The distribution of bioavailable copper in Puget Sound surface waters

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Abstract

Surface water samples were collected from distinct locations throughout Puget Sound and from the Lake Washington-Lake Union-ship canal area. Samples were analyzed using ICP-MS and CLE/AdCSV for particulate copper, dissolved copper, free copper, and organic copper-binding ligands. Copper concentrations were highest in South Sound and the shore of Portage Bay and lowest in Admiralty Inlet. Admiralty Inlet had the lowest concentrations of ligands, leaving a greater proportion of its dissolved copper in the free, toxic form relative to dissolved copper in South Sound and at the shore of Portage Bay. Relative to the pelagic North Pacific Ocean, only Admiralty Inlet showed similar concentrations of particulate copper while particulate copper in South Sound and the shore of Portage Bay was nearly ten times higher.

Introduction

Trace metals such as manganese, iron, cobalt, nickel, zinc, cadmium, and copper play important biological roles, often by forming cofactors in enzymes and providing structure to proteins. The micronutrient copper (Cu) is present at trace concentrations (< 0.1 μmol/L) in surface waters due to uptake by plankton and continuous export of Cu-containing biomass to deeper waters. Marine organisms use trace amounts of Cu in several cellular processes including nitrification, methane oxidation, and organic nitrogen utilization (Morel and Price 2003). In phytoplankton, Cu is the metal center in metalloproteins such as plastocyanin, cytochrome oxidase, and multicopper ferroxidase (Twining and Baines 2013).

Due to Cu's scarcity in the open ocean, organisms have evolved efficient mechanisms for concentrating it (Morel and Price 2003). Therefore, even though Cu is an essential micronutrient for prokaryotes and eukaryotes, it can also be toxic to marine and aquatic organisms even at extremely low concentrations. Seawater Cu concentrations higher than those to which organisms are adapted can be extremely detrimental. For example, cyanobacteria are particularly sensitive to Cu toxicity, having evolved in a sulfidic ocean with Cu concentrations much lower than those observed today (Saito et al. 2003).

Cu toxicity affects a variety of organisms. Concentrations of free, unbound Cu^{2+} as low as 10^{-12} mol/L can be toxic to phytoplankton, with a spectrum of sensitivity ranging from cyanobacteria, having the greatest sensitivity, to diatoms, having the least (Brand et al. 1986). The larvae of several zooplankton are also susceptible to Cu^{2+} toxicity at a concentration of 10^{-11} mol/L (Buck and Bruland 2005). Juvenile salmonids exhibit avoidance behavior when exposed to sub-lethal concentrations of dissolved Cu in both seawater and freshwater (Sommers et al. 2016), and modeling suggests that, with chronic early life stage exposure to these concentrations, body size is reduced (O'Neill et al. 2015). In freshwater, the olfactory capacity of salmon, essential for detection of predators and prey, is reduced by short-term exposure to Cu (O'Neill et al. 2015). Cu toxicity thus has the potential to endanger the survival of a variety of economically and ecologically important marine organisms within Puget Sound.

It is necessary to understand Cu speciation, or the distribution of Cu amongst chemical species, in order to evaluate its bioavailability and toxicity. Dissolved Cu binds with a variety of compounds including organic and inorganic ligands (ions or molecules which bind to transition metal ions to form complexes). In seawater, > 99% of the total dissolved Cu (as well as other trace metals including iron, cobalt) is bound to organic complexes (Fig. 1; Millero et al. 2009).

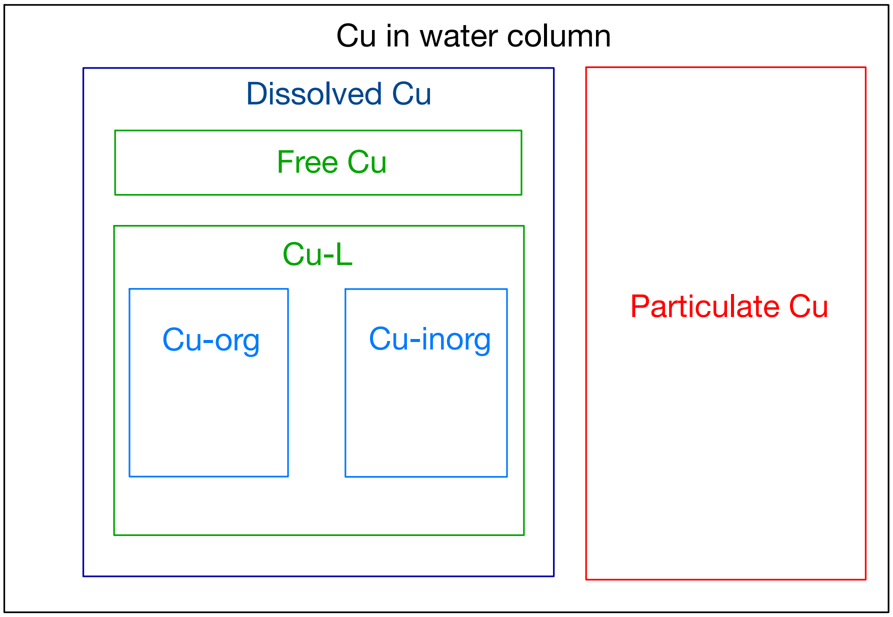

Fig 1. Pools of Cu (proportions of boxes not to scale). Cu may be particulate or dissolved (in this study, < 0.2 µm

pore diameter). Dissolved Cu may be a free ion ("Free Cu") or part of a complex with ligands ("Cu-L"). Cu-ligand complexes may include organic ligands ("Cu-org") or inorganic ligands ("Cu-inorg").

The most bioavailable form of dissolved Cu is the free, uncomplexed ion, Cu^{2+}. Cu toxicity is thus a function of the concentration of the labile or free fraction of dissolved Cu, and not of the total dissolved Cu. Ligand binding strength varies, and weakly-bound Cu-ligand complexes can also comprise part of this labile fraction of total Cu. Strong Cu-ligand complexes, in contrast, are difficult to take up by microorganisms due to thermodynamic or kinetic constraints (Millero et al. 2009). If most of the dissolved Cu is in an inert form, there is no threat of Cu toxicity even with a high concentration of total dissolved Cu. Although Cu^{2+} is the most bioavailable form of Cu, much of the dissolved Cu in seawater is complexed by organic ligands. In a study of Cu complexation in the Northeast Pacific, for example, over 99.7% of total dissolved Cu shallower than 200 m was bound to organic ligands (Coale and Bruland 1988). Strong Cu-binding organic ligands tend to dominate Cu speciation in estuarine and coastal waters. In this case, most of the dissolved Cu is tied up in a form that is inert relative to the free Cu^{2+} ion and inorganic Cu complexes (Buck and Bruland 2005) and thus relatively non-toxic to marine organisms. Particulate Cu is another important source of bioavailable Cu because it can become available to phytoplankton within days. Cu in this phase is solubilized in certain redox conditions or as a result of processes such as photochemistry, binding to other soluble phases, and organic matter remineralization (Berger et al. 2008). Thus, in order to determine the amount of total bioavailable Cu, it is necessary to measure several concentrations: the concentration of total dissolved Cu, the concentration of ligands bound to that Cu (in order to then determine the portion of free, uncomplexed and weakly-bound Cu), and the labile portion of particulate Cu.

