

# Techniques for determination of trace metals in small samples of size-fractionated particulate matter: phytoplankton metals off central California

Jay T. Cullen<sup>1</sup>, Robert M. Sherrell<sup>\*</sup>

*Institute of Marine and Coastal Sciences, Rutgers University, 71 Dudley Road, New Brunswick, NJ 08901-8521, USA*

Received 5 May 1999; accepted 2 July 1999

---

## Abstract

The oceanic biogeochemical cycles of many trace elements are dominated by their association with the growth, death, consumption and sinking of phytoplankton. The trace element content of marine phytoplankton reflects nutritional status, species composition, surface area to volume ratios, and interactions with bioactive and toxic elements in the ambient seawater. Despite the ecological and environmental importance of trace element assimilation by autotrophs, there are few modern measurements of trace elements in phytoplankton assemblages from the natural environment. Here we introduce a new method for collection and analysis of size-fractionated particulate samples from practical seawater volumes. We pay particular attention to accurate determination of trace element filter blanks which are typically the limiting factor for analysis of such samples. Metals were determined at very low detection limits by high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) for 11 elements (Ag, Al, Cd, Co, Cr, Cu, Fe, Mn, U, Zn and P, which is used as a biomass normalizer) in three types of polymer filters (0.45, 5.0, and 53  $\mu\text{m}$  pore size) and a quartz fiber filter (0.8  $\mu\text{m}$  pore size). To place these new determinations in a practical context, results are presented for a vertical profile of samples filtered from 1–4 l of coastal seawater (0.3–1.0 mg total solid dry weight) at a station off central California. The results demonstrate that the blanks of the evaluated filter types, precleaned appropriately, are sufficiently low to allow accurate determination of the trace metal content of three size-classes of phytoplankton. At the Pacific station, measured phytoplankton Zn content (as Zn/P) agrees with values predicted from single-species culture studies growing at seawater Zn concentrations expected for coastal waters. The new method has utility as a generally applicable and simple size fractionation technique, and allows determination of natural and pollutant elements in small samples of phytoplankton and particles in coastal, estuarine and offshore marine regimes. © 1999 Elsevier Science B.V. All rights reserved.

*Keywords:* trace metals; phytoplankton; filtration; size-fractionation

---

## 1. Introduction

The vertical and horizontal distributions of many trace elements in the ocean are determined by association with the cycle of growth, sinking and reminer-

---

<sup>\*</sup> Corresponding author. Tel.: +1-732-932-6555 ext. 252; Fax: +1-732-932-8578; E-mail: sherrell@imcs.rutgers.edu

<sup>1</sup> E-mail: cullen@imcs.rutgers.edu.

alization of marine phytoplankton (Collier and Edmond, 1984; Morel and Hudson, 1984; Donat and Bruland, 1995). The importance of these organisms to trace metal biogeochemical cycles lies in their ability to incorporate trace elements in tissues and inorganic hardparts at concentrations of  $\sim 10^{-5}$ – $10^{-6}$  g (g dry weight) $^{-1}$  from seawater concentrations of  $10^{-7}$ – $10^{-9}$  g (g seawater) $^{-1}$ . Phytoplankton represent important vectors for trace elements in the marine ecosystem, both by sinking of living and detrital organic material and through the transfer of elements to higher trophic levels (Reinfelder and Fisher, 1994). Modeling efforts to gain quantitative understanding of the trophic transfer of trace elements in polluted coastal waters, or the biogeochemical cycling of trace elements in oceanic environments, require empirical data quantifying the assimilation of trace elements by phytoplankton.

Knowledge of the trace element composition of phytoplankton as it relates to marine biogeochemical cycles and pollutant transport has resulted largely from work carried out in trace element-buffered laboratory culture studies (Brand, 1992; Brand et al., 1983; Morel and Hudson, 1984; Sunda, 1991; Sunda and Huntsman, 1998). Although this work has been enormously useful in understanding aspects of the fundamental controls on phytoplankton metal uptake, laboratory studies are generally carried out with a single species of phytoplankton under unrealistically controlled conditions. Extrapolation of results based on laboratory cultures to the complex physical and chemical environment of oceanic surface waters is largely untested. High quality measurements of the trace element content of natural phytoplankton assemblages are necessary to critically evaluate cellular metal concentrations and elemental flux estimates based on results of laboratory culture work. The trace element composition of marine phytoplankton is difficult to determine for a number of practical reasons. The concentrations of phytoplankton in oceanic surface waters can be exceedingly low (Karl and Lukas, 1996), requiring processing of large volumes or, alternatively, the analytical challenge of working with small sample sizes. Techniques relying on sensitive analytical methods may be stymied by filter blanks, since phytoplankton cannot in general be separated from the filter media. In addition, the possible contamination of phytoplankton samples by

terrigenous particles and authigenic oxides with associated non-biogenic trace elements can make isolating the biogenic metal component a difficult task. Finally, trace element contamination of the sample from ship, investigator, sampling apparatus, and reagents used during processing and analyses can compromise attempts to determine natural phytoplankton trace element quotas.

Another difficulty in determining trace element quotas in phytoplankton is that biomass must be determined, and the most robust measure of biomass, organic carbon, may not be determined on the same samples as are trace elements, because filters with low trace element blanks are generally composed of hydrocarbon polymers. Although it may be possible to estimate biomass from chlorophyll *a* (chl*a*) extracted from plastic filters, chl*a*:C ratios can vary by more than an order of magnitude depending on phytoplankton species, growth rate, light availability, and nutrient status (Banse, 1977; Geider et al., 1998). If plastic filters are required to achieve low metal blanks, metal:biomass must be determined by normalization to another major biochemical element such as P, with C:P either assumed equal to the Redfield value, or determined independently. Accordingly, one of the goals of this paper is to determine whether quartz fiber filters, which have much lower metal blanks than typical glass fiber filters (e.g., Whatman GF/F, the standard filter used for particulate organic carbon (POC) determination; Bishop et al., 1985; Buesseler et al., 1995), can be used for direct determination of metal:C ratios at practical particle loadings. A disadvantage of quartz filters is that they are available in only one nominal pore size and therefore can be used only to determine “less than” size fractionation information when used in combination with various pore size prefilters.

These challenges are partly to blame for the paucity of recent determinations of phytoplankton metal content for any marine environment. The few published high quality analyses of trace elements in coastal and open ocean phytoplankton were completed 15–25 years ago (Martin and Knauer, 1973; Martin et al., 1976; Bruland et al., 1978; Collier and Edmond, 1984). Most of these studies relied on net tows (44–76  $\mu\text{m}$  nylon mesh size) to concentrate phytoplankton from large volumes of surface water. These large size-fraction samples likely include zoo-

plankton and undersample many classes of phytoplankton, including some classes of diatoms, that contribute substantially to vertical fluxes in the ocean. More recently, investigators have employed continuous shipboard centrifugation (Schübler and Krelling, 1993; Helmers, 1996) and large volume in situ pumping (Sherrell, 1991; Sherrell and Boyle, 1992; Sherrell et al., 1998; Buesseler et al., 1995; Bishop, 1999), to concentrate particulate material from oceanic surface waters. While both of these methodologies can return large (5–100 mg) trace element-clean particulate samples, the time and equipment required can limit their utility for studies requiring high spatial and/or temporal sampling resolution. Importantly, there are presently no reports of size-fractionated oceanic particulate trace metals using any technique.

Here we describe a procedure for rapid collection of trace element-clean, size-fractionated phytoplankton and particle samples at sea, followed by elemental analysis using plasma source mass spectrometry. We report the first comprehensive and accurate determinations of blank values for 11 elements of importance to phytoplankton physiology and toxicology in four different filter types. Filter blanks for trace elements are compared to field samples from the California coastal upwelling region, and to expected natural concentrations from other oceanic regimes. This information is used to establish the filter loading necessary to achieve acceptable signal/blank ratios across a range of marine environments. The elements determined in this study include those that act as micronutrients (P, Fe, Mn, Cu, Zn), that may be toxic to phytoplankton at sufficiently high concentrations (Cu, Zn, Cr, Cd, Ag), that have no known biological function (U), and that are primarily associated with terrigenous particles or authigenic oxides (Al, Fe, Mn) and can therefore be used to estimate the contribution of non-biological particles to the overall particulate metal composition. Note that several metals fall into more than one classification. Required nutrient metals can become toxic at higher concentrations, and some important nutrient metals are also found in high concentrations in terrigenous particles or oxides (e.g., Fe), phases which may overwhelm the biogenic fraction of these metals, even at low concentrations of non-biogenic particles.

