Manuscript in preparation for Marine Chemistry

Assessment of the stability, sorption, and exchangeability of marine dissolved and colloidal metals

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Abstract

The size partitioning of dissolved trace metals is an important factor for determining reactivity and bioavailability of metals in marine environments. This, alongside the advent of more routine shipboard ultrafiltration procedures, has led to increased attention in determining the colloidal phase of metals such as Fe in seawater. While clean and efficient filtration, prompt acidification, and proper storage have long been tenets of trace metal biogeochemistry, few studies aim to quantify the kinetics of colloidal exchange and metal adsorption to bottle walls during storage and acidification. This study evaluates the effect of storage conditions on colloidal size partitioning, the kinetics of colloid exchange over time, and the timescale of bottle wall adsorption and desorption for dissolved Fe, Cu, Ni, Zn, Cd, Pb, Mn and Co. We report that preservation of dissolved size partitioning is possible only for Fe and only under frozen conditions. All metals except Mn and Cd show regeneration of the colloidal phase following its removal in as short as 14 hours, validating the importance of prompt ultrafiltration. Adsorption of metals to bottle walls is a well-known sampling artifact often cited for Fe and assumed to be potentially significant for other metals as well. However, only Fe and Co showed significant proclivity to adsorption onto low density polyethylene bottle walls, sorbing a maximum of 91 and 72% over 40 months, respectively. After 20 weeks of acidification neither Fe nor Co desorbed to their original concentrations, leading to an acidified storage recommendation of 30 weeks prior to analyses following storage of unacidified samples for long periods of time. This study provides empirical recommendations for colloidal and dissolved trace metal methodology while also paving the way for much-needed future methods testing.

Introduction

Colloids represent a dynamic class of compounds that exert control on the fate (transport, reactivity, and bioavailability) of trace metals in seawater. A colloid is distinguished from a truly dissolved species (here called "soluble" species) based on its size, where the transition from soluble to colloidal compounds theoretically occurs when the internal characteristics of a compound become significantly differentiated from the solution such that an interface is established [*Wells*, 2002]. This surficial interface is critical for the adsorption of trace metals, and given the larger relative surface area of colloids compared to their particulate analogs, colloids can serve as an important adsorptive sink for metals from the dissolved phase. Moreover, given the physical inclination of colloids to aggregate [*Honeyman and Santschi*, 1988], adsorption followed by colloidal aggregation can serve as an important output vector of metals from the ocean when they ultimately sink as particles to the sediments. Thus, colloids play an important role in marine biogeochemistry as an intermediary in the continuum of size fractions in seawater and, as aggregators, as a part of the "scavenging" removal of elements from the ocean.

Additionally, the reactivity and bioavailability of trace metals in the ocean is closely tied to their physicochemical speciation. For iron (Fe), which is the best studied of the micronutrient metals, species in the smallest soluble size fraction (typically <3 nm) have been established as the most bioavailable to marine phytoplankton, although larger Fe colloids (3-200 nm) are also thought to be bioavailable, depending on their chemical composition [*Chen and Wang*, 2001; *Chen et al.*, 2003; *Hassler et al.*, 2011]. The colloidal distributions of other metals have been investigated in some earlier studies but remain poorly constrained in open ocean regions, with significant variability dependent on ultrafiltration method used and region studied [*Buesseler et al.*, 1996; *Wen et al.*, 1999; *Doucet et al.*, 2007]. However, it remains clear that the size and chemical composition of colloids play important roles in the bioavailability and reactivity of dissolved trace metals in seawater.

Over the last few decades, several isolation methods have been applied to the sampling of marine colloids: solid-phase extraction [*Louchouarn et al.*, 2000], flow field-flow fractionation (FFFF) [*Stolpe et al.*, 2010; *Baalousha et al.*, 2011], chromatography [*Minor et al.*, 2002], gel filtration and stirred-cell ultrafiltration [*Guo and Santschi*, 2007], as well as more recent methods such as Vivaspin centrifuge ultrafilters [*Schlosser et al.*, 2013]. However, ultrafiltration methods such as Anopore filtration (0.02 μm pore size cutoff) and the widely used cross flow filtration (1-1000 kDa) are now among the more common methods for metal colloid studies as they allow large quantities of water to be filtered at once over a large range of sizes [*Buesseler et al.*, 1996; *Guo and Santschi*, 2007; *Fitzsimmons and Boyle*, 2014b]. These have led to a multitude of global ocean investigations of the size partitioning of dissolved Fe (synthesized in [*Fitzsimmons* *and Boyle*, 2014a; *von der Heyden and Roychoudhury*, 2015]), which have shown that the size partitioning varies spatially and is critically dependent on which pore size cutoff is used, since the size distribution of Fe colloids is itself dynamic [*Stolpe et al.*, 2010; *Baalousha et al.*, 2011]. Unfortunately, few studies have compared colloidal metal concentrations across a range of ultrafiltration pore sizes in seawater at the same sites [*Larsson et al.*, 2002; *Ingri et al.*, 2004; *Fitzsimmons and Boyle*, 2014a], likely because of the time consuming nature of ultrafiltration itself. This would help reveal the true size distribution of marine colloidal metals.

As is true for any operationally-defined method, it is important to rigorously calibrate the method and test for potential artifacts so that different users can expect to acquire the same results. For colloid ultrafiltration methods, methodological artifacts can arise after initial particulate removal via bulk filtration (> 0.2 μm pore size), which is required since particles can clog ultrafilters and/or serve as sorptive surfaces, both before and during the slow ultrafiltration process. Some of these artifacts were reviewed during an intercomparison exercise early in the development of colloidal methods [*Buesseler et al.*, 1996] as well as during later studies [*Chen et al.*, 2004; *Schlosser et al.*, 2013; *Fitzsimmons and Boyle*, 2014b]. For metals, the most significant concerns addressed were contamination and recovery differences across ultrafilters [*Reitmeyer et al.*, 1996]. While a subset of these ultrafilters have been vetted and are in regular use today [*Larsson et al.*, 2002; *Fitzsimmons and Boyle*, 2014a; *Fitzsimmons et al.*, 2015b], potential artifacts still exist and warrant further constraint, including the underexplored kinetics of colloidal exchange with other solution components and/or with bottle walls, which are especially critical when it can take tens of minutes to many hours to ultrafilter a single seawater sample.

Previous studies have demonstrated that sorption and desorption from colloidal complexes is highly dependent on pH, ionic strength of the solution, temperature, and particularly the composition of particles, leading to exchange on the timescale of hours to days [*Scheinost et al.*, 2001; *Roberts et al.*, 2003]. Marine colloids are thought to be primarily organic in nature, comprising a combination of humic-type substances and products of biological activity such as exopolymeric substances [*Gaffney et al.*, 1996; *Ron and Rosenberg*, 2001] and, more generically, dissolved organic matter (DOM) [*Santschi*, 2018]. Dissolved organic carbon (DOC) in particular is largely colloidal in size and, as such, is subject to rapid turnover, forming an important part of the global carbon cycle [*Baskaran et al.*, 1992; *Benner et al.*, 1992; *Santschi et al.*, 1995; *Guo and Santschi*, 1997; *Opsahl and Benner*, 1997; *Wen et al.*, 1997]. If metals are bound to or otherwise incorporated within these organic colloids, they would be more vulnerable to exchange over short timescales.

Sorption of trace metals to sub-sample bottle walls during storage also represents a potential artifact affecting measurements in seawater. The loss of various trace metals to bottle walls during storage is well documented ([*Massee et al.*, 1981], references therein), leading to the standardization of storage procedures that typically involve acidification of samples prior to analysis [*Pellenbarg and Church*, 1978; *Bruland and Rue*, 2001; *Lohan et al.*, 2005]. Furthermore, the bottle material and physicochemical speciation of each metal are known to affect the overall bottle wall sorption loss [*Starik et al.*, 1963; *James and Healy*, 1972a; *Pellenbarg and Church*, 1978; *Fischer et al.*, 2007]. While previous studies have elucidated losses of dissolved, soluble, and colloidal Fe to bottle wall sorption [*Schlosser et al.*, 2011; *Fitzsimmons and Boyle*, 2012], no recent rigorous kinetic testing has been performed to examine adsorptive loss of other trace metals to bottle walls. Moreover, trace metals such as manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), and cadmium (Cd) each have different inorganic speciation in seawater compared with Fe [*Byrne*, 2002], as well as varying degrees of complexation to ligands [*Bruland et al.*, 2013]. Thus, we might expect that adsorption and subsequent desorption of these metals would be different from Fe and dependent on their speciation at seawater pH, leading to important considerations of the timing of sample acidification and analysis, and for colloids, the slow ultrafiltration process. Naturally, these concerns raise questions of whether seawater samples can be preserved for ultrafiltration back in the lab, when more time and supplies would allow for better streamlining of the ultrafiltration process.

Here, we report the results of four experiments designed to answer questions related to the kinetics of colloidal metal exchange and adsorption to bottle walls, as well as the efficacy of preserving the colloidal size partitioning of metal samples: 1) a "Colloid Preservation Experiment" to assess the ability to preserve multi-metal marine colloidal size partitioning under room temperature and frozen conditions over several weeks, 2) a "Colloid Exchangeability Experiment" to assess the timescale of colloid re-formation rates from the soluble phase at room temperature over several days, 3) a "Bottle Adsorption Kinetics Experiment" to assess the timescale of adsorption onto low density polyethylene (LDPE) bottle walls using two different bottle volumes, and 4) a "Bottle Desorption Kinetics Experiment" to assess the timescale of desorption from the same LDPE bottle walls. We analyzed these experimental treatments for their Fe concentrations but also, for the first time, extended our measurements to the size partitioning of Mn, Co, Ni, Cu, Zn, Cd, and lead (Pb). The goal of these experiments was to provide recommendations regarding sample storage prior to acidification, constraints on how long samples can be stored before ultrafiltration and the influence of the ultrafiltration processing time itself, in order to avoid artifacts during future field studies.

Methods

Sample collection

Water used for these experiments was collected on three research cruises: 1) in September 2015 during the U.S. Arctic GEOTRACES GN01 cruise (Station 52: 77.50°N, 148.01°W, 100 m depth) aboard the USCGC *Healy* for the Bottle Adsorption/Desorption Kinetics Experiments, 2) in January 2016 in the Southern Ocean during the Palmer Long-Term Ecosystem Research (Pal-LTER) cruise aboard the ARSV *Laurence Mckinley Gould* (Station 600.040: -64.93°N, 64.40°W, 1320 m depth for the Colloid Preservation Experiment; and Station 200.000: -67.77°N, -69.95°W, 682 m depth for the Colloid Exchangeability Experiment), and 3) in June 2018 on a Galveston Bay cruise aboard the R/V *Trident* (Station 3, 29.45°N, 94.87°W, 1 m depth) for the Colloid Preservation Experiment. Briefly, samples collected aboard the ARSV *Laurence M. Gould* were collected using a trace metal clean CTD rosette loaded with 12 x 12 L Niskin-X bottles from stations close to the coast thought to be rich in colloids. Samples collected aboard the USCGC *Healy* followed established trace metal sampling protocols [Cutter et al., 2010] using a trace-metal clean Seabird CTD rosette mounted with 24 x 12 L GO-FLO (General Oceanics) bottles. The surface-water samples from Galveston Bay were collected using a trace-metal clean PTFE double diaphragm pump (Cole Parmer) fitted with acid-cleaned Bev-A-Line tubing (1/4 in, Cole Parmer) at stations thought to be rich in colloids. In all cases, samples were initially filtered through 0.2 μm AcroPak-200 polyethersulfone membrane filter capsules (Pall) and collected, following three 10% volume rinses, in LDPE Nalgene bottles previously cleaned using hydrochloric acid (HCl, trace metal grade, 1 M) and ultrapure Milli-Q (MQ, Millipore) water at 60°C [Fitzsimmons and Boyle, 2012].