Unfortunately, national regulations for Cu toxicity are based only on the concentration of total dissolved Cu (Buck and Bruland 2005).

Despite the majority of dissolved Cu being associated with organic ligands, small changes to the free Cu can have large effects on toxicity. The threshold for Cu toxicity varies between phytoplankton phyla (Buck and Bruland 2005; Millero et al. 2009), and a Cu^{2+} concentration of 10^{-11} mol/L can inhibit the growth rates of cyanobacteria, begin to reduce the viability of phytoplankton species including diatoms, and evoke signs of sensitivity in zooplankton larvae (Buck and Bruland 2005). This concentration is a useful and broadly applicable Cu toxicity threshold in the environment.

The threat of Cu toxicity is elevated in estuaries such as the Puget Sound where total dissolved Cu may be higher due to anthropogenic inputs. Cu is used in building materials, automobile parts, pesticides, plumbing components, and as a toxicant in anti-fouling paint for boat hulls (O’Neill et al. 2015). In the Puget Sound, a region subjected to ongoing urbanization, there is potential for Cu toxicity due to elevated non-point sources of Cu from brake pads and subsequent runoff and from anti-fouling paint. Despite Cu’s harmful biological effects and prevalence as a pollutant, knowledge of Cu distributions and especially of the effects of ligand complexation on Cu toxicity in the Puget Sound is incomplete. Previous studies have assessed the distribution of Cu in central Puget Sound sediments (Bloom and Crecelius 1987) and discovered strong complexation of Cu^{2+} with organic ligands in Hood Canal of Puget Sound (Jacquot et al. 2014). However, data is lacking on surface water concentrations of dissolved Cu in the greater Puget Sound region.

This study investigates the distribution of dissolved and particulate Cu in surface waters of Puget Sound and the Lake Washington-Lake Union-ship canal area with respect to proximity to point sources and residence times (flushing rates) of water. Even at high Cu concentrations, adequate concentrations of organic Cu-binding ligands have been shown to buffer Cu toxicity (Earley et al. 2014). Therefore, since Cu speciation is so instrumental in determining its toxicity, a second objective of the study is to investigate how free Cu concentrations vary with organic ligand concentrations. It is predicted that total dissolved Cu and particulate Cu concentrations will be higher at sites proximal to point sources of Cu with high residence times than at sites distal to point sources of Cu with low residence times. As a secondary hypothesis, it is predicted that free Cu ion (Cu^{2+}) concentrations will decrease with increasing concentrations of organic Cu-binding ligands. Consequently, even in areas with high concentrations of total dissolved Cu, the presence of high concentrations of Cu-binding ligands may buffer or eliminate Cu toxicity.

Methods

Oceanographic context/sampling region

A total of six surface seawater samples were collected from distinct locations throughout Puget Sound ranging from South Sound to Central Sound. Five of the samples were collected aboard the R/V *Rachel Carson* on cruises RC0016 and RC0017 on January 11th to 18th, 2019 (Fig. 2). The vessel was equipped with a CTD, and measurements of salinity, temperature, pH, and florescence were taken at every location sampled. Samples were collected from sites in South Sound, Lake Washington, Lake Union, Portage Bay, and Admiralty Inlet. The final sample was taken near the shore of Portage Bay in a small marina and was collected by hand on February 7th.

Fig. 2. (Top) map of all sampling sites and (bottom) a zoomed in view of Lake Washington, Portage Bay and Lake Union sampling sites.

Sample name	Location	Latitude	Longitude	Date collected
SB01	South Sound	47° 4' 14.89" N	122° 54' 35.43" W	1/13/19
SB02	Lk. Washington	47° 37’ 49.18” N	122° 15’ 37.76” W	1/11/19
SB03	Lk. Union	47° 38’ 14.27” N	122° 20’ 04.83” W	1/11/19
SB04	Portage Bay	47° 38’ 55.31” N	122° 18’ 49.76” W	1/11/19
SB05	Admiralty Inlet	47° 56' 0.50" N	122° 29' 40.68" W	1/18/19
SB06	Portage Bay shore	47° 39' 2.81" N	122° 18' 49.73" W	2/7/19

Table 1. Dates and locations of sample collection.

Sample collection and storage

A trace metal clean pole sampler was used to collect samples from the ship’s rail during the cruises. The pole sampler was equipped with an acrylic support plate for the bottle and was fixed to the end of a fiberglass pole (Fig. 3). Samples were stored in 500 mL fluorinated low-density polyethylene (FLDPE) bottles and filtered within a week of collection using 47 mm polycarbonate track etch (PCTE) filters with a pore diameter of 0.2 µm (Pall Corporation). The filters were stored dry and frozen at -20 °C for later particulate leaching. 60 mL of filtrate was acidified to pH 1.8 with 2 mL/L (120 µL) concentrated optima HCl (Fisher Scientific) and saved for later analysis of dissolved trace metals. The remainder of filtrate (about 440 mL) was stored frozen at -20 °C in 500 mL FLDPE bottles and saved for later voltammetric analysis of Cu-binding ligands (Fig. 4.)

Fig. 3. Pole sampler in use to collect surface seawater samples from the R/V *Rachel Carson*. Photo by Julian Sachs.

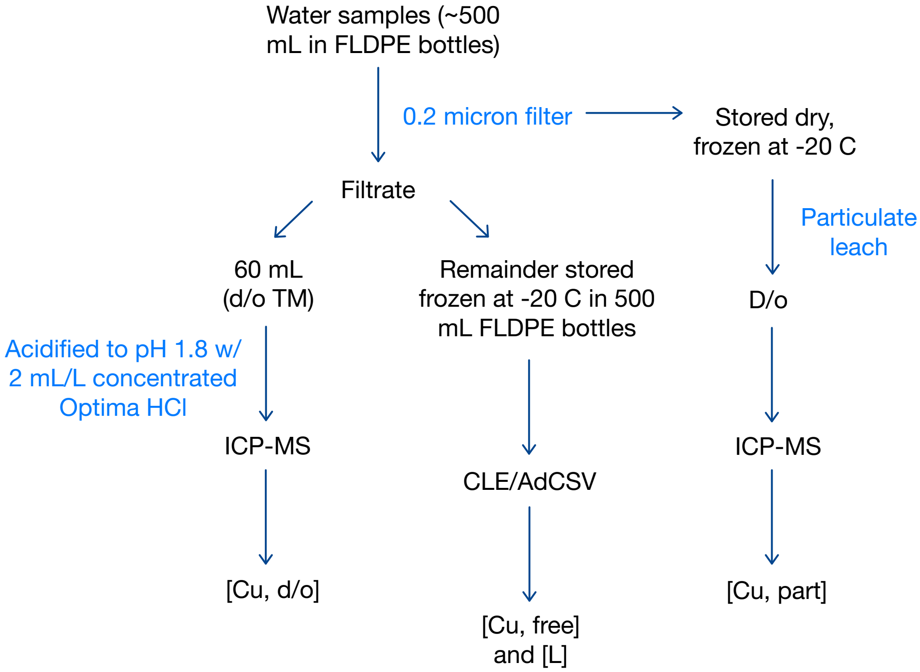

Fig. 4. Flow chart of sample analysis. D/o = dissolved, part = particulate, L = ligands, TM = trace metals.