We demonstrate the novelty of the new method by presenting the first trace element data for size-fractionated oceanic particles, collected at a station off the central California coast. We evaluate the consistency of the data, the contribution of filter blanks to overall measurement uncertainty, and the magnitude of determined particulate Zn:biomass relative to values derived from culture studies. The success of this application indicates that the method can be applied to determine phytoplankton metals over a wide range of polluted and pristine marine environments. The filter blanks reported here serve as a guide for investigators interested in metal concentrations in any kind of natural particles, and the in-line size-fractionating filtration procedure could be used by investigators interested in other aspects of phytoplankton or particle composition. The small sample sizes and efficient methods for processing and analysis of samples make this method appropriate for large temporal/spatial studies in which many samples must be analyzed.

## 2. Methods and materials

### 2.1. Reagents

Trace metal clean 32 mol l<sup>-1</sup> hydrofluoric acid (HF; Baseline, SeaStar Chemical) and 16 mol l<sup>-1</sup> nitric acid (HNO<sub>3</sub>; Baseline, SeaStar Chemical) were used for all digestion procedures. Deionized distilled water (ddH<sub>2</sub>O) was prepared from house deionized water with finishing deionizing columns and glass distillation. The primary standards were obtained from High-Purity Standards (Charleston, SC) and were used to make working standard mixtures in ultrapure 10% HNO<sub>3</sub> (v/v). Preparation of standards and samples in 10% HNO<sub>3</sub> was found to reduce sample introduction problems and control instrument blank during sample analysis by high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS).

### 2.2. Acid cleaning of materials

All materials and apparatus used for sampling were of polypropylene, polyethylene, or polystyrene and were leached in 1 mol l<sup>-1</sup> HCl (reagent grade)

at 60°C for at least 24 h prior to use. Teflon digestion vials (15 ml screw-cap; Savillex, Minnetonka, MN) were cleaned by boiling in aqua regia for 4 h, rinsing with ddH<sub>2</sub>O, and sealed refluxing of 1 ml of 16 mol l<sup>-1</sup> (reagent grade) HNO<sub>3</sub> on a hotplate set at 210°C for an additional 4 h. Following acid leaches or boiling steps all materials were rinsed thoroughly (five times) with ddH<sub>2</sub>O and left to dry in a Class 100 laminar flow bench.

### 2.3. Filter selection criteria

We opted to develop a single in-line filter stack approach whereby the volume filtered would be limited by the most easily clogged filter of the set (Fig. 1). Filter materials were selected to maximize flow rate and volume through the stack before clogging, with seawater passing sequentially through either a stack of three 47-mm filters (53 μm Pecap polyester, then 5 μm Poretics polycarbonate, then 0.45 μm Supor polysulfone-ester; Fig. 1a) or two 47-mm filters (53 μm polyester, then 0.8 μm nominal quartz microfiber; Fig. 1b), each stack held in a polypropylene in-line filter holder (Micro Filtration Systems). Having sequential filters in an in-line holder rather than performing independent filtrations through each filter vastly simplifies field operations in which many such samples must be collected while minimizing handling and potential contamination of individual filters. A potential complication with this approach is that by orienting filters back to back in the in-line holder particles could adhere to the overlying filter upon disassembly of the stack, thus over-estimating concentrations on the larger size-fraction and under-estimating those in the smaller size class. While coarse-grid separators between each filter type could help limit particle transfer between size classes, particle loss on the separators could lead to error in particulate metal concentrations.

### 2.4. Filter blanks

The types of filters analyzed for trace metal content are summarized in Table 1. Polyester filters were cut under Class 100 clean conditions from 1 × 10 m rolls obtained from the manufacturer (Tetko, Briarcliff Manor, NY) using an acid-cleaned 47 mm diameter acrylic template, acrylic cutting

board and a stainless steel scalpel. Other filter types were pre-cut by the manufacturer. Batches of 100 filters were precleaned in 1-l HDPE bottles filled with 1 mol l<sup>-1</sup> HCl (BDH Reagent Grade), sealed in two layers of plastic, and heated in an oven at 60°C for 24 h. Using more concentrated acids to preclean filters was either no more effective at reducing trace element blanks (e.g., 6 mol l<sup>-1</sup> HCl) or damaged the structure of filters (1 mol l<sup>-1</sup> HNO<sub>3</sub>; J.T. Cullen, unpublished data). Filters were then rinsed five times with ddH<sub>2</sub>O and dried individually in acid-cleaned Petri slides (Millipore) in a class 100 clean bench. For blank determinations, filters were cut using the above method and one half (50 ± 4% by mass) of each blank filter was adhered to the wall of a sealed 15 ml screwcap Teflon vial, as is routine for samples. Comparisons of half and whole filter blanks indicate that cutting was not a significant source of contamination (J.T. Cullen, unpublished data). The half-filters were digested by refluxing in a mixture of 950 μl of 16 mol l<sup>-1</sup> HNO<sub>3</sub> and 50 μl 32 mol l<sup>-1</sup> HF on a hotplate at 120°C for 4 h. This procedure leaves filters partially digested but has been demonstrated previously to effect a complete digestion of suspended marine particulate matter (Sherrell, 1991; Sherrell and Boyle, 1992). After cooling, remaining filter fragments were removed from vials to the extent possible, using acid-cleaned Tefzel forceps. Digests were then evaporated just to dryness on a hotplate at 40°C under glass chambers that were continually flushed with filtered air (0.2 mm Gelman Supor-100 cartridge), then were redissolved in 1 ml 0.1 mol l<sup>-1</sup> HNO<sub>3</sub>.

### 2.5. Size-fractionated particle sampling

Particulate samples were collected from California coastal surface water at 36°36.52'N, 122°00.34'W (near Monterey Bay) by hydrocast from the R/V *Pt. Sur* on April 29, 1997. The in-line filter stacks (Fig. 1) were used in concert with a rosette holding 12 10-l Niskin bottles (General Oceanics, Miami, FL) to collect suspended particulate material. Prior to leaving port, the neoprene O-rings in each sampling bottle were replaced with blue fluorosilicone, internal springs were replaced with new C-Flex tubing (Cole-Parmer) and bottles were leached for 24 h with 0.1 mol l<sup>-1</sup> reagent grade HCl, then rinsed with

