

# Transition metal availability to speleothems controlled by organic binding ligands

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## Abstract



Speleothems are important archives of Quaternary palaeoclimate. However, climate proxies based on trace elements in speleothems are currently limited to the metals which exhibit simple partitioning ( $Mg^{2+}$ ,  $Sr^{2+}$ ,  $Ba^{2+}$ ). This study aims to expand understanding of the processes controlling the divalent first row transition metals ( $M^{2+}$ ) in these systems. Adsorptive cathodic stripping voltammetry was used to determine Cu speciation in speleothem dripwater, stream and pool samples from five limestone caves located in diverse climatic settings. Our results demonstrate that Cu binding and stabilisation by organic ligands (L) is a universal property of cave waters, which decreases the available Cu concentration ( $[Cu']$ ) by  $\sim 5$  orders of magnitude relative to total Cu concentration ( $[Cu_T]$ ). Furthermore,  $[Cu']$  does not change meaningfully with increases in either  $[Cu_T]$  or  $[L]$ , meaning that  $Cu^{2+}$  substitution in  $Ca^{2+}$  valence sites in precipitating  $CaCO_3$  speleothems is likely to be inhibited by organic complexation. We suggest that the residence time of speleothem thin water films (1/drip rate), the dissociation rates of labile metal-organic complexes, and the stability of adsorbed (ternary) metal-organic complexes will determine  $M^{2+}$  incorporation in speleothems.

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## Introduction

The pervasiveness of natural organic matter (NOM) in aquatic systems has been established for decades (Oliver *et al.*, 1983; Filella, 2009). Equally well established is the interaction between trace metals and NOM ligands, which dominates metal speciation and bioavailability in aquatic environments with pH values typical of cave waters (pH  $\sim 8$ ) (Tipping and Hurley, 1992).

It is possible to classify different metals according to their NOM binding affinity, with Cu and Ni showing particularly high binding strength among the transition metals (Warnken *et al.*, 2007; Levy *et al.*, 2012). The interaction between Cu, ligands and inorganic ions in water can be summarised using the following mass balance (charges omitted for simplicity with exception of  $Cu^{2+}$ ):

$$[Cu_T] = [CuL] + [Cu'] \quad \text{Eq. 1}$$

$$[Cu'] = [Cu^{2+}] + [CuX_{IN}] \quad \text{Eq. 2}$$

Where  $[ ]$  denotes concentration,  $Cu_T$  is the total copper in the analysis (no differentiation based on filtration, majority of samples in this study were unfiltered),  $CuL$  is the Cu fraction bound by organic ligands,  $Cu^{2+}$  is the hydrated 'free' form of the copper cation,  $CuX_{IN}$  is the fraction of copper held in simple inorganic complexes (*e.g.*,  $CuCl^+$ ,  $CuHCO_3^+$  etc), and

$Cu'$  is the 'available copper' calculated as the sum of  $Cu^{2+}$  and  $CuX_{IN}$ .  $CuX_{IN}$  is considered to be labile because the stability of inorganic transition metal complexes (which dissociate rapidly in the presence of a suitable sink) is much lower than metal-organic complexes (which dissociate much more slowly (Shafaei Arvajeh *et al.*, 2013)).

The transport of metals by colloidal and dissolved NOM has been shown to be important in shallow near surface systems (Hartland *et al.*, 2012). However, the aforementioned research has to some extent 'muddied the waters', with the role of NOM often becoming equated with soil flushing in the speleothem literature and not being recognised for its pervasive influence both in metal transport and metal distribution between solution and crystal.

While organic complexation is very important for transmission of metals in karst aquifers (Hartland *et al.*, 2011), strong binding of metals by NOM also creates an 'aqueous sink' for divalent metal ions which is likely to inhibit  $M^{2+}$  substitution for  $Ca^{2+}$  in calcite lattice sites (Hartland *et al.*, 2014). Thus, it is not appropriate to interpret the changing concentration of transition metals in dripwaters (and by extension speleothems) in terms of simple partitioning (as is routinely and correctly done with regard to the alkaline earth metals (Huang and Fairchild, 2001; Day and Henderson, 2013)).

