Preservation and Determination of Trace Metal Partitioning in River Water by a Two-Column Ion Exchange Method

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A one-step preconcentration method using two columns in series was used to partition trace metals in natural waters into several operationally defined fractions. The water passed through a Chelex-100 cation exchange column to collect dissolved labile trace metals and then through an AG MP-1 macroporous resin column to collect trace metals complexed with natural organic matter. A third fraction (inert) was obtained from the difference between total dissolved trace metal concentrations, determined after UV irradiation of a separate aliquot, and the sum of the fractions retained on the two columns. This analytical scheme was successfully applied to the determination of Al, Cd, Co, Cu, Fe, Mn, Ni, Pb, V, and Zn in river waters collected from across the state of Texas. Trace metal partitioning between fractions was found to be stable for at least 8 days after the addition of ammonium acetate buffer (pH 5.5). In most samples collected from relatively pristine sections of Texas rivers, the sum of fractions retained on the two columns accounted for better than 80% of the total dissolved trace metal concentrations. In rivers where anthropogenic inputs were more evident (San Antonio and Trinity Rivers), the inert fraction became pronounced, in some cases accounting for up to 95% of the total dissolved concentrations. The preconcentrate is free of matrix interference and can be easily analyzed by atomic absorption spectrometer or inductively coupled plasma mass spectrometer. Using a 1-L sample allows preconcentration factors of 150 or higher for both labile and organic fractions, and therefore, trace metal concentrations and speciation in natural waters at ambient levels can be accurately and precisely determined.

Reliable measurements of dissolved trace metal concentrations are needed when the behavior of trace metals in rivers are studied. However, obtaining such data in unpolluted river systems is very difficult due to both problems of sample contamination during collection, handling, and analysis[1–3] and problems with detection limits of analytical instruments. Concern has been expressed[2,4,5] that much of the trace metal data for world rivers published prior to the 1980s may be incorrect. In the past 20 years, improvements in instrumentation and the introduction of clean procedures, which minimize contamination from sampling and sample treatments, have allowed more accurate measurement of trace metal concentrations in natural waters. However, attention to detail and specialized methods are still necessary to get reliable data and these methods have not been used in most routine monitoring efforts conducted or sponsored by environmental protection agencies.

Trace metals can exist in a number of physicochemical forms or species in natural waters. These include free aquatic ionic forms, dissolved inorganic or organic complexes, complexes with colloidal or particulate matter (inorganic or organic), and complexes associated with the biota. The speciation can have a major influence on the transport, behavior, bioavailability, and toxicity of a trace metal. It has been suggested that the manner in which chemicals partition among the dissolved, particulate, and colloidal phases affects both their chemical and physical behavior in aquatic environments.[2,6] Thus, all three phases need to be considered in aquatic environment studies.

The free ionic form of a number of trace elements (e.g., Cd, Cu, Pb, Zn) has been found to be considerably more toxic to aquatic biota than complexed, colloidal, or particulate forms.[6] However, for elements such as Hg and Sn, the methylated forms are more toxic than the ionic forms.[7] In any case, bioavailability and toxicity of a trace metal is dependent on the chemical and physical form of the metal, which is affected by water chemistry (e.g., pH, temperature, salinity)[8,9] in addition to other factors. In freshwater systems, organic matter plays an important role, through complex formation, in controlling the fraction of trace metals that is bioavailable.[10–15] This is especially true for Cu, Fe,

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Ni, Pb, and Al and, to a lesser extent, Cd and Mn. Some studies have suggested that labile metal fractions are the most toxic,[16,17] where labile fractions were determined using anodic stripping voltammetric (ASV) techniques. At the same time, several studies have related bioavailability or toxicity of trace metals to fractions retained on different ion exchange resins.[10–12,14,18,19] For example, Figura and McDuffie[20,21] determined lability of metals using voltammetric and ion exchange (Chelex-100) techniques and operationally defined metal speciation into very labile, moderately labile, slowly labile, and inert species. The labile fractions of trace metals determined by ion exchange methods are generally greater than those obtained by voltammetric techniques owing to the longer contact times in ion exchange preconcentration.[22]

For ion exchange, Chelex-100 is the most commonly used resin as cationic exchanger. The use of Chelex-100 has been well characterized by many previous studies, for example Pai et al.,[23] Pai,[24] and Figura and McDuffie.[20,21] At the same time, several resins have been used to isolate humic materials or to separate metal complexes from waters. These include C18,[25,26] cellulose with immobilized chelating groups,[10,19] Amberlite XAD resins,[10,14,19,26–28] and anion macroporous resin AG MP-1.[29] XAD resins are best known for their ability to isolate humic substances from waters.[30] XAD 7 resins have been used to preconcentrate trace metals from natural waters,[27,28] but their recoveries showed no advantages when compared with Chelex-100 preconcentration. A combination of Chelex-100 and AG MP-1[29] appeared to be able to separate labile (Chelex) and organically bound (AG MP-1) metal fractions. However, as used by Liu and Ingle,[29] the technique did not have detection limits low enough to determine trace metals in unpolluted waters and only three metals were studied.