Ultrafiltration methods

In each of the colloidal preservation and exchangeability experiments, standard protocol for the ultrafiltration of seawater was completed using a trace metal clean cross flow filtration system (CFF) [*Fitzsimmons and Boyle*, 2014b]. This CFF system employed a Millipore Pellicon XL regenerated cellulose filter with a nominal weight cutoff of 10 kDa (~3 nm for spherical proteins, [*Erickson*, 2009]) and a Cole Parmer Masterflex pump with all fluorinated ethylene propylene (FEP) tubing. Prior to the research cruises, new Pellicon filters were first cleaned with 1 L of 0.24 M HCl (Optima Fisher Scientific), which was allowed to recirculate through the system for 1 hour. The system was then flushed with at least 500 mL of 0.024 M HCl and stored in 0.0024 M HCl until use. Prior to each use, 500 mL of dilute (~0.005 M) HCl was circulated through the system followed by 100-500 mL of sample seawater (as much as the experimental volume allowed) to condition the system and allow for an accurate flow rate calibration. Total flow rate was calibrated to 25 mL/min, and permeate and retentate flows were subsequently equalized to 12.5 mL/min and recorded for accurate determination of the colloid concentration factor and recovery.

In this paper, all ultrafiltration via CFF was "single-pass," meaning that the retentate solutions were not recycled back into the feed solution for cumulative ultrafiltration; instead, we treat all results as traditional filtrations but with a small pore size, which minimizes artifacts associated with common CFF approaches that pass sample retentate over the same filter multiple times. Critically, by maximizing flow rate through our CFF system, we minimize the height of our concentration polarization layer and associated filtration biases that were observed in early CFF ultrafiltration experiments for metals [*Buffle et al.*, 1992], and also by keeping sample volumes low and acid cleaning well between samples, we avoid variations in the permeate metal concentrations over time, which were so often observed in earlier studies (e.g. [*Wen et al.*, 1996]). In our system, soluble metal (sMe) concentrations are therefore defined as the concentration measured in the permeate solution, or what passes through the 10 kDa (~0.003 μm) membrane. Colloidal metal (cMe) concentrations are calculated as the mass balance difference between dissolved metal (dMe) and sMe concentrations ([cMe] = [dMe] – [sMe]), which assumes that any metal lost to the CFF system (during imperfect recovery from the CFF system) is lost from the colloidal size fraction only, not the soluble size fraction. This was confirmed in *Fitzsimmons and Boyle* [2014a] for Fe using Pellicon CFF filters, where Fe losses were attributed mostly to colloidal Fe trapping inside the membrane itself. The cMe fraction is calculated as the cMe concentration divided by the dMe concentration (%cMe = [cMe]/[dMe]). The recovery is calculated as in *Fitzsimmons and Boyle* [2014a] and assesses the metal yield in the permeate + concentrated retentate solutions compared to the initial dMe concentration fed into the CFF system.

Experimental methods

1. Colloid preservation experiment - Water collected from a near-bottom depth of 1320 m near Palmer Station (West Antarctic Peninsula) was used to assess whether seawater samples can be preserved over time for cMe size partitioning determination (Figure 1). Four 250 mL bottles, two 60 mL bottles, and one 4 L bottle of 0.2 μm -filtered (Acropak 200, Pall) seawater were filled directly from a trace metal clean Niskin-X bottle following three 10% rinses of all bottles, caps, and threads. The two 60 mL bottles were immediately acidified and represent the initial dissolved metal concentrations (T0). The 4 L bottle was left at room temperature for immediate ultrafiltration into two 60 mL permeate (P T0) and two 60 mL retentate (R T0) solutions. Two 250 mL bottles were preserved in the dark (black bag) at room temperature (RT), and the remaining two 250 mL bottles were preserved in the dark at -20°C (frozen) and thawed to room temperature before ultrafiltration.

To assess the colloidal size partitioning, ultrafiltration of the 250 mL preserved (frozen or RT) samples was completed after 7 days (T7) and after 21 days (T21), using the CFF methods above. Each time point yielded one 60 mL bottle of non-filtered, “poured” seawater, to record the dMe concentrations remaining in the bottle at that time point (minus any adsorption to bottle walls), one 60 mL bottle that was filled with CFF-ultrafiltered permeate solution (P), which contains no colloids and only truly soluble species, and one 60 mL bottle that was filled with ultrafiltered retentate solution (R), which contained some soluble species and the concentrated colloids (for CFF recovery determination).

We repeated this experiment with additional replicates and time points using the same procedure but with surface water collected from Galveston Bay. To better constrain the variability imparted by the CFF ultrafiltration itself, triplicate initial dMe (T0) were collected as well as triplicate CFF-ultrafiltered permeate (P T0) and retentate (R T0) samples. Replicate 250 mL samples were stored frozen and at room temperature and were then ultrafiltered after 2 days in duplicate (T2), after 9 days (T9, only one replicate each), and after 23 days (T23, one replicate for room temperature, duplicate for frozen treatment). All samples in both experiments were acidified to 0.012 M HCl (Optima) following their final filtration.

2. Colloid exchangeability - Water collected from a near-bottom depth (682 m) in Marguerite Bay on the West Antarctic Peninsula was used to determine colloid metal exchangeability ("re-formation") kinetics using the methods illustrated in Figure 2. Water was filtered directly from a trace metal clean Niskin-X bottle through a 0.2 μm filter (Acropak 200, Pall) into a 4 L LDPE bottle following three 10% rinses of the bottle, cap, and threads. This filtered seawater was immediately sub-sampled into two 60 mL bottles for dMe determination (T0), whilst additional seawater was immediately ultrafiltered into two 60 mL bottles for permeate (P T0) and two 60 mL bottles for retentate (R T0), as well as one 2 L bottle of just permeate solution. The 2 L bottle was the “incubation bottle” in which colloids were initially removed but might be re-forming ("exchanging with" or "aggregating from" the soluble phase) over time. This bottle was stored in the dark at room temperature until further processing at each time point.

After 14 hours, seawater from the bulk 2 L permeate sample was poured into 60 mL bottles (P T14 “pour”) to capture the dMe concentrations in the bulk 2 L bottle, which excludes any metals lost to bottle wall adsorption. Solution from the 2 L bulk bottle was ultrafiltered again into permeate and retentate fractions (PP, PR at T14). This procedure was repeated at 24, 48, and 70 hours (T24, T48, and T70, respectively). All 60 mL samples were subsequently acidified to 0.012 M HCl (Optima).

3. Adsorption/desorption - Adsorption and desorption of metals was tested using LDPE bottles of two volumes, 250 mL and 125 mL, to quantify any effect of surface area to volume ratio (SA:V) on metal adsorption. Seawater for these experiments was collected at 100 m depth in the Western Arctic Ocean.

For the adsorption experiment (Figure 3), seawater was filtered from a GO-FLO bottle through a 0.2 μm filter (Acropak-200, Pall) into a trace metal clean 10 L carboy, homogenized, and then immediately subsampled into eighteen 250 mL bottles and eighteen 125 mL bottles following three 10 % rinses of the insides, caps, and threads, with no headspace. For the initial time point (T0), three 250 mL and three 125 mL bottles were immediately acidified (0.024 M HCl, Optima). The remaining bottles were stored unacidified at room temperature in the dark (black bags) for different time periods during which metals could adsorb to the bottle walls. At each subsequent time point, three 250 mL and three 125 mL samples were poured into new, acid-cleaned 250 and 125 mL bottles, respectively, and acidified, which leaves any metals adsorbed to the original bottle walls in the original bottles. This was repeated at 4, 8, 16, and 24 hours. Additionally, three 250 mL and three 125 mL samples were set aside as "long term" storage time points. They were transferred to new bottles and acidified (0.024M HCl, Optima) at ~40 months.

For the desorption experiment (Figure 3), water collected from the same depth was 0.2 μm filtered from a separate GO-FLO bottle and placed into another 10 L carboy, homogenized, and then directly sub-sampled into twelve 250 mL bottles and twelve 125 mL bottles following three 10% rinses of the insides, caps, and threads, with no headspace. All bottles were left at room temperature in the dark, unacidified (to allow for metal adsorption to bottle walls) for ~22 months until acidification (0.012 M HCl, Optima). Immediately following acidification, two 250 mL and two 125 mL bottles were poured off into four fresh 250 mL bottles, representing the initial time point (T0). This T0 time point captured any dMe remaining in solution as well as any dMe that had rapidly desorbed from bottle walls flowing acidification. This process of pouring into a new clean bottle was repeated after 1, 3, 7.5, 12, and 20 weeks to measure the kinetics of metal desorption from bottle walls as a function of bottle SA:V ratio.

Metal analysis

Samples were analyzed for Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb at least 1 month following acidification using an offline isotope dilution and pre-concentration method on a SeaFAST-pico system (ESI, Omaha), modified from *Lagerström et al.* [2013]. This method is described for Zn in *Jensen et al.* [2019] but is expanded here for the other metals. Briefly, a 10 mL sample aliquot was weighed and spiked with an isotope spike mixture containing [^{57}Fe] = 480.5 nM, [^{62}Ni] = 1455.8 nM, [^{65}Cu] = 687.5 nM, [^{68}Zn] = 709.6 nM, [^{206}Pb] = 44.7 nM, and [^{114}Cd] = 449.6 nM and loaded into the SeaFAST system. For monoisotopic metals Mn and Co, a standard was created where [Mn] = 452.80 nmol/kg, and [Co] = 12.01 nmol/kg, and this standard was used to create a 6-point standard curve spanning 0 to 10 nmol/kg for Mn and 0 to 0.27 nmol/kg for Co. It is important to note here that samples were not UV oxidized and thus the Co measured represents the "labile" fraction. Samples were then buffered in line with an ammonium acetate buffer (Optima, Fisher Scientific) prepared to pH ~7.4, which when mixed with the 10 mL of spiked sample at a flow rate of 350 μL/min results in a buffered sample pH of 6.2 ± 0.3 [*Sohrin et al.*, 2008]. This solution was immediately loaded onto a column containing Nobias-chelate PA1 resin and rinsed with ultrapure water to remove salts. The sample was subsequently back-eluted with 10% (v/v) nitric acid (Optima, Fisher Scientific) to yield 400 μL of eluent, representing a 25-fold sample pre-concentration. These eluents were analyzed in low (Pb and Cd) and medium (Fe, Zn, Ni, Mn, Cu, and Co) resolution on a Thermo Element XR high resolution inductively coupled plasma mass spectrometer (HR-ICP-MS) at Texas A&M University in the R. Ken Williams Radiogenic Isotope Facility. Table 1 summarizes the detection limit and reference sample analyses to demonstrate the accuracy and precision of the measurements.

Results

1. Colloid Preservation Experiment: Can soluble/colloidal size partitioning be preserved over time by storing seawater frozen or at room temperature?

The goal of this experiment was to assess whether the natural size partitioning of marine metals into soluble and colloidal fractions could be preserved prior to ultrafiltration over a timescale of weeks under room temperature or frozen conditions. This would allow seawater to be collected in the field, stored using an ideal preservation method, and then ultrafiltered back in the laboratory when more time allows for optimal ultrafiltration conditions. This experiment was completed twice: first using water from the West Antarctic Peninsula continental shelf (the Southern Ocean experiment using near-bottom seawater in Palmer Canyon), and then with increased experimental replication using surface water from Galveston Bay, Texas.

In the Southern Ocean experiment (Figure 4; salinity = 34.70, T = 1.48°C), the samples stored at room temperature exhibited a statistically significant decrease (*t*-test, two-tailed, heteroscedastic, $p < 0.05$) in dFe concentration of 75% after just 7 days, likely from adsorption of dFe to bottle walls (see Adsorption Experiment results below), and continued to decrease through 21 days. In contrast, when stored frozen, the initial dFe concentration was fairly well preserved over 21 days, with no significant decrease (<5%), suggesting that wall adsorption does not occur to a significant extent if seawater is frozen immediately. Additionally, when frozen the dFe size partitioning was also well preserved compared to initial ultrafiltration. This can be seen by relatively constant sFe concentrations over time and comparable % cFe after 7 days (63%) and even 21 days (67%), compared to the initial partitioning (62 ± 2%). These results suggest that freezing filtered seawater can preserve both the dFe concentration and its size partitioning into soluble/colloidal fractions relatively well (compared to samples ultrafiltered immediately) over the timescale of weeks. In contrast, storing samples at room temperature allows for wall adsorption that prevents the preservation of dFe concentrations over time.