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The aim of this paper is to establish the fact that metal binding by NOM ligands in the waters that form speleothems dictates that the activity of  $[M'] \ll [M_T]$ ; and therefore, the formation of  $\text{CaCO}_3/\text{Ca(M)CO}_3$  solid solutions cannot be treated using partition coefficients unless the role of organic ligands is properly accounted for (Fairchild *et al.*, 2012). As a model system we use Cu, which has a strong binding affinity for NOM, to demonstrate this point and present a new hypothesis on the climatic significance of transition metal variations in speleothems.

## Methods and Materials

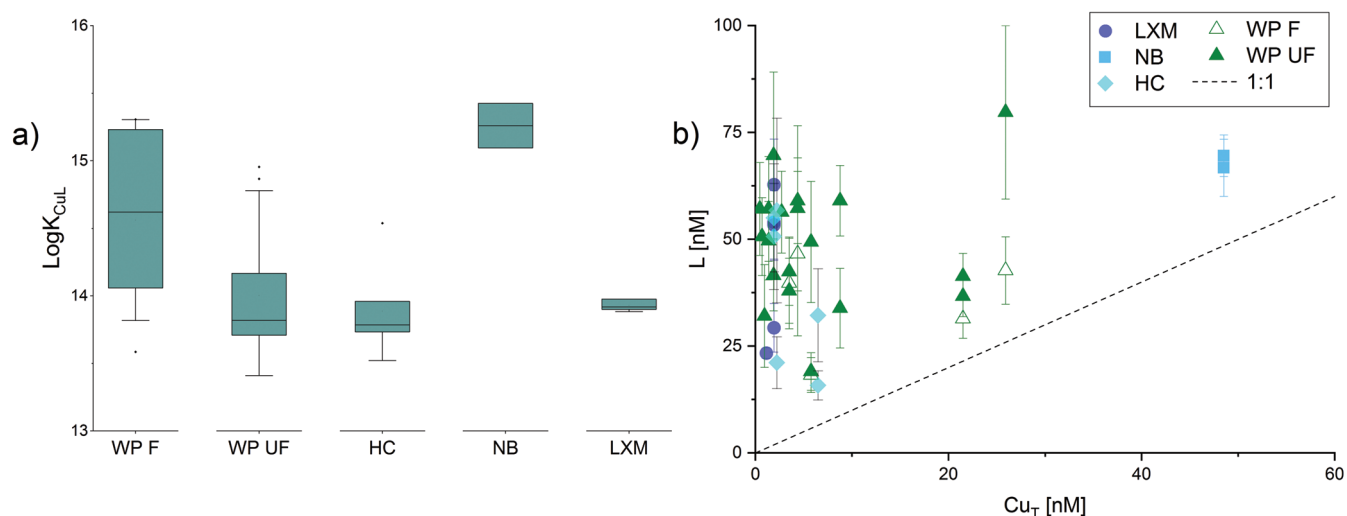
**Study sites.** Samples of cave water were collected in acid washed, low density polyethylene (LDPE) bottles (Hartland *et al.*, 2011) direct from active speleothem-forming drip points (including one pool and one stream sample) in five New Zealand caves. The caves were located across a latitudinal gradient: 38 °S Waipuna Cave (n = 24; 21 drip samples, 3 stream samples), Waitomo, subtropical podocarp forest; 40 °S Nettlebed Cave (n = 2) and Hodges Creek Cave (n = 6), temperate Beech forest; and 45 °S Calcite Cave (n = 2) and Dave's Cave (n = 3; 2 drips, 1 pool), Mt Luxmore, Fiordland, alpine tussock grassland. Unless otherwise noted drip samples were collected from actively-depositing stalactites. With the exception of Nettlebed, New Zealand's deepest cave (889 m), developed in Ordovician marble, all other caves are developed in Oligocene shallow marine limestone. Upon collection, water samples were chilled to  $<4$  °C, then frozen (within 48 hours) and stored at  $-4$  °C prior to analysis.