This present work, which was modified after that of Liu and Ingle,[29] used an analytical scheme based on a weakly acidic (cation) ion exchange column coupled with a strong basic (anion) ion exchange column. These two columns retain different physicochemical forms of soluble trace metals. Soil humic acid (purchased commercially) was used to simulate organic complex formation between natural organic matter and trace metals in water because humic substances contribute to ∼50% of the dissolved organic carbon in natural waters and are known to form complexes with trace metals[10–12,31]

The main goal of this study was to develop an ion exchange procedure for trace metal speciation that is inexpensive, repeatable, and applicable to many elements at ambient levels and that can be applied to large-scale sampling programs when immediate sample processing is not possible. To do this, fractionation of trace metal species was tested in laboratory model solutions and the stability of the fractionated speciation was examined in field samples. A great advantage of this proposed technique is that the speciation information can be obtained and each fraction can be concentrated simultaneously prior to instrumental analyses. A third fraction was obtained by preconcentrating and measuring total dissolved metal concentration from a separate aliquot. This is an important improvement compared to the work by Liu and Ingle,[29] in which the third fraction was estimated from analyzing the effluent after the sample had passed through the columns. To determine trace metal concentrations at ambient levels, a large-volume field sample was used (500–1000 mL) in this study, giving a concentration factor of up to 150. This improved detection limit allowed more trace elements to be analyzed than would have been possible by direct analyses. The method was used to determine the speciation of Al, Cd, Co, Cu, Fe, Mn, Ni, Pb, V, and Zn in Texas rivers.

EXPERIMENTAL SECTION

Materials. All plastic tubing and bottles for sampling, storage, and processing were acid-cleaned (nitric and hydrochloric acids, Instra-analyzed grade, J.T. Baker). All reagents (HNO_3, NH_4OH, CH_3COOH) used for sample preservation and processing were either purchased as doubly quartz distilled grade (Seastar or J.T. Baker) or were purified, if necessary, by means of ion exchange[23] in our laboratory. Distilled water was sub-boiling quartz distilled.

Model Solutions. Experiments were carried out in the laboratory using commercially isolated humic acid (HA, Aldrich Chemical) in distilled water spiked with trace metals under various conditions in order to investigate the efficiency of the two-column design. Model solutions of metal concentrations 0–20 μg/L, HA concentrations 0–40 mg/L, and equilibrium pH 4–9 were allowed to equilibrate (18–48 h) before the pH was adjusted to 5.5 ± 0.3 with ammonium acetate buffer solution (1 M). Thereafter, the fractions of trace metals were separated by the two-column technique.

Columns and Preconcentration. Aliquots of 2.0 g of Chelex-100 (100–200 mesh) and 0.5–2.0 g of AG MP-1 (100–200 mesh), both weighed out directly from original packing, were separately slurry packed into Poly-Prep columns (Bio-Rad) in 2 N HNO_3. Resins were converted to NH_4^+ and OH^- forms, respectively, by washing each column with 10 mL of 1 M NH_4OH solution. After being washed with distilled water, the two columns were connected with the Chelex-100 column on top. Each sample was then connected to a column set through a 50-cm section of silicone tubing, with the sample bottle being set ∼30 cm higher than the top of the column set, flow was initiated by gravity, and flow rates

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were controlled at 2−3 mL/min by two-way stopcocks. After each sample passed through the column sets, the columns were disconnected and washed with distilled water (2 × 5 mL). Major ions (Na, Ca, Mg, K) were separated from target elements by washing the columns with 5 × 4 mL of 1 M ammonium acetate at pH 5.5. After washing again with distilled water (2 × 5 mL), trace metals were eluted by 7 mL of 2 N HNO_3. This procedure resulted in preconcentration factors of up to 150 with a 1000-mL sample and has the advantage of one-step preconcentration yielding two fractions of trace metals, in a medium free from matrix interference.

Bed volumes of the columns were 1−3.5 mL for Chelex-100, varying throughout the sample passing process, and 0.9−3.6 mL for AG MP1 (0.5−2.0-g packing amounts), giving contact times of 5−20 s at flow rates of 2−3 mL/min. The species separated by the described ion exchange method are operationally defined as labile (Chelex-100), organic (AG MP-1), and inert (with respect to Chelex-100 and AG MP1 resins). The labile metal fractions we obtained would be comparable to the sum of "very labile" and "moderately labile" fractions described by Figura and McDuffie.[21]

Field Sampling. A "clean" sampling and analysis procedure, as outlined in U.S. EPA method 1669 and other reports[1,3] was closely followed in this study. Field samples were taken from Texas rivers during June 1997 and August 1998 on trips lasting 1−3 days. At each sampling location, a 1000-mL (polyethylene) sample and a 500-mL (Teflon) sample, each filtered on-site through an acid-cleaned Gelman 0.45-μm polyethersulfone capsule filter with 600-cm^2 filtration area, were collected. Extra samples, as well as field blanks (laboratory distilled water), were taken at selected locations to serve as quality control aliquots and for storage effect studies. Field blanks provided verification of the cleanness of the sampling system, and replicate samples were utilized as duplicates and spike aliquots. Extra replicates were taken in order to conduct storage experiments.

Field Sample Storage Experiments. On two occasions, field replicates were collected from Texas rivers with differing geochemical characteristics, and tests were run to examine effects of sample storage and pretreatments on the distribution of trace metal species. The first batch of field replicates (June 1997 from the Brazos and Colorado Rivers) received two different pretreatments 2−3 h after sample collection. For each sampling location, a set of six replicates was buffered at pH 5.5 by the addition of 30 mL of ammonium acetate (1 M NH_4OAc) into 1000 mL of the samples and the other set of six replicates was acidified to pH <2 with the addition of HNO_3. Acidified samples were buffered to pH 5.5 just prior to the preconcentration procedure. One sample from each set of replicates was preconcentrated on days 0, 1, 2, 3, 5, and 7 with the first set being processed just 3 h after collection. Another set of storage experiments was carried out in August 1997 when replicate samples were collected from the Sabine and Neches Rivers. Three replicates from each river were buffered to pH 5.5 within 4 h after collection, and three others were stored without any treatment until just prior to the preconcentration procedure, at which time they were buffered to pH 5.5. Samples were preconcentrated on different days (up to 8) after collection and compared to test for storage effects on trace metal speciation.

