton Applied Research Model 374 polarographic analyzer. A deposition potential of -0.3 V was used. The linear ramp potential scan began at the deposition potential, and ended at 0.1 V. The oxidation potential for copper varies from approximately -0.064 V to 0.042 V depending upon the nature of the sample. Deposition time was 100 seconds, scanning rate was set at 0.002 V/.5 sec. pulse. Samples were stirred gently with a magnetic stirring bar during deposition state. All samples were purged of oxygen by bubbling ultrapure nitrogen gas through the cell for 10 minutes prior to analysis.

A 0.03M ammonium acetate solution was used in all standards and samples to insure a conductive media and buffer against pH shifts during analysis. This reagent was prepared by bringing 23.125 g of ammonium acetate crystals up to 100 ml volume and adjusted to pH = 7 with air

distilled ammonium hydroxide. One hundred l of this solution was added to 10 ml of standard or sample.

Standard Preparation. Standard stock solutions of 1.0 and 10.0 mg/l Cu were made by diluting a 1000 mg/l commercial AAS standard by 100 and 1000 in deionized water. Instrument standards were made daily by pipetting small amounts (10-100 l) of stock material in 10 mls deionized water. Standards were checked for total copper by AAS. Current readings for individual standards and samples were obtained by taking a mean of a minimum of 2 scans for each sample. Instrument blank values were obtained with a 0.03M solution of ammonium acetate in deionized water.

Precision. It is possible to determine precision, but not accuracy or recovery for DPASV, because of the complexation which may be occurring when copper spikes are made to river water. Estimates of precision were made by running a series of tests on:

1. A 0.010 mg/l Cu standard,
2. a river water samples spiked with 0.010 mg/l Cu,

each in 5 replicates. These 10 samples were run with a standard curve. Mean instrument response, standard deviations, and coefficients of variations were determined for each standard. Detection limits were determined as the lowest standard run which exceeded twice the range of blank values observed.

Complexometric Titration of River Water

Complexation titration curves were generated for river samples collected April 19, stations 1-6, and for river samples collected June 13, and August 2, stations 2-6. Each curve was generated by making successive spikes of a copper standard, ranging from 0.010 to 0.300 mg/l Cu, to a cell containing 10 mls of river water and 0.03M ammonium acetate. DPASV determinations were made following each spike. Complexometric titrations were made on each of two replicate samples collected at each station. To determine linearity of instrument response to copper, five successive standard series were generated in deionized water (assumed no complexation), ranging from 0.010 to 0.300 mg/l Cu.

Copper Binding Experiments with Specific Ligands

Bound copper determinations were made by adding successive spikes of copper, ranging from 0.010 to 0.300 mg/l, to separate solutions containing $2x10^{-5}M$ KPO_4, $2x10^{-5}M$ $Na_4P_2O_7$, $4x10^{-4}M$ Na_2CO_3, $1.3x10^{-3}M$ KCl, $5x10^{-6}M$ K·Oxalate, $5x10^{-6}M$ Glycine, $5x10^{-6}M$ Na·Citrate, and $5x10^{-6}M$ Na·EDTA. Each experiment was repeated twice, and a mean was taken of the instrument response. Concentrations of apparent bound copper were made by comparing instrument response in the presence of the specific ligands to instrument response in deionized water. Bound copper values were calculated as the difference between the amount of reducible metal and total amount added.

Theoretical complexation curves for each of the ligands were generated using a chemical equilibrium computer program, MINEQL, to be compared with measurements. Documentation for this program is available in Westall et al. (1976).

RESULTS

Chemistry

Precision and Accuracy

The results of precision and accuracy experiments for total copper are presented in Table 3. River water samples spiked with 0.020 mg/l copper, and 0.020 mg/l standards demonstrated similar instrument response. Spiked river water samples which were diluted by 50% demonstrated slightly higher than expected absorbance, resulting in a concentration of 0.012 $\pm$ 0.0044 mg/l (95% confidence interval). This may be due to a masking effect present in full strength river water, or instrument drift during analysis. Detection limits for this series was determined to be 0.005 mg/l. Coefficients of variation (S.D./$\bar{X}$ x 100%) were 10.48 and 16.93% for the 0.020 standard and river water sample, respectively. The result of 5 determinations on the 0.0185 mg/l EPA quality control sample were 0.017 $\pm$ 0.0018 mg/l (95% confidence interval).

The results of precision tests for DPASV measurements are presented in Table 4. Mean current readings for spiked river water samples were smaller than those for the 0.010 mg/l standard indicating either complexation in this sample, or the presence of interfering compounds which mask copper in the sample. Coefficients of variation for 0.010 mg/l standard and spiked river water samples were 19.52 and 19.74%, respectively. Detection limit was determined to be 0.001 mg/l.

Total Copper

Total copper concentrations in Green River water ranged from below detection limits (0.005 mg/l) to 0.032 mg/l. Considerable variation exists between station and sampling period. In April samples, high values were seen at station 1 (0.018 mg/l) and station 5 (0.007 mg/l). No detectable copper was found at stations 3, 4, and 6. In June samples, highest copper concentrations were observed at station 6 (0.025 and 0.032 mg/l). Station 4 showed total copper levels of 0.005 an 0.010 mg/l; no detectable copper was found at any other station in June. August sampling resulted in relatively higher concentrations of total copper, ranging from nondetectable to 0.021 in stations 4 and 5 and 0.024 in station 6. These data are presented in Table 5.

Table 3. Means, standard deviations, and confidence intervals in absorbance units (a.u.) for a 0.020 mg/l Cu standard, 0.020 mg/l spiked river water, and an EPA quality control sample.

Sample	$\bar{x}$ (a.u.)	S.D.[1]	n	C.V.(%)[2]	Conc. (mg/l) + 95% C.I.[3]
0.020 mg/l Cu Std.	0.019	0.002	5	10.48	-
River water + 0.020 mg/l Cu	0.020	0.003	5	16.93	0.021 $\pm$ 0.0070
1/2 river water + 0.020 mg/l Cu	0.010	0.002	5	18.86	0.012 $\pm$ 0.0044
0.0185 mg/l Cu EPA Std.	0.016	0.002	5	5.7	0.017 $\pm$ 0.0018

[1] S.D. = standard deviation

[2] C.V. = coefficient variation

[3] C.I. = confidence interval

Table 4. Means, standard deviations, and 95% confidence intervals for peak current values of 0.010 mg/l Cu standard in deionized water and river water.

Sample	$\bar{x}$ (nA)*	S.D.	C.I.	n	C.V.(%)
0.010 mg/l Cu std.	715	140	441-989	5	19.52
0.010 mg/l Cu in river water	575	113	353-797	5	19.74

*nA - nanoamps

Table 5. Total copper in replicate Green River water samples.

Station	Total copper concentration (mg/l) (4/19/82)	(6/13/82)	(8/2/82)
1	0.018,0.017		
2	0.006,0.006	<0.005,<0.005	<0.010,<0.010
3	<0.005,<0.005	<0.005,<0.005	<0.010,0.011
4	<0.005,<0.005	0.010,0.005	0.011,0.021
5	0.006,0.007	<0.005,0.005	0.021,0.021
6	<0.005,<0.005	0.025,0.032	0.024,0.020

For most sampling collections, duplicate samples agree closely. Differences are seen at station 4 and 6 for both June and August collections. These differences may have been caused by lack of homogeneity within the sample. When collected samples were allowed to sit for a period without agitation, a sediment was visible at the bottom of the container. The presence of copper in association with these sediments may account for some variability. Samples were not filtered prior to analysis. Total nonfilterable (TNFR) values are presented along with other water quality data for samples collected along with the total copper samples in Appendix 1.

Reducible Copper

Reducible copper in river water ranged from nondetectable to 0.004 mg/l, or in comparison to total copper, up to 29% of the total copper present. No distinct trends were observed in these data with respect to station or sampling trip. The results of duplicate samples are presented in Table 6.

Both the total, and reducible copper determinations for river water samples are near or below detection limits for the respective instruments. Coefficients of variation for polarograph values are relatively high at these levels. Furthermore, the determinations were made by comparing instrument response in river water to instrument response to standard curves generated in deionized water. Shuman and Cromer (1979) warn against comparing peak heights from solutions of grossly different composition. Consequently, the percentage values presented in Table 12 may lack accuracy and should be used for obtaining relative trends only.

A comparison of reducible copper and total copper values for each station give indications of sources of copper inputs to the Green River. Most copper inputs come from down river areas. Station 6 demonstrated the greatest concentration of metals with no detectable levels of reducible metals. Stations 3, 4, and 5 also showed relatively high total copper values during August, with the presence of small amounts of reducible copper. The one exception to the trend appeared in Station 1 during the April sampling period, which demonstrated unusually high levels of total copper.