To compare these results to a seawater sample with different physicochemical Fe speciation, the experiment was repeated in Galveston Bay (Figure 4; salinity = 26.50, T = 30.93°C) with two improvements: 1) more size partitioning replicates at each time point to improve statistical strength in comparisons between time points, and 2) more sampling time points: T2 at 2 days, T10 at 10 days, and T23 at 23 days, which is most similar to the T21 time point from the first Southern Ocean experiment. Unlike in the Southern Ocean experiment, dFe concentrations were not well preserved even when frozen (Figure 4a, right), dropping 17% from 2.36 ± 0.04 nmol/kg (n = 3) to 1.99 nmol/kg in the frozen treatment after 10 days; this is similar magnitude to the dFe loss observed in the room temperature treatment. However, despite these dFe losses, the size partitioning of dFe was well maintained over time in both the room temperature and frozen treatments, with the exception of two likely contaminated permeate samples (RT T2 and frozen T10, which were anomalous for several metals). This difference between experiments suggests that variation in the physicochemical speciation of Fe between sites can affect adsorption to bottle walls and/or long-term colloid exchangeability with other species in solution, both of which would affect the ability to preserve a sample over time. However, both experiments together suggest that storing samples frozen can fairly well preserve dFe size partitioning into soluble and colloidal phases.

Like Fe, Mn and Co both show little sorption loss to bottle walls (<10%) in the frozen treatment compared to room temperature (Figure 4), making the frozen storage method the best method for size partitioning preservation of these metals. However, while Zn, Ni, Cu, Cd, and Pb display minimal change in their dissolved concentrations over time, they all suffer reductions in permeate "soluble" concentrations, usually even by 7 days. Thus colloids were being generated in solution due to aggregation over the duration of the experiment. This was especially true for Cd, which showed substantial colloidal aggregation from 0 to >20% colloidal Cd at the Galveston Bay site. However, in the Southern Ocean experiment, this was also true for Ni and particularly true for Zn and Pb, where the colloid fraction increased from ~20% to >90% by 21 days. Interestingly, this effect was more prominent for frozen samples (Figure 4d and e, respectively).

What explains the poor preservation of dMe size partitioning for all metals besides Fe, Mn, and Co under frozen conditions? Several factors must be considered, including metal sorption to bottle walls (which could favor soluble or colloidal metals), natural colloidal aggregation over time (which could be dependent on preservation conditions including temperature), and artifacts related to the ultrafiltration process. While we explore bottle wall adsorption and colloidal formation kinetics in detail in the next two experiments, we will first preview some patterns here based on these initial Colloid Preservation Experiments.

Sorption to bottle walls was evident in the room temperature treatments for dissolved Fe, Zn, Co, Pb, Ni, and Mn, but was much more severe for Fe and Co (66% and 69% after 21 days, respectively) than for Zn (16%), Pb (25%), Ni (6%), and Mn (4%). We also note that these statistics are for the Southern Ocean samples, which in general showed more bottle wall adsorption than the Galveston Bay samples. This can be attributed to differences between sites in either initial sample temperature and/or physicochemical metal speciation. It is known that sorption of metals to bottle walls is an exothermic process that is hastened in samples kept at colder temperatures [*Bartell et al.*, 1951; *Fitzsimmons and Boyle*, 2012]. It is not clear whether smaller soluble-sized or colloidal-sized species are favored during bottle wall adsorption, since for Co and Mn the dissolved and permeate concentrations appeared to decrease by similar amounts, implicating soluble Co and Mn in wall sorption, but for the other metals (Zn, Ni and Pb in particular) the permeate concentrations decreased much more than the dissolved concentrations, possibly implicating wall sorption of the soluble phase or colloid formation. Additionally, soluble-colloidal exchange cannot be precluded, making any pattern difficult to attribute specifically to adsorption of soluble or colloidal metals alone.

It should also be noted that for the two metals with the greatest ingrowth of colloids over time (Zn and Pb) in the Preservation Experiment, the recovery of these metals through the CFF system also decreased over time from >90% in samples ultrafiltered immediately (T0) to <30% recovery after 21 days for the Southern Ocean samples. Thus, some of the “colloidal ingrowth” in these experiments could be misattributed and instead be caused by increasing soluble metal losses to the CFF system over time. The behavior of metals other than Fe in these CFF filters has not been well constrained, as the original CFF methods development and testing focused solely on Fe [*Fitzsimmons and Boyle*, 2014b]. In fact, an earlier study by [*Wen et al.*, 1996] using a recirculating CFF system with different ultrafilter materials found molecular weight shifts in trace metal colloids during storage at room temperature, indicating potential aggregation over the course of hours at room temperature. However, even within our own experiments, it would appear that aged samples do not perform as reliably in the CFF system compared to fresh samples, since reductions in recovery were observed for all of the dissolved metals measured in the Southern Ocean experiment as a result of sample storage. In contrast, CFF recovery was generally higher and overall much more constant in the higher temperature Galveston Bay samples. Notably, the differences in recovery under frozen or room temperature conditions was not statistically different for any of the metals, so while we suggest here that there is a sample temperature effect on CFF recovery, which may help explain the differences between the Southern Ocean vs. Galveston Bay results, we cannot conclusively prove temperature causation.

In summary, the differences in dissolved metal concentrations and size partitioning preservation between the Southern Ocean and Galveston Bay samples can likely be attributed to two major factors: natural physicochemical speciation differences between samples and ultrafiltration artifacts. Natural physicochemical speciation refers to the native physical and chemical forms that the colloids originally had in seawater. In the Southern Ocean, previous studies point to potential sedimentary sources and/or resuspension supplying both particulate and dissolved Fe to Palmer Canyon bottom waters [*Sherrell et al.*, 2018], as well as a high abundance of Fe-binding ligands with benthic sources [*Gerringa et al.*, 2008] that may serve to stabilize dFe. In contrast, in an estuarine environment such as Galveston Bay, we would expect many inorganic dFe nanoparticles coated in humic-like organics [*Powell et al.*, 1996; *Wen et al.*, 2008]. These two samples thus have quite different physicochemical metal speciation and thus it is not surprising that they behave differently upon preservation or when processed using our CFF system. Furthermore, prior studies have suggested that Fe solubility increases at colder temperatures and that dFe samples that were frozen and thawed have a higher solubility [*Schlosser et al.*, 2012]. In this case, Fe would be more prone to stay solubilized under frozen conditions but also natively in a cold climate. This would in turn play into any filtration artifacts, which may be influenced by the original physicochemical speciation of the sample, as well as how it behaves when aged/warmed over time [*Schlosser et al.*, 2011].

2. Colloid Exchangeability Experiment: Do colloids "grow back" after removal via ultrafiltration, and if so, how fast?

The goal of this experiment was to determine the rate of potential colloid re-formation (aggregation from soluble phase) at room temperature over 70 hours after all colloids were removed from a near-bottom Southern Ocean sample via ultrafiltration. Aggregation of material from the soluble to the colloidal size fraction could be stimulated by interactions with the bottle walls, organic coagulation [*Wilkinson et al.*, 1997], gel formation [*Chin et al.*, 1998], and/or the so-called "Brownian pumping" of soluble-sized species [*Honeyman and Santschi*, 1989; *Wen et al.*, 1997]. At each time point after colloids were removed from the permeate solution at T0, any potential adsorption to bottle walls was monitored by pouring off a subsample of the original permeate solution, and then this original permeate solution was also re-ultrafiltered to assess whether colloids had re-formed over time. If there was no generation of colloids, the re-ultrafiltered permeate should have equaled the "poured" permeate concentration at each time point. This was only true for dissolved Mn and Cd, which had negligible colloids in the original sample, and no additional colloids formed over the 70 hour time period that was studied (Figure 5d, h).

However, for Fe, Co, Ni, Cu, Zn, and Pb, additional colloids were generated by aggregation over the 70 hour experiment after they were removed at T0 (Figure 5). For Fe, the Southern Ocean sample originally had 58% of its dFe in the colloidal size fraction (cFe = 0.75 nmol/kg), and after the colloids were removed at T0, cFe returned to 40% of dissolved after only 14 hours (cFe = 0.21 nmol/kg) and eventually exceeded the original fraction to 74% after 70 hours (cFe = 0.46 nmol/kg). Additional results from an identical experiment at Station ALOHA (22.75°N, 158.00°W, 24 September 2013, 800 m; sampling methods in [*Fitzsimmons et al.*, 2015a]) demonstrated that Fe colloids can re-aggregate in as little as 3 hours and approach the initial % colloidal partitioning after only 22 hours (Figure 5e). This supports the conclusion that cFe aggregation occurs almost immediately and continues over time, likely attributed to interactions between the soluble and colloidal organic compounds that complex Fe in seawater, as well as possible self-assembly [*Ding et al.*, 2008]. While we do not have direct chemical speciation measurements at these near-bottom study sites, the only available literature data from surface waters of the West Antarctic Peninsula and coastal Antarctica indicate that ligands that may form complexes with Fe are present but are often saturated and thus not uniformly in excess of Fe in this region in the surface ocean [*Buck et al.*, 2010; *Thuróczy et al.*, 2012].

The colloidal Fe re-aggregation kinetics pattern was replicated for Ni and Zn, except that for Ni and Zn both the colloidal concentrations and % colloidal Ni and Zn exceeded the original levels by 14 and 24 hours, respectively; in contrast, it took Fe 70 hours to exceed the original 58% cFe. Thus, Ni and Zn were particularly affected by the removal of colloidal species at the start of the experiment, and their re-aggregation kinetics were especially fast. This must be related to Ni and Zn themselves and/or the organic ligands that bind them [*Van den Berg and Nimmo*, 1987; *Bruland*, 1989], since Fe, Cu, and Pb did not behave similarly in the same samples. For Cu and Pb, colloids did re-aggregate by 14 hours following colloid removal, but cCu never reached its original % colloidal Cu, even after 70 hours, while dPb quickly returned to the size partitioning of the original samples.

Thus, it appears that the metals studied here that have a substantial natural colloidal fraction in seawater (Fe, Cu, Zn, Ni, and Pb) will re-aggregate to form colloids in as short as 14 hours. However, the extent to which those colloids re-form varies, as often the re-formed % colloidal metal and the colloidal metal concentrations do not match the initial results. We hypothesize that organic aggregation/coagulation kinetics are involved, since the one element most likely to exist as a free cation (Mn, [*Oldham et al.*, 2017]) showed no colloidal character in these samples. Fe and Cu are both thought to be bound >99.9% by organic ligands in seawater [*Rue and Bruland*, 1995; *Wu and Luther III*, 1995; *Moffett and Dupont*, 2007], yet Cu did not re-form colloids to the same extent as Fe. Our knowledge of organic complexation of some of the other metals, like Zn, Cd, and Pb, however, is too poor to speculate further on the direct pathways of colloidal aggregation, except to say that organic aggregation is likely involved and our data demonstrate the extent to which these metals can re-aggregate.

3. Adsorption Experiment: How quickly are dissolved metals lost from solution to bottle wall adsorption in the absence of seawater acidification?

The goal of this experiment was to quantify the rate of adsorptive loss of metals onto LDPE bottle walls over 24 hours, which is a relevant timescale during which filtered seawater might be stored in holding bottles awaiting further processing. The results are shown in Figure 6, where the % dMe change from the initial concentration (indicated by a grey bar) are shown. Triplicate sampling allowed obviously contaminated samples to be removed from the averages used to calculate % metal loss to bottle walls.