Sample Treatments for Texas River Sampling. This two-column technique for trace metal speciation was applied to 141

river water samples from eight major Texas rivers (Sabine, Neches, Trinity, Brazos, Colorado, Guadalupe, San Antonio, Nueces) during 1997 and 1998. Filtered field samples in 1000-mL polyethylene bottles were buffered on the day of sampling with 30 mL of 1 M ammonium acetate buffer solution at pH 5.5. For total dissolved trace metal concentrations, samples in the 500-mL Teflon bottles were acidified with 1 mL of concentrated HNO_3, followed by UV irradiation (200 W for 24 h[32]) to decompose organic matter. Before preconcentration, ∼1.5 mL of 21% ammonium hydroxide solution was added to the UV-irradiated samples along with 30 mL of 1 M ammonium acetate buffer solution and the pH was adjusted to pH 5.5 ± 0.3 with HNO_3 or NH_4OH.

Analyses. The preconcentration procedure allowed a large suite of metals to be determined by inductively coupled plasma mass spectrometry (ICPMS, Hewett-Packered 4500). However, some metals still had to be determined by graphite furnace atomic absorption spectrometry (GFAAS, Perkin-Elmer 3030 with Zeeman correction) due to sensitivity (e.g., Cd) or mass interference (e.g., Fe) problems. Samples were also analyzed without preconcentration with an axial viewed inductively coupled plasma atomic emission spectrometer (ICP-AES, Perkin-Elmer OPTIMA 3300DV) in order to verify the recoveries (when possible) and the capacity of the column settings. Quality assurance information, such as method detection limits (derived from 3 times standard deviation of six replicate field samples) and a summary of analysis of a reference material (river water SLRS-3 from NRCC) are listed in Table 1. A set of field blank results (from one sampling trip on which three different sets of sampling materials, including tubings and filters, were used) is listed in Table 1 to illustrate the levels of cleanness of the entire operational system. The three different field blanks were obtained after laboratory distilled water was brought to the field, pumped through tubings and capsule filters that are used for sampling, and treated as field samples. Therefore, field blank data included all possible contamination from material used, cleaning procedures, added reagents, preconcentration, and analysis. The blank values were generally 10% or less of typical river concentrations for all elements.

The fractions obtained from the two columns are operationally defined as the labile (Chelex retained) and the organic (AG MP-1 retained) fractions whereas total dissolved trace metals were determined on separate UV-irradiated samples. The difference between total dissolved and the sum of the labile plus organic fractions (a fraction not retained by either Chelex or AG MP-1 at pH 5.5) is an operationally defined dissolved inert fraction with respect to Chelex-100 and AG MP-1 columns.

RESULTS AND DISCUSSION

Column Recoveries. Regardless of the partitioning of trace metals, metals mixed with HA could be recovered almost entirely by the two-column system (Table 2). However, the amount of AG MP-1 required depended on the amount of HA added. This was revealed by a series of experiments in which resin weight and the amount of HA added were varied, and the effluents were analyzed in order to obtain a mass balance of spiked metal. In summary, a positive relationship was found between the amount of HA and the weight of AG MP-1 needed to recover metal−HA

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Table 1. Method Detection Limits (MDL) for This Study and Recoveries for a River Water Reference Material (SLRS-3)[a]

	Al	Cd	Co	Cu	Fe	Mn	Ni	Pb	V	Zn
MDL	0.2	1	0.01	0.06	0.3	0.1	0.1	10	0.5	0.1
SLRS-3 recovery ($N = 18$)										
certified	31	13	0.027	1.35	100	3.9	0.83	68	0.3	1.04
measured (av)	30.8	12.8	0.027	1.35	98.2	3.8	0.78	70	0.30	1.14
standard deviation	2.4	0.9	7.8	0.09	4.2	0.2	0.06	9	0.01	0.12
recovery (%)	99	99	98	100	98	97	94	103	102	110
field blank										
A-labile	0.08	0.3	0.000	0.000	0.04	0.01	0.01	2.7	0.00	0.02
A-organic	0.10	0.1	0.000	0.004	0.01	0.00	0.00	1.0	0.01	0.01
B-labile	0.08	0.3	0.000	0.000	0.02	0.01	0.00	3.0	0.00	0.03
B-organic	0.17	0.1	0.000	0.016	0.01	0.00	0.00	1.2	0.01	0.03
C-labile	0.10	0.1	0.000	0.000	0.03	0.00	0.00	2.6	0.00	0.01
C-organic	0.14	0.2	0.000	0.016	0.02	0.00	0.00	1.1	0.01	0.01

[a] Concentrations are ng/L for Cd and Pb and μg/L for other elements.

Table 2. Results of Cu Recoveries[a] of Two-Column Method under Different Experimental Conditions

HA (mg)	resin wt (g)	[Cu], % labile	org-1	org-2	effluent	total recovery
2	2/1.0	54	41	1	nd[b]	96
5	2/1.0	38	55	3	nd	96
10	2/1.0	26	73	2	nd	101
10	2/1.5	24	74	3	nd	101
20	2/0.5	11	70	5	12	98
20	2/1.0	18	68	3	13	102
20	2/1.5	12	86	4	nd	102
40	2/1.5	10	81	3	5	99
40	2/2.0	11	83	4	nd	98

[a] Resin weight lists weights for Chelex/AG MP-1; Cu labile/Chelex retained fraction; Cu Org-1/AG MP-1 fraction eluted by 2 N HNO₃; Cu Org-2/AG MP-1 fraction eluted by mixing stripping solution; and Cu effluent/Cu fraction not retained by either column. [b] nd, not detected.