Complexometric Titrations in Green River Water

The relationship between DPASV instrument response and copper concentration is linear over a range of standards, from 0.010 to 0.300, in deionized water (Fig. 13). Complexometric titrations in river water resulted in both linear and curvilinear relationships. For most curvilinear relationships, proportionally greater reductions in instrument response were seen at the lower concentrations, resulting in an increasing slope with increasing concentration of copper.

Table 6. Reducible copper concentrations in replicate Green River water samples. Percent of total copper is in parentheses.

Station	4/19/82	6/13/82	8/4/82
1	0.004,0.003 (22)		
2	<0.001,<0.001 (-)	<0.002,<0.002 (-)	0.002,0.002 (-)
3	<0.001,<0.001 (-)	<0.002,<0.002 (-)	0.002,0.002 (18)
4	<0.001,<0.001 (-)	<0.002,<0.002 (-)	0.002,0.002 (13)
5	0.002,<0.001 (29)	<0.002,<0.002 (-)	<0.001,<0.001 (-)
6	<0.001,<0.001 (-)	<0.002,<0.002 (-)	<0.001,<0.001 (-)

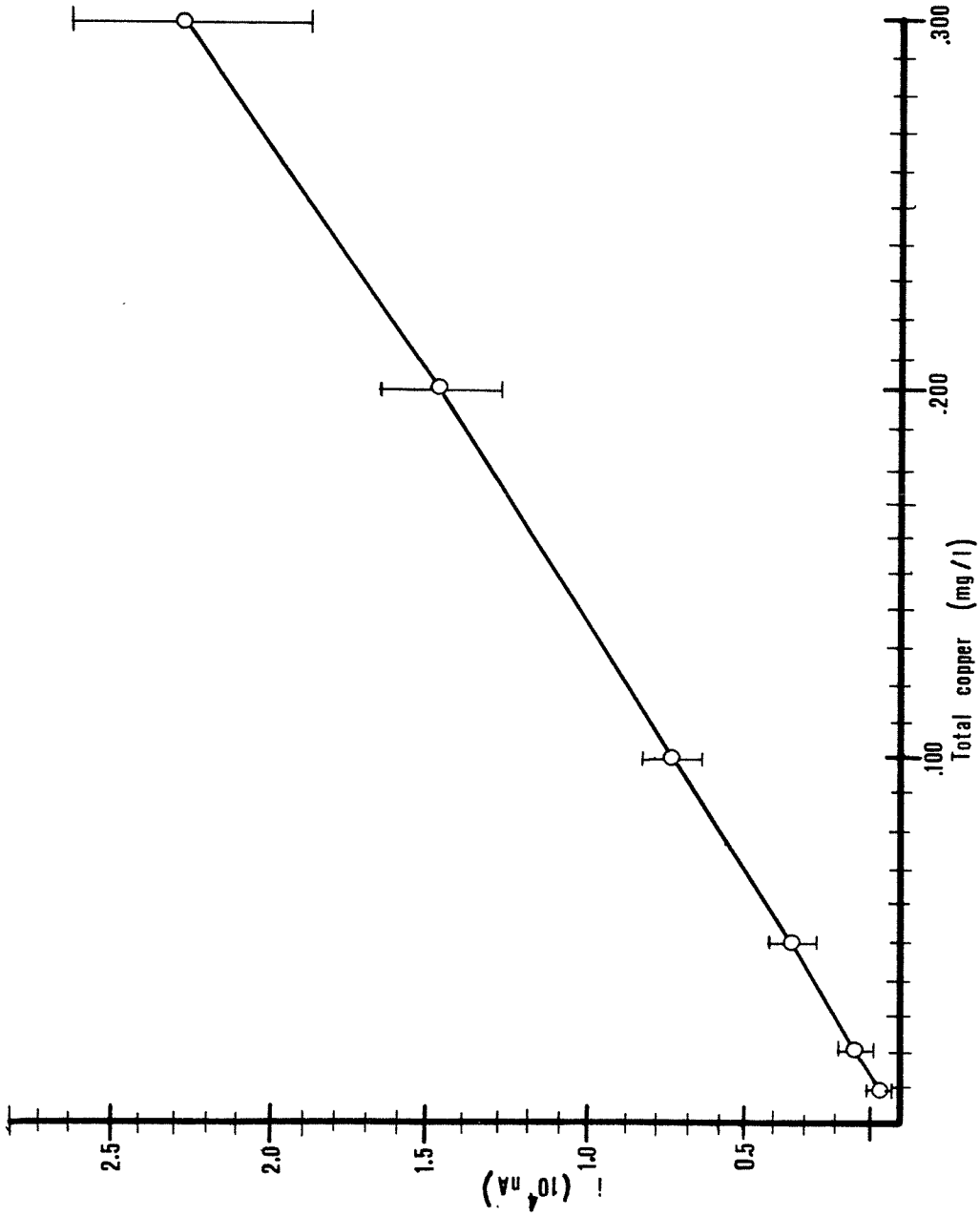

Fig. 5. Means and standard deviations for instrument response to standards (n=5).

April Samples. Figure 6 presents instrument response for titrations of station 1-6 against copper additions. Stations 1-5 demonstrated slowly increasing slopes with increasing concentrations. No distinct endpoint was visible for any titration. Station 6 demonstrated the most linear of the curve, with a reduced slope. Peak current values for station 6 were similar to the other stations up to 50 mg/l Cu, but decreased markedly above this. Peak current values for 0.300 mg/l were less than half those for the other stations.

June Samples. Peak current values for additions of copper in river water samples collected in June are presented in Figure 7. As for April samples, stations 2, 3, and 4 demonstrated curves of increasing slope with concentration, and no clear endpoint was observed in the range of copper additions. A much greater spread was observed in appar- ent complexation for collections made in June, than those made in April. Station 6 again demonstrated a linear, lower sloped curve than the rest of the stations. Station 5 demonstrated a possible endpoint, although this is somewhat of a tenuous conclusion since an additional point(s) above 0.200 mg/l would be needed to clarify this, and the change in slope at the endpoint is not very great. Stations 5 and 6 demonstrate the greatest amounts of apparent complexation.

August Samples. Samples collected in August demonstrated similar apparent complexation characteristics for stations 2, 3, and 5 as in other collections. Station 2 demonstrated the typical increasing slope described earlier. No distinct endpoints were observed in any of these samples. Stations 6 and 4 both demonstrated reduced peak current values when compared with other stations. The data for these stations are presented as peak current values in Figure 8.

Several trends are evident from looking at the figures. Stations 2 and 3 demonstrated the least amount of apparent complexation. For the majority of the curves generated, no clear endpoint to the complexometric titration was evident, although the shapes of most curves suggested that successive additions of copper resulted in a decrease in the proportion being complexed. Linearity of instrument response was achieved between 0.050 and 0.100 mg/l total copper for most samples. Station 6 invariably showed linear instrument response to spikes of copper, with a flattened slope. The cause for this dramatic increase in apparent complexation over the other stations is not known. Results presented in the next section show reduced instrument response in the presence of elevated electrolyte concentration (KCl). Relatively high conductivity values were observed at station 6 (see Appendix Table A.1), most likely the result of mixing with seawater. The presence of increasing electrolyte concentration in the sample may have affected instrument response in some manner, resulting in an increase in apparent complexation.