Sample analysis

Particulate metals

Particulate trace metals on the 0.2 μm filters were digested using a weak acid leach (25% HAc) with a mild reducing agent (0.02 M hydroxylamine hydrochloride) and a short heating step (10 min at 90°C) (Fig. 4). This "HAc_{T+R}" leach modifies the leach of Chester and Hughes (1967) and was found to solubilize more of the labile particulate metal phase than the original leach (Berger et al. 2008).

The particulate leach was performed for ten samples: six seawater samples (collection and storage described above), three filter blanks, and one sample of Arizona test dust as a reference material. Filters from seawater samples, filter blanks, and the reference material were placed into 15 mL acid-cleaned centrifuge tubes and 5 mL of leach solution (25% HAc + 0.02 M hydroxylamine hydrochloride) was added to each sample. A hot block was heated to 90°C and samples were heated for 10 minutes, then allowed to sit for 3 hours and 50 minutes at room temperature. Samples were then centrifuged for five minutes and syringe-filtered (one for each seawater sample, one for the reference material, and one for all three blanks) with acid-cleaned syringes twice, prior to being placed on the hot block at 135°C until the supernatant evaporated (about 15 hours). Leach reagents preparation and leach performance were conducted in accordance with "HAc leaches of reference materials for dust" (Rachel Shelley, pers. comm).

The evaporated particulate samples were re-suspended in 9.6 mL HNO_3^-, the ICP-MS carrier solution, and 200 μL In and 200 μL Rh were added as internal standards for a final volume of 10 mL. In addition to the leached particulate samples, three blanks and four mixed element standards were analyzed via ICP-MS. Internal consistency blanks and internal consistency standards (“ccb” and “ccv”) were made to account for instrument drift and were analyzed between every ~10 samples. Trace metals were analyzed using an iCap-RQ in KED mode and data was quantified using a standard curve.

Dissolved metals

The filtered seawater samples that had been acidified to pH 1.8 were analyzed for dissolved trace metals (Fig. 4). 200 µL Rh was added as an internal standard to 15 mL of each sample, and samples were pre-concentrated via seaFAST, a trace metal clean automated preconcentration system. The seaFAST system concentrated 10 mL of sample after being buffered to pH ~6 onto a Nobias PA1 resin, and then eluted 200 µL of sample using 5% HNO_3^-. The eluent was diluted to a final volume of 3 mL and was analyzed on the iCap-RQ in the same way as the particulate samples.

Organic Cu speciation

Samples from South Sound, Admiralty Inlet, and the shore of Portage Bay were chosen to be analyzed for Cu-binding ligands and $[Cu]_{free}$ using competitive ligand exchange/adsorptive cathodic stripping voltammetry (CLE/AdCSV) (Fig. 4). In this method, the well-characterized ligand salicylaldoxime (SA) is added to the sample to compete with natural ligands for complexation with Cu. Cu that is bound to SA absorbs to the mercury drop electrode, but the natural ligands do not. Natural Cu-binding ligands are titrated with incrementally-added Cu, until all of the natural ligands are bound with Cu. At this point, a linear relationship between current and added Cu indicates when the natural ligands have been out-competed by SA (Buck and Bruland 2005). Any deviation from linearity represents competition with SA for the added Cu.

For each of the three samples, 10 to 12 standard additions of Cu were used to create a titration curve. An initial potential of -150 mV was applied for a 300 s deposition time while the sample was stirred, followed by 15 s of quiet time with no stirring and then a -600 mV final potential. As the potential changed, the change in cathodic stripping current resulting from reduction of free Cu in the Cu-SA complex was recorded. Peak heights were obtained using ECDsoft and ProMCC was used to calculate $[Cu]_{free}$, natural Cu-binding ligand concentrations, and ligand binding strengths (Omanović et al. 2015). Each sample was solved for either one or two ligand classes and whichever result gave the lowest root mean square error was chosen. For all samples analyzed, the smallest error was found by solving for a single ligand class.

Results

ICP-MS measurements of particulate and dissolved metals

Location	**Station**	**Concentration (nmol/L)**								
		[Cu]	**[V]**	**[Mn]**	**[Fe]**	**[Co]**	**[Ni]**	**[As]**	**[Cd]**	**[Pb]**
South Sound	SB01	37.22	51.56	539.35	15497.20	2.00	1.58	7.71	0.17	7.47
Lake Washington	SB02	8.11	7.20	1089.68	4714.22	0.83	3.02	4.06	0.05	5.22
Lake Union	SB03	17.91	11.69	1107.88	6352.62	1.07	3.63	5.85	0.07	11.85
Portage Bay	SB04	19.90	10.85	1145.02	5526.63	1.04	4.94	4.25	0.06	7.77
Admiralty Inlet	SB05	4.00	7.73	691.61	1577.59	1.46	0.74	1.11	0.15	2.65
Portage Bay shore	SB06	43.84	9.74	627.36	5590.04	0.71	3.78	5.99	0.07	15.94
North Pacific		4.45			0.54	0.02	2.79		0.08	0.05

Table 2. Concentrations in nmol/L of particulate trace metals measured by ICP-MS. North Pacific concentrations are measured “acid dissolvable” trace metals at the surface, averaged across all stations (Ezoe et al. 2004).

Location	Station	Concentration (nmol/L)										
		[Cu]	[V]	[Cr]	[Mn]	[Fe]	[Co]	[Ni]	[Zn]	[As]	[Cd]	[Pb]
South Sound	SB01	2.05	4.02	0.03	3.12	5.34	0.05	0.74	3.72	0.01	0.04	0.01
Lake Washington	SB02	2.44	1.03	0.03	0.03	3.81	0.01	0.57	1.54	0.01	0.00	0.01
Lake Union	SB03	3.09	1.01	0.03	0.08	6.81	0.01	0.57	2.36	0.01	0.00	0.02
Portage Bay	SB04	2.61	0.96	0.03	0.04	4.72	0.01	0.54	1.33	0.01	0.00	0.01
Admiralty Inlet	SB05	0.90	4.29	0.03	0.44	3.86	0.02	0.88	0.84	0.01	0.05	0.01
Portage Bay shore	SB06	6.47	1.05	0.03	0.52	10.92	0.01	0.64	6.78	0.01	0.00	0.05
North Pacific		3.42				0.52	0.01	2.73	3.67		0.05	0.04

Table 3. Concentrations in nmol/L of dissolved trace metals measured by ICP-MS. North Pacific concentrations are measured dissolved trace metals at the surface, averaged across all stations (Ezoe et al. 2004).