Table 3. Recoveries of Trace Metals Retained by Chelex and AG MP-1 Columns under Various Conditions[a]

	Cd	Cu	Ni	Pb	Zn	V	Co
average (%)	96.7	97.1	93.7	103.2	103.3	91.9	94.3
standard deviation	2.9	5.8	8.3	5.7	5.3	5.5	5.6

[a] Experimental conditions covered: metal concentrations, $2-20$ μg/L; HA concentrations, $2-40$ mg/L; pH, $1-9$; total sample number, 29.

complexes. It was found that 1 g of AG MP-1 resin, of original moisture content, had enough capacity to retain metal−HA complexes in a solution containing 10 mg of HA, and 1.5 and 2 g of AG MP-1 resin were needed to retain metal−HA complexes with up to 20 and 40 mg of HA, respectively.

In this study, only one stripping solution (2 N HNO₃) was used to elute metals from both columns. This is in contrast to an earlier report[29] that different solutions were required to elute different metals. We found that 2 N HNO₃ would elute most (details below) of the metal off the AG MP-1 column without washing much organic matter into the final preconcentrate, which was helpful for analysis by either ICPMS or GFAAS. In a test, a mixture of ammonium nitrate/EDTA/ammonium solution was used to strip metal−HA complexes off AG MP-1 resin,[29] following the leaching by HNO₃. It was concluded that unless a very large amount of HA (>20 mg in a model solution) was retained on AG MP-1 column, 7 mL of 2 N HNO₃ was sufficient to elute more than 90% of the metals retained, as illustrated by the Cu data shown in Table 2. Only Cu data are shown for illustration because it is the most difficult metal to recover due to its strong organic complexation. Table 3 shows average recoveries (seven metals) from laboratory samples (metal/HA solutions) for the sum of the two columns after eluted using HNO₃ only.

Metal Speciation in Model Solutions. Results shown in Tables 2 and 3 indicate that the proposed two-column preconcentration setup was able to differentiate metals in labile and organically bound fractions. Before conducting experiments to investigate effects of different conditions on the distribution of metal species, precision was estimated to be ~5% or better for each metal fraction by analyzing three replicate model solutions of the same conditions (pH, metal, and HA concentrations). Variation of the labile and organic fractions retained by the two columns can be seen under different experimental conditions. Figure 1 clearly shows that the labile metal fractions decreased with increasing HA concentrations. Thus, the organic metal fractions positively correlated with HA concentration. Since humic substances are present in most natural aquatic environments, and they make up ~50% of dissolved organic carbon (DOC) in surface waters, it becomes evident that the degree of organic complexation strongly depends on DOC concentration and can be estimated by ion exchange techniques. Comparing the extent of organic complexation at the same metal concentration and HA content, the organic fractions in aqueous phase for different metals are in the following order: Cu > Pb > Ni > Co = Zn = Cd.

Based on the two-column preconcentration technique, Cd, Co, and Zn had insignificant percentages of organic fractions when HA concentrations were <40 mg/L. This was found to be true as equilibrium pH ranged from 4 to 8. However, equilibrium pH had some effects on the distribution of labile and organic fractions of Cu, Ni, and Pb, as shown in Figure 2. Analytically, an effect of pH on the distribution of metal species exists, indicating that the addition of buffer solution did not produce "pH 5.5 trace metal speciation". Here, higher equilibrium pH enhanced organic

Table 4. Summary Statistics for Replicate Samples (*N* = 6) Processed on Different Days[a].

	total dissolved	Colorado River, buffered			Colorado River, acidified			total dissolved	Brazos River, buffered			Brazos River, acidified		
		labile	organic	sum	labile	organic	sum		labile	organic	sum	labile	organic	sum
Al av	1.44	0.36	0.55	0.90	0.57	0.38	0.95	1.65	0.41	0.51	0.92	0.71	0.32	1.03
std dev		0.07	0.17	0.24	0.13	0.13	0.25		0.06	0.09	0.14	0.20	0.08	0.27
Cd av	4.89	1.64	0.38	2.02	2.12	0.10	2.22	7.04	3.73	0.06	3.79	4.31	0.02	4.33
std dev		0.43	0.53	0.61	0.86	0.07	0.91		1.38	0.09	1.35	0.97	0.04	0.93
Co av	0.048	0.031	0.013	0.044	0.034	0.007	0.041	0.084	0.043	0.021	0.063	0.056	0.012	0.067
std dev		0.003	0.003	0.003	0.003	0.000	0.003		0.008	0.001	0.007	0.009	0.001	0.009
Cu av	0.66	0.31	0.38	0.69	0.40	0.27	0.67	1.10	0.51	0.55	1.06	0.60	0.37	0.97
std dev		0.02	0.03	0.03	0.02	0.01	0.03		0.08	0.05	0.12	0.06	0.01	0.06
Fe av	2.93	1.36	1.04	2.40	1.99	0.56	2.54	1.75	0.34	0.42	0.76	0.61	0.23	0.84
std dev		0.10	0.08	0.12	0.20	0.05	0.21		0.05	0.08	0.09	0.12	0.08	0.19
Mn av	0.79	0.81	0.00	0.81	0.75	0.00	0.75	0.31	0.30	0.00	0.31	0.31	0.00	0.31
std dev		0.04	0.00	0.04	0.05	0.00	0.05		0.04	0.00	0.04	0.01	0.00	0.01
Ni av	0.79	0.60	0.22	0.81	0.73	0.19	0.92	1.28	0.90	0.33	1.23	1.23	0.14	1.37
std dev		0.03	0.02	0.03	0.03	0.20	0.22		0.03	0.01	0.04	0.11	0.01	0.11
Pb av	35.19	26.17	1.61	27.77	25.54	0.62	26.16	25.32	11.43	2.29	13.71	12.19	1.28	13.48
std dev		4.21	0.80	4.36	7.51	0.24	7.74		3.86	0.67	4.41	1.97	0.61	1.84
V av	3.33	2.98	0.25	3.23	2.38	0.70	3.08	5.29	4.88	0.37	5.25	4.21	1.19	5.40
std dev		0.24	0.01	0.23	0.19	0.10	0.15		0.21	0.02	0.20	0.47	0.22	0.37
Zn av	0.54	0.48	0.07	0.55	0.45	0.01	0.46	0.38	0.29	0.08	0.37	0.28	0.02	0.31
std dev		0.05	0.02	0.06	0.01	0.00	0.01		0.06	0.01	0.07	0.03	0.00	0.03

[a] Concentrations are ng/L for Cd and Pb and μg/L for the other elements.