Prior studies on bottles made of plastics other than LDPE indicate that the higher SA:V of smaller bottles will cause greater adsorptive Fe losses [*Fischer et al.,* 2007; *Fitzsimmons and Boyle,* 2012]. Indeed, the SA:V ratio of our 125 mL bottles is higher than our 250 mL bottles (0.838 cm^{-1} versus 0.760 cm^{-1}), providing more area per volume onto which metals can adsorb. The Adsorption Experiment data here on LDPE bottles show that, as expected, more adsorption occurred in the smaller 125 mL bottles compared to the 250 mL bottles for Co, Cu, Zn and Ni over 40 months. DFe also showed higher % loss onto the 125 mL bottles until 40 months, when surprisingly the adsorption onto 250 mL bottle walls (91% loss) surpassed that on the 125 mL bottle walls (75%). This could indicate that the dependence of bottle sorption on SA:V ratio is not the most significant factor in sorption, particularly when the SA:V ratios are comparable, as in our 125 and 250 mL bottles. However, these results do support prior suggestions that if storage in a plastic bottle over time is necessary, it is preferable to use larger bottle sizes (lower SA:V) to help reduce metal sorption losses to bottle walls.

Prior studies also suggest that metal sorption to bottle walls appears to be affected by bottle material. For example, Fe adsorbed the most over ~72 hours in quartz bottles (99%, [*Fischer et al.,* 2007]), compared to HDPE bottles (29-60%, [*Schlosser et al.,* 2011; *Fitzsimmons and Boyle,* 2012]), and PTFE bottles (40-60%, [*Schlosser and Croot,* 2008]) in prior studies. Adsorption onto LDPE bottles had not been explored previously, despite their being the bottle of choice for marine trace metal studies since the GEOTRACES era [*Cutter et al.,* 2010], yet the second result of our Adsorption Experiment showed that LDPE bottle walls did promote some wall adsorption of dFe over 24 hours, which continued until 40 months after sample collection for both sizes. Our data show that sorption losses from unacidified samples to LDPE bottles are generally low over the first 24 hours and only statistically significant for dissolved Fe, Co, Ni, Zn, and Cd after 40 months (Figure 6). Previously, dFe was adsorbed to HDPE bottle walls at a ratio of 0.878% per SA:V (cm^{-1}) ratio [*Fitzsimmons and Boyle,* 2012] over 15.5 hours, which would predict that 0.20 and 0.15 nmol/kg of dFe would have adsorbed onto 125 mL and 250 mL HDPE bottle walls by 16 hours. However, only 0.16 and 0 nmol/kg were actually adsorbed, respectively, onto the LDPE bottle walls used in this study after 16 hours, indicating that adsorption of metals to LDPE bottle walls is lower than adsorption to HDPE bottle walls, even with identical acid cleaning.

The third result of the Adsorption Experiment was that wall adsorption to LDPE bottles can now be extended to other metals beyond Fe, with significant losses observed over just 16 hours for dissolved Co, Ni, Cu, Zn, and Cd (Figure 6). While wall losses of dFe have been studied in the past, losses of the other metals have been poorly constrained in any bottle type. Of the available data, one study showed that dCu had an adsorptive loss of 1.6% onto fluorinated ethylene (poly)propylene Nalgene bottles over the course of 2 hours, while dCd showed almost no adsorption in seawater samples spiked with Cu, Cd, and Pb [*Cuculić and Branica*, 1996]. No conclusive kinetic data for dissolved Ni, Mn, Zn, or Co exists from prior studies despite the fact that we now routinely collect and measure these dissolved metals in seawater. In this study, dissolved Fe, Co, and Zn showed the largest losses over 40 months in both bottle sizes, ranging from 22 to 91% loss (Figure 6 a,b,c). Ni and Cu also showed modest losses over this timescale (6 to 8% and 4 to 9% loss, respectively), despite showing almost no loss for Ni after 24 hours. Wall losses of dissolved Mn and Cd were negligible (<5%), even after being left unacidified for 40 months.

4. Desorption Experiment: Once samples have been acidified, how quickly do metals desorb from bottle walls?

The goal of this experiment was to examine the kinetics of metal desorption from 125 and 250 mL LDPE bottle walls and thus assess how long samples that are thought to have suffered losses to bottle walls must be acidified to desorb the metals. We set up both the Adsorption and Desorption Experiments using seawater from the same 100 m depth in the Arctic Ocean so that we could compare concentrations at time points in both experiments to the same concentration (T0 of adsorption experiment, Figure 6, grey bar), which was the initial filtered seawater acidified immediately upon collection. The Desorption Experiment bottles were left unacidified for 22 months during which time metals were allowed to adsorb onto the bottle walls.

The Adsorption Experiment showed that dissolved Fe, Co, Ni, Cu and Zn were significantly adsorbed to LDPE bottle walls after spending 40 months unacidified; however, the Desorption Experiment showed that the desorption kinetics of each of these metals back to their initial concentration varied significantly. For instance, 30-40% of the dissolved Fe and Co that had adsorbed over 40 months desorbed immediately, but remained 7-19% lower than their initial concentrations 20 weeks after acidification (Figure 6a, b); thus, we never recovered all of the lost Fe or Co. DFe also showed some temporally variable desorption or exchange, as it desorbed to within 11% of the initial concentration by 12 weeks only to fall again to 19% below the initial concentration at 20 weeks. In contrast, dissolved Ni, Cu, and Zn all desorbed back to within ±10% of the original sample concentration immediately upon acidification. However, all three were still significantly different from the initial concentration after 20 weeks in the 250 mL bottles only. Dissolved Cd and Mn had insignificant desorption rates, but they also had insignificant adsorption to bottle walls, indicating that the timescale of acidification plays little to no role in the storage and analyses of these two metals.

Discussion and Implications

Based on the experimental results described above, we first make several recommendations for the collection and handling of dissolved and particularly colloidal trace metals samples in seawater. Then, we discuss some implications for the physicochemical speciation of dissolved metals in the ocean.

We recommend that:

1. Seawater should only be preserved for Fe size partitioning, not the size partitioning of any of the other metals, and only if stored seawater samples are preserved frozen at -20°C immediately upon collection;
2. Careful attention be given to the source and thus potential native conditions/physicochemical speciation of samples analyzed for metal size partitioning, as this appears to affect the preservation of metal speciation over time as well as colloid recovery during the ultrafiltration process;
3. Filtered seawater samples that require ultrafiltration should be ultrafiltered as soon as possible but no later than 24 hours after initial filtration in order to avoid wall adsorption to bottle walls and/or exchange between the soluble and colloidal size fractions;
4. Seawater samples that will be stored in 125 mL or 250 mL LDPE bottles should be acidified as soon as possible (within a few hours at most) to avoid adsorptive wall losses of dissolved Fe and Co, which are difficult to recover;
5. Seawater samples that have suffered significant wall losses should not be analyzed for dissolved Fe or Co concentrations unless they have been acidified for at least 30 weeks, which is 1.5 times the maximum desorption timescale that we tested for dissolved Fe and Co;

6. If analyzing metals other than dissolved Fe and Co, 12 weeks of acidification is sufficient for complete desorption of most metals, and analysis after shorter timescales is acceptable if samples were not left unacidified for very long.

Additionally, looking across the experiments, a few conclusions about natural marine colloidal metal size partitioning can also be made. First, the colloidal preservation experiment showed contrasting size partitioning and preservation behavior of metals collected from different locations (West Antarctic Peninsula vs. Galveston Bay), suggesting that the inherent differences in physicochemical speciation at different locations largely affect the kinetics of metal exchangeability in seawater. As noted above, this is particularly noticeable in the preservation of dZn size partitioning (Figure 4d), where a statistically significant aggregation into colloids in both the frozen and room temperature treatment was observed in the Southern Ocean bottom water, while there was no discernible change in the % cZn over the same timescale in Galveston Bay estuarine surface water. This pattern was also true for Cu and Ni (Figure 4 b-c), indicative of a more stable equilibrium between the soluble and colloidal phases in Galveston Bay waters.

We also found that colloids tended to be more abundant in Galveston Bay, consistent with the literature [*Guo and Santschi*, 1997; *Wen et al.*, 1999], suggesting a permanent sedimentary, riverine, or *in situ* flocculation source that maintains the presence of colloids at this estuarine site. The fact that metal preservation was so consistent in Galveston Bay, with little apparent wall loss or exchange, indicates that the ligands or other compounds involved in forming colloidal metals in Galveston Bay are very different from those in the Southern Ocean samples. The prior work of *Wen et al.* [1999] in the Galveston Bay estuary showed high % colloids for Fe, Cu, Zn, and Pb (55 to 91%) as well as an average 55% contribution of colloids to the organic carbon phase into which many metal-binding ligands might fall. *Wen et al.* [1999] further estimated that only 1% of the surface sites in the very abundant colloidal organic carbon (COC) phase would be sufficient to complex all trace metals, which is supported by a strong correlation between COC and colloidal Co, Ni, Cu, Zn, Cd. *Wen et al.* [1999] also report a large contribution of freshwater colloids associated with both metal and organic carbon phases in Galveston Bay, which is important as river water appears a major component of the Galveston Bay water collected during this study (salinity 26.5). This contribution from rivers has been shown to be a significant source of colloidal metals such as Fe, Ni and Cu in particular (Powell et al., 1996), which when combined with the increased colloidal complexation capacity observed between COC and some metals, may be responsible for the different partitioning behaviors we observed in our two preservation experiments.

Second, we may interpret the differences in colloidal behavior in the Southern Ocean vs. Galveston Bay experiments as anecdotal evidence that cold seawater samples do not perform as well in the CFF system. As noted above, the temperature and salinity of a sample will have an effect on the overall initial physicochemical speciation of the sample. For example, the Southern Ocean metals other than Fe, in general, did not have well preserved size partitioning, while Galveston Bay (25°C warmer and fresher) showed much better stability of size partitioning of these same metals over time. Additionally, the frozen and then thawed samples showed relatively poorer recovery in the Galveston Bay experiment, perhaps a function of having been stored at a colder temperature. Previous studies have shown that frozen and thawed samples may increase the solubility of Fe over time [*Schlosser et al.*, 2011], which would have a direct effect on speciation measurements and recovery.

Third, while it is common practice to freeze and thaw unacidified samples as a way of preserving DOC concentrations [*Fellman et al.*, 2008], ligand concentrations [*Apte et al.*, 1990], and even chemical speciation of trace metals when immediate analyses are not possible [*Capodaglio et al.*, 1995; *Buck et al.*, 2012], there may actually be artifacts in physicochemical speciation that arise simply from the freeze-thaw process itself. Previous studies have found that some sampling and storage artifacts, such as bottle adsorption, are in fact increased under cold conditions [*Fitzsimmons and Boyle*, 2012]. While we observed no change in dFe concentration or size partitioning using frozen samples over 3 weeks in the Colloid Preservation Experiments, we cannot rule out that the process of freezing and thawing may have some effect on the kinetics of exchange between soluble and colloidal compounds in any seawater sample. This could include the possibility of self-aggregation or self-assembly [*Ding et al.*, 2008; *Verdugo et al.*, 2008] that may be a side effect of the freezing process or a natural function of these organic colloids.

Fourth, the results of all of our experiments show that the kinetics of colloidal exchange and metal adsorption are variable across different metals. Many factors contribute to this variability, such as as the chemistry of the metal, the characteristics of the solution (in this case unacidified seawater) including organic ligand concentrations, the properties of the container, and other external factors such as temperature [*Massee et al.*, 1981]. We can apply some of these parameters directly to our study. For instance, adsorption to plastics such as polyethylene is initially motivated by hydrophobic and/or van der Waal's attraction of metal complexes with the hydrophobic plastic surface, perhaps encouraging greater adsorption of metals bound by organic compounds with hydrophobic "tails." Indeed, metals like Fe, Co, Zn, and Cu, all of which are organically complexed in seawater [*Bruland et al.*, 2013], showed more significant (4-91%) adsorption in our experiments over 40 months and even 24 hours compared to metals commonly found as free ions in seawater like Mn [*Byrne*, 2002]. However, experiments from decades ago have shown that sustained cationic adsorption to plastic surfaces is a product of ion exchange with hydroxide groups in the inner charged layer of polyethylene's double charged surface layer

[*Beneš and Smetana*, 1969]. Thus, it is expected that hydrolyzed metals will adsorb more rapidly compared to free ions [*Starik et al.*, 1963; *James and Healy*, 1972b] [*Starik et al.*, 1963; *James and Healy*, 1972a], leading us to hypothesize that hydrolysable metals or metals with significant organic complexation, like Fe, will show a greater loss to bottle walls over time. Addtionally, organic complexation may indicate a proclivity to enter the colloidal phase [*Dammshäuser and Croot*, 2012], as demonstrated for metals such as Fe where colloids are a significant component of the dissolved phase [*Fitzsimmons and Boyle*, 2014a]. There is often more consistent partitioning between soluble and colloidal phases for organically complexed metals such as Ni and Cu across the global ocean (Jensen and Fitzsimmons, unpublished). Thus, any ingrowth of colloids over 70 hours is significant. Likewise, metals such as Mn that typically exist largely or entirely in the soluble phase showed no colloidal growth and were preserved for the dissolved phase under frozen conditions.