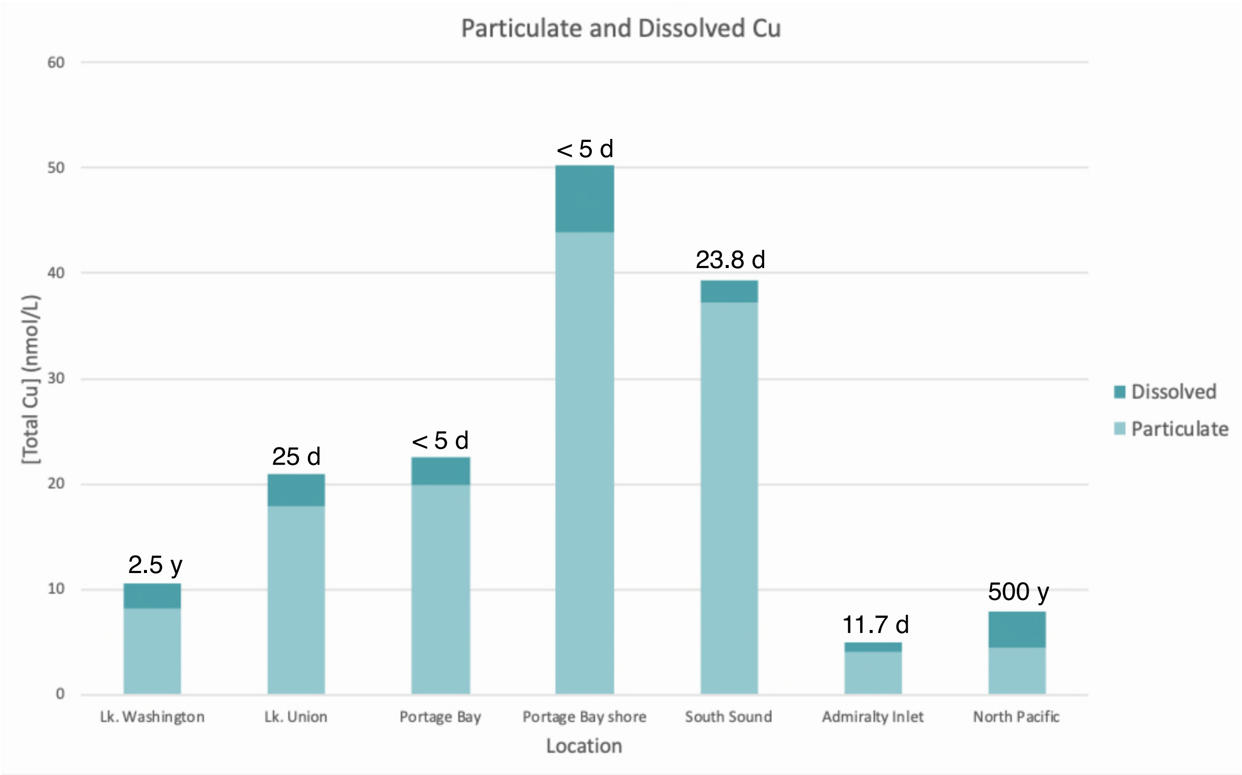

Fig. 5. Concentrations in nmol/L of dissolved and particulate Cu at each station, measured by ICP-MS. North Pacific values are measured dissolved and "acid dissolvable" (particulate) trace metals at the surface, averaged across all stations (Ezoe et al. 2004). Approximate residence times are given above the bar of the corresponding location (King County 2003, 2018; Babson et al. 2006).

Particulate Cu ranged from 4.00 to 43.84 nmol/L and was lowest in Admiralty Inlet and highest in South Sound and the Portage Bay shore (Table 2.) A similar pattern was observed in the dissolved Cu concentrations except for in South Sound, where concentrations were similar to those observed in Lake Washington, Lake Union, and Portage Bay (Fig. 5, Table 3).

Location	[Cu] (nmol/L)	% Particulate	% Dissolved
South Sound	39.27	94.79	5.21
Lk. Washington	10.55	76.90	23.10
Lk. Union	20.99	85.29	14.71
Portage Bay	22.51	88.41	11.59
Admiralty Inlet	4.90	81.60	18.40
Portage Bay shore	50.31	87.13	12.87
Open ocean	7.87	52.38	47.62

Table 4. Concentration of total Cu (dissolved plus particulate) measured by ICP-MS and percent belonging to the particulate or dissolved phase. Most of the Cu measured was derived from the particulate phase. The North Pacific percentages were originally described as a ratio of acid dissolvable to dissolved concentrations averaged throughout the water columns studied, equal to 1.1 ± 0.3 (n = 177).

Most of the Cu measured by ICP-MS was derived from the particulate phase (Fig. 5, Table 4). The fraction of total Cu found in the particulate and dissolved phases varied between samples, but particulate Cu always represented at least 76.90% of the analyzed Cu (Table 4). The lowest dissolved Cu was 0.90 nmol/L in Admiralty Inlet. This concentration was over six times lower than the highest concentration, 6.47 nmol/L at the Portage Bay shore. The lowest particulate Cu (4.00 nmol/L) was also found in Admiralty Inlet. This concentration was over nine times lower than the concentration found in South Sound (37.22 nmol/L) and almost 11 times lower than the concentration found at the Portage Bay shore (43.84 nmol/L).

CLE/AdCSV measurements of Cu and Cu-binding ligands

Sample	Location	[Cu] (nM)	$[\mathrm{Cu}]_{\mathrm{free}}$ (M)	$-\log[\mathrm{Cu}]_{\mathrm{free}}$	% free	[L1] (M)	log(K1)
SB01	South Sound	2.05	2.37E-15	14.62	1.16E-04	4.05E-08	13.54
SB05	Admiralty Inlet	0.90	2.42E-15	14.62	2.68E-04	9.72E-09	13.82
SB06	Portage Bay shore	6.47	6.23E-15	14.21	9.63E-05	3.94E-08	13.72

Table 5. Concentrations of total Cu in nmol/L, concentrations of free Cu in mol/L, concentrations of free Cu expressed as -log([$\mathrm{Cu}_{\mathrm{free}}$]) where [$\mathrm{Cu}_{\mathrm{free}}$] is in mol/L, percent of free Cu comprising the total Cu, concentrations of ligands in mol/L, and ligand binding strengths expressed as log(K_1). $[\mathrm{Cu}]_{\mathrm{free}}$ includes the free ion Cu^{2+} and inorganic complexes containing Cu^{2+}.