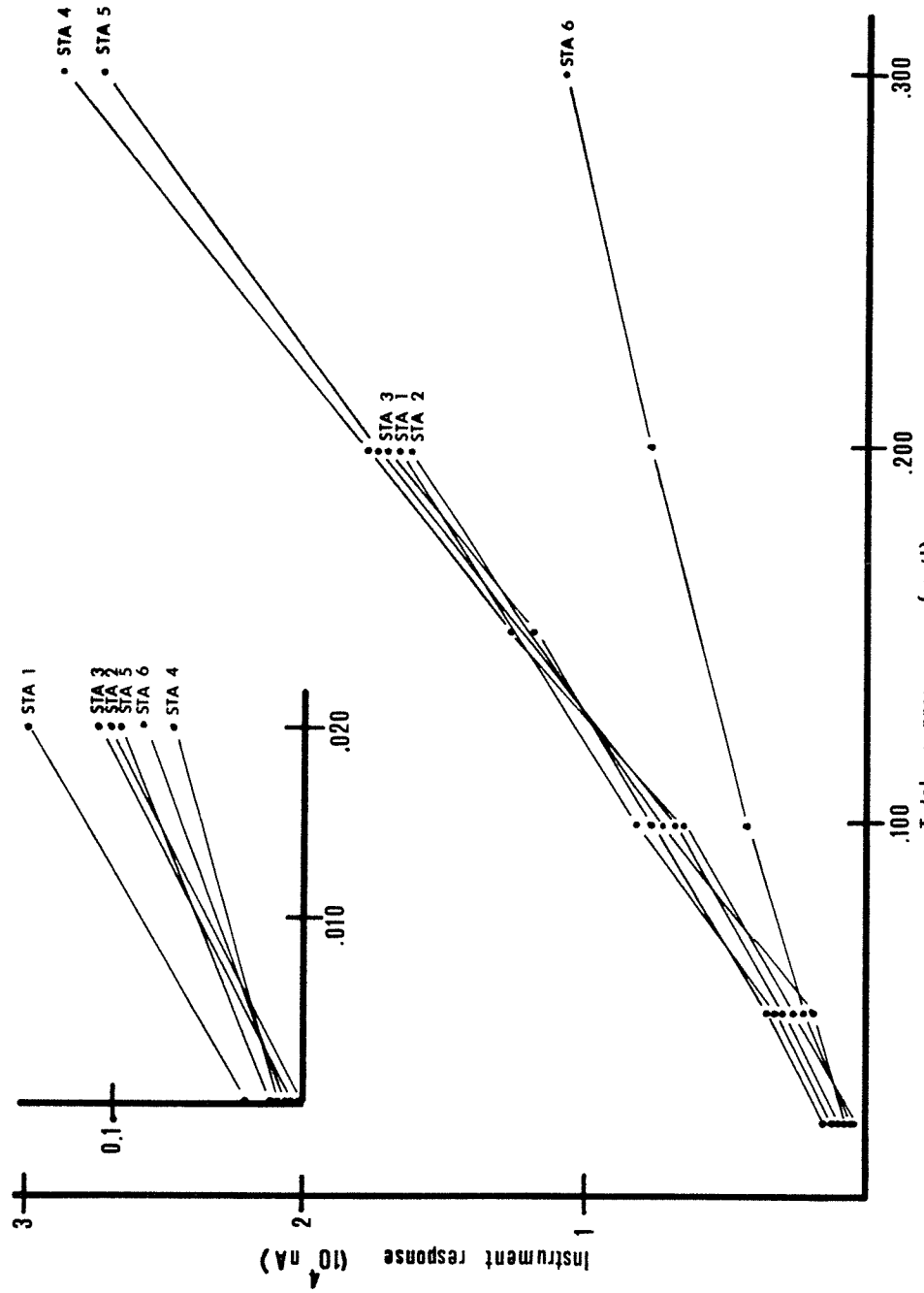

Fig. 6. Results of complexometric titrations in Green River water, April 19 samples.

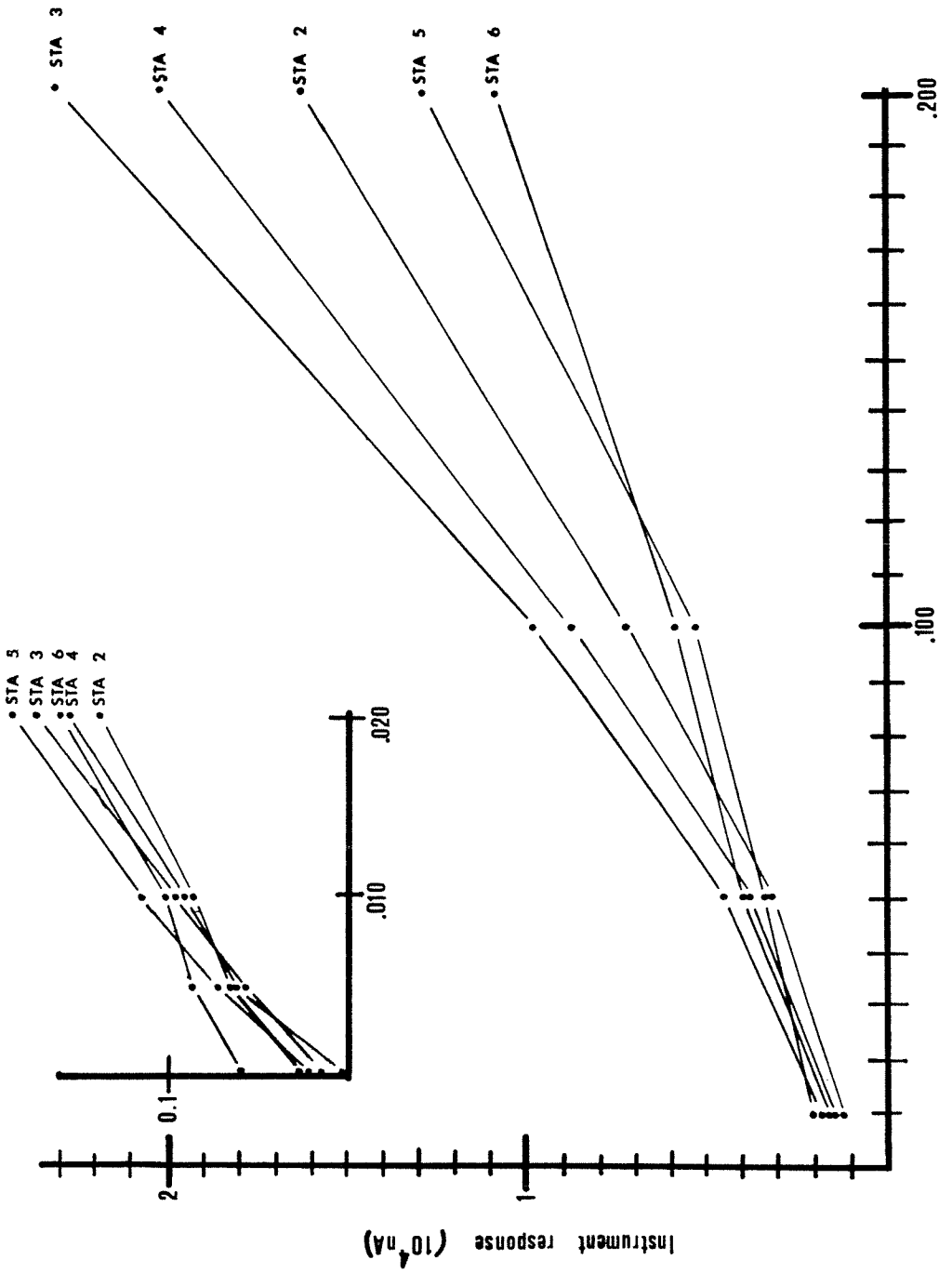

Fig. 7. Results of complexometric titrations in Green River water, June 14 samples.