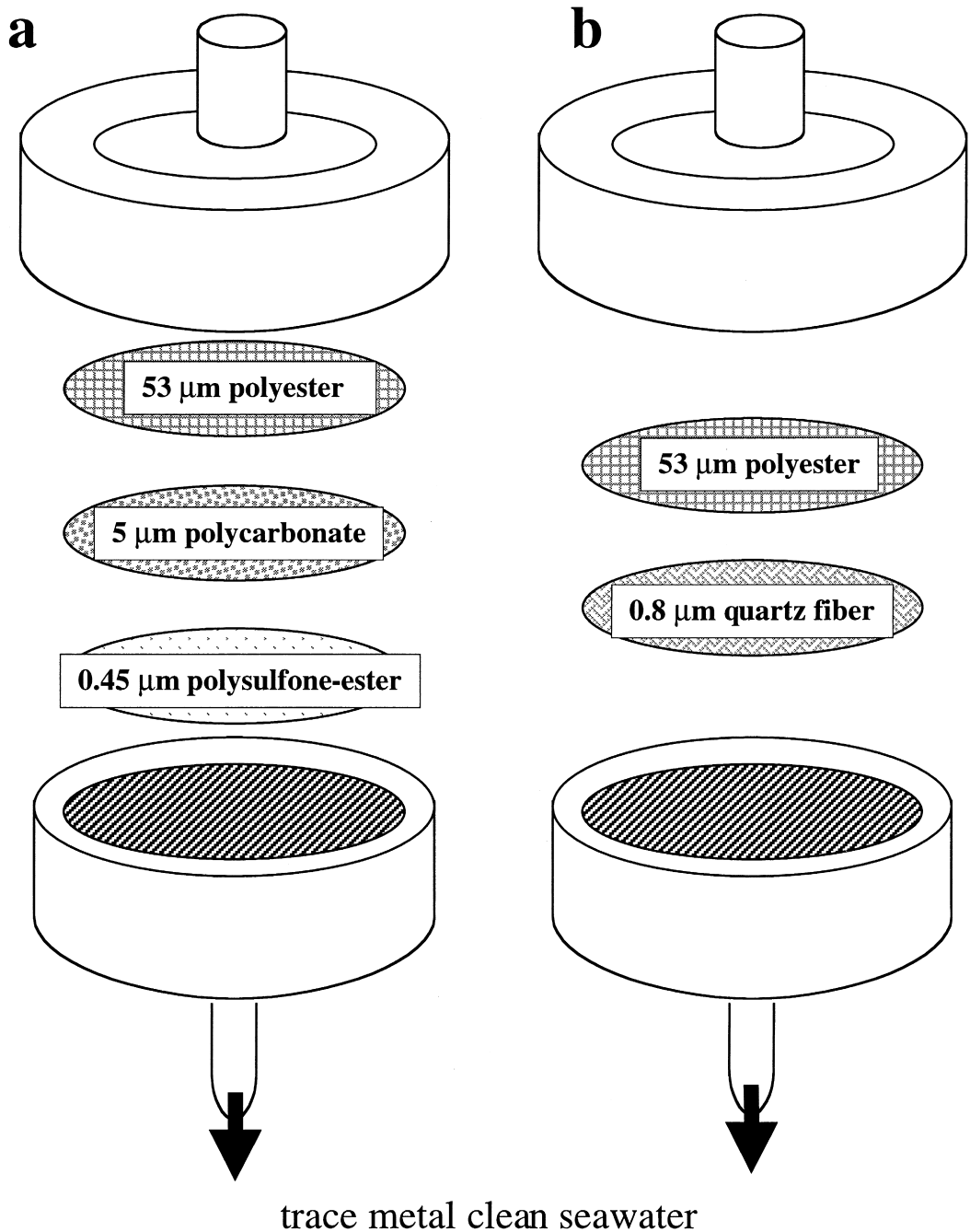


Fig. 1. Schematic of polypropylene in-line filter holders (Micro Filtration Systems) with (a) stack of three hydrocarbon filters (53  $\mu\text{m}$  polyester, 5  $\mu\text{m}$  polycarbonate, and 0.45  $\mu\text{m}$  polysulfone-ester), and (b) stack of two filters (53  $\mu\text{m}$  polyester and 0.8  $\mu\text{m}$  quartz fiber) used in the study to obtain trace metal clean, size-fractionated marine particles.

ddH<sub>2</sub>O and offshore (10 km) surface seawater before use. Water samples were collected during rosette

retrieval by slowing the hydrowire speed to 5 m min<sup>-1</sup> and tripping sample bottles on the upcast to

minimize the potential for contamination from sacrificial Zn anodes and instrument payload at the rosette's base. Upon return to deck after a hydrocast, the Niskin bottles were overpressured through their vent caps with 0.2  $\mu\text{m}$  filtered air ( $< 100$  mm Hg, endcaps retained by internal stretched tubing) fed through trace metal clean polyethylene tubing fitted to nylon compression fittings. Seawater from each Niskin flowed by way of C-Flex tubing (Cole-Parmer) through either a stack of three 47-mm (Fig. 1a) or two 47-mm filters (Fig. 1b), each stack held in the polypropylene in-line filter holder. Subsamples of the filtrate from the hydrocarbon filter stack were retained for dissolved trace metal analysis. Contamination from ship and/or investigator was minimized because the entire sample stream was closed to the atmosphere. Potential loss of particles to container walls was minimized by avoiding multiple transfers before filtration (Bishop, 1999). After filtration, in-line holders were processed in a class 100 clean bench. Gentle suction was applied to the base of filter holders to remove residual seawater from the

holder headspace while the air drawn through the filter was kept to a minimum; filters were not rinsed to avoid loss of labile elements (Collier and Edmond, 1984). Filter stacks were removed into acid-cleaned polystyrene Petri slides, sealed in two layers of plastic zip closure bags and stored at  $-20^\circ\text{C}$  until analysis. In the laboratory on shore, filters were subsampled frozen over dry ice in a class 100 clean bench by cutting them as for blank filters. For each sample and 10 blank filters (five each from two different lots; 10 from one lot for QM-A filters), half of each filter was digested, evaporated to dryness, and redissolved as for blank filters.

## 2.6. Instrumentation and analyses

Digests of samples and filter blanks, and digest blanks (acid only), were analyzed for 11 trace elements by magnetic sector HR-ICP-MS using a novel but simple combination of internal and external standardization, described in detail elsewhere (J.T. Cullen et al., in preparation). Briefly, each sample is pre-

Table 1

Summary of digest and filter trace element blanks ( $n = 10$ ), and comparison to a 10-m sample collected off central California coast, April 1997

Element	Digest blank		Supor <sup>®</sup> -450 (0.45 $\mu\text{m}$ ) <sup>a</sup>				Poretics PC (5 $\mu\text{m}$ ) <sup>b</sup>			
	pmol/filter <sup>c</sup>	SD	Blank		Sample		Blank		Sample	
			pmol/filter	SD	pmol/filter <sup>f</sup>	%UB <sup>h</sup>	pmol/filter	SD	pmol/filter <sup>f</sup>	%UB <sup>h</sup>
Ag	0.093	0.002	0.423	0.113	1.03	32.8	0.337	0.043	0.377	34.5
Al	40.8	0.815	1300	415	25,000	2.3	500	222	130,000	0.5
Cd	0.089	0.018	1.20	0.472	68.3	2.1	0.396	0.075	12.9	1.7
Co	0.068	0.017	1.51	0.574	44.3	3.9	0.660	0.222	26.9	2.5
Cr	1.92	0.077	91.8	33.6	14.3	708	260	110	170	195
Cu	0.629	0.031	18.2	7.12	1200	1.8	14.5	7.66	260	8.8
Fe	0.179	0.072	560	241	15,000	4.8	240	115	40,000	0.9
Mn	0.910	0.055	27.5	6.41	3200	0.6	6.47	2.67	3000	0.3
P	50.0	8.233	1900	1040	170,000	1.8	440	93.5	52,000	0.5
U	0.017	0.004	0.161	0.120	3.07	11.7	0.026	0.015	1.37	3.3
Zn	1.07	0.061	122	41.7	701	17.9	24.7	8.74	396	6.6

<sup>a</sup>Supor-450 (Gelman Sciences) 47 mm 0.45  $\mu\text{m}$  polysulfone-ester filters (Lot # 9219, 3991).

<sup>b</sup>Osmonics Poretics 47 mm 5.0  $\mu\text{m}$  polycarbonate membrane filters (Lot # A154DM11B019, A154D011A008).

<sup>c</sup>Tetko PeCap<sup>®</sup> 53  $\mu\text{m}$  polyester square weave (Lot # 9504870012).

<sup>d</sup>Whatman QM-A 0.8  $\mu\text{m}$  quartz fiber filters (Lot # 711512).

<sup>e</sup> $n = 5$ , represents blank derived from digest acids and Teflon vial normalized to 1/2 filter per digest equivalent.

<sup>f</sup>Collected 04/28/97 at  $36^\circ36.520'N$ ,  $122^\circ00.339'W$  from 10 m depth, 1.25 l processed through the filter stack, corrected for digest and filter blank.

<sup>g</sup>Sample as in (f), 10.43 l processed through the QMA filter corrected for digest and filter blank.

<sup>h</sup>%UB = % blank uncertainty =  $(3 \times \text{SD of filter blank}) / (\text{sample}) \times 100$ .

pared with a 1 ppb addition of In that acts as an internal standard to which signal intensities for each element are normalized, controlling for instrument sensitivity drift during a run. A multi-element external standard curve also containing 1 ppb In is used to calculate trace element concentrations after subtraction of instrument blank. Standard additions to selected samples demonstrate that simple external standardization provides accurate results. Digest blanks are subtracted from sample and filter blanks to correct for blank derived from digestion acids and Teflon vials. Detection limits, calculated for this method as  $3 \times$  the standard deviation of a 10% HNO<sub>3</sub> solution, were less than 2 nmol l<sup>-1</sup> for all elements of interest. Overall analytical precision was  $\pm 5$ –10% based on five replicate analyses of a single sample digest solution, depending on the element and signal intensity. Analyte blank derived from the sample introduction system can be a limiting factor in the determination of low-level filter blanks. Our method incorporates copious rinsing of the instrument's inlet system and interface region (torch and cones) with a

combination of HF, HCl and HNO<sub>3</sub> between all samples and standards. Blank solutions (10% HNO<sub>3</sub>) are analyzed periodically throughout the run to verify maintenance of low and constant blank for the easily contaminated elements (Fe, Al, Zn and Cu).