**Experimental. 1. Adsorptive cathodic stripping voltammetry (AdCSV).** To determine Cu speciation parameters Adsorptive Cathodic Stripping Voltammetry (AdCSV) with salicylaldoxime (SA) was used as the synthetic complexing agent (Lucia *et al.*, 1994). Prior to analysis, each unfiltered sample was thawed at 4 °C overnight and brought up to room temperature ( $22 \text{ °C} \pm 1$ ). For the Cu ligand titrations, 12 aliquots (4 mL each) of each sample were separately transferred into acid washed and pre-conditioned Teflon vials. Afterwards, 15  $\mu\text{L}$  of 3 M KCl (Metrohm), used as a supporting electrolyte (final sample salinity of 0.08 ppt), and 100  $\mu\text{L}$  of 1 M EPPS buffer (Sigma Aldrich, stock solution 1 M in Milli-Q adjusted to pH 8 with NaOH) were added to each sample – all

titrations reported here were performed at a pH of  $8.0 \pm 0.2$ , making direct comparison of Cu speciation parameters possible (Sander *et al.*, 2005). This step was followed by increasing Cu additions (ranging from 0 to 300 nM), calculated as a geometrically spaced series (*i.e.* logarithmic scale) (Garnier *et al.*, 2004), of a known Cu standard (Fisher Scientific, prepared by dilution of a 15.74 mM atomic absorption Cu standard in 1 % quartz distilled HCl) to the 12 aliquots (Sander *et al.*, 2007). A 20 min equilibration period was then implemented to allow the Cu to bind with the natural organic ligands present in solution. Subsequently, SA (Acros Organics; SA: 98 %) was added to the aliquots at a final concentration of 5  $\mu\text{M}$ . Finally, samples were left to equilibrate for a minimum of 12 hours, which was found to result in stable Cu-SA peaks (Sander *et al.*, 2007). Once equilibrated, samples were analysed by AdCSV using a Metrohm 663 VA stand connected to a PGSTAT10 (Eco Chemie) potentiostat interfaced with GPES v4.9 software. The three electrode configuration of the system included a hanging mercury drop electrode (HMDE) as the working electrode, an Ag|AgCl|3 M KCl reference electrode, and a platinum counter electrode. The system was operated in differential pulse mode at room temperature. The deposition on a medium-sized Hg drop was set at  $-0.15$  V for 30–180 s (deposition times varied for different samples according to their initial  $[\text{Cu}_T]$ ), followed by a 5 s equilibrium time and a cathodic scan from  $-0.15$  to  $-0.6$  V at 20 mV/s. The potential step size was 5 mV, the modulation time 0.05 s, the interval time 0.1 s, and the differential pulse amplitude was 25 mV.

Prior to the AdCSV measurement each sample was de-aerated for 120 s with  $\text{O}_2$ -free  $\text{N}_2$ . Measurements were carried out in triplicate, using a freshly generated mercury drop for each replicate. If sample volume was sufficient, samples were run in duplicate (unfiltered) and/or were filtered (0.2  $\mu\text{m}$ ; using an acid washed syringe and an acrodisc filter). All sample handling and electrochemical measurements were conducted inside a clean air bench following trace metal clean procedures (Sander *et al.*, 2005; Powell, 2015).