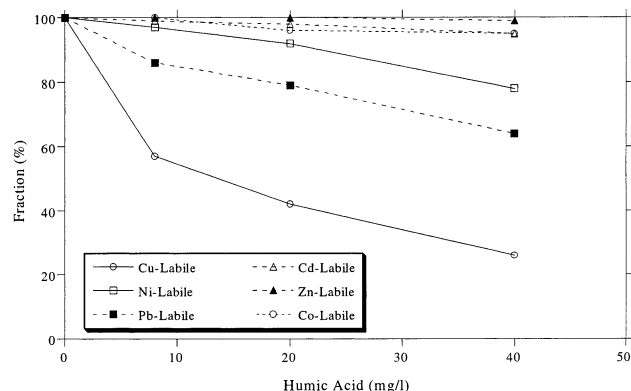**Figure 1.** Changes in trace metal (Cd, Co, Cu, Ni, Pb, and Zn, 20 μg/L each) fractions with various HA concentrations in the model solution. Shown in the graph are the labile fractions only, with the remaining percentages being the organic fractions. The illustration clearly demonstrates that the labile metal fractions decrease with increasing HA concentrations; consequently, the organic fractions have a positive relationship with HA concentrations.

Figure 2. Changes in the labile and organic Cu, Ni, and Pb fractions at different equilibrium pH. Higher equilibrium pH promotes organic complexation and thus results in increasing organic fractions with increasing pH.

complexations such that, between pH 4 and 8, the differences in organic fractions were ~10% for Cu and Ni and ~20% for Pb.

Storage of Field Samples. The effects of storage on metal speciation must be known if large-scale sampling is to be conducted. Table 4 shows the variability in trace metal concentrations in the labile and organic fractions over 7 days for the June 1997 samples after buffering or acidification. We found that the distribution between the labile and organic fractions of most metals did not change for at least 7 days if samples were buffered at pH 5.5 soon after collection. Concentration changes found were due mostly to method variability. Furthermore, the elements that did appear to change (Al, Cd, Fe, Pb) were at concentrations very close to the method detection limits. Acidification to pH <2 caused some increases in the labile fractions for all elements measured, regardless of the length of storage, but did not decompose organic complexes completely. For example, after 7 days at pH 2, Al, Co,
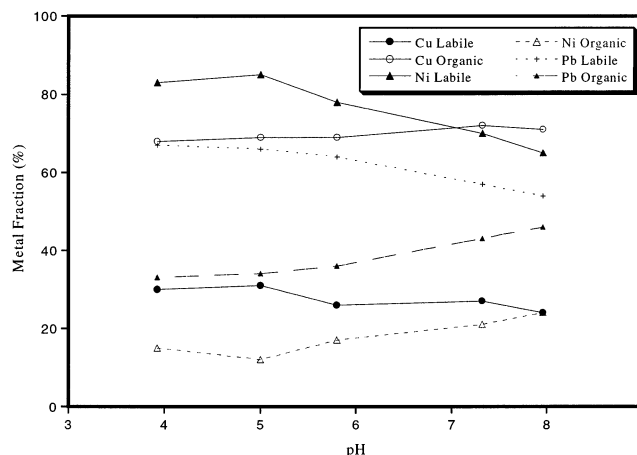
Cu, Fe, Ni, and V organic fractions that had been 20−60% of the total had decreased to 10−30%.