CLE/AdCSV revealed concentrations of total organic Cu-binding ligands and ligand binding strengths, allowing calculation of the concentrations of free Cu (Cu^{2+}), the most toxic form of Cu. Due to the high volumes needed and the lengthy nature of this analysis, samples from three distinct locations were chosen to be analyzed. Samples from South Sound, Admiralty Inlet, and Portage Bay shore were selected based on the ICP-MS results. South Sound and Portage Bay shore showed the highest total Cu concentrations (39.27 and 50.31 nmol/L, respectively), while Admiralty Inlet showed the lowest total Cu concentration (4.90 nmol/L).

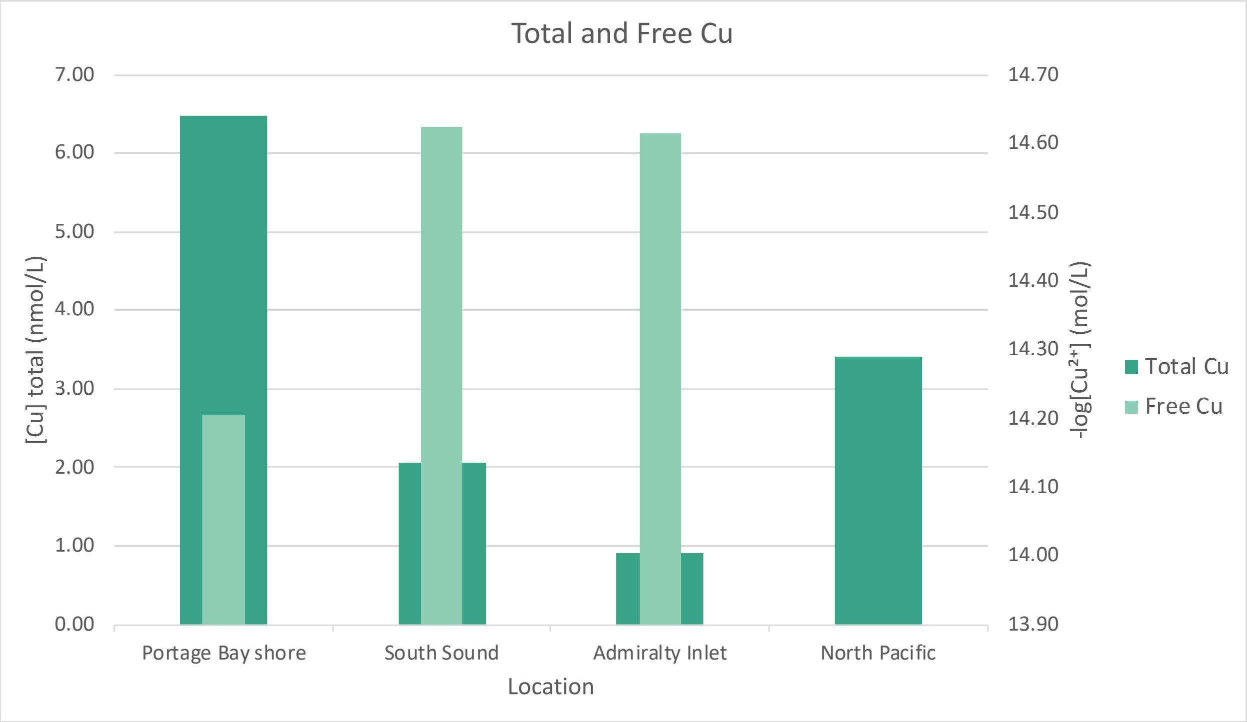

Fig. 6. Concentrations in nmol/L of total dissolved Cu (left axis) and free Cu (right axis, expressed as -log([Cu_{free}]) where [Cu_{free}] is in mol/L) at each station, measured by CLE/AdCSV. While total Cu varies significantly between stations, levels of free Cu are nearly equivalent at South Sound and Admiralty Inlet. North Pacific concentrations is measured dissolved Cu at the surface, averaged across all stations (Ezoe et al. 2004).

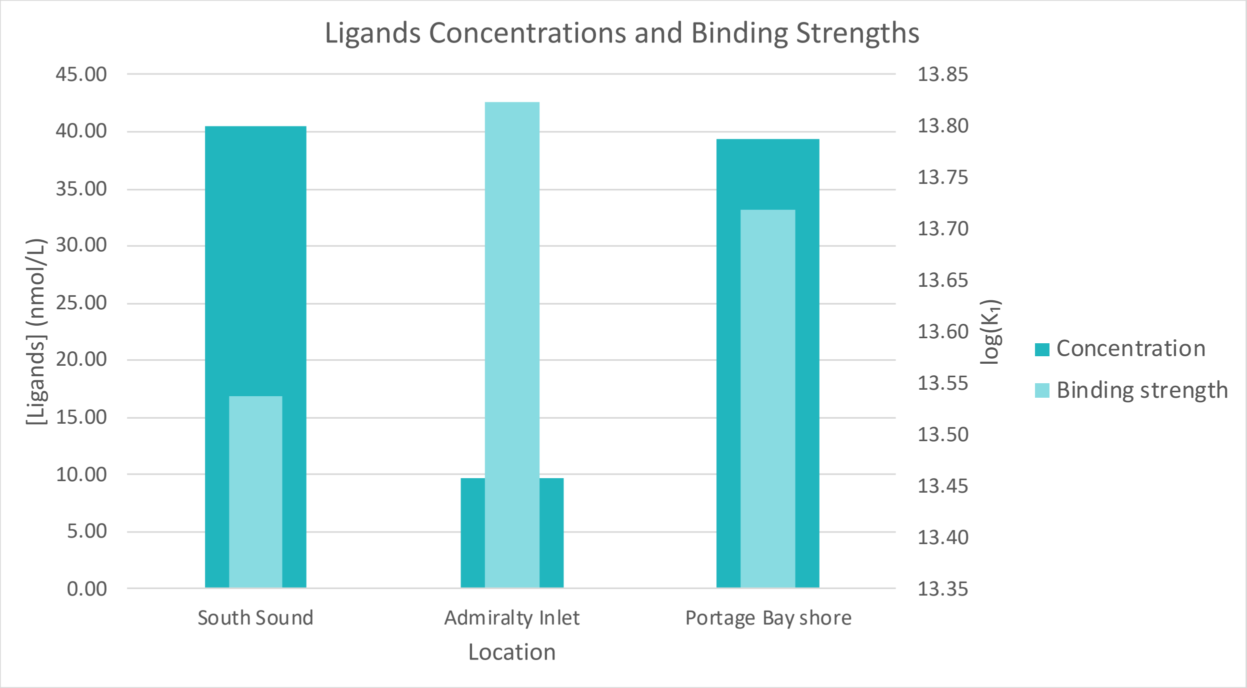

Fig. 7. Concentrations in nmol/L of Cu-binding ligands at three stations are plotted on the left axis. Ligand binding strengths are expressed as log(K_1) and plotted on the right axis. Ligand concentrations are very similar at South Sound and Portage Bay shore, but these measurements deviate significantly at Admiralty Inlet. Binding strengths do not vary much between stations.