**2. Calculation of Cu speciation parameters.** After completion of the titration, the ProMCC software (Omanović *et al.*, 2010) was used to calculate conditional formation constants (K), Cu-binding ligand concentrations ( $[L]$ ), and  $[\text{Cu}']$  (sum of  $[\text{Cu}^{2+}]$  and  $[\text{CuX}_{\text{IN}}]$ ) of each solution. Conditional stability constants (input data for ProMCC) of



**Figure 1** (a) Box plots of conditional stability constants ( $\text{logK}_{\text{CuL}}$ ) of  $\text{CuL}$  complexes in cave samples. Values calculated using ProMCC (Omanović *et al.*, 2015). Error bars are 1 standard deviation. (b) Correlation between the total Cu concentration ( $[\text{Cu}_T]$ ) and corresponding Cu ligand concentration. For all samples which plot above the 1:1 dashed diagonal line, one can assume that all available Cu is strongly complexed to organic ligands. Vertical error bars represent the fitting error in  $[L_1]$ . WP = Waipuna Cave (n = 24), F = filtered, UF = unfiltered; HC = Hodges Creek Cave (n = 6); NB = Nettlebed Cave (n = 2); LXM = Mt Luxmore Caves; Daves Cave (n = 3) and Calcite Cave (n = 2).

the CuSAx-complex of the samples were adjusted according to the approach of Lucia *et al.* (1994). The inorganic side reaction coefficient ( $\alpha$ -Cu-inorg) for Cu was adjusted for each sample separately using Visual MINTEQ 3.0 with set input variables: pH of 8.0, a temperature of 21 °C, and measured concentrations of Cu, Ca, Mg, K, Na, Cl, SO<sub>4</sub>, HCO<sub>3</sub> and CO<sub>3</sub>. Potential inaccuracy introduced by using adjusted conditional constants for CuSA and inorganic side reaction coefficients are assumed to be fully within experimental uncertainty.

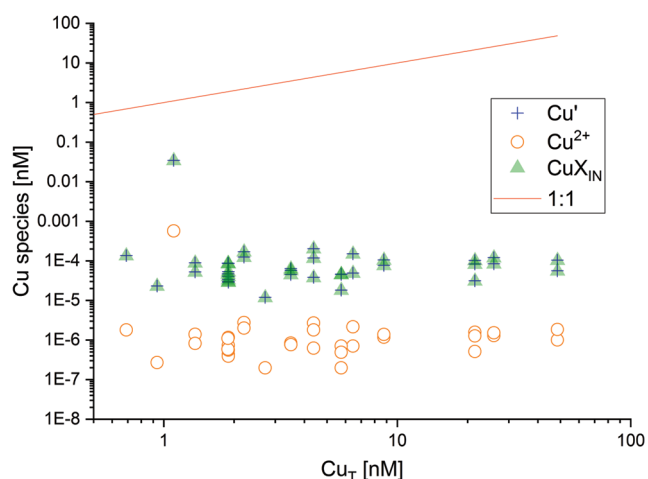
## Results and Discussion

For all titrations, the fitted data could be adequately explained by a one ligand model. This modelling does not inform ligand composition, beyond identifying the stability of the metal-organic complexes (see below). Titrations demonstrated the presence of a strong organic ligand class (*i.e.* L<sub>1</sub>), ranging from 15.8 – 79.7 nM, with Cu-binding capacities of logK<sub>CuL</sub> 13.4 – 15.4. Ligand concentrations ranged from as low as [L<sub>1</sub>] = 15.8 ± 3.4 nM (Hodges Creek Cave), to 79.7 ± 20.3 nM (Waipuna Cave). Ligand concentrations differed between unfiltered and filtered (0.2 µm) Waipuna Cave samples, with filtered samples usually having lower [L<sub>1</sub>] and higher logK<sub>CuL</sub> than unfiltered ones (Fig. 1a). This indicates the presence of organic ligands in the size fraction above 0.2 µm, consistent with previous studies which identified Cu associated with coarse colloidal NOM in cave systems (Hartland *et al.*, 2011, 2012). Further, the ligand concentrations were always in excess of the total Cu concentrations ([Cu<sub>T</sub>]) (Fig. 1b), indicating that all Cu was present in complexes with organic ligands.