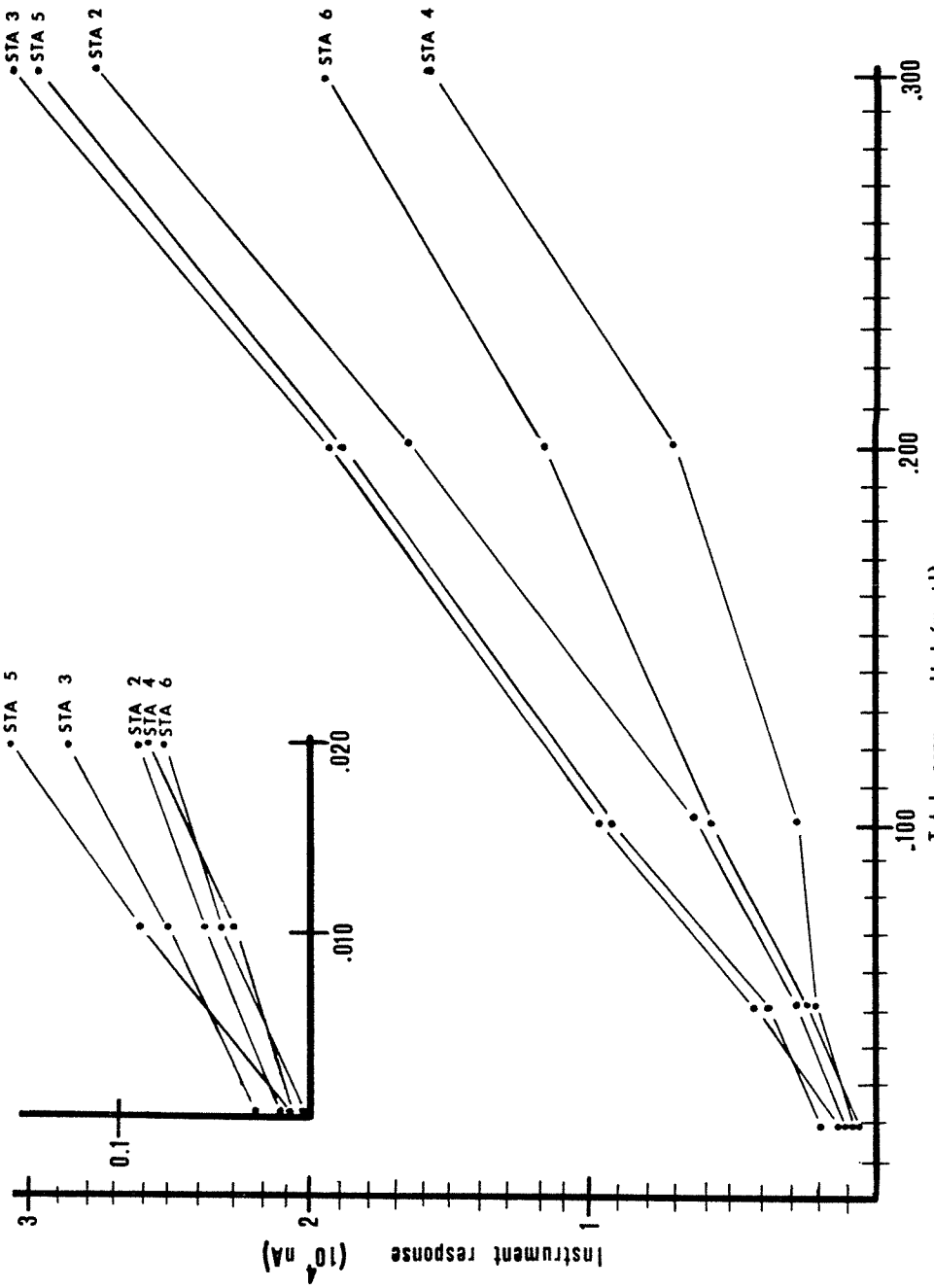

Fig. 8. Results of complexometric titrations in Green River water, August 2 samples.

Copper Binding Experiments with Specific Ligands

Instrument response to copper spikes of 0.050, 0.100, 0.200, and 0.300 mg/l copper in the presence of various ligands is presented in Table 7. P_2O_7 demonstrated the greatest reduction in peak current values over PO_4 and CO_3. CO_3 and PO_4 did not seem to have an affect on instrument response. Of the organic ligands, EDTA and citrate demonstrated lower peak current values than glycine or oxalate. There did not seem to be any simple relationship between log K value of the copper complex and peak current values of the organic ligands.

Raw instrument response values for copper in the presence of each of these ligands were compared to standard curves without the ligand to determine apparent complexation for each ligand. A comparison of these values with theoretical calculations on MINEQL demonstrate that instrument response generally determined a smaller concentration of ligand-bound copper than calculations allowed for. For PO_4, glycine, and oxalate, peak current values demonstrated no apparent complexation (Figs. 9, 10, and 11). Calculations from MINEQL allow for a small amount of complexing. Measurements with P_2O_7, CO_3, citrate, and EDTA (Figs. 12, 13, 14, and 15), all resulted in signficant apparent complexation, however none of these were as high as indicated by MINEQL. Most surprising is the metal chelator EDTA which is known to form very strong complexes with copper (log K = 19.3). From the figures, it can be seen that the instrument is responding to approximately 0.025 mg/l copper at the addition of 0.100 mg/l total copper. At 0.300 mg/l, the instrument is responding to 0.060 mg/l reducible copper. MINEQL calculations showed that 100% of all copper additions to 0.300 mg/l should be chelated to EDTA at equilibrium.

The presence of KCl of 1.3×10^{-1}M depressed peak current values by approximately 50%. Calculated values of bound copper in the presence of 1.3×10^{-1}M ranged from 2.0×10^{-9} to 1.8×10^{-8} mg/l for copper additions from 0.010 to 0.300 mg/l. This amount of complexation should be indistinguishable to the instrument. However, peak current values, when compared to instrument responses to copper without KCl present, indicated that approximately 1/2 of the copper at 0.010 to 0.300 mg/l would be apparently bound. It is unlikely that the presence of KCl would cause this amount of complexation to occur. It is most likely that the low peak current values are due to a direct effect of increasing ionic strength on instrument sensitivity. This data suports the observation of decreased slopes in complexometric titrations of station 6, a brackish water sample.

DISCUSSION AND CONCLUSIONS

The results presented here indicate that a general relationships may exist between reducible copper and toxicity, however, these results are confounded by lack of instrument quantification of complexing with known ligands, and sources of interference and masking which are not totally

Table 7. The effects of organic and inorganic ligands on peak current values at 0.050, 0.100, 0.200, and 0.300 mg/l total copper.

Copper ligand	Log K	Conc. (M)	Peak current value (nA) 0.050 mg/l Cu	0.100 mg/l Cu	0.200 mg/l Cu	0.300 mg/l Cu
Deionized H_2O	-	-	5,080	10,040	20,720	31,020
PO_4	15.61	$2x10^{-5}$	5,150	9,810	19,850	-
P_2O_7	7.97	$2x10^{-5}$	4,650	9,720	18,330	-
CO_3	6.34	$4x10^{-4}$	5,200	9,920	20,163	-
Glycine	8.42	$5x10^{-6}$	3,895	8,173	17,660	27,080
Oxalate	5.10	$5x10^{-6}$	3,980	8,910	18,160	27,310
Citrate	19.9	$5x10^{-6}$	2,580	6,403	14,437	22,077
EDTA	19.47	$5x10^{-6}$	324	1,399	4,205	7,612
KCl	0.32	$1.3x10^{-3}$	4,375	9,450	20,020	-
KCl	0.32	$1.3x10^{-2}$	-	4,510	8,365	-
KCl	0.32	$1.3x10^{-1}$	-	5,490	10,480	-

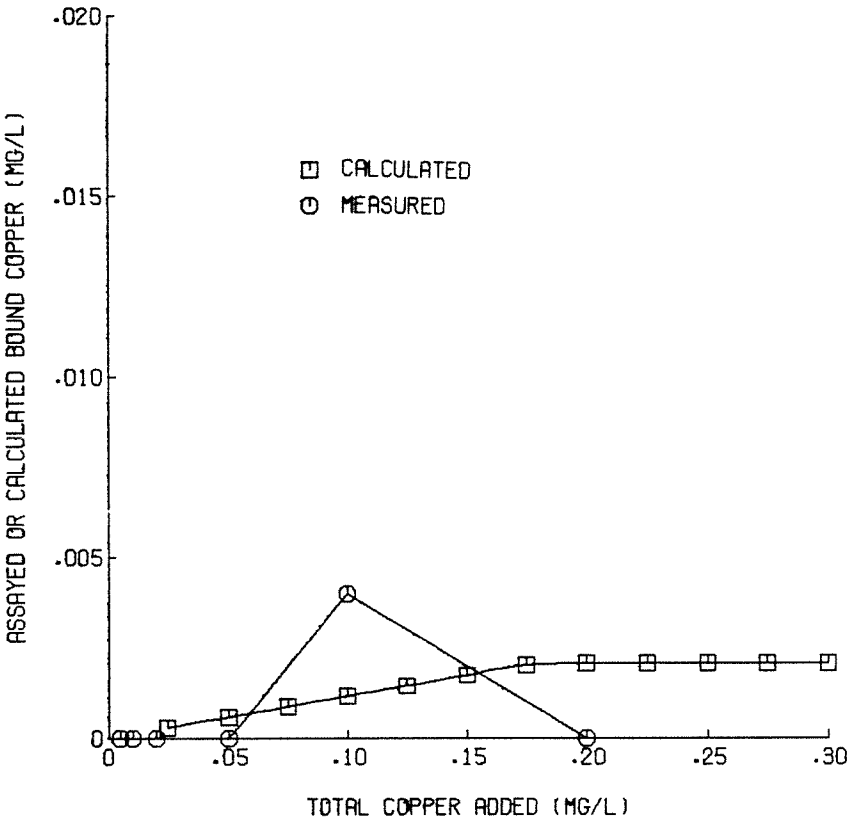

Fig. 9. A comparison of measured versus calculated bound copper in the presence of 2×10^{-5} M PO_4.