Subsamples (1 cm<sup>2</sup>, 7.6% by area) of quartz fiber filters were fumed under HCl overnight and analyzed for organic C and N on a CHN-analyzer (Carlo-Erba model NA 1500 Series 2).

### 3. Results

Blank values for 11 trace elements in the four filter types investigated, as well as blanks contributed by digest acids and/or leaching from Teflon digestion vials, are summarized in Table 1. The arithmetic mean concentrations extracted from digest blanks and filters, expressed as picomole per 47 mm filter area, are reported with the calculated standard deviation ( $n = 10$ ). Trace element content of a particulate sample collected at a depth of 10 m by

PeCap <sup>®</sup> polyester (53 μm) <sup>c</sup>				Whatman QM-A (0.8 μm) <sup>d</sup>			
Blank		Sample		Blank		Sample	
pmol/filter	SD	pmol/filter <sup>f</sup>	%UB <sup>h</sup>	pmol/filter	SD	pmol/filter <sup>g</sup>	%UB <sup>h</sup>
219	3.00	1.60	562	10.1	0.871	17.1	15.3
33,000	3400	370,000	2.8	4,700,000	530,000	4,800,000	33.1
5.75	0.499	18.2	8.2	31.8	6.85	824	2.5
142	1.67	63.4	7.9	38.0	2.98	531	1.7
1500	142	570	74.8	7900	282	2300	36.7
158	6.06	970	1.9	221	25.8	3400	2.3
11,000	85.2	140,000	0.2	86,000	5204	800,000	2.0
1,700,000	77,000	160,000	144	4000	93.7	68,000	0.4
1,900,000	110,000	340,000	97	480,000	37,624	2,600,000	4.3
4.45	0.193	2.82	20.5	77.8	3.04	336	2.7
2800	48.8	982	14.9	26,000	4113	10,000	123

filtering 1.25 l of seawater through a size fractionation filter stack (0.45  $\mu\text{m}$  Supor polysulfone; 5  $\mu\text{m}$  Poretics polycarbonate; 53  $\mu\text{m}$  Pecap polyester) and 10.43 l through a 0.8  $\mu\text{m}$  nominal QM-A and 53  $\mu\text{m}$  Pecap polyester filter stack are shown for comparison to the absolute filter blanks and their standard deviation.

### 3.1. Digest blanks

After rigorous vial cleaning, the blank leached from Teflon vials and contributed by the HF and  $\text{HNO}_3$  during digestion was generally less than 10% of the filter blanks for polycarbonate membrane filters (the cleanest analyzed), but exceeded the instrumental blanks by > 10-fold in all cases. Digest blanks for all elements were equivalent to < 50 pmol filter<sup>-1</sup> and usually much lower, indicating that the main source of blank in the digestion procedure was the filter material. The blank was found to be dependent on digestion temperature, with higher concentrations extracted from the Teflon vials at higher digestion temperatures, possibly because thermal expansion allowed acid contact with contaminant metals within the vial walls (J.T. Cullen, unpublished data; Takenaka et al., 1997). Overall, digest acids and Teflon vials (after sufficient precleaning) were not an important source to the filter blanks determined in this study, but were subtracted from total filter digest concentrations.

### 3.2. Evaluation of filter blank corrections

To estimate conservatively the utility of different filter materials as media for particulate trace metal determination, we present results in a fashion (termed % uncertainty of blank or %UB) which takes into consideration the absolute metal blanks, inter-filter blank variability, and variable extent of particle loading on filters in the field. We define %UB as three times the standard deviation of the filter blank, expressed as a percent of the blank-corrected sample concentration on each filter (Table 1, where %UB is calculated for a sample from 10 m depth).

Our method of estimating the contribution of blank variability to the overall uncertainty of the determined sample concentrations may be an overestimate because it is based on the  $3\sigma$  uncertainty of

the blank. In other words, a 30% RSD for the blank results in a %UB about equal to the blank concentration as a percentage of sample concentration. This estimation is intended to allow for possible variations among lot numbers which may be encountered by other investigators, and to provide a conservative guideline as to the importance of filter blanks and their variability. The %UB information can be used by other investigators as a first order approximation of the particle loading necessary to achieve a desirable sample/blank ratio on different filter types.

#### 3.2.1. Polysulfone-ester 0.45 $\mu\text{m}$ (Gelman, Supor)

Polysulfone-ester filters exhibited low absolute blank values for most of the elements determined (Ag, Cd, Co, Cr, Cu, Mn, U and Zn < 120 pmol filter<sup>-1</sup>). Notable exceptions were Al, Fe and P (1300, 560 and 1900 pmol filter<sup>-1</sup>). This analysis demonstrates that %UB was < 5% for Al, Cd, Co, Cu, Fe, Mn and P and < 33% for Ag, U and Zn (Fig. 2a). A notable exception was Cr, with %UB of 708%. We note, however, that no significant differences were observed for metal blanks between the two lot numbers of polysulfone-ester filters used in this study.

#### 3.2.2. Polycarbonate 5 $\mu\text{m}$ membrane (Poretics)

Polycarbonate membrane filters exhibited the lowest trace element blanks among the four filter types under the digestion conditions used in this study (Tables 1 and 2). Highest blanks were observed for Al (500 pmol filter<sup>-1</sup>), Cr (260 pmol filter<sup>-1</sup>), Fe (240 pmol filter<sup>-1</sup>) and P (440 pmol filter<sup>-1</sup>). The %UB, as defined above but in this case applying to the 5–53  $\mu\text{m}$  fraction of the vertical profile at each depth, was < 10% for all elements except Ag, Cr and Cu (Fig. 2b). The high trace element blank/sample ratio for Ag on this size fraction results in part from the exceedingly low concentrations of particulate Ag found in this size fraction (0.3 pmol filter<sup>-1</sup>). As with the 0.45  $\mu\text{m}$  Supor filter, Cr was the most compromised by filter blank, with %UB of 195%. A subset of published trace element blank data determined for polycarbonate filters of various pore-sizes is compared with our results in Table 2. Significant steps toward minimizing absolute metal blanks are realized through the combination of our cleaning, handling and analytical