Conditional stability constants (logK<sub>CuL</sub>) ranged from as low as 13.4 ± 0.2 from Waipuna Cave to 15.4 ± 0.2 in samples from Nettlebed Cave. The determined stability constants indicate Cu complexation with organic ligands, such as thiols, carboxylic acids, hydroxyl and amine functional groups, which have very high stability constants (logK<sub>CuL</sub> of 14–16) (Sander and Koschinsky, 2011). Further, logK<sub>CuL</sub> values generally increased between unfiltered and filtered samples, which suggests that Cu complexes with nano-scale organic ligands are slightly more stable than Cu complexes with colloids > 0.2 µm (Hartland *et al.*, 2012).

Available Cu concentrations in solution (sum of [Cu<sup>2+</sup>] and [CuX<sub>IN</sub>]) were low, ranging from 11.9 fM to 34.5 pM, some five orders of magnitude lower than [Cu<sub>T</sub>] (Fig. 2). Indeed, [Cu'] only increased weakly with higher [Cu<sub>T</sub>], indicating that the scarcity of Cu<sup>2+</sup> due to the action of binding ligands is a universal feature of cave dripwaters. Aside from the significance of this finding for trace element signals in speleothems (Fairchild *et al.*, 2012), this finding also has significance for geomicrobiology because at biologically available Cu concentrations (Zhao *et al.*, 2016) below 10<sup>-15</sup> M, Cu is an inhibitor (*e.g.* microbial activity/growth) for some marine microorganisms (Sunda and Huntsman, 1995). We also note that [L] was uncorrelated with the total organic carbon (TOC) concentration – dripwater TOC mainly correlates with soil-derived organic matter based on numerous fluorescence studies (Liao *et al.*, 2018) – in the samples (Fig. S-1), and therefore cannot exclude the possibility that microbes in cave systems secrete ligands in order to regulate copper bioavailability (Peña *et al.*, 1998), thereby contributing to the pool of Cu binding ligands in the system.

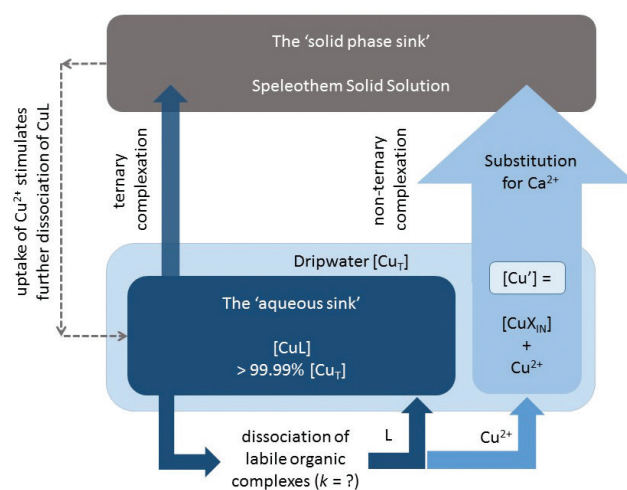
From a review of the literature, the evidence suggests that Cu<sup>2+</sup> adsorption to calcite is favoured (Elzinga and Reeder, 2002) but inhibited by formation of solution complexes with organic ligands (Lee *et al.*, 2005), *i.e.* the 'aqueous sink' (Fig. 3). Previous experimental work (Lee *et al.*, 2005) also indicates that ternary (metal-organic) complexes (Fein, 2002) at the calcite surface have lower stabilities than non-ternary complexes,



**Figure 2** Available (Cu'), inorganic (CuX<sub>IN</sub>) and free (Cu<sup>2+</sup>) copper concentrations plotted against [Cu<sub>T</sub>] in cave samples calculated using ProMCC (Omanović *et al.*, 2015). [Cu'] are consistently low despite widely varying [Cu<sub>T</sub>] demonstrating the dominant influence of organic ligands in these samples.

and this is supported by empirical evidence showing large increases in the metal/NOM ratio between dripwaters and speleothems (Hartland *et al.*, 2014).