Whereas total dissolved Cu in South Sound was 2.05 nmol/L, more than double the 0.90 nmol/L found in Admiralty Inlet, free Cu concentrations at these stations were nearly identical ($2.37*10^{-15}$ mol/L at South Sound and $2.42*10^{-15}$ mol/L at Admiralty Inlet) (Table 5). Compared to these stations, the Portage Bay shore sample showed much higher concentrations of both total Cu, at 6.47 nmol/L, and free Cu, at $6.23*10^{-15}$ mol/L (Table 5, Fig 4).

Concentrations of Cu-binding ligands in South Sound and Portage Bay shore ($4.05*10^{-8}$ and $3.94*10^{-8}$ mol/L, respectively) were over four times higher than the concentration found in Admiralty Inlet ($9.72*10^{-9}$ mol/L). [L_1] in Table 5 denotes that only a single class of ligands was detected and its concentration measured. Ligand binding strengths did not vary much between stations; for South Sound, Admiralty Inlet, and Portage Bay shore, $\log(K_1)$ values were 13.54, 13.82, and 13.72, respectively (Fig. 7). The conditional stability constant, K_1, is defined:

$$Cu + L \rightleftarrows CuL \tag{1}$$

$$K_1 = [CuL]/[Cu][L] \tag{2}$$

and describes the strength with which the organic ligands bind Cu, while taking into account the effects of the seawater matrix on the binding strength (hence the “conditional” binding strength).

Discussion

Considerations and uncertainties

This dataset shows some of the first combined data on particulate and dissolved Cu along with its organic speciation in Puget Sound. In order to accurately interpret the data, several possible sources of error that might have occurred during data collection must first be considered. The water sample intended to be taken from station SB02 in Lake Washington was actually taken from a nearby station, MB10, which was over a submerged coal car. It is possible that extra metals from the coal car were present in the water column, leading to particulate and dissolved concentrations not representative of the lake as a whole. During the cruise, there were instances of the pole sampler (Fig. 3) touching the metal floor of the vessel, LDPE bottles touching metal objects on the deck and being placed on the counter without a protective bag, and the bottle cap of SB04 being touched with work gloves, all of which could have led to contamination of samples with trace metals.

Analysis of ICP-MS data resulted in negative concentrations for particulate chromium, particulate zinc, and particulate and dissolved silver. This may be a result of how blanks were measured and analyzed. To account for contamination in particulate samples, for example, three filters were analyzed and subtracted from the measured particulate concentrations. For particulate ^{52}Cr, the standard deviation of the counts per second measured by ICP-MS for the three filter blanks is more than twice the mean counts per second. Thus, blank values for some metals were quite high and might have contributed to these anomalous values.

Sources of metals and ligands

Residence time of water, along with removal processes such as biological uptake and particle sorption, dictates the standing stock of trace metals and ligands that are measured in a given location. Surface water in Admiralty Inlet has a mean residence time of 11.7 days whereas surface water in South Sound has a mean residence time of 23.8 days (Babson et al. 2006). Since the residence time is over twice as long in South Sound as in Admiralty Inlet, dissolved and particulate metals and ligands may be more likely to accumulate in the South Sound. Meanwhile, the input and mixing of "clean" water from the Pacific Ocean through Admiralty Inlet, evidenced by similar concentrations of particulate Cu in Admiralty Inlet and the North Pacific (Table 2, Fig. 5), may quickly remove metals and ligands at this site and dilute higher metal concentrations with the relatively lower levels of metals in ocean waters. These processes may explain South Sound's higher levels of particulate Cu, dissolved Cu, and organic ligands relative to Admiralty Inlet (Table 2, Table 3, Table 5). Iron, cobalt, particulate arsenic, particulate lead, and other metals were also higher in South Sound than in Admiralty Inlet (Table 2, Table 3). The particulate iron concentration was nearly ten times higher in South Sound than in Admiralty Inlet but the concentration of dissolved iron, a marker of sample contamination, was not substantially elevated (Table 2, Table 3). This indicates that the high level of particulate iron is likely a result of pollution and not of sample contamination. Lead often has an anthropogenic source and its relatively high values in South Sound, Lake Union, Portage Bay, and especially the shore of Portage Bay indicate sources of pollution at these locations. The high concentrations of metals such as Cu and lead at the Portage Bay shore may result from this location's proximity to streets and consequent accumulation of runoff and pollutants from the surrounding urban environment. Portage Bay and its shore must have a short residence time on the order of days since the flux of water from Lake Washington (residence time 2.5 years) into Portage Bay is equivalent to the flux out of Portage Bay into Lake Union (residence time 5 to 25 days), and Portage Bay has a smaller volume than both lakes (King County 2003, 2018). Despite Portage Bay's high flushing rate, analysis of the sample taken from its shore revealed markedly high metal concentrations relative to other locations: the highest particulate Cu (43.84 nmol/L) and lead (15.94 nmol/L) and the second-highest particulate arsenic (5.99 nmol/L) (Table 2). This finding emphasizes the impact of the Portage Bay shore's proximity to roads.

The expected relationship between residence time and metal concentrations does not hold at the fresh water stations. The average water residence time of Lake Washington is 2.5 years, which is relatively rapid for a lake (King County 2003). Residence time in Lake Union is much shorter, ranging from 5 days in late autumn and winter to over 25 days in summer (King County 2018). However, relative to Lake Washington, concentrations of trace metals in Lake Union are greater for particulate metals, and equal or greater for dissolved metals (Table 2, Table 3). The only exception is dissolved vanadium, which has a concentration of 1.03 nmol/L in Lake Washington and 1.01 nmol/L in Lake Union (Table 3). This indicates that there may be elevated point sources in Lake Union relative to Lake Washington, or perhaps pollution is more diluted in Lake Washington due to its much greater basin size and lower commercial and boating activity.