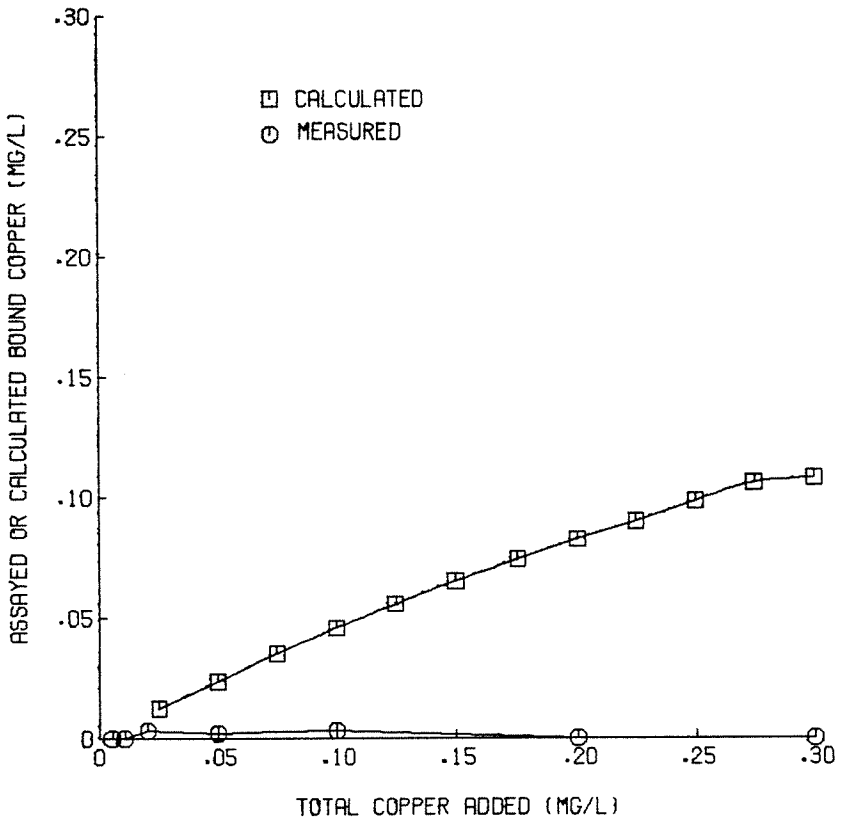

Fig. 10. A comparison of measured versus calculated bound copper in the presence of 5×10^{-6} M glycine.

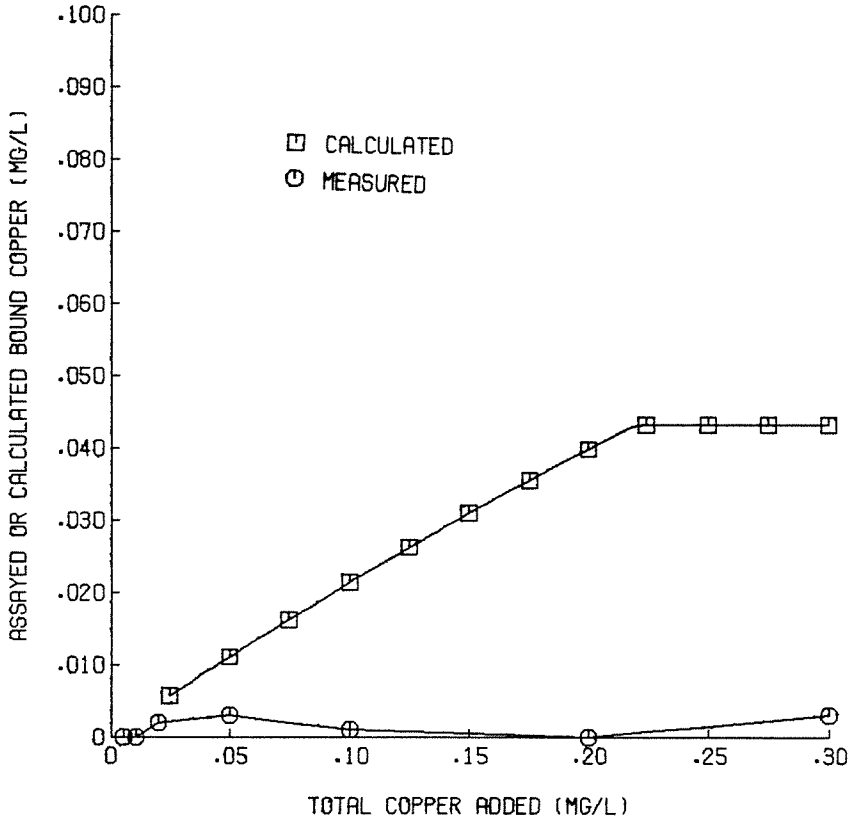

Fig. 11. A comparison of measured versus calculated bound copper in the presence of 5×10^{-6} M oxalate.

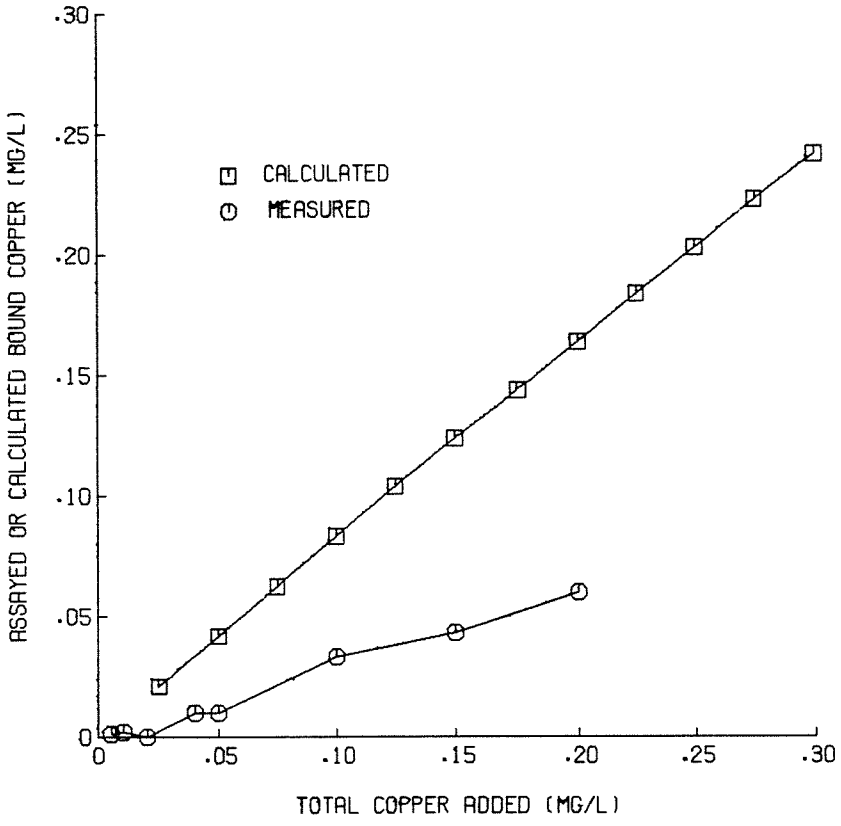

Fig. 12. A comparison of measured versus calculated bound copper in the presence of 2×10^{-5} M P_2O_7.

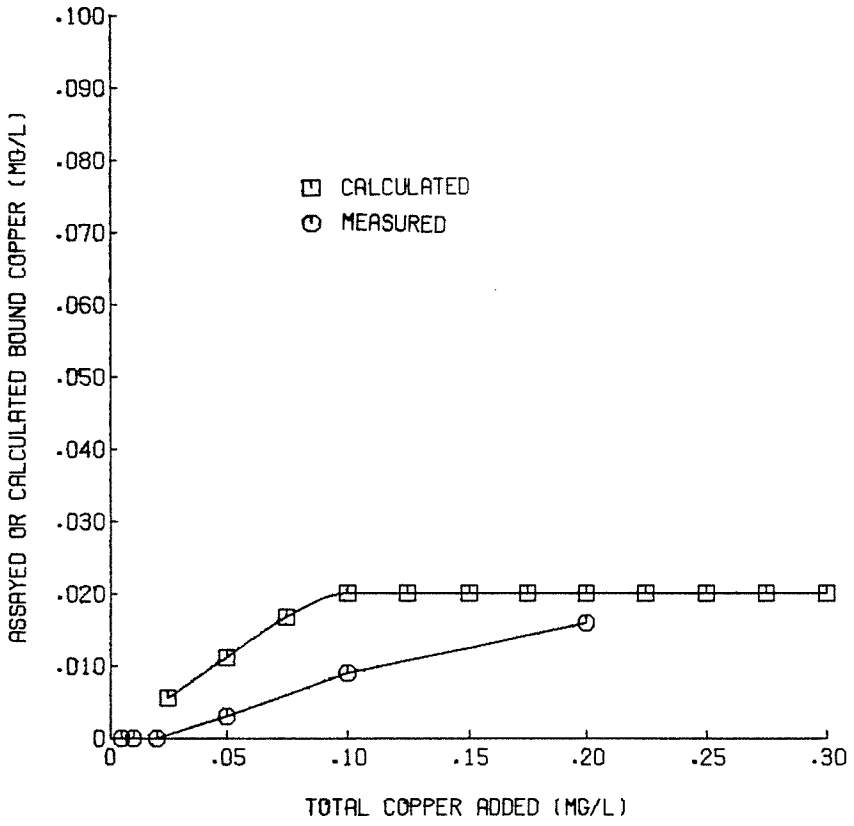

Fig. 13. A comparison of measured versus calculated bound copper in the presence of 4×10^{-4} M CO_3.