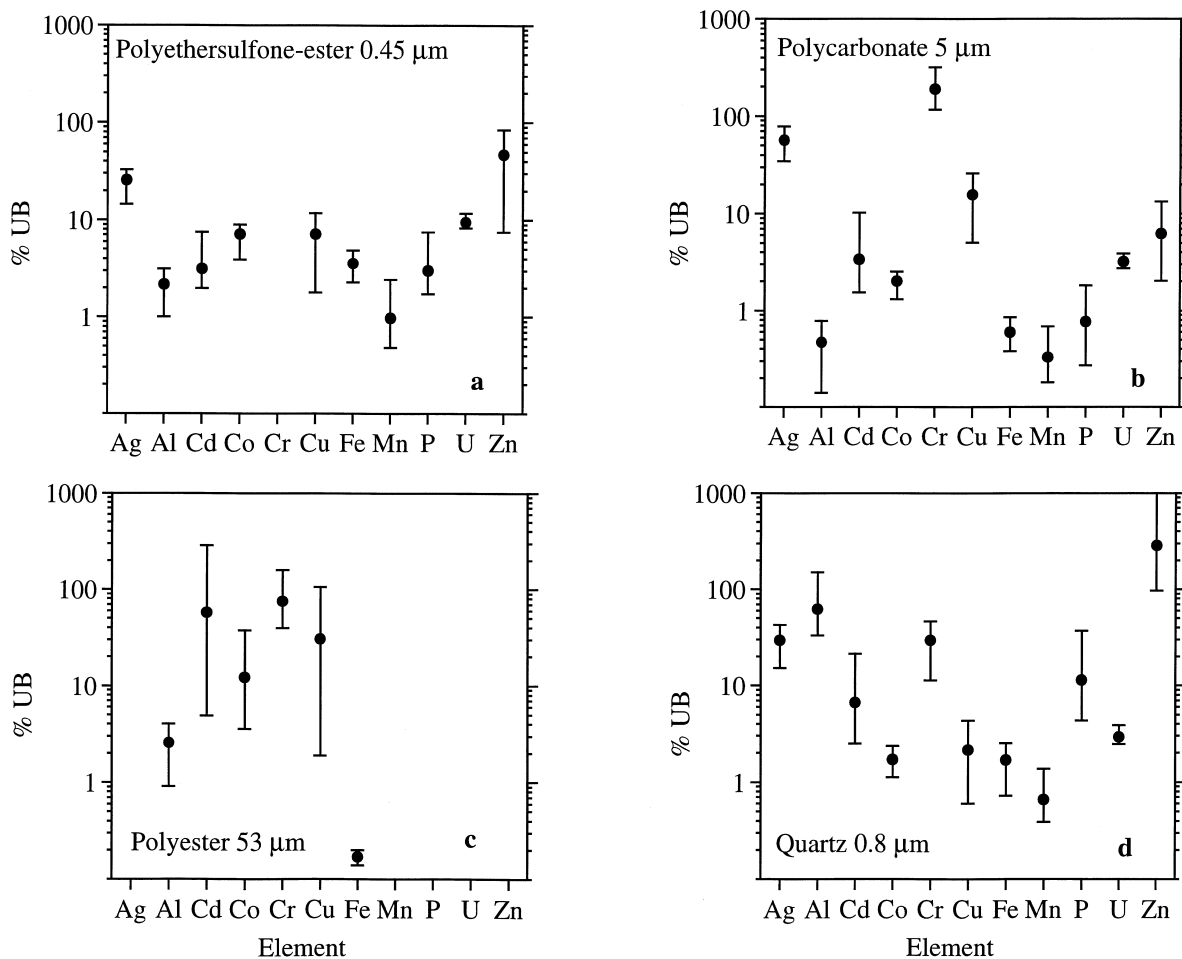


Fig. 2. Comparison of % uncertainty of the blank ( $\%UB = [3 \times SD \text{ of blank}] / [\text{blank-corrected sample}] \times 100$ ) for 47 mm diameter 0.45  $\mu\text{m}$  polysulfone-ester (a), 5  $\mu\text{m}$  polycarbonate (b), 53  $\mu\text{m}$  polyester (c), and 0.8  $\mu\text{m}$  quartz fiber (d) filters. Note this expression of blank contribution to sample measurement uncertainty incorporates absolute metal blanks, inter-filter blank variability, and variable extent of particle loading on filters (see text). Frames a, b and c represent size-fractionated particles filtered from 1–4 l seawater and frame d represents particles filtered from 10.43 l seawater; all samples collected from six depths at a coastal California station. Error bars represent range of %UB for variable filter loading at different depths. Values not plotted for Ag, Mn, P, U and Zn for 53  $\mu\text{m}$  polyester filter (c) because blank corrections yielded unmeasurable sample concentrations for all samples below 10 m.

methods for all elements in polycarbonate filters except Cr and Mn. No significant differences in trace metal blanks were found for the two lot numbers of polycarbonate filters used in this study.

### 3.2.3. Polyester square weave 53 $\mu\text{m}$ (Tetko, Pecap)

Polyester filters had significantly higher trace element blanks than either polysulfone or polycarbonate membrane filters. Very high blanks were determined for Al, Fe, Mn and P (33 000, 11 000, 1 700 000, and

1 900 000  $\text{pmol filter}^{-1}$ , respectively; Table 1). Comparing filter trace element blanks to the > 53  $\mu\text{m}$  field sample reveals high blank to sample ratios (> 100%) for all elements except Al (9%), Cd (32%), Cu (16%) and Fe (8%) at this level of filter loading (1.25 l filtered). However, the variability of blank among filters was low (mean = 10% RSD). Using the %UB criterion as above, Ag, Cr, Mn, P, U and Zn were greater than 75% on average (Fig. 2c). We surmise that the resin used in manufacture of the

Table 2

Summary of reported trace element blanks for polycarbonate filters normalized to 47 mm diameter filter size

Element	This study		Sherrell, 1991 <sup>a</sup> pmol/filter	Bruland et al., 1994 <sup>b</sup> pmol/filter	Landing and Lewis, 1991 <sup>c</sup>		Wallace et al., 1977 <sup>d</sup>	
	pmol/filter	SD			pmol/filter	SD	pmol/filter	SD
Ag	0.337	0.043						
Al	505	222	2000		878	409	≤ 322	
Cd	0.396	0.075	0.098	4.01	49.9	9	1.85	0.89
Co	0.660	0.222	4.00		9.16	1.7		
Cr	260	110					167	33
Cu	14.5	7.66	30.1		156	9.5	62.8	19
Fe	240	115		1359	312	27	2489	627
Mn	6.47	2.67	10.0	4.00	12.2	3.6	25.1	18
P	440	93.5						
U	0.026	0.015						
Zn	24.7	8.74	40.1	89.0	145	28	79.7	26

<sup>a</sup>142 mm 1.0 μm polycarbonate filters (Nuclepore) precleaned 1 mol l<sup>-1</sup> HCl at 60°C overnight, digested in HNO<sub>3</sub> and HF at 90°C for 4 h.

<sup>b</sup>Sum of acetic acid leachable and refractory fractions for acid-precleaned 0.3 μm 142 mm diameter polycarbonate filter (Nuclepore).

<sup>c</sup>142 mm 0.4 μm polycarbonate filters (Nuclepore) precleaned 3 mol l<sup>-1</sup> HNO<sub>3</sub> at room temperature for one week; blank reported as sum of sequential leaches in 25% acetic acid, 2 mol l<sup>-1</sup> HCl + 1 mol l<sup>-1</sup> HNO<sub>3</sub> and HCl/HNO<sub>3</sub>/HF (*n* = 5–7).

<sup>d</sup>47 mm 0.4 μm polycarbonate (Nuclepore) precleaned overnight in equal volumes of 1 mol l<sup>-1</sup> HNO<sub>3</sub> and 1 mol l<sup>-1</sup> HF and digested following low temperature ashing (*n* = 10).

fibers from which these filters are woven was of a much lower purity than those used for the two smaller pore-size filters. The high but relatively constant blank of the polyester filter suggests that with high filter loadings, most of the trace elements of interest could be determined in > 53 μm particles. This point is important because the large and chain-forming diatoms which often dominate bloom events off central California and in other coastal regions tend to be trapped in the > 53 μm size-fraction.