The observed range of total dissolved Cu was 0.90 to 6.47 nmol/L (Table 3, Fig. 5), which encompasses the range seen in Hood Canal: 2.03 ± 0.049 to 2.74 ± 0.12 nmol/L (Jacquot et al. 2014). The range of bioavailable Cu is $2.37*10^{-15}$ to $6.23*10^{-15}$ mol/L (Table 5). This range is lower than the range found in Hood Canal, where the maximum bioavailable Cu value was $1.36*10^{-12}$ mol/L (Jacquot et al. 2014). The higher free Cu in Hood Canal is likely a combination of the sometimes higher Cu concentrations, as well as the lower ligand concentrations that were observed in that study. This suggests that high levels of organic matter observed in Portage Bay and South Sound are buffering the free Cu in this study. The range of total dissolved Cu found in this study and in Jacquot et al. (2014) is much lower than that seen in San Francisco Bay, where dissolved Cu ranged from 17.9 to 49.6 nmol/L (Table 3, Buck and Bruland 2005). The range of bioavailable Cu ($2.37*10^{-15}$ to $6.23*10^{-15}$ mol/L) in this study is also lower than the range found in San Francisco Bay, where the maximum bioavailable Cu value was $5.01*10^{-14}$ mol/L (Table 5, Buck and Bruland 2005). San Francisco Bay is known to have high point sources of Cu pollution from relic military ships that contain high levels of Cu from now illegal Cu-based antifouling paint (Buck and Bruland 2005). This, combined with the low residence times of some parts of San Francisco Bay – as long as five months in its southern reaches (Conomos 1979) – likely allows it to accumulate anthropogenic Cu, leading to a saturation of the natural organic compounds that bind Cu and higher free Cu levels.

A concentration of 0.582 nmol/L of total dissolved Cu has been measured in the northeast Pacific Ocean at 20 m depth and more recently, an average of 4.45 nmol/L was measured across the surface waters of several stations in the North Pacific (Coale and Bruland 1988; Ezoe et al. 2004). Typically, in the open ocean in both the Atlantic and Pacific dissolved Cu is approximately 0.5 nmol/L (Schlitzer et al. 2018). The lowest and highest values of dissolved Cu found in this study (0.90 and 6.47 nmol/L) are both higher than the referenced pelagic values (Table 3, Fig. 5). Whereas in this study particulate Cu comprised 76.90 to 94.79% of total Cu (dissolved plus particulate), it comprised an average of 52.38% of Cu measured in the North Pacific (Table 4, Ezoe et al. 2004). This difference, alongside higher dissolved Cu concentrations, may indicate that the Puget Sound is polluted with particulate and/or dissolved Cu from either point or non-point sources. However, the source of the Cu is difficult to determine with this work and studies that focus on temporal sampling for total Cu might disentangle some of these prospective sources.

Influence of ligands on Cu toxicity

While the absolute concentration of Cu in the South Sound, Admiralty Inlet, and Portage Bay shore samples is important for understanding total Cu loading to Puget Sound, the concentration of free Cu is more indicative of the toxic or bioavailable fraction of total Cu. Depending on the concentration and strength of the natural organic ligands present, it is possible for concentrations of free Cu to be low despite high concentrations of total dissolved Cu. Thus, natural organic matter can buffer Cu toxicity in these systems. This buffering effect of organic ligands is illustrated through comparison of the Admiralty Inlet sample to the South Sound and Portage Bay shore samples. Admiralty Inlet showed the lowest concentration of total dissolved Cu, 0.90

nmol/L (Table 3). However, this sample's concentration of ligands, 9.72 nmol/L, was more than four times lower than concentrations of Cu-binding ligands in South Sound (40.5 nmol/L) and Portage Bay shore (39.4 nmol/L) (Fig. 7, Table 5). Thus, due to the relative dearth of ligands in Admiralty Inlet, there was a much greater proportion of dissolved Cu in the free form at this site. Admiralty Inlet showed the highest percent free value: $2.68*10^{-4}$, over twice that of South Sound ($1.16*10^{-4}$) and Portage Bay shore ($9.63*10^{-5}$) (Table 5).

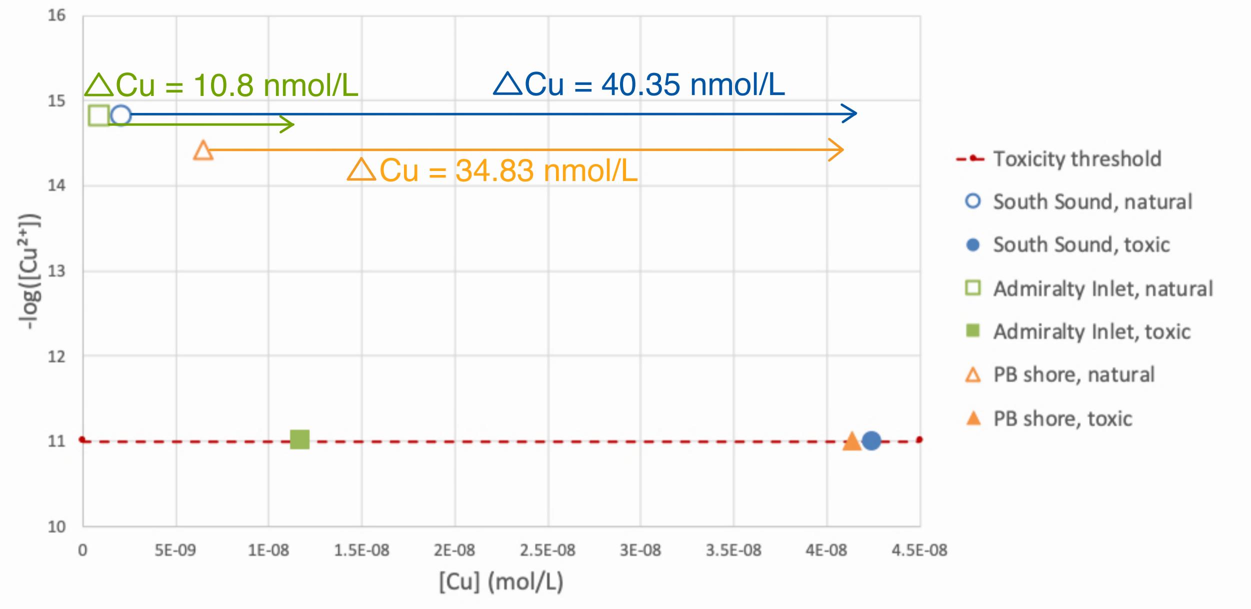

Fig. 8. Bioavailable Cu expressed as -log([Cu^{2+}]) on the y-axis as a function of total Cu in mol/L on the x-axis. The dashed line at -log([Cu^{2+}]) = 11 represents the threshold for Cu toxicity (Buck and Bruland 2005). Values are plotted as circles, squares, and triangles which correspond to South Sound, Admiralty Inlet, and the Portage Bay shore, respectively. Measured data are plotted as "natural" concentrations with unfilled shapes. Filled shapes represent hypothetical "toxic" values of Cu at these stations, calculated using measured values for ligand concentrations and binding strengths (Moffett and Dupont 2007). Arrows extend from unfilled shapes to filled shapes and corresponding ΔCu values represent the increase in [Cu] necessary to reach the toxicity threshold given the Cu-binding ligands present at each location. At every station, an increase in [Cu] by at least an order of magnitude is necessary to reach the toxicity threshold.