Finally, very little work, to the authors' knowledge, has directly examined metal desorption kinetics from sample bottle walls, although early studies stressed the importance of acid cleaning sampling containers and materials before use to prevent desorption of metal contaminants from bottle manufacture [*Batley and Gardner*, 1977; *Ross*, 1986]. It is well known that protons significantly disrupt the binding of metals to common ligand groups [*Doucet et al.*, 2007], making acidification commonplace in long-term sample storage. We found that while adsorption was significant for Fe and Co over even 24 hours, desorption remained slow and incomplete after 20 weeks. In contrast, Cu did not show significant adsorption, but also did not completely desorb over the full 20 weeks. Therefore, we recommend preventing samples from experiencing significant adsorption to bottle walls, since it can be hard to recover these metals even after extended time acidified. In the event that bottle wall adsorption does occur, we recommend allowing samples to sit acidified for at least 30 weeks in order to maximize the probability of metal remobilization.

Conclusions

The accurate determination of trace metal concentrations and physicochemical speciation in seawater has progressed over the last decades as clean sampling and storage procedures have been increasingly optimized. However, there are still challenges and unanswered questions regarding filtration and sample processing that warrant further examination, particularly in the context of multi-elemental analyses. For instance, while ultrafiltration to determine the colloidal concentrations of trace metals has received increased attention in the oceanographic literature ([*Guo and Santschi*, 2007] and references therein), the timescale of storage prior to filtration and the potential effects this has on measured size fractionation remain poorly known. Additionally, important contributing factors to measuring dissolved and colloidal metal concentrations over time will include sampling and storage artifacts such as adsorption to bottle walls. Combining these issues, this study sought to answer four major questions: 1) Can we preserve the size partitioning of trace metals over time under frozen or room temperature conditions?, 2) Do colloids "grow in" over time following removal, and if so, how fast?, 3) How fast do metals adsorb to bottle walls in the absence of acidification?, and 4) Once samples are acidified, how quickly do metals desorb from bottle walls? The results of these experiments allowed us to make several recommendations involving sample storage and proposed timing of ultrafiltration and analyses. For example, it is evident from the Colloid Preservation Experiment that only the size partitioning of Fe (and perhaps labile Co) can be preserved under frozen conditions. Notably, this experiment also demonstrated that the natural physicochemical speciation of waters will give rise to differential exchange between the soluble and colloidal phases and thus effect their ability to be preserved.

In the Colloid Exchangeability Experiment, it was clear that in most cases metal colloids (once removed) do grow back in via aggregation from the soluble phase, often exceeding the initial colloidal fraction. Prior work suggested that metal-organic complexation in seawater may impart a proclivity for trace metals to form colloids [*Dammshäuser and Croot*, 2012]. Thus it is not surprising that Fe, Ni, Cu and Zn, which are all thought to be organically complexed in seawater, regenerated colloids in as short as 14 hours, whereas Mn, which is thought to exist mostly as a free cation and weak chloride complexes in solution, did not aggregate into colloids. It is difficult to conclude from this study why colloids might re-form and reach higher proportions compared to their original natural partitioning, but future work might combine chemical speciation measurements with these colloid re-aggregation kinetics to investigate the mechanisms responsible for re-aggregation.

The results of the Bottle Adsorption and Desorption experiment simply add to what we already knew about adsorption kinetics of Fe, namely that the adsorption rate is dependent on both bottle material and surface-to-volume ratio It is evident that smaller bottles with higher SA:V ratios promote more sorption, not just for Fe, but also for Co, Ni, Cu and Zn. We also saw less sorption using LPDE bottles compared to previous studies such as [*Fischer et al.*, 2007; *Schlosser et al.*, 2011; *Fitzsimmons and Boyle*, 2012] that used HDPE bottles. Even so, over 24 hours, dissolved Fe, Co, Cu, and Zn all showed significant adsorption to LDPE bottle walls, demonstrating that any further sample processing, such as ultrafiltration, should occur as soon as possible after sample collection in order to avoid metal adsorption artifacts.

Based on the results of the desorption experiment we conclude that 20 weeks (5 months) may not be long enough to completely desorb dissolved Fe and Co (the two metals that were the most susceptible to adsorption) from LDPE bottles. However, it is important to note that our samples were stored unacidified for 22 months prior to acidification, which is much longer than most oceanographers wait to acidify after sample collection. It would be informative to conduct experiments to learn whether storage time prior to acidification plays a role in setting the desorption time scale. Finally, because we observed different behaviors and different kinetics for different metals in different water types, our recommendations on how to avoid artifacts in trace metal speciation measurements should be supplemented with further experimentation under one's "local" conditions of water chemistry and temperature when sample processing and sample acidification cannot be conducted as soon as possible after collection.

Acknowledgements

We would like to thank the Captain and crew of the USCGC *Healy,* the ARSV *Gould*, and the R/V *Trident*; Dave Kadko, Greg Cutter, Hugh Ducklow, and Robert Sherrell for cruise leadership and sampling opportunities; Gabi Weiss, Simone Moos, Amber Annett, Janelle Steffen, and Nate Lanning for sample collection at sea and processing ashore; and Luz Romero for assistance with ICP-MS analyses. This work was supported by NSF OCE 1434493 and 1713677 to JNF, NSF OCE 1355833 to WML (*Healy)*, a Texas A&M T3 Triad to JNF (*Trident*), and a Texas Sea Grant "Grants-In-Aid of Graduate Research" award 2018SP-S-Jensen-GIA (*Trident*) to LTJ.

Figure captions

Table 1. Summary of SAFe D1, blank, and limit of detection (LOD) results from this study compared to the May 2013 consensus values reported by GEOTRACES. All values are reported in nmol/kg.

Figure 1. Schematic of the Colloid Preservation Experiment methods. All samples came from the same, homogenized seawater sample (a single Niskin-X bottle in the Southern Ocean or a carboy in Galveston Bay). Blue bottles indicate samples frozen at -20°C, while black bottles indicate samples kept at room temperature in the dark. "Pour" indicates samples that were directly poured from the corresponding 250 mL sample. "CFF" indicates when samples were ultrafiltered to permeate (P) or retentate (R) solutions. The Southern Ocean experiment had three timepoints: T0, T = 7 days, T = 21 days, while the Galveston Bay experiment had four timepoints: T0, T = 2 days, T = 9 days, T = 23 days.

Figure 2. Schematic of the Colloid Exchangeability Experiment methods. All samples came from the same, original Niskin-X 12 L bottle. "Pour" indicates that samples were directly poured from the corresponding 2 L sample. "CFF" indicates when samples were ultrafiltered to permeate (P) or retentate (R) solutions. All time points originated from the same, homogenized 2L permeate sample, which had the colloids removed by ultrafiltration at T = 0.

Figure 3. Schematic of the Adsorption (left) and Desorption (right) Experiment methods. The water for both was taken from the same depth but two separate GO-Flo bottles. The time points for the Adsorption Experiment are in hours, except for the final time point of 40 months. Dark blue bottles indicate transfer to new, acid-cleaned bottles. Red (outlined) bottles indicate acidification. Note that the adsorption experiment was performed in triplicate while the desorption experiment was performed in duplicate, both over two bottle sizes: 125 mL and 250 mL.

Figure 4. Summary of all results of the Colloid Preservation Experiment. The left hand and right hand columns represent the initial Southern Ocean and subsequent Galveston Bay experiments, respectively. The legend for all treatment types is included in panel (a), where black is the initial seawater sample. The black line separates where the left-hand axes (concentration) are applicable to the “Dissolved” and “Permeate” groups of data from where the right-hand axes (percentage) are applicable to the “% Colloids” and “% Recovery” groups of data. The red error bars represent standard deviation over replicate bottles within a given time point. The frozen 9 day time point in the Galveston Bay experiment are removed for permeate concentration, % colloids and % recovery due to clear contamination of the permeate solution across all metal types. * indicates a result that is significantly different from the initial (T0) result, used a two-tailed heteroscedastic t-test ($p<0.05$).

Figure 5. Summary of all results of the Colloid Exchangeability Experiment across all metals. The legend for all treatment types is included in panel (a), where black bars are the initial permeate solution concentration re-poured at each subsequent time point to monitor for wall loss (compared to the white bar at T0, which is the initial permeate soluble metal concentration). White bars at non-zero timepoints are the re-ultrafiltered soluble concentrations, and grey bars are the re-ultrafiltered colloidal concentrations, obtained by subtracting the white bar from the black bar. Overlaid in red is the % colloidal metal (cMe/dFe; right axis) at each time point. The T0 time point contains the initial size partitioning of the seawater sample, and the initial % colloids is indicated by the dashed red line. Panel E shows unpublished data for Fe from a prototype of this experiment using water from 800 m depth at Station ALOHA, showing similar trends for Fe across a comparable timescale.

Figure 6. Results from the Adsorption (left) and Desorption (right) Experiments. Water was collected at 100 m depth in the Arctic Ocean and filtered immediately upon collection and stored

in the dark at room temperature for 22 months. The grey bar indicates the average value of the initial dissolved metal concentration for the seawater used in these experiments, and the width reflects the standard deviation in those initial measurements. 125 mL sample treatments are shown as a closed circle, while 250 mL sample treatments are shown as an open circle. The annotations refer to the % change in concentration from the average initial dMe concentration (grey bar) at select time points. * indicates a result that is statistically significant from the initial (T = 0) adsorption result (grey bar), used a two-tailed heteroscedastic t-test ($p<0.05$). Asterisk above the data is for 250 mL treatment and below is for the 125 mL treatment. Pb has been excluded to due poor experimental reproducibility.