#### 3.2.4. Quartz fiber 0.8 μm (Whatman QM-A)

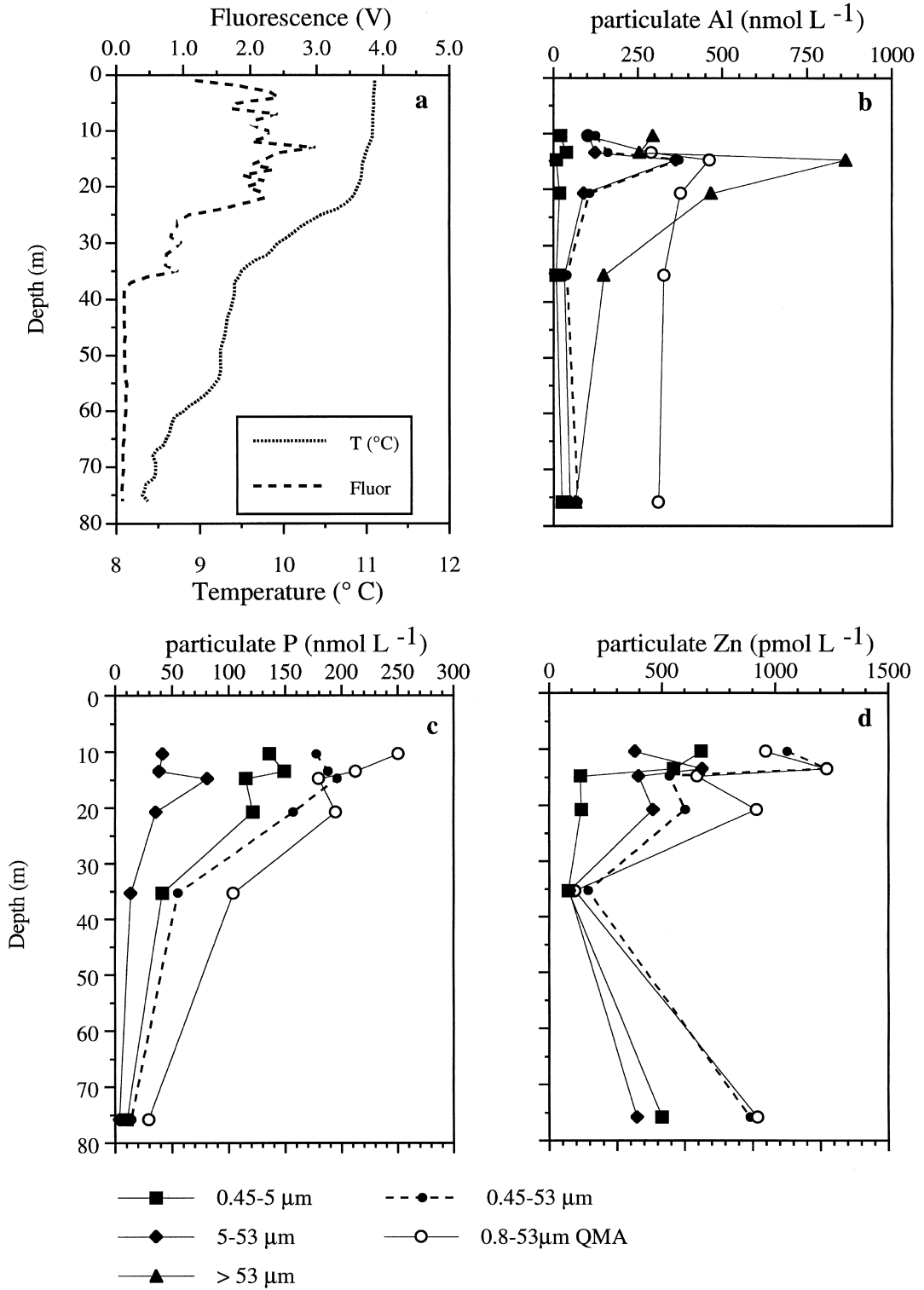
Quartz microfiber filters had, on average, the highest determined trace element blanks of all filter types tested in this study. Particularly high blank values were found for Al, Cr, Fe, P and Zn (11 000 000, 7900, 86 000, 480 000 and 26 000 pmol filter<sup>-1</sup>). Blanks for Cu (220 pmol filter<sup>-1</sup>) and Co (40 pmol filter<sup>-1</sup>) were similar to those found for 53 μm polyester filters. However, only the blanks for Al (98%), Cr (78%) and Zn (73%) were greater than 30% of a typical particulate sample (10 m) filtered

from 10.43 l of coastal seawater. Interestingly, blank variability was quite low (mean = 5% RSD) so that with the exception of Al (33%), Cr (37%) and Zn (124%), all other elements had %UB less than 25% of the sample signal for a complete depth profile (Fig. 2d). The combination of low blank variability and the ease with which larger water volumes can be filtered in this clogging-resistant filter allow reasonably accurate determination of most elements in this 0.8–53 μm fraction.

#### 4. Particulate elemental profiles from central California coastal waters

To demonstrate the utility of these filter combinations for size-fractionated plankton collection, and to compare results from polymer filters with those for quartz filters, we employed both techniques to collect particles from the water column approximately 10 km off the coast of central California. Because this paper focuses on the efficacy of the technique,

Fig. 3. Vertical profiles of (a) temperature and fluorescence and particulate (b) Al, (c) P and (d) Zn for various size fractions collected by in-line filtration from Niskin bottles at a station 10 km offshore near the southern end of Monterey Bay, California, 29 April, 1997. Dashed line is sum of 0.45–5 and 5–53 μm size fractions from plastic filters. Chl *a* fluorescence from CTD sensor shown (a) to identify phytoplankton-rich euphotic zone. Bottom depth 82 m.



we report results for three representative elements from the full suite of eleven. Particulate Al is a tracer of terrigenous particles, P is a proxy for total biomass, and Zn is a contamination-prone essential micronutrient. A complete treatment of phytoplankton concentrations for all measured elements and size fractions requires substantial discussion and will be presented elsewhere (Cullen and Sherrell, in preparation).

#### 4.1. Aluminum

Particulate Al concentrations displayed a marked subsurface maximum at approximately 16 m (Fig. 3b). A generous estimate of biogenically associated Al (Al organic carbon<sup>-1</sup> = 3 mg g<sup>-1</sup>; Moran and Moore, 1988) accounted for < 10% of our particulate Al signal. We therefore assume that particulate Al is dominated by terrigenous clay particles. Because these particles are typically small (1–10 μm), it is surprising that most of the Al is in the largest size fraction, with successively lower concentrations in the smaller size fractions (maximum concentrations are ~ 860 nmol l<sup>-1</sup>, ~ 360 nmol l<sup>-1</sup>, and ~ 40 nmol l<sup>-1</sup>, for the > 53, 5–53, and 0.45–5 μm fractions, respectively). We speculate that large biogenic particles effectively scavenge small clay particles in this environment (Deuser et al., 1983), so that most of the Al is aggregated with organic material caught in the largest size fraction. The vertical distribution of terrigenous particles may therefore reflect an accumulation horizon for such aggregates (Jackson and Burd, 1998), although it could also derive from lateral injection of resuspended sediment from the nearby continental shelf. The particulate Al profile collected from 10.43 l on the nominal 0.8 μm QM-A filter (53 μm prefilter not analyzed) agreed well in surface waters with Al determinations on the parallel summed 0.45–53 μm hydrocarbon filter stack, but exceeded by several-fold this latter measurement in subsurface waters (Fig. 3b). Differences in particulate Al on the two filter types likely result either from differences in loading and effective size cutoff for the two filter types, or from the large value for %UB on 0.8 μm QM-A filters (Table 1). The contribution of terrigenous particles to overall particle composition at this station is substantial; suspended mass of clays is estimated at 60–400 μg l<sup>-1</sup>

(assuming Al is 8% of average crustal particles; Wedepohl, 1995), compared to biogenic suspended mass of ~ 100–800 μg l<sup>-1</sup>. To determine the concentrations of metals in biogenic material, the metals associated with terrigenous particles must be estimated and subtracted.

#### 4.2. Phosphorous

Particulate P decreased with depth, consistent with maximal biomass concentrations (as chl *a* fluorescence) in the upper 20 m (Fig. 3 a and c). Particulate P could not be determined accurately for the polyester > 53 μm size fraction because P blanks for polyester filters were prohibitively high, given the moderate loading of the filter stacks (1–4 l of seawater). The particulate P vertical profile measured on 0.8–53 μm QM-A filters was qualitatively similar to the 0.45–5 and 5–53 μm profiles. However, up to 80% more particulate P was retained by the QM-A than the summed 0.45–53 μm size-fractions despite the larger 0.8 μm nominal pore size of the QM-A. A possible explanation for this observation is that the nominal pore size of the QM-A decreases markedly as the filter becomes heavily loaded (as ours were), thus retaining a larger fraction of < 0.45 μm particulate material than the moderately loaded 0.45 μm polysulfone filter (Horowitz et al., 1996). Alternatively, the faster flow rates through the QM-A filter stack may have reduced the retention of large, fragile aggregates by the 53 μm filter, allowing a portion of this material to be caught on the QM-A filter underneath. By using P as a proxy for biomass and assuming Redfield elemental ratios, we calculate suspended POC concentrations of 3–24 μmol l<sup>-1</sup> at this site, excluding the > 53 μm size-fraction. This range of values is in good agreement with POC concentrations of 4–25 μmol l<sup>-1</sup> determined directly (CHN analyzer) on subfractions of the QM-A filters (data not shown). The assumption that particulate P is a good estimate of organic P is justified because terrigenous particles contribute < 1% of total P (calculated from Al, assuming Al = 8% of clay mass and P = 700 ppm in clays; Ruttenberg, 1993). Measured C/P ratios on the quartz filters varied from 82–112 (mol mol<sup>-1</sup>; data not shown) in the upper 40 m high-chlorophyll waters at this station, bracketing the classical Redfield ratio of 106,

and suggesting that most of the suspended organic matter in the upper waters consisted of phytoplankton cells.