Another set (August 1997) of experiments was carried out in which buffered and raw (both filtered) samples were preconcentrated on different days after sample collection to further examine changes in the distribution of trace metals due to storage. Figure 3 shows trace metal concentrations for buffered samples and for samples untreated until preconcentration, over a storage time of up to 8 days. For samples that were buffered after collection, results of variations in trace metal speciation confirmed the findings of our June 1997 experiments: there was no concentration change and speciation shift. Pb was the only exception, in which variability in both total dissolved concentration and speciation had no obvious trend. However, when buffered and untreated aliquots were compared, large differences in the distribution between labile and organic fractions were found.
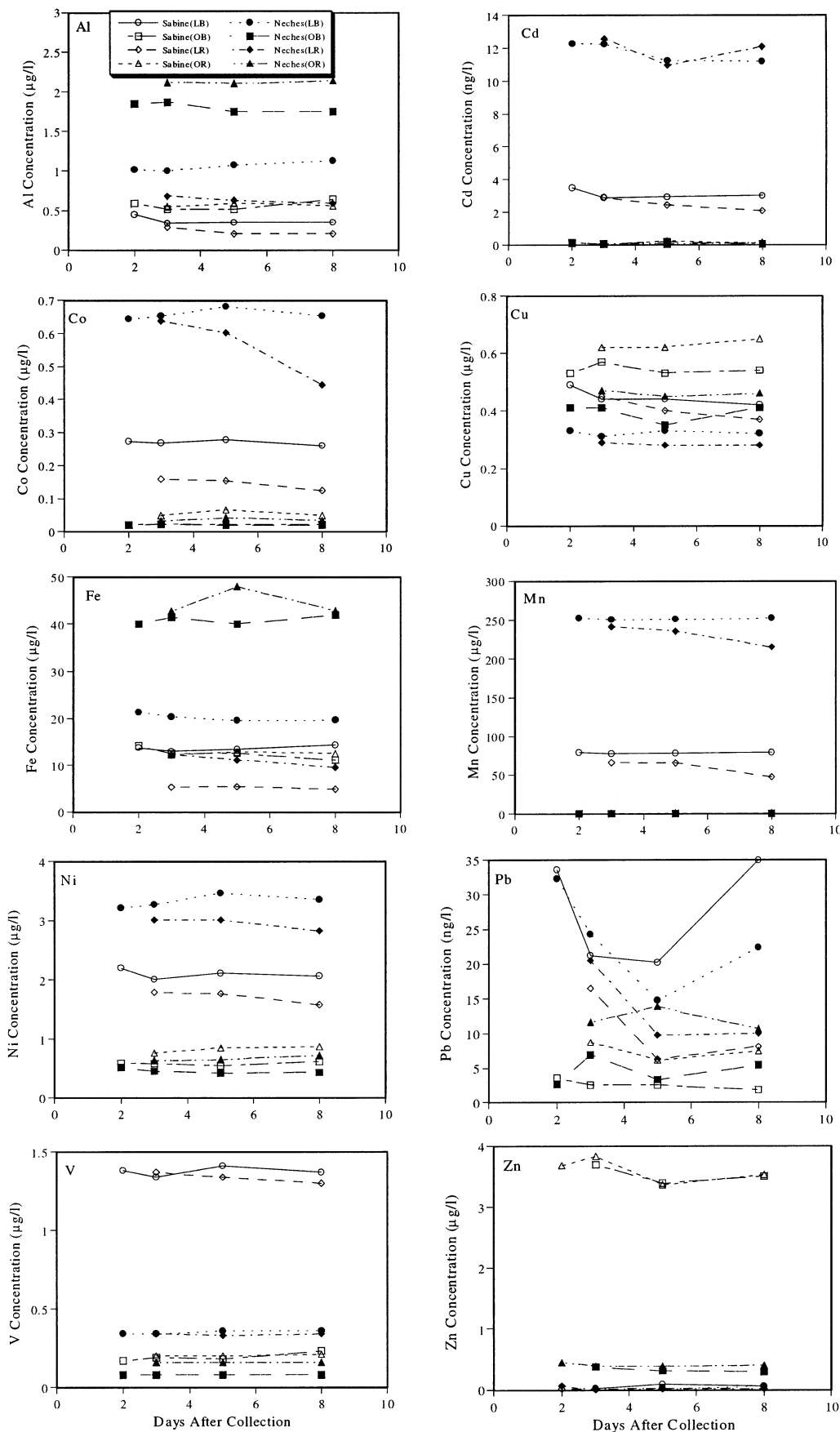
Figure 3. Variability of trace metal distribution in replicate samples from different Texas rivers. Samples were either buffered on the day of collection (buffered) or stored until the day of preconcentration (raw). LB, labile fraction buffered; OB, organic fraction, buffered; LR, labile fraction, raw; OR, organic fraction, raw. Raw samples were buffered just prior to preconcentration. Results show that buffered replicates provided stability of metal partitioning for at least 8 days.
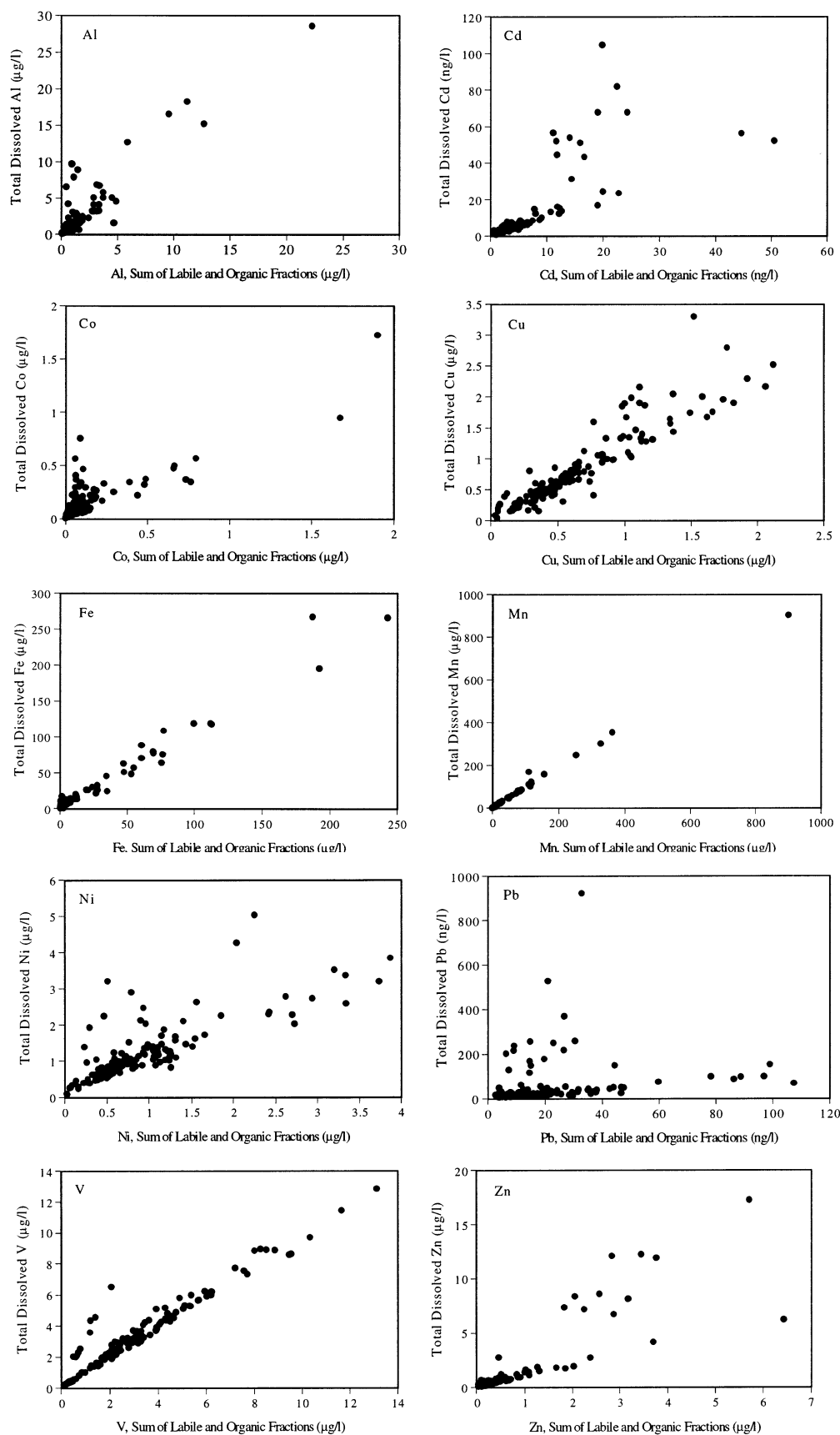
Figure 4. Comparison of total (UV-irradiated) trace metal concentration and the sum of the labile plus organic fractions obtained from the two-column preconcentration procedure ($N = 141$). Data with excess total dissolved concentrations are samples from certain geographic areas (mostly San Antonio and mid-Trinity Rivers). In less human-influenced rivers, the sum of the labile plus organic fractions accounted for most of the total dissolved metals (80−100%).