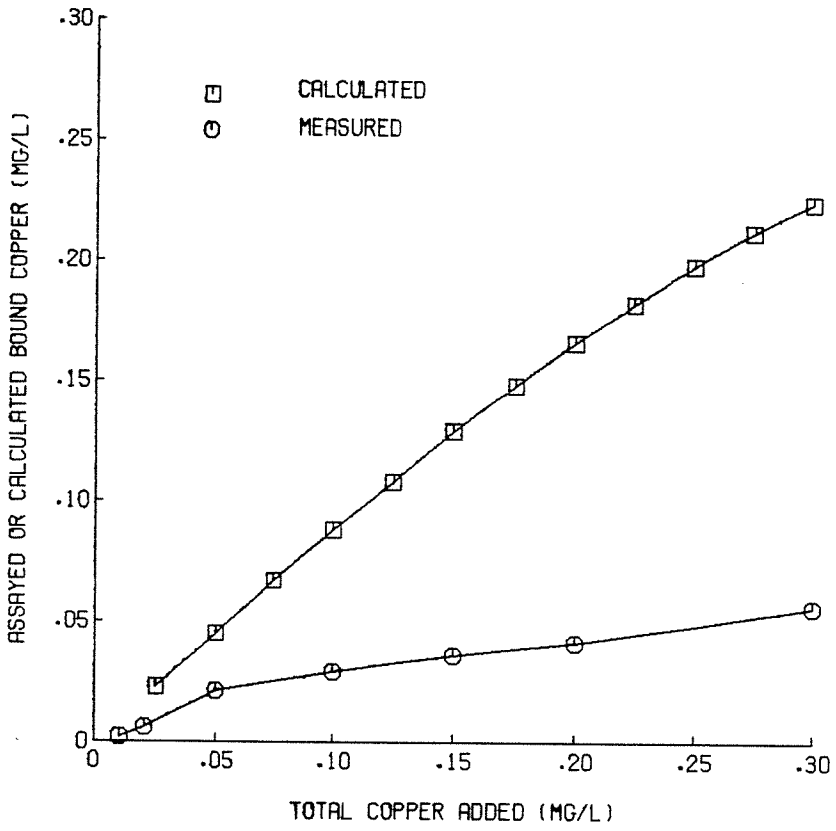

Fig. 14. A comparison of measured versus calculated bound copper in the presence of 5×10^{-6} M citrate.

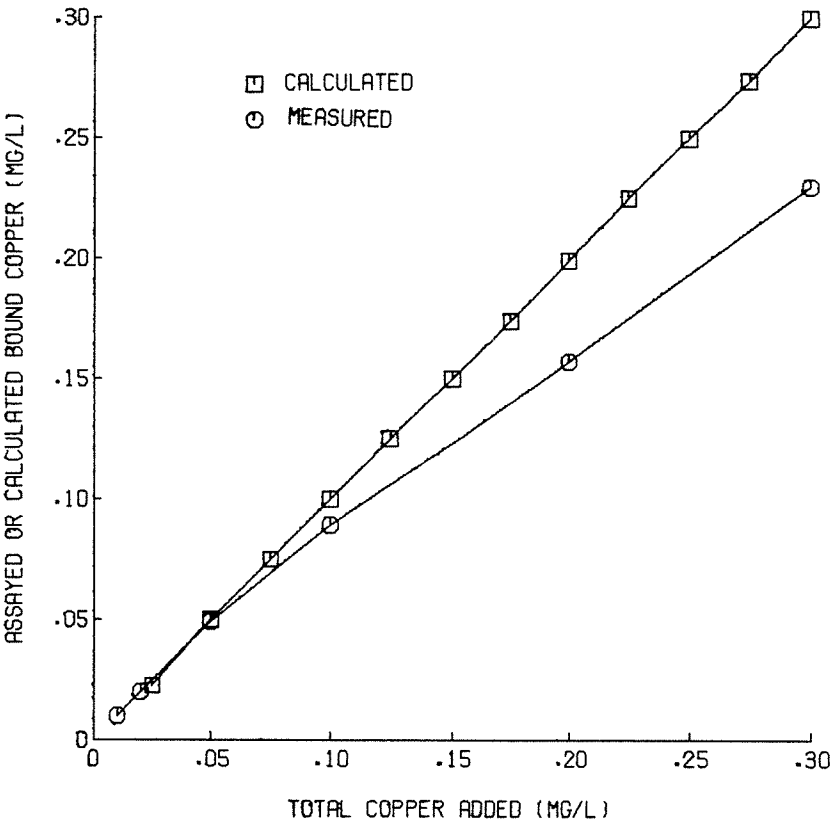

Fig. 15. A Comparison of measured versus calculated bound copper in the presence of 5×10^{-6} M EDTA.

understood. There are several technical aspects to DPASV that can be discussed in terms of the assumptions regarding the use of this technique. Many of these technical aspects can and probably did affect reducible copper measurements. A discussion of these is presented below.

Selection of Supporting Electrolyte

Changes in pH will affect conditional formation constants of all copper complexes. Decreased binding is observed at low pH because of competition of hydrogen ions with copper for binding sites. Because the purpose of these experiments was to investigate natural complexation of river water, it was necessary to utilize a supporting electrolyte which would buffer against pH shifts during analysis, and would hold pH at approximately that of the natural water in the study area. A 0.03M ammonium acetate solution was chosen for this purpose because of its buffering capacity at pH of 7, and its reported success in copper complexing investigations previously conducted in this laboratory (Felton and McClain 1979). Varying the amount of electrolyte from 0.01 to 0.06 M had little effect on instrument response in preliminary experiments. Polarography requires a 10^{-3}M minimum concentration of supporting electrolyte, and recommends 0.1 to 0.3M for copper analysis (Princeton Applied Research application note P-2).

Investigations with MINEQL on the effects of the ammonium acetate on copper speciation in the cell were revealing. Even in the presence of 2×10^{-5}M PO_4, 2×10^{-5}M P_2O_7, and 4×10^{-4}M CO_3, 99.5% of all the copper was in association with NH_4^+. Other ligands tested were able to compete successfully with the buffer for the Cu^{++}. It was assumed that all of the copper associated with the NH_4^+ was electrochemically labile, and was seen by the instrument during the scans.

Several investigators have reported using CO_2/N gas systems to maintain pH during analysis to avoid interactions with metal ions. Because deposition and scanning phases require that no disturbance to the hanging drop mercury electrode occur, gas could not be bubbled through the test samples during the entire analysis, but would have to be maintained at exact partial pressures in the space above the cell during these phases of analysis.

Diffusion Rates, Deposition Time, and Dissociation of Copper Complexes During Analysis

A critical stage in the analytical procedure, referred to as the deposition phase, is when copper is reduced and deposited onto the mercury electrode. A review of literature on the use of DPASV to measure copper complexation in natural waters indicates no standard procedure for setting the instrument deposition period. Reported times vary from 80 seconds to 5 minutes. The choice of 100 seconds for this study was

considered an optimum while trying to balance the potential for dissociation of stable copper complexes and loss of instrument sensitivity. Shuman and Woodward (1977), Shuman and Cromer (1979), Wilson et al. (1980) and Chau et al. (1974) have used deposition times greater than the 100 seconds used in these experiments to measure complexation of naturally occurring organic matter.

Although the deposition time scale may affect the degree of dissociation of complexes, another consideration may be the actual residence time within the space affected by the applied deposition potential. In the absence of stirring during the deposition phase, instrument responses were severely decreased. This would imply that all space in the cell is not equally affected by the applied potential during deposition. The rate of stirring would affect the residence time of an individual molecule, with lower stirring rates resulting in longer residence times and faster stirring rates resulting in shorter residence times.

The event of dissociation then, of any particular copper complex in the cell could be described as a function of a) the kinetics of dissociation of the particular complex, b) the rate of diffusion of the copper complex, and c) the residence time spent in the space affected by the deposition potential. Chau et al. (1974) report that only copper complexes with log-stability constants above approximately 13 are not reduced by the instrument. This study demonstrated a significant amount of dissociation even of the EDTA-copper complex during analysis (log-stability constant approximately 19).

Sources of Interference

Interferences to instrument response are of 4 types. The first of these is the formation of inter-element compounds within the hanging drop of mercury. Such compounds will depress the current peak and tend to affect those metals with the smallest, or most negative redox potentials. This is because at their low redox potentials, there is a greater potential for other metals to be deposited along with the metal of interest. Shuman and Woodward (1977) reported that the formation of Cu_aZn_b compounds can interfere with the analysis of both compounds. For the data presented here, this type of interference was not considered a problem since the deposition potential used (-0.3 V) was higher than redox potentials for other materials.