#### 4.3. Zinc

The suspended particulate Zn (Fig. 3d) decreases with depth as for P, but is somewhat elevated at the deepest sample (~6 m above bottom). Particulate Zn concentrations were similar in the 0.45–5 and 5–53  $\mu\text{m}$  fractions (80–700 pM). Zn could not be determined accurately on the polyester > 53  $\mu\text{m}$  fraction because blank corrections were large given the volume of seawater filtered (1–4 l). The particulate Zn profile obtained from the 0.8–53  $\mu\text{m}$  QM-A sample matched well the summed 0.45–53  $\mu\text{m}$  size fractions; discrepancies were substantially smaller than those for P. The increased Zn content of near-bottom samples is not caused by increased terrigenous (Al-rich) or biogenic (P-rich) particles (Fig. 3b and c). It is likely that resuspended bottom particles are enriched in authigenic oxides or detrital organics carrying adsorbed non-biogenic Zn. The organic Zn/P ratio (particulate Zn and P for terrigenous inorganic components always < 10% for P and < 37% for Zn) on 0.45–53  $\mu\text{m}$  plastic and 0.8–53  $\mu\text{m}$  quartz filter size-fractions is plotted along with depth profiles of organic C/N ratio measured on the 0.8–53  $\mu\text{m}$  quartz fiber filters (Fig. 4). C/N values of ~ 5.5 in these surface waters, where  $\text{NO}_3^-$  concentrations were ~ 17  $\mu\text{mol l}^{-1}$  (data not shown), are near Redfield and are consistent with phytoplankton growing under luxuriant  $\text{NO}_3^-$  availability (Geider et al., 1998). Large diatoms (typically *Chaetoceros* spp., *Nitzschia* spp.) dominate upwelling-driven bloom events off central California (Tortell et al., 1997; Hutchins and Bruland, 1998). Calculated Zn:C using P as a biomass proxy and assuming Redfield elemental ratios yields values of  $1\text{--}5 \times 10^{-5}$  mol mol<sup>-1</sup> for all size fractions. In metal uptake experiments conducted on phytoplankton of various species in culture, Zn quotas are a relatively tight sigmoidal function of  $\text{Zn}^{2+}$  in the media. The quotas we determine agree with those determined in the relatively invariant cellular Zn portion of this curve, at  $\text{Zn}^{2+} = 0.01\text{--}1.0$  nM, about the expected concentrations of non-organically complexed Zn for these coastal waters. The Zn content of the phytoplankton assemblage at this station is therefore consistent with

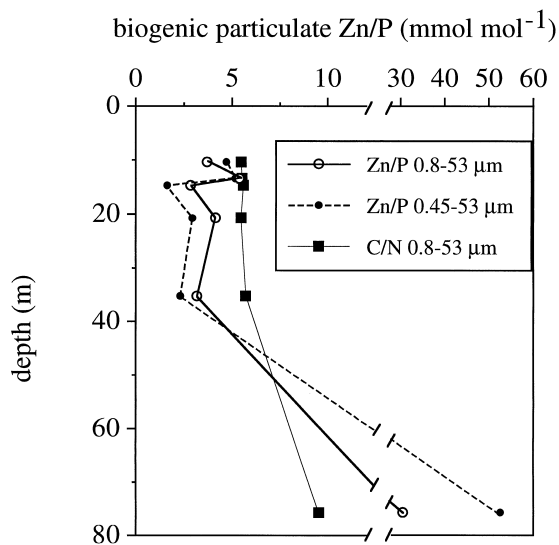


Fig. 4. Particulate organic Zn/P (0.45–53  $\mu\text{m}$  pooled plastic filter and 0.8–53  $\mu\text{m}$  quartz filter size-fractions, both corrected for estimated aluminosilicate Zn and P fractions) and particulate organic C/N (0.8–53  $\mu\text{m}$  quartz) profiles at coastal California station. Deepest sample (76 m) off-scale values are Zn/P = 52 mmol mol<sup>-1</sup> for 0.45–53  $\mu\text{m}$  fraction, and Zn/P = 31 mmol mol<sup>-1</sup> for 0.8–53  $\mu\text{m}$  fraction.

uptake at natural Zn concentrations in seawater; we speculate that other particle types contribute only minor fractions to the total particulate Zn concentrations. Zn quotas in phytoplankton from Zn-polluted waters are expected to be higher, although there is evidence that Zn uptake is competitive with Mn uptake, so that Zn uptake could be suppressed in very high dissolved Mn waters proximal to estuarine or sediment  $\text{Mn}^{2+}$  sources (Sunda and Huntsman, 1998).

## 5. Summary and application to other marine environments

We have developed an efficient method for collection of size-fractionated phytoplankton samples that are suitable for trace metal analysis from 1–10 l samples of waters with POC > 3  $\mu\text{mol l}^{-1}$ . Trace element compositional analysis of size-fractionated phytoplankton populations is a valuable augmentation of the 15–25-year-old set of measurements which form the basis for our current understanding of natural phytoplankton trace element quotas (Martin

and Knauer, 1973; Martin et al., 1976; Bruland et al., 1978; Collier and Edmond, 1984). The ability to determine metal quotas as a function of size is important because metal uptake processes may depend on taxonomic differences and/or on surface area to volume ratios, and because phytoplankton-mediated vertical fluxes of metals depend in part on cell size (Fisher et al., 1983; Fisher and Reinfelder, 1995).

The results of this field investigation serve as a feasibility guide for investigators interested in determining metal concentrations in small samples of any natural particles. The trace element composition of the materials investigated here varied considerably among filter types. Polycarbonate membrane filters generally have the lowest metal blanks, followed closely by polysulfone-ester depth filters. However, despite acid cleaning, blanks for both Ag and Cr compromised attempts to quantify these metals in particles collected on these two filter types from productive coastal waters (POC  $3\text{--}30 \mu\text{mol l}^{-1}$ ). Some elements could be determined accurately on the  $53 \mu\text{m}$  polyester filter using 1–4 l volumes (Al, Cd, Cu and Fe), but roughly 10-fold larger filtered volumes would be required to measure accurately the particulate Co, Cr, P, U and Zn in these waters, and Mn and Ag are virtually undeterminable due to excessively high filter blanks. Using our precleaning, sampling and analytical methods, quartz fiber filters were found to be suitable for determination of metals in marine phytoplankton with the added advantage that biomass (as POC) can be determined on the same sample. Despite high Ag, Al, Cr and Zn blank/sample ratios for quartz fiber filters, the high loading achieved by filtration of  $\sim 10 \text{ l}$  in productive coastal waters allows metal/C and C/P ratios to be determined on sub-fractions of the same filter. Further information about the composition of each size fraction could be obtained, for example, by pigment analysis or genetic identification of the phytoplankton taxa and biodebris contributions present on the same filters.

In more offshore environments, surface water POC and phytoplankton biomass may be two orders of magnitude lower than in the productive waters sampled here (Karl and Lukas, 1996; Sherrell et al., 1998). We estimate that by increasing to  $10 \text{ l}$  the volume through the size-fractionating filter stack, all

elements except Ag, Cr and Zn could be determined in particles  $0.45\text{--}53 \mu\text{m}$  in size (with less than 50% blank correction, assuming similar metal/biomass ratios). However, many trace metals in these environments could not be measured on the  $53 \mu\text{m}$  polyester and  $0.8 \mu\text{m}$  quartz fiber filters without filtering impractically large volumes (50–100 l) or scaling down filter size with associated increases in filtration time. Placing a 25-mm filter holder containing a  $53\text{-}\mu\text{m}$  polyester filter in series with a 47-mm holder for the  $0.45\text{--}53 \mu\text{m}$  fraction would increase particle loading for the largest size-fraction and improve sample/blank for Mn, P and Zn. Further reduction in filter blanks may result from less rigorous sample digestion techniques, but experiments would need to be performed to verify dissolution of all phases of interest.