Table 5. Summary Statistics of Distribution of Trace Metals (Average Percentage of Each Fraction and Concentration Ranges) in Eight Texas River Waters Collected during 1997−1998 (pH 5.8−8.5; DOC 0.8−11.7 mg/L; Salinity 0−4)[a]

		labile	organic	inert
Al	av (std dev), %	37 (23)	27 (17)	36 (28)
	concn range	nd−7.2	nd−15.1	nd−28.4
Cd	av (std dev), %	75 (22)	2 (3)	23 (22)
	concn range	1−50	nd−12	1−85
Co	av (std dev), %	50 (28)	13 (12)	37 (28)
	concn range	nd−1.88	nd−0.09	nd−0.66
Cu	av (std dev), %	38 (10)	43 (14)	20 (18)
	concn range	nd−1.44	nd−1.07	nd−1.77
Fe	av (std dev), %	28 (16)	32 (19)	40 (28)
	concn range	nd−66.9	nd−19.5	nd−79.2
Mn	av (std dev), %	95 (8)	1 (1)	4 (8)
	concn range	0.2−901.8	nd−0.3	nd−57.8
Ni	av (std dev), %	53 (19)	21 (11)	26 (22)
	concn range	nd−3.4	nd−1.6	nd−2.8
Pb	av (std dev), %	49 (28)	17 (13)	35 (33)
	concn range	nd−105	nd−60	nd−890
V	av (std dev), %	79 (18)	11 (11)	10 (17)
	concn range	nd−10.7	nd−3.4	nd−4.4
Zn	av (std dev), %	63 (24)	7 (6)	31 (25)
	concn range	nd−6.4	nd−1.6	nd−11.5

[a] Concentrations are ng/L for Cd and Pb, and μg/L for other elements. [b] nd, not detected.

On the basis of changes in distribution and concentration with time of storage of our August 1997 experiments, the 10 trace metals determined in this study can be classified into three groups. (1) No change: Cd and V. The sum of labile plus organic fractions did not differ between buffered and raw samples. Changes in the labile and organic metal concentrations over time were within method variability. (2) Partitioning shifts: Al, Cu, Ni, Pb, and Zn. These metals had a constant sum of labile plus organic fractions but the labile fractions became smaller in untreated samples and in general continued to decrease over time. (3) Net loss: Co, Fe, and Mn. These elements showed large decreases when the samples were not preserved by the addition of buffer solution. Most of the losses occurred from the labile fractions, while the organic fractions had small increases or no change. It is believed that these decreases were due to loss to the container walls. Although flocculation that causes neither resin to retain some fractions is possible, it is unlikely in this case because only a small number of metals are involved.

Field duplicate samples were taken at selective locations as quality control samples. The magnitudes of variations between duplicate samples were similar to those observed in our storage experiments, in which all field duplicate samples had variations of <10%, with all samples subject to two-column preconcentration being processed within 8 days from sample collection. This further confirmed the stability of metal speciation by our preservation method and the precision of the preconcentration procedure.

Application of the Two-Column Method to Natural Waters. We applied the method described above to samples from eight of the major rivers in the state of Texas. Figure 4 shows a comparison between total dissolved metal concentrations (from UV-irradiated samples) and the sum of labile plus organic metal fractions (from field-buffered samples). As the figures show, more than 90% of the samples lie close to 1:1 line with the sum of the
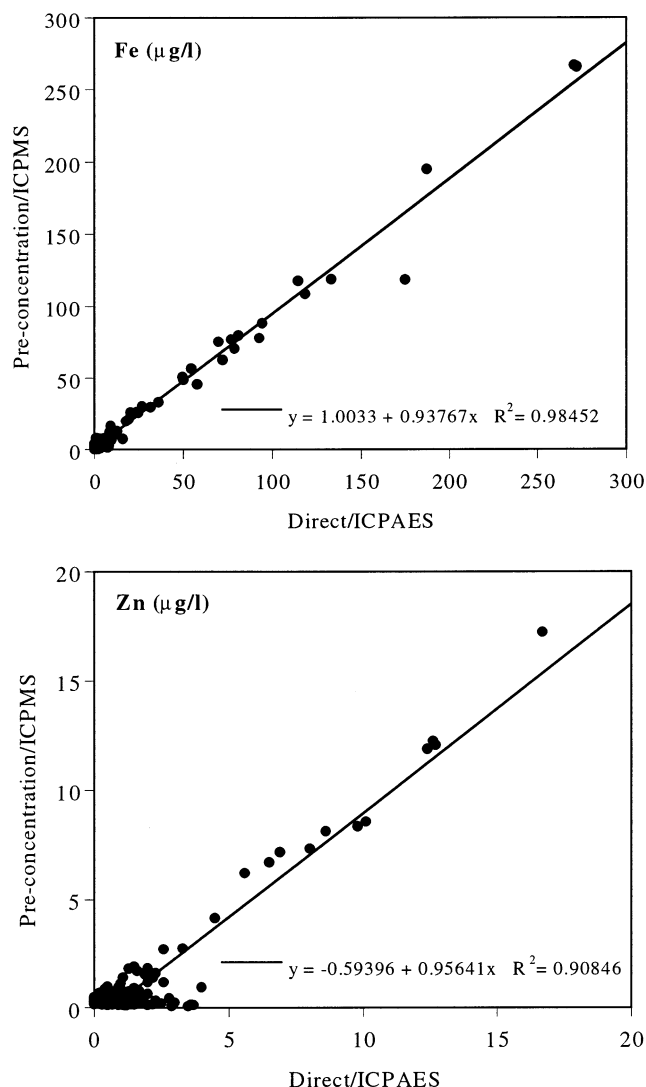