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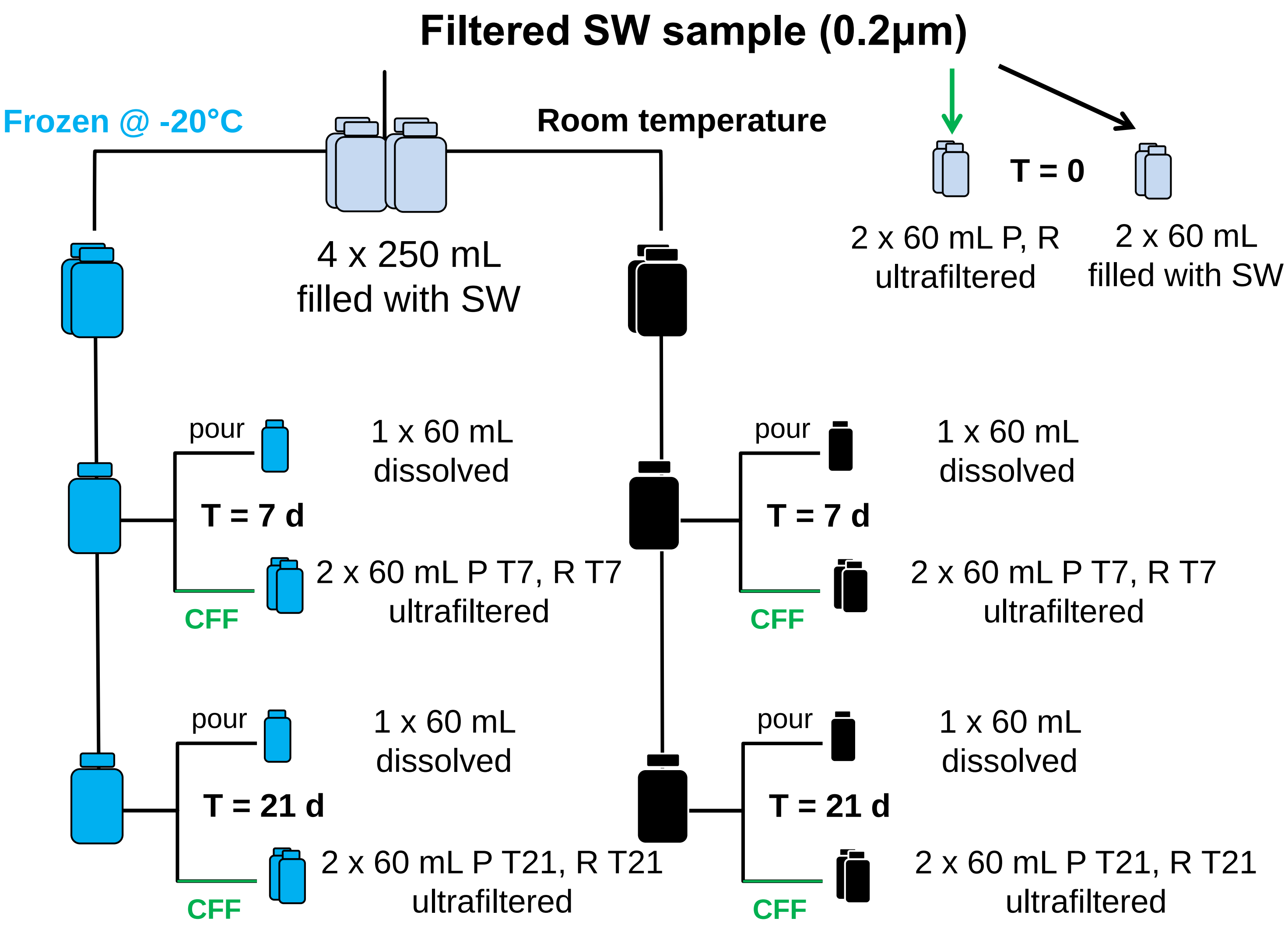
Figure

Parameter	**dFe**	**dZn**	**dNi**	**dCu**	**dCd**	**dPb**	**dMn**	**dCo**
SAFe D1 mean	0.604	7.395	8.584	2.087	1.045	0.023	0.409	0.032
Stdev	0.063	0.212	0.098	0.063	0.006	0.003	0.009	0.001
n	8	10	10	10	10	10	9	8
Consensus	0.670	7.400	8.580	2.270	0.991	0.0277	0.35	0.045
Consensus stdev	0.040	0.350	0.260	0.110	0.031	0.0026	0.05	0.005
Mean blank	0.056	0.069	0.014	0.008	0.0003	0.0002	0.002	0.0004
Blank stdev	0.010	0.011	0.003	0.003	0.0001	0.00004	0.001	0.0002
LOD	0.031	0.033	0.010	0.008	0.0002	0.00012	0.002	0.0005
n	4	4	4	4	4	4	4	4

Figure 1



Colloid Preservation Experiment

Figure 2

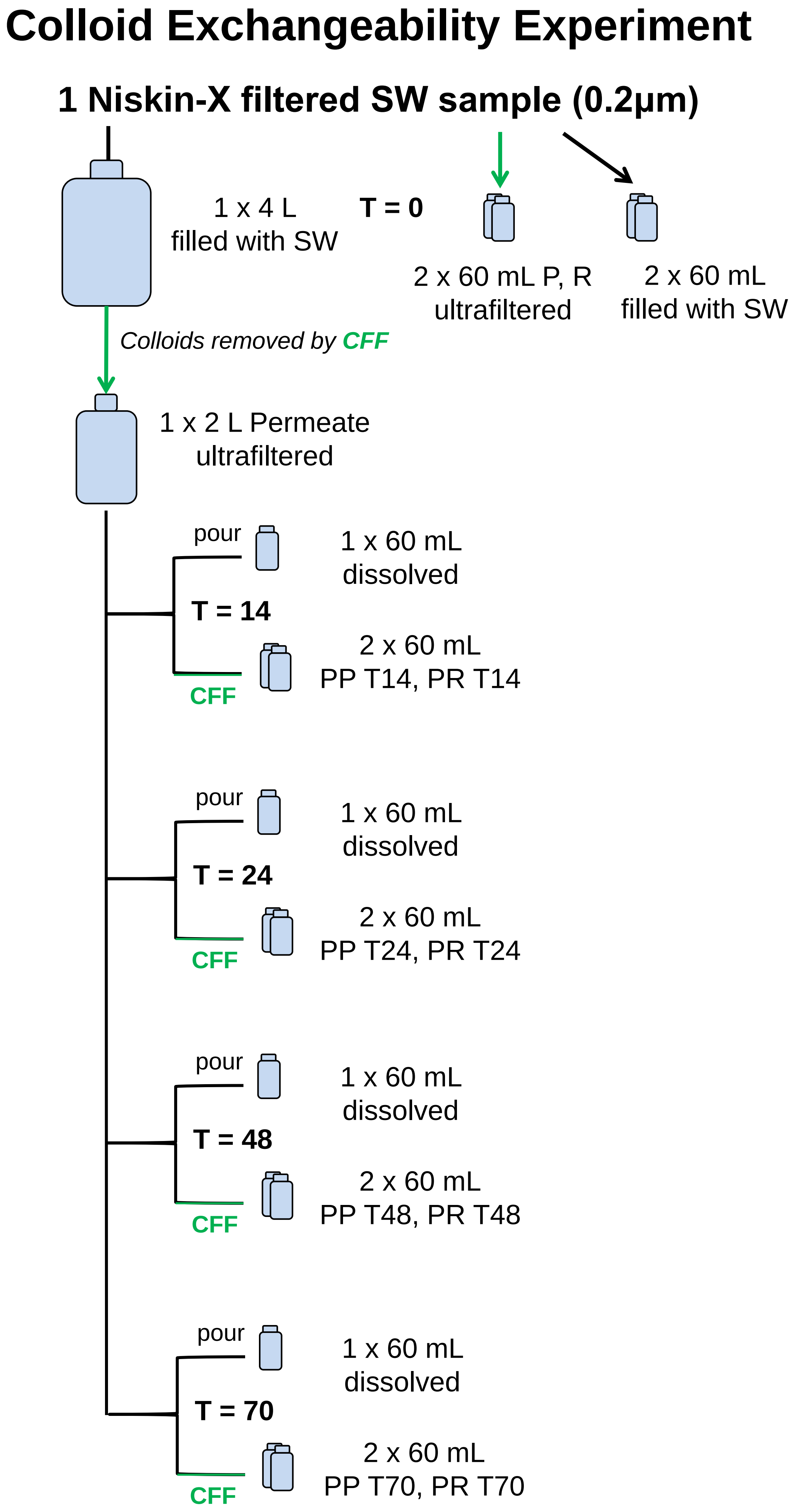

Colloid Exchangeability Experiment
1 Niskin-X filtered SW sample (0.2µm)
1 x 4 L
filled with SW
T = 0
2 x 60 mL P, R
ultrafiltered
2 x 60 mL
filled with SW
Colloids removed by CFF
1 x 2 L Permeate
ultrafiltered
pour
1 x 60 mL
dissolved
T = 14
2 x 60 mL
PP T14, PR T14
CFF
pour
1 x 60 mL
dissolved
T = 24
2 x 60 mL
PP T24, PR T24
CFF
pour
1 x 60 mL
dissolved
T = 48
2 x 60 mL
PP T48, PR T48
CFF
pour
1 x 60 mL
dissolved
T = 70
2 x 60 mL
PP T70, PR T70
CFF

Figure 3

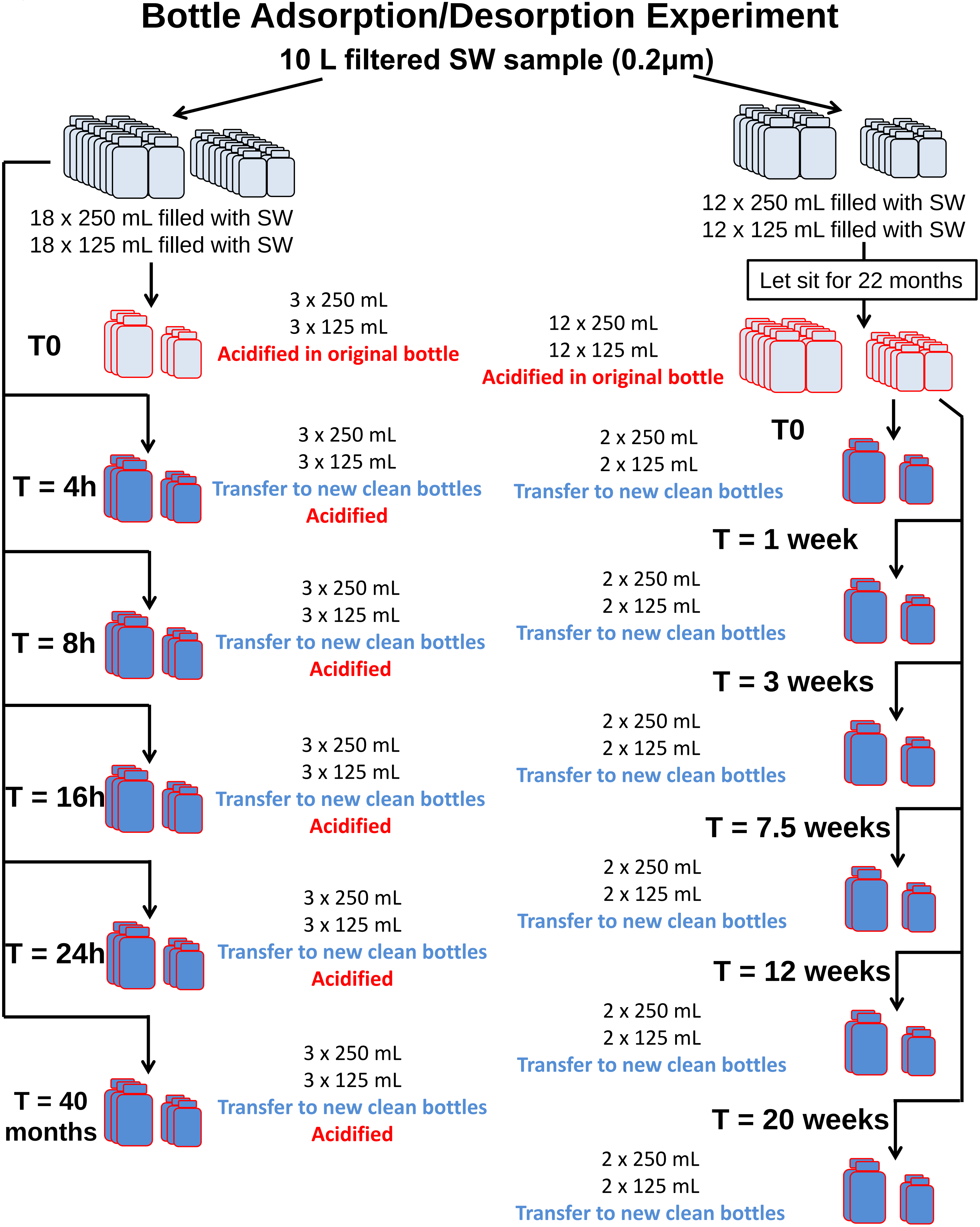
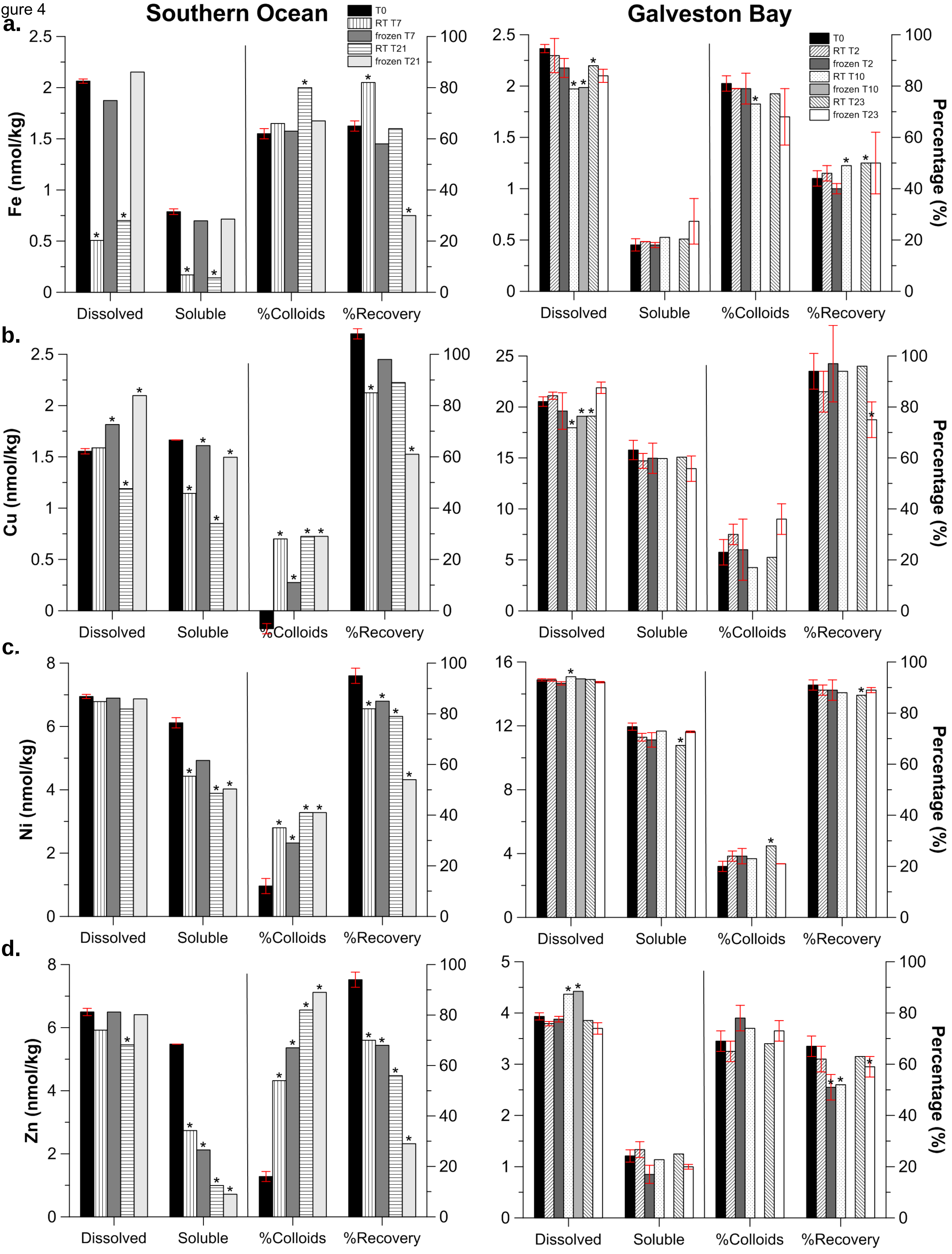