Bioavailable Cu was found to be below 10^{-11} mol/L, the conservative threshold for toxicity (Buck and Bruland 2005) at South Sound, Admiralty Inlet, and the Portage Bay shore. Concentrations and binding strengths of ligands were used to predict the total dissolved Cu concentration necessary to reach the toxicity threshold (Moffett and Dupont 2007). At every station, the concentration of total dissolved Cu would need to increase by at least an order of magnitude in order to reach the toxicity threshold (Fig. 8). This is important, because periodic inputs of Cu could have the potential to exceed this toxicity threshold, however relatively high Cu loadings would be required.

Particulate trace metals can be solubilized and made available to phytoplankton on a timescale of days via reduction-oxidation reactions, photochemistry, organic matter remineralization, or other processes (Berger et al. 2008). These processes could lead to Cu toxicity at sites such as the Portage Bay shore, where it was calculated that an addition of 34.83 nmol/L of dissolved Cu would result in meeting the toxicity threshold (Fig. 8). This could be achieved if the 43.84 nmol/L of particulate Cu measured at the site were solubilized (Table 2). The particulate Cu measured at South Sound, a concentration of 37.22 nmol/L, is also close to the calculated value of additional dissolved Cu needed to reach the toxicity threshold, 40.35 nmol/L (Table 2, Fig. 8). If the measured particulate Cu is labile, then the South Sound and Portage Bay shore sites are at risk of Cu toxicity.

Future research

Often, monitoring water quality with respect to trace metal contaminants such as Cu consists only of measuring the total metal concentrations. Ligand concentrations are essential to understanding the toxicity of Cu, but measuring them is time-consuming and difficult. Future research could test an alternative approach: measuring dissolved organic carbon (DOC) as a proxy for ligands. Since most Cu-binding ligands are organic, ligand concentrations may vary as a function of DOC in a reliable way. Current models have not successfully used the relationship to predict Cu toxicity in estuarine waters (Pearson et al. 2017). This could be an important component to add to future monitoring studies, and might be key for understanding the role of Cu toxicity on marine organisms in Puget Sound.

Conclusion

Consistent with the primary hypothesis, dissolved and particulate Cu were higher at South Sound, where residence time is higher, than at Admiralty Inlet, where residence time is lower. Concentrations of ligands, particulate metals except manganese, and dissolved manganese, iron, cobalt, and zinc were also higher at South Sound than at Admiralty Inlet. However, the inverse of this relationship occurred between Lake Union and Lake Washington. Thus, it is likely a combination of residence time as well as other uptake and/or abiotic scavenging that contribute to the observed Cu distributions.

The secondary hypothesis, that free Cu ion (Cu^{2+}) concentrations would decrease with increasing concentrations of organic Cu-binding ligands, was not consistent with findings; free Cu did not decrease with increasing Cu-binding ligands in a consistent way. For example, the Portage Bay shore sample had an intermediate concentration of ligands but had nearly three times as much free Cu as the other two sites analyzed. However, ligands did appear to buffer Cu toxicity, since free Cu was on average about six orders of magnitude lower than total dissolved Cu. Future research on Cu toxicity and assessments of water quality should consider the effects of Cu-binding ligands since they have potential to greatly reduce bioavailable dissolved Cu. The sources of these ligands might also be insightful for determining Cu toxicity, as many natural ligands (such as humic substances) can prevent toxicity and some organisms might also be actively producing organic Cu ligands as a means for preventing toxicity (Buck and Bruland 2005).

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Appendix

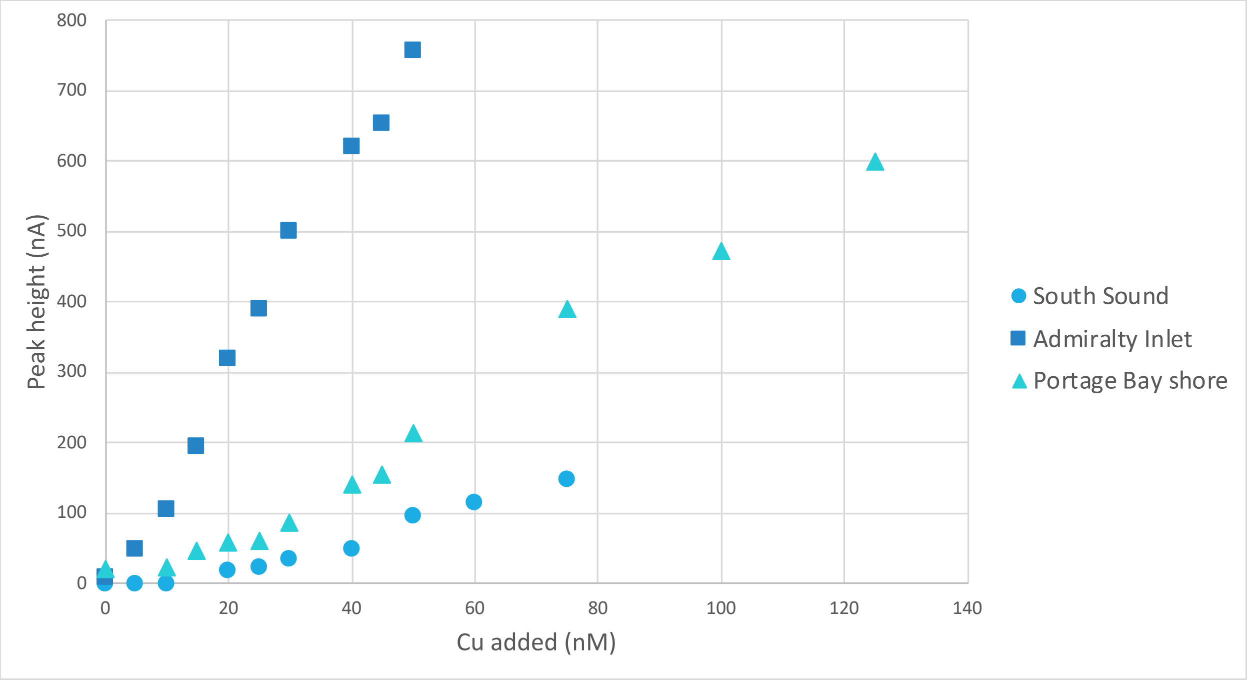

Fig. 9. Cu titrations performed during CLE/AdCSV analysis for samples from South Sound (circles), Admiralty Inlet (squares), and Portage Bay shore (triangles). Cu added is plotted in nmol/L on the x-axis and the corresponding peak height is plotted in nanoamps on the y-axis.

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