$$y = 1.0033 + 0.93767x \quad R^2 = 0.98452$$

$$y = -0.59396 + 0.95641x \quad R^2 = 0.90846$$

Figure 5. Comparison of results of metal concentrations obtained from independent analytical methods (ICPMS after two-column preconcentration and ICP-AES direct measurements) to ensure the capacity and accuracy of the preconcentraiton methods used.

labile and organic fractions accounting for 80−100% of the total dissolved trace metal concentrations. However, in a few samples, the sum of the labile and organic fractions was much less than the total dissolved value. These outliers are samples collected from the San Antonio River and the mid-Trinity River (below the Dallas−Ft. Worth metropolis), two areas where anthropogenic influences are likely. The columns gave low recoveries for Cd, Co, Cu, Ni, Pb, and Zn in these samples.

The low recoveries of elements referred to above suggests that an inert dissolved fraction is present. As seen in Figure 4, sometimes this inert fraction accounted for as much as 95% of the total dissolved concentrations (e.g., Co and Pb) and the sum of labile plus organic fractions accounted for less than 50% for all six metals in these samples. We suspect that EDTA or other ligands that form strong complexes with trace metals probably caused the observed elevated inert fraction. Preliminary study on the total EDTA concentrations in Texas river waters sampled with the same trace metal speciation study, using a modified method described by Bergers and de Groot[33] on a Shimadzu HPLC with

a SPD-M10AV diode array detector, showed that average EDTA concentrations in Texas river waters were 50 nM in the Trinity River, 200 nM in the San Antonio River, and ~10 nM or lower for the other rivers. The retained organic fraction was probably mostly metal complexes with natural organic ligands (e.g., humic materials). In our samples, the organic fraction accounted for the majority of dissolved Cu (Table 5), whose strong complexes with natural organic matter (humic material) is well known, while Cd, Mn, and V had very small natural organic fractions (<10%). Organic fractions for Al, Co, Fe, Ni, Pb, and Zn ranged from 10 to 60%. The labile fraction accounted for almost all the dissolved Mn in river waters in Texas. The inert fraction was important for Al, Cd, Co, Cu, Fe, Ni, Pb, and Zn only in waters collected at locations known to be influenced by human activities.

Comparison between Independent Methods. Most of the trace metal concentrations were determined by more than one instrumentation techniques (AAS, ICPMS, ICP-AES), with or without pretreatments. In ICP-AES determinations, samples were acidified as required for the EPA total dissolved metal definition and analyzed without preconcentration. The Figure 5 data for Fe and Zn, two elements with higher concentrations in river waters, show the column design used in this study gives good recovery of these metals. The good agreement suggests that, with the operational settings used in this study, the two-column procedure using 2 g of Chelex-100 and 1 g of AG MP-1 resin is able to cover a wide range of metal concentrations in natural waters. The lower parts of these figures should not be compared directly because the differences in detection limits between those two methods are generally 100-fold or higher.

CONCLUSIONS

The one-step two-column preconcentration technique developed for this work is suitable for determining the partitioning of dissolved trace metals in natural water at background concentrations. Furthermore, we found that adding ammonium acetate buffer (pH 5.5) can preserve the trace metal distribution in filtered river waters for at least 8 days. Preservation by acidification, as described in U.S. EPA methods 1600 series for total dissolved trace metal analysis, will cause the distribution to shift toward labile fractions and, thus, is not suitable for speciation studies. Samples without any treatment or preservation undergo speciation change over a time period of a few days and can lose mass over time.

In this study, we obtained both total dissolved metal concentrations and the distribution between operationally defined labile, organic, and inert fractions in Texas river waters. The organic fractions are most likely metal complexes with natural organic matter, while the inert fractions are probably metal complexes with anthropogenic ligands that form very strong metal complexes. The metal partitioning found in this study is similar to that found in some earlier work on the trace metal speciation, even though a different analytical scheme was applied.[20,21,34] Agreement between the ICP-AES direct analysis and the AAS/ICPMS method with preconcentration for high concentrations of total dissolved metals indicates that the preconcentration technique applied here works well over a large concentration range. Furthermore, the preconcentration factors possible with the method described here result in much lower detection limits and thus more reliable data at low concentrations. This is important for better understanding trace metal geochemistry at ambient levels and improving knowledge of processes that control trace metal speciation in natural waters.

(33) Bergers, P. J. M.; de Groot, A. C. *Water Res.* **1994**, *28*, 639−642.
(34) Batley, G. E.; Florence, T. M. *Mar. Chem.* **1976**, *4*, 347−363.