Bottle Adsorption/Desorption Experiment
10 L filtered SW sample (0.2μm)
18 x 250 mL filled with SW
18 x 125 mL filled with SW
T0
3 x 250 mL
3 x 125 mL
Acidified in original bottle
T = 4h
3 x 250 mL
3 x 125 mL
Transfer to new clean bottles
Acidified
T = 8h
3 x 250 mL
3 x 125 mL
Transfer to new clean bottles
Acidified
T = 16h
3 x 250 mL
3 x 125 mL
Transfer to new clean bottles
Acidified
T = 24h
3 x 250 mL
3 x 125 mL
Transfer to new clean bottles
Acidified
T = 40 months
3 x 250 mL
3 x 125 mL
Transfer to new clean bottles
Acidified
12 x 250 mL filled with SW
12 x 125 mL filled with SW
Let sit for 22 months
12 x 250 mL
12 x 125 mL
Acidified in original bottle
T0
2 x 250 mL
2 x 125 mL
Transfer to new clean bottles
T = 1 week
2 x 250 mL
2 x 125 mL
Transfer to new clean bottles
T = 3 weeks
2 x 250 mL
2 x 125 mL
Transfer to new clean bottles
T = 7.5 weeks
2 x 250 mL
2 x 125 mL
Transfer to new clean bottles
T = 12 weeks
2 x 250 mL
2 x 125 mL
Transfer to new clean bottles
T = 20 weeks
2 x 250 mL
2 x 125 mL
Transfer to new clean bottles

Figure 4


Southern Ocean
Galveston Bay
a.
b.
c.
d.
T0
RT T7
frozen T7
RT T21
frozen T21
T0
RT T2
frozen T2
RT T10
frozen T10
RT T23
frozen T23
Fe (nmol/kg)
Cu (nmol/kg)
Ni (nmol/kg)
Zn (nmol/kg)
Percentage (%)
Dissolved
Soluble
%Colloids
%Recovery
*

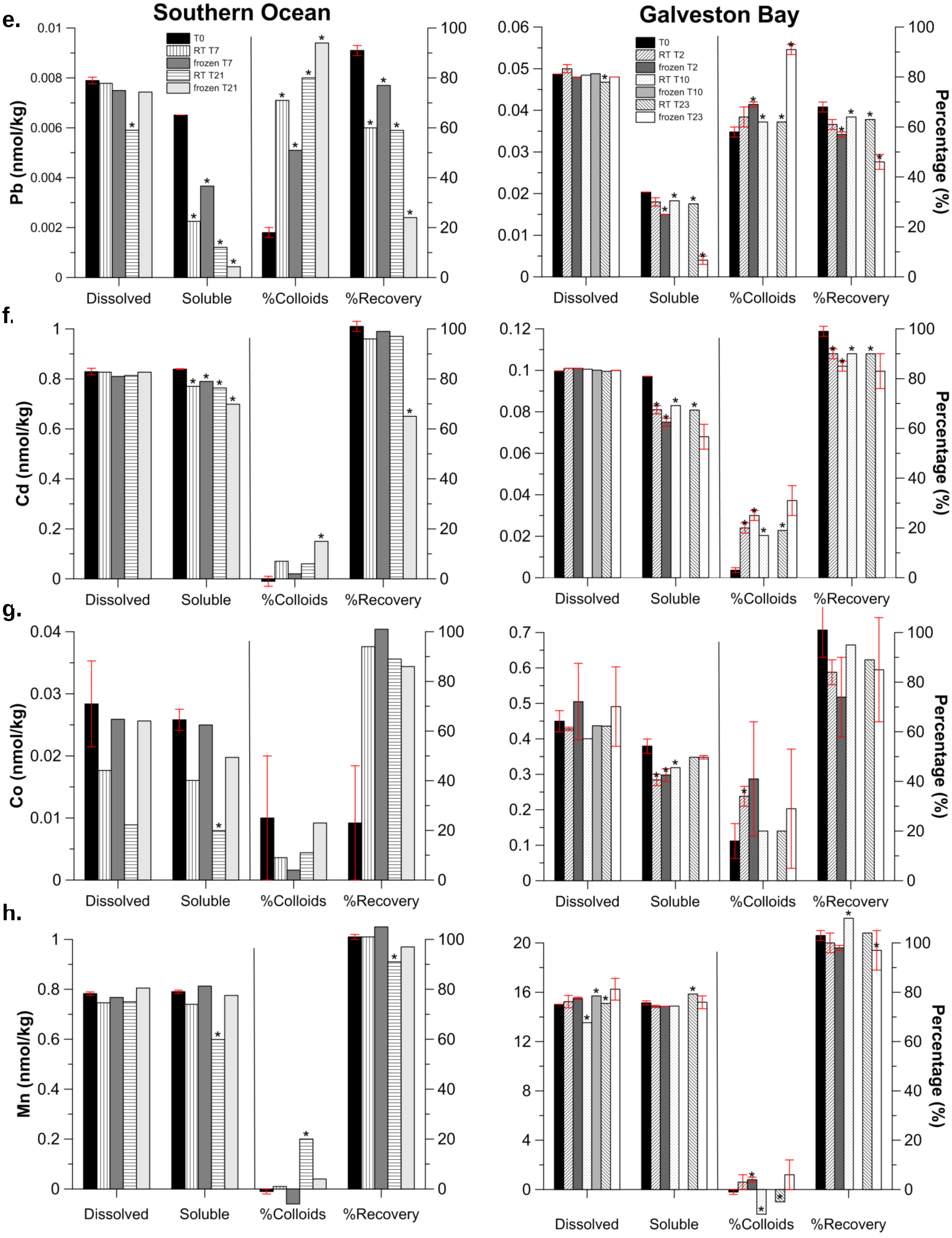

e.
Southern Ocean
Galveston Bay
Pb (nmol/kg)
T0
RT T7
frozen T7
RT T21
frozen T21
RT T2
frozen T2
RT T10
frozen T10
RT T23
frozen T23
f.
Cd (nmol/kg)
g.
Co (nmol/kg)
h.
Mn (nmol/kg)
Percentage (%)
Dissolved
Soluble
%Colloids
%Recovery
*