The second type of interference may occur when redox potentials for two forms occur so close together that the two curves overlap, obscuring the measurement of one. This can occur between two different elements, or between two oxidation states of the same element. The only element with a similar oxidation potential to copper is bismuth, which is not considered a potential contaminant. In this study, the occurrence of double peaks and shoulders which tended to obscure copper measurements, especially in samples collected from bioassays, demonstrated the

presence of some interfering agent. The role of a stable cuprous compound in the formation of double peaks has already been discussed.

A third type of interference is the presence of organic or inorganic impurities which may associate with the mercury drop electrode surface, and inhibit the deposition or stripping of metals. Several investigators have theorized about the effects of adsorbed fulvics on the mercury electrode (Wilson et al. 1980; Shuman and Cromer 1979). Cominoli et al. (1980) reported adsorption effects due to the presence of fulvic acids at deposition potentials less than -0.6V. Such adsorbed fulvics may alter pH in the space immediately surrounding the mercury drop, thereby affecting oxidation potentials of diffusion rates of the metals. Saar and Weber (1982) pointed out that due to the effects of adsorbed fulvic acid at the mercury electrode, standard curves generated in the absence of fulvic acid will lead to inaccurate results for samples containing these compounds. For this investigation, the relatively low organic content of Green River water coupled with the relatively high deposition potential (-0.3V) probably resulted in minimal adsorption interference.

The fourth, and probably most significant type of interference from the standpoint of these data are the effects of natural compounds which affect the current values directly, but do not deposit, or oxidize in the analysis. Such interference may tend to change the shape of the baseline activity curve, displacing it to the right or left, or raising or lowering it. This type of phenomenon was observed in samples collected from bioassay waters, where it appeared that baseline activity was shifted to the left, obscuring the copper peak. Another effect may be to alterations in peak heights due to changes in conductivity in the sample itself. For example, complexometric titrations with copper and KCl resulted in depressed peak heights. Calculations demonstrated only trivial amounts of copper binding to Cl^- in these titrations. Station 6 samples, which came from the Duwamish Waterway, and demonstrated increased conductivity, also showed greatly reduced instrument response when compared to other river samples.

A study of the various scans for PO_4, P_2O_7, CO_3, and KCl demonstrated that the presence of each of these in the test water can affect baseline activity curves. 2×10^{-5}M PO_4 tended to shift the baseline curve to the left (anodic), however this shift was not enough to affect the formation of a distinct copper peak. The presence of 2×10^{-5}M P_2O_7 did not seem to affect the shape of position of the baseline curve, however the oxidation potentials for copper was displaced negative at the lower concentrations of copper, and approached normal values with increasing additions of copper. This phenomenon was observed in river water also. The presence of 4×10^{-4}M CO_3 did not seem to affect oxidation potentials in any manner, however 2×10^{-2}M CO_3 resulted in severe increases in baseline activity to completely mask any copper spikes to 0.300 mg/1. This may have been the result of pH affects associated with the high concentrations of CO_3. KCl tended to shift copper oxidation peaks to the

left (-0.124 V) and increase baseline values to the right of the copper peak; distinct copper peaks were evident, however.

Determinations of Reducible Copper

For most analytical techniques, the general procedure for determining an unknown involves either the generation of a standard curve, made by adding known concentrations of the compound to be assayed to an uncontaminated blank media, or by the method of standard additions. For assays in which compounds in the sample may mask or inhibit instrument response, the method of standard additions is preferred. For DPASV analysis, there appears to be problems in both of these approaches. The effects of increased electrolyte (KCl) on instrument response has been documented. The fact that downstream samples tended to show reduced current values may not be related to complexation at all, but may simply reflect the increased conductivity, or presence of salts. This especially is evident for station 6. Consequently, comparing natural water samples to standards generated in deionized water may lead to inaccurate determinations.

The alternative is to rely upon standard additions to the sample containing an unknown concentration. This method, known as standard additions, or internal standards, can be used provided the instrument's response to successive additions is linear over a range of concentrations which include the unknown. For Green River water samples, linearity was not observed in the range bracketing the river water concentrations.

Complexing Capacity Determinations

The concept of a single complexing capacity for a receiving water is appealing from a regulatory standpoint. For DPASV measurements, this involves complexometric titrations, as were carried out in Green River water. The endpoint of the titration, or the point on the curve where the slope changes sharply, may be determined without any regard to the relative instrument response between samples, or for that matter, the slope of the titration curve. In order for an accurate determination of the endpoint, several requirements must be met regarding the chemistry of the complexes under study, and the manner in which the titration is carried out.

The first of these is that complexation must occur rapidly. For copper, the reaction kinetics are not considered a problem, considering the 10 minute purge times before analysis. It seems likely that most of the complexation will have occurred in this period. Slow reactions are of little interest from the standpoint of protection of the fish anyway, since fish residing in the vicinity of the outfall would be exposed shortly after discharge anyway. In the case where slow reactions do

exist at significant concentrations, the endpoint of the titration would be moved to the left, resulting in a conservative estimate of complexing capacity.

The second requirement would be that the reactions for nonelectrochemically labile forms proceed stoichiometrically. Fulfillment of this requirement in a complex natural water is not as easily met. It has been suggested by Schwarzenbach and Flaschka (1969) that NH_3 and CN^- can displace the water molecules in the hydration sheath surrounding the copper ion. Consequently, the complexation reaction may take place in several stages. The result is that at any particular point in the titration curve, several different intermediate complexes may be present in large concentrations. The endpoint is not easily recognized under these circumstances, and may be drawn out over a large concentration. Furthermore, an excess of the copper must be present to saturate all binding sites. Also, for fulvic acids in natural waters, it is most likely that all copper binding sites are not identical in any particular sample. Gamble et al. (1978) have demonstrated that copper will bind to the stronger fulvic acid sites first, followed by the weaker sites; initial K values were as much as 10-40 times higher than the average K values. This shifting of K values and complexing by stages could account for lack of linearity observed in the titration curves, and would result in a complexing curve lacking a distinct endpoint.

The third requirement, that the complex under question must be stable, has already been discussed in terms of dissociation during the analysis. Since the term labile, or more precisely electrochemically labile, is defined operationally, it would be more appropriate to require that a complex must be labile or nonlabile; either the copper is reduced entirely or is not reduced entirely for any particular species. This requirement is probably met for many of the less stable ligands. Data collected on EDTA and citrate, however, suggest that very stable complexing agents will undergo partial dissociation during the analysis. Saar and Weber (1982) reported that data which is uncorrected for species dissociation may underestimate complexation by 22%. In another paper, Bhat et al. (1981) successfully used equations proposed by Shuman and Cromer (1979) in combination with a computer program which compensates for instrument response to kinetic dissociation to determine conditional formation constants for fulvic-copper complexes. The result of dissociation is again, a more conservative estimates of the complexing capacity.

The fourth requirement is that for the titration procedure to clearly show an endpoint, copper spikes must be continued to be carried out beyond the reaction endpoint. For organic ligands present in trace amounts, this procedure is feasible and realistic. For naturally occurring inorganic ligands, which may be present at several orders of magnitude greater than the copper concentrations, this may be analytically impossible due to instrument limitations. Saturation of binding for

CO_3, for example, is extremely variable depending upon pH and the presence of competing ions.

The fifth requirement for correct endpoint determination is that the instrument exhibit a directly proportional response to the uncomplexed metal, so that when complexation sites are saturated, the binding curve is linear. For ionic copper in deionized water this requirement is fulfilled.

Even if the endpoint was easily seen on Green River water samples, its biological significance is not evident. Shuman and Cromer (1979) reported that the tendency for copper-organic complexes to dissociate during deposition can have significant effects on accurate determinations of endpoints. The magnitude of conditional formation constants of naturally-occurring fulvic and humic acids (Florence and Batley 1980; Shuman and Woodward 1977) most likely results in considerable dissociation of copper complexes of these humid materials during deposition, resulting in very conservative complexing capacity values generated from water samples containing these ligands.

Nonetheless, investigators have reported linear titration curves with distinct enpoints in natural water (Srna et al. 1980; Shuman and Woodward 1977; Chau et al. 1977; Florence and Batley 1977). Srna et al. (1980) concludes that the use of DPASV results in a good relative indicator of the ability of natural water to bind copper, but not an absolute indicator. It is likely that dissociation of copper complexes and the lack of distinct endpoints may contribute significantly to errors in estimation of complexing capacities.