With simple modifications to the sampling and analytical techniques described here, size-fractionated phytoplankton and particulate concentrations for a broad suite of metals can be determined in a range of oceanic regimes, from productive coastal and estuarine environments to oligotrophic offshore waters. These methods would also be useful, possibly in a scaled-down version, for determination of phytoplankton metals in ship-board incubation or in situ enrichment experiments to study size-class dependence of phytoplankton–metal interactions. The development of methods for washing natural samples to remove extracellular adsorbed metals (Hudson and Morel, 1989) without contaminating natural particulate samples or leading to loss of more labile elements (for example P) would complement our methods by improving estimates of intracellular metal concentrations.

### Acknowledgements

We thank Lex van Geen, Paul Field and the captain and crew of the R/V *Pt. Sur* for their assistance in collecting and analyzing these samples. The manuscript was improved by the careful reviews of Mark Wells and two anonymous reviewers. This work was supported by NSF Chemical Oceanography award OCE-9720692 to RMS.

## References

- Banse, K., 1977. Determining the carbon-to-chlorophyll ratio of natural phytoplankton. *Mar. Biol.* 41, 199–212.
- Bishop, J.K.B., 1999. Transmissometer measurement of POC. *Deep-Sea Res.*, Part I 46, 355–369.
- Bishop, J.K.B., Schupack, D., Sherrell, R.M., Conte, M., 1985. A multiple-unit large-volume in situ filtration system for sampling oceanic particulate matter in mesoscale environments. In: Zirino, A. (Ed.), *Mapping Strategies in Chemical Oceanography*. American Chemical Society, Washington, DC, 209 (1985) 155–175.
- Brand, L.E., 1992. Minimum iron requirements of marine phytoplankton and the implications for the biogeochemical control of new production. *Limnol. Oceanogr.* 36, 1756–1771.
- Brand, L.E., Sunda, W.G., Guillard, R.R.L., 1983. Limitation of marine phytoplankton reproductive rates by zinc, manganese, and iron. *Limnol. Oceanogr.* 28, 1182–1198.
- Bruland, K.W., Knauer, G.A., Martin, J.H., 1978. Cadmium in northeast Pacific waters. *Limnol. Oceanogr.* 23, 618–625.
- Bruland, K.W., Oriens, K.J., Cowen, J.P., 1994. Reactive trace metals in the stratified central North Pacific. *Geochim. Cosmochim. Acta* 58, 3171–3182.
- Buesseler, K.O., Andrews, J.A., Hartman, M.C., Belostock, R., Chai, F., 1995. Regional estimates of the export flux of particulate organic carbon derived from thorium-234 during the JGOFS EqPac program. *Deep-Sea Res.*, Part II 42, 777–804.
- Collier, R., Edmond, J.M., 1984. The trace element geochemistry of marine biogenic particulate matter. *Prog. Oceanogr.* 13, 113–199.
- Deuser, W.G., Brewer, P.G., Jickells, T.D., Commeau, R.F., 1983. Biological control of the removal of abiogenic particles from the surface ocean. *Science* 219, 388–391.
- Donat, J.R., Bruland, K.W., 1995. Trace elements in the ocean. In: Salbu, B., Steinnes, E. (Eds.), *Trace Metals in Natural Waters*. CRC Press, pp. 247–281.
- Fisher, N.S., Reinfelder, J.R., 1995. The trophic transfer of metals in marine systems. In: Tessier, A., Turner, D.R. (Eds.), *Metal Speciation and Bioavailability in Aquatic Systems*. Wiley, pp. 363–406.
- Fisher, N.S., Burns, K.A., Cherry, R.D., Heyraud, M., 1983. Accumulation and cellular distribution of  $^{241}\text{Am}$ ,  $^{210}\text{Po}$ , and  $^{210}\text{Pb}$  in two marine algae. *Mar. Ecol. Prog. Ser.* 11, 233–237.
- Geider, R.J., MacIntyre, H.L., Kana, T.M., 1998. A dynamic regulatory model of phytoplanktonic acclimation to light, nutrients, and temperature. *Limnol. Oceanogr.* 43, 679–694.
- Helmers, E., 1996. Trace metals in suspended particulate matter of Atlantic Ocean surface water (40°N to 20°S). *Mar. Chem.* 53, 51–67.
- Horowitz, A.J., Lum, K.R., Garbarino, J.R., Hall, G.E.M., Lemieux, C., Demas, C.R., 1996. Problems associated with using filtration to define dissolved trace element concentrations in natural water samples. *Environ. Sci. Technol.* 30, 954–963.
- Hudson, R.J.M., Morel, F.M.M., 1989. Distinguishing between extra- and intracellular iron uptake in marine phytoplankton. *Limnol. Oceanogr.* 34, 1113–1120.
- Hutchins, D.A., Bruland, K.W., 1998. Iron-limited diatom growth and Si:N uptake ratios in a coastal upwelling regime. *Nature* 393, 561–564.
- Jackson, G.A., Burd, A.B., 1998. Aggregation in the marine environment. *Environ. Sci. Technol.* 32, 2805–2814.
- Karl, D.M., Lukas, R., 1996. The Hawaii Ocean Time-series (HOT) program: background, rationale and field implementation. *Deep-Sea Res.*, Part II 43, 129–156.
- Landing, W.M., Lewis, B.L., 1991. Collection, processing, and analysis of marine particulate and colloidal material for transition metals. *Geophys. Monogr.* 63, 263–272.
- Martin, J.H., Bruland, K.W., Broenkow, W.W., 1976. Cadmium transport in the California current. In: Windom, H.L., Duce, R.A. (Eds.), *Marine Pollutant Transfer*. Heath, pp. 159–184.
- Martin, J.H., Knauer, G.A., 1973. The elemental composition of plankton. *Geochim. Cosmochim. Acta* 37, 1639–1653.
- Moran, S.B., Moore, R.M., 1988. Evidence from mesocosm studies for biological removal of dissolved aluminium from seawater. *Nature* 335, 706–708.
- Morel, F.M.M., Hudson, R.J.M., 1984. *The Geobiological Cycle of Trace Elements in Aquatic Systems: Redfield Revisited*, pp. 251–281.
- Reinfelder, J.R., Fisher, N.S., 1994. The assimilation of elements ingested by marine planktonic bivalve larvae. *Limnol. Oceanogr.* 39, 12–20.
- Ruttenberg, K.C., 1993. Reassessment of the oceanic residence time of phosphorus. *Chem. Geol.* 107, 405–409.
- Schüßler, U., Kremling, K., 1993. A pumping system for underway sampling of dissolved and particulate trace elements in near-surface waters. *Deep-Sea Res.*, Part I 40, 257–266.
- Sherrell, R.M., 1991. Collection of oceanic suspended particulate matter for trace metal analysis using a new in situ pump. *Geophys. Monogr.* 63, 285–294.
- Sherrell, R.M., Boyle, E.A., 1992. The trace metal composition of suspended particles in the oceanic water column near Bermuda. *Earth Planet. Sci. Lett.* 111, 155–174.
- Sherrell, R.M., Field, M.P., Gao, Y., 1998. Temporal variability of suspended mass and composition in the Northeast Pacific water column: relationships to sinking flux and lateral advection. *Deep-Sea Res.*, Part II 45, 733–761.
- Sunda, W.G., 1991. Trace metal interactions with marine phytoplankton. *Biol. Oceanogr.* 6, 411–442.
- Sunda, W.G., Huntsman, S.A., 1998. Control of Cd concentrations in a coastal diatom by interactions among free ionic Cd, Zn, and Mn in seawater. *Environ. Sci. Technol.* 32, 2961–2968.
- Takenaka, M., Hayashi, M., Suzuki, I., Yamada, Y., Takamatsu, K., Kageyama, M., 1997. Evaluation of a mirror-polishing technique for fluorocarbon polymer surfaces for reduction of contamination from containers used in ultratrace analysis. *Anal. Chem.* 69, 972–976.
- Tortell, P.D., Reinfelder, J.R., Morel, F.M.M., 1997. Active uptake of bicarbonate by diatoms. *Nature* 390, 243–244.
- Wallace, G.T., Fletcher, I.S., Duce, R.A., 1977. Filter washing, a simple means of reducing blank values and variability in trace metal environmental samples. *J. Environ. Sci. Health, Part A* 12, 493–506.
- Wedepohl, K.H., 1995. The composition of the continental crust. *Geochim. Cosmochim. Acta* 59, 1217–1232.