Figure 5

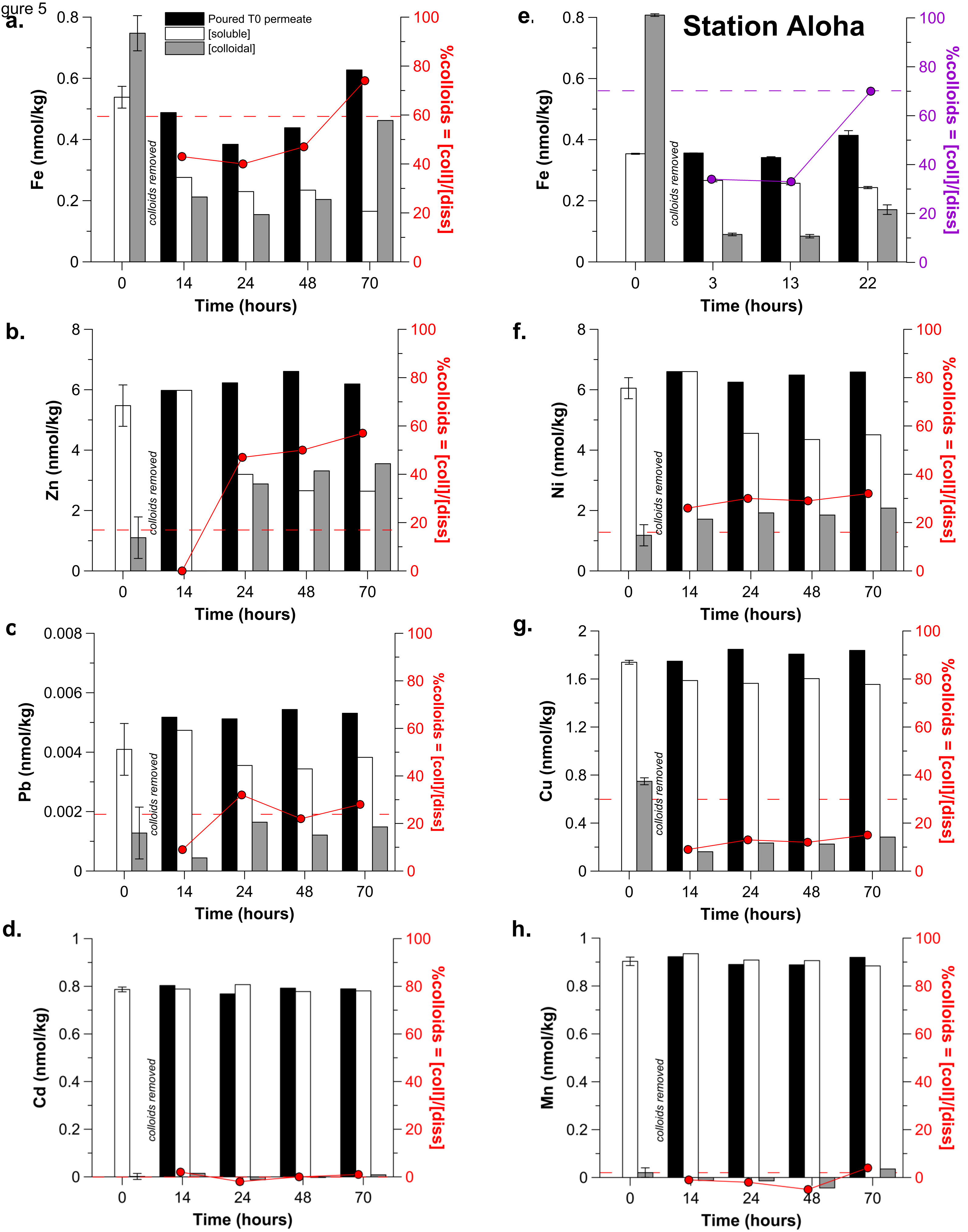
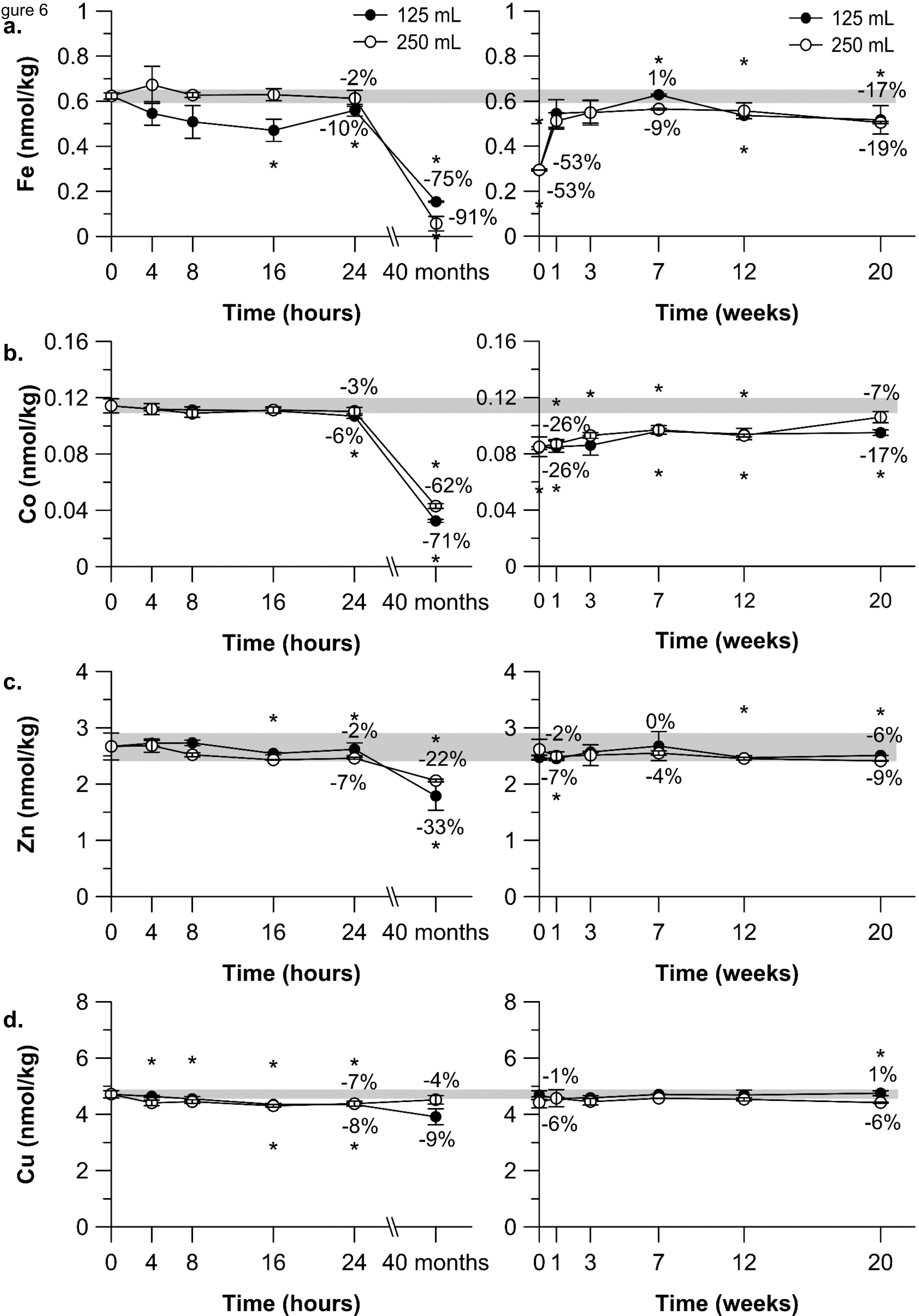
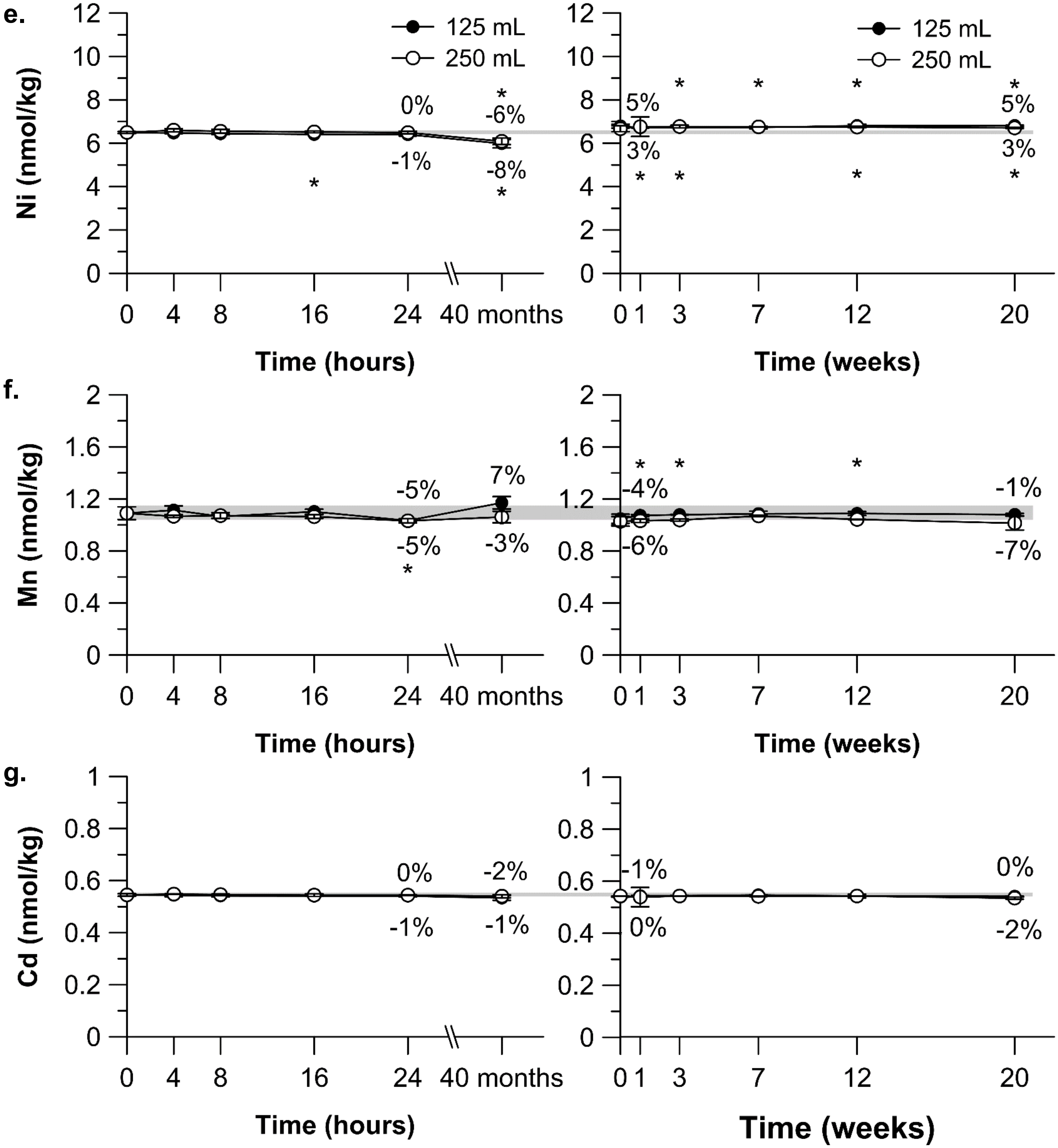

a.
Poured T0 permeate
[soluble]
[colloidal]
Fe (nmol/kg)
0
0.2
0.4
0.6
0.8
%colloids = [coll]/[diss]
0
20
40
60
80
100
colloids removed
0
14
24
48
70
Time (hours)
e.
Station Aloha
Fe (nmol/kg)
0
0.2
0.4
0.6
0.8
%colloids = [coll]/[diss]
0
20
40
60
80
100
colloids removed
0
3
13
22
Time (hours)
b.
Zn (nmol/kg)
0
2
4
6
8
%colloids = [coll]/[diss]
0
20
40
60
80
100
colloids removed
0
14
24
48
70
Time (hours)
f.
Ni (nmol/kg)
0
2
4
6
8
%colloids = [coll]/[diss]
0
20
40
60
80
100
colloids removed
0
14
24
48
70
Time (hours)
c
Pb (nmol/kg)
0
0.002
0.004
0.006
0.008
%colloids = [coll]/[diss]
0
20
40
60
80
100
colloids removed
0
14
24
48
70
Time (hours)
g.
Cu (nmol/kg)
0
0.4
0.8
1.2
1.6
2
%colloids = [coll]/[diss]
0
20
40
60
80
100
colloids removed
0
14
24
48
70
Time (hours)
d.
Cd (nmol/kg)
0
0.2
0.4
0.6
0.8
1
%colloids = [coll]/[diss]
0
20
40
60
80
100
colloids removed
0
14
24
48
70
Time (hours)
h.
Mn (nmol/kg)
0
0.2
0.4
0.6
0.8
1
%colloids = [coll]/[diss]
0
20
40
60
80
100
colloids removed
0
14
24
48
70
Time (hours)

Figure 6


a.
Fe (nmol/kg)
125 mL
250 mL
-2%
-10%
-75%
-91%
1%
-9%
-53%
-53%
-17%
-19%
*
0 4 8 16 24 40 months
Time (hours)
0 1 3 7 12 20
Time (weeks)
0 0.2 0.4 0.6 0.8 1
b.
Co (nmol/kg)
0 0.04 0.08 0.12 0.16
-3%
-6%
-62%
-71%
-26%
-26%
-7%
-17%
c.
Zn (nmol/kg)
0 1 2 3 4
-2%
-7%
-22%
-33%
-2%
-7%
0%
-4%
-6%
-9%
d.
Cu (nmol/kg)
0 2 4 6 8
-7%
-8%
-4%
-9%
-1%
-6%
1%
-6%


e.
Ni (nmol/kg)
125 mL
250 mL
0%
-1%
*
-6%
-8%
*
5%
3%
*
*
*
*
*
*
*
5%
3%
*
Time (hours)
0
4
8
16
24
40 months
Time (weeks)
0 1 3 7 12 20
f.
Mn (nmol/kg)
-5%
-5%
*
7%
-3%
*
-4%
-6%
*
*
-1%
-7%
Time (hours)
Time (weeks)
g.
Cd (nmol/kg)
0%
-1%
-2%
-1%
-1%
0%
0%
-2%
Time (hours)
Time (weeks)