From the standpoint of the regulatory agency, the concept of a receiving water complexing capacity is appealing. The considerable time and expense involved with conducting bioassays could be greatly reduced with the availability of such techniques. There is some potential for DPASV to fill this gap. However, the development of a standard procedure for such a regulatory "number" for all waters may result in nothing more than a relative measure, and be no more useful than attempting to predict long term impact from acute toxicity tests, albeit with considerably less cost. In the development of such a procedure, the following considerations should be made:

1. The selection of an appropriate buffer. Consideration should be given to its copper complexation characteristics.
2. The generation of standardized instrument set-up procedures, including deposition potential, and deposition time. In addition, newer instruments allow the operator to adjust scanning rates, pulse height, and pulse duration. These factors all can influence instrument sensitivity.
3. The careful calibration of the complexing capacities with biological response tests. Such a biological complexing capacity test should be conducted with a laboratory cultured stock of test animals. The particular test animal should be one which can be tested

in large sample sizes, at small expense, and be continuously available. Possible test organisms include Daphnia sp. (Andrew et al. 1977), algae (Sunda and Lewis 1978; Guy and Rosskean 1980) or bacterial species (Davey et al. 1973; Gillespie and Vaccaro 1978).

Conclusions

1. The use of DPASV for making determinations of reducible or 'labile' copper in natural waters is limited due to the affects of naturally occurring agents which may affect instrument responses or linearity.

2. The use of DPASV to establish a relative complexing capacity index for natural waters is possible, but the biological significance of these determinations are not clear. The development of these values may provide a simple relative indicator of the complexing characteristics of natural water.

3. The recommendation of substituting DPASV analysis for bioassays studies conducted with the receiving water in question can not be made at this time.

4. It is recommended that further studies be conducted with a reference organism in water of widely variable complexing characteristics to develop a biological complexing capacity value.

SUMMARY

1. A need for inexpensive approaches for the determination of biologically available forms of copper has been established.

2. The use of differential pulse anodic stripping has been proposed as an inexpensive, simple approach for determination of biologically available forms of copper.

3. The objective of this study was to investigate the DPASV measurement and its application to investigation of copper speciation in a river environment. Areas and investigation included:

   a. Analytical measurements of copper in the presence of naturally-occurring ligands in river water.
   b. Analytical measurements of copper in the presence of specific copper ligands to determine their effect on instrument response.

4. Investigation on the use of DPASV measurements to determine reducible copper concentrations in natural waters demonstrated:

a. Naturally-occurring ligands or other compounds may affect linearity of instrument response to copper spikes, with no distinct endpoint observed.
b. Naturally-occurring ligands or other components may directly affect peak current values other than by complexation with copper.

5. Investigations on the effects of specific organic and inorganic ligands on instrument response demonstrated:

   a. Each single substance tested affected the copper current peak, either by displacing the redox potential to the negative, or by affecting the instrument baseline curve.
   b. DPASV measurement of copper in the presence of these ligands always resulted in an underestimation of the amount of complexed copper. This was most likely due to dissociation of the complex during analysis.

6. Conclusions on the use of DPASV to measure complexation in natural waters were:

   a. The use of DPASV for making reducible copper determinations in natural waters is limited, due to the affects of naturally occurring agents which may affect instrument response or linearity.
   b. The use of DPASV to establish a relative complexing capacity index for natural waters may be possible, however the biological significance of these determinations are not clear.
   c. The recommendation of substituting DPASV analysis for bioassay studies conducted with the receiving waters in question cannot be made at this time.
   d. Further studies should be conducted with a reference organism in water of widely variable complexing characteristics to develop a biological complexing capacity value.

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APPENDIX

Water Quality and Metal Data for the Green River

Table A.1. Water quality data for the Green River.

		pH	Conductivity (mhos)	TNFR[1] (mg/l)	TOC[2] (mg/l)
April 19 -	Station 1	7.7,7.5	32,29	2.2,2.9	1.1,1.2
	2	7.6,7.7	39,38	1.0.0.6	1.3,1.5
	3	7.9,7.9	41,41	2.7,1.4	1.4
	4	7.5,7.5	50,50	8.3,2.4	1.6,1.4
	5	7.5,7.5	79,79	4.9,5.6	6.7,5.4
	6	7.4,7.5	9500,9700	4.9,11.1	1.9,2.1
June 13 -	Station 2	7.2,7.2	72,70	0.0,0.5	1.2,1.2
	3	7.3,7.2	72,78	2.8,3.1	1.7,2.3
	4	7.4,7.6	98,94	4.2,5.3	3.0,3.3
	5	7.4,7.4	420,450	4.9,5.7	2.5,2.9
	6	7.6,7.5	24,000, 24,000	23.5,23.6	2.0,2.5
August 2 -	Station 2	7.5,7.6	115,95	0.5,1.1	1.4,1.4
	3	7.4,7.6	140,140	1.9,2.9	1.9,1.6
	4	7.6,7.5	119,123	4.2,4.2	1.9,2.1
	5	7.6,7.6	220,200	9.1,8.6	3.2,3.3
	6	7.8,7.9	18,000, 16,000	8.6,6.4	3.1,2.9

[1]Total nonfilterable residue.

[2]Total organic carbon.

Table A.2. Total metals data for the Green River.

		Zn (mg/l)	Cd (mg/l)	Pb (mg/l)	Cu (mg/l)
April 19 - Station	1	<.010,<0.010	0.0005,0.0006	<0.002,<0.002	0.018,0.017
	2	<0.010,<0.010	<0.0002,<0.0002	<0.002,<0.002	0.006,0.006
	3	<0.010,<0.010	<0.0002,<0.0002	<0.002,<0.002	<0.005,<0.005
	4	<0.010,<0.010	<0.0002,<0.0002	<0.002,<0.002	<0.005,<0.005
	5	0.015,0.014	<0.0002,<0.0002	<0.002,<0.002	0.006,0.007
	6	0.025,0.026	<0.0002,<0.0002	<0.007,<0.002	<0.005,<0.005
June 13 - Station	1				
	2	<0.020,<0.020	<0.0005,<0.0005	<0.002,<0.002	<0.005,<0.005
	3	<0.020,<0.020	<0.0005,<0.0005	<0.002,<0.002	<0.005,<0.005
	4	<0.020,<0.020	<0.0005,<0.0005	<0.002,<0.002	<0.010,0.005
	5	<0.020,<0.020	<0.0005,<0.0005	<0.002,<0.002	<0.005,<0.005
	6	0.020,0.020	<0.0005,<0.0005	<0.002,<0.002	0.025,0.032
August 2 - Station	1				
	2	<0.010,<0.010	<0.005,<0.005	<0.002,0.003	<0.005,<0.005
	3	<0.010,0.011	<0.005,<0.005	<0.002,<0.002	<0.005,<0.005
	4	0.011,0.021	<0.005,<0.005	<0.002,<0.002	<0.005,0.006
	5	0.021,0.021	<0.005,<0.005	<0.002,<0.002	<0.005,<0.005
	6	0.024,0.020	<0.005,<0.005	<0.002,<0.002	0.006,0.010

Table A.3. Reducible metals data for the Green River.

		Zn (mg/l)	Cd (mg/l)	Pb (mg/l)	Cu (mg/l)
April 19 -	Station 1	0.028,0.017	<0.001,<0.001	<0.001,0.002	0.004,0.003
	2	0.006,0.008	<0.001,<0.001	0.001,<0.001	<0.001,<0.001
	3	0.005,0.004	<0.001,<0.001	<0.001,<0.001	<0.001,<0.001
	4	0.012,0.008	<0.001,0.0017	<0.001,0.003	<0.001,0.001
	5	0.013,0.016	<0.001,0.001	<0.001,0.005	0.002,<0.001
	6	0.013,0.015	<0.001,<0.001	<0.003,0.003	<0.001,<0.001
June 13 -	Station 2	0.004	<0.001	<0.001	<0.002
	3	<0.001	<0.001	<0.001	<0.002
	4	<0.001	<0.001	<0.001	<0.002
	5	<0.001,<0.001	<0.001,<0.001	<0.001,<0.001	<0.002,<0.002
	6	<0.001,<0.001	<0.001,<0.001	<0.001,<0.001	<0.002,<0.002
August 2 -	Station 2	0.008,0.004	<0.001,<0.001	<0.001,<0.001	<0.005,<0.005
	3	0.002,0.009	<0.001,<0.001	<0.001,<0.001	<0.005,<0.005
	4	0.003,0.010	<0.001,<0.001	<0.001,<0.001	<0.005,<0.005
	5	0.007,0.007	<0.001,<0.001	<0.001,<0.001	<0.005,<0.005
	6	0.012,0.010	0.001,0.001	<0.001,<0.001	<0.005,<0.005