If we accept that the inorganic pathway (*i.e.* Cu<sup>2+</sup> substitution) is the dominant mechanism for Cu incorporation in speleothems, it follows that under conditions of continuous calcite precipitation, Cu<sup>2+</sup> adsorption to calcite will proceed at the rate dictated by the kinetics of CuL dissociation (Warnken *et al.*, 2007). We can expect that the rapid adsorption (Elzinga and Reeder, 2002; Lee *et al.*, 2005) of newly dissociated Cu<sup>2+</sup> to the calcite surface (*i.e.* the 'solid phase sink') will further drive this dissociation process, likely profoundly changing the resulting stable metal isotope fractionation compared with inorganic laboratory experiments (Schott *et al.*, 2014) (Fig. 3). With the exception of sub-aqueous calcite deposition (typically characterised by very long water residence times), the time available for dissociation is the residence time of the crystallising fluid, which may be approximated by the interval between drips. This opens the possibility of reconstructing the palaeo-discharge of speleothem dripwaters based on the metal accumulation (and metal isotope fractionation) patterns of speleothems (if the dissociation rate constants of metal-organic complexes and fractionation factors can be determined (Fig. 3)).



**Figure 3** Conceptual diagram illustrating the influence of Cu ligands on the incorporation of Cu in speleothems.



## Conclusions

We have shown that dissolved ligands (<0.2  $\mu\text{m}$ ) are the primary agents controlling the speciation of Cu in speleothem-forming cave waters. Indeed, complexation by strong binding ligands drives consistently low concentrations of [Cu] despite widely varying [Cu<sub>r</sub>]. We argue that dominant organic complexation of divalent transition metals (e.g., Ni<sup>2+</sup>, Co<sup>2+</sup>, Cu<sup>2+</sup>, and Zn<sup>2+</sup>) and other metals able to bind to organic ligands (e.g., Cd<sup>2+</sup> and Pb<sup>2+</sup>) in the 'aqueous sink' leads to a range of signals in speleothems controlled by the dissociation rates of these complexes, the residence time of the dripwaters (1/drip rate) and the compatibility of M<sup>2+</sup> in lattice valence sites. Through this study we aim to increase awareness in the speleothem community of the central importance of metal complexation by organic ligands for understanding trace element signals in speleothems. Future research should aim at understanding the significance of these processes for speleothem palaeoclimate records, and the geomicrobiology of karst environments.

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## Additional Information

Supplementary Information accompanies this letter at <http://www.geochemicalperspectivesletters.org/article1824>.



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### Supplementary Information

The Supplementary Information includes:

- Table S-1
- Figure S-1

### Supplementary Table

**Table S-1** Results of AdCSV measurements on cave water samples.

Sample name	Date	Rep	Method	pH	Cu [nM]	L [nM]	error	logK	error	[Cu <sub>XIN</sub> ]	Cu <sup>2+</sup> [nM]	Cu' [nM]
WP1-1	18/10/2016	1	unfiltered	8.3	2.72	56.4	9.6	13.8	0.10	4.5E-05	7.7E-07	4.5E-05
WP1-1	20/04/2017	1	unfiltered	8	3.50	42.4	7.8	13.8	0.12	8.3E-05	1.3E-06	8.4E-05
WP1-1	20/04/2017	2	unfiltered	8	3.50	38.0	7.6	14.2	0.16	4.5E-05	7.1E-07	4.6E-05
WP1-1	20/04/2017	3	filtered	8	3.50	39.8	10.7	14.1	0.21	7.6E-05	1.2E-06	7.7E-05
WP1-2	18/10/2016	1	unfiltered	7.9	1.88	41.6	8.3	13.9	0.12	3.8E-05	6.3E-07	3.8E-05
WP1-2	18/10/2016	2	unfiltered	7.9	1.88	69.7	19.5	13.7	0.17	3.1E-05	5.1E-07	3.1E-05
WP1-2	16/11/2016	1	unfiltered	8.3	0.45	57.1	10.9	13.6	0.12	1.2E-05	2.0E-07	1.2E-05
WP1-2	21/03/2017	1	unfiltered	8.1	25.91	79.7	20.3	14.2	0.21	6.2E-05	8.4E-07	6.3E-05
WP1-2	21/03/2017	2	filtered	8.1	25.91	42.7	7.9	15.3	0.31	5.6E-05	7.6E-07	5.7E-05
WP1-2	20/04/2017	1	unfiltered	8.1	5.75	49.4	14.2	13.9	0.24	1.2E-04	1.5E-06	1.2E-04
WP1-2	20/04/2017	2	unfiltered	8.1	5.75	19.1	4.4	14.9	0.26	4.6E-05	5.9E-07	4.7E-05
WP1-2	20/04/2017	3	filtered	8.1	5.75	18.3	4.1	14.6	0.22	8.6E-05	1.1E-06	8.7E-05
WP1-3	18/10/2016	1	unfiltered	8.1	0.93	32.0	12.0	13.4	0.20	7.6E-05	1.2E-06	7.7E-05
WP1-3	16/11/2016	1	unfiltered	8.3	1.36	49.7	9.2	13.8	0.14	4.5E-05	4.9E-07	4.5E-05
WP1-3	16/11/2016	2	unfiltered	8.3	1.36	57.1	12.3	13.6	0.16	1.8E-05	2.0E-07	1.8E-05
WP1-3	15/12/2016	1	unfiltered	8.2	0.69	50.6	9.1	13.7	0.11	2.3E-05	2.7E-07	2.3E-05
WP1-3	21/03/2017	1	unfiltered	8.1	8.76	59.0	8.3	13.8	0.11	8.8E-05	1.4E-06	8.9E-05
WP1-3	21/03/2017	2	unfiltered	8.1	8.76	33.9	9.3	14.6	0.22	5.2E-05	8.2E-07	5.2E-05



WP1-3	20/04/2017	1	unfiltered	8	4.38	57.3	19.3	13.7	0.24	1.3E-04	1.8E-06	1.4E-04
WP1-3	20/04/2017	2	unfiltered	8	4.38	59.0	10.0	13.8	0.15	1.0E-04	1.4E-06	1.1E-04
WP1-3	20/04/2017	3	filtered	8	4.38	46.6	19.2	13.6	0.27	2.0E-04	2.7E-06	2.0E-04
WP Stream	21/03/2017	1	unfiltered	8	21.48	41.3	5.4	14.8	0.14	1.2E-04	1.8E-06	1.2E-04
WP Stream	21/03/2017	2	unfiltered	8	21.48	36.7	4.8	15.0	0.22	1.0E-04	1.6E-06	1.0E-04
WP Stream	21/03/2017	3	filtered	8	21.48	31.4	4.6	15.2	0.27	8.2E-05	1.3E-06	8.3E-05
CC1	Mar-15	1	unfiltered	7.7	1.89	53.4	8.0	14.0	0.10	2.8E-05	4.0E-07	2.9E-05
CC1	Mar-15	2	unfiltered	7.7	1.89	29.3	5.7	14.0	0.12	5.3E-05	7.3E-07	5.3E-05
DC Pool	Mar-15	1	unfiltered	7.9	1.89	54.0	8.9	13.9	0.10	3.3E-05	4.4E-07	3.4E-05
DC Pool	Mar-15	2	unfiltered	7.9	1.89	62.8	10.7	13.9	0.10	3.0E-05	3.9E-07	3.0E-05
DC2	Mar-15	1	unfiltered	7.9	1.10	23.4	1.3	13.9	0.42	3.4E-02	5.7E-04	3.5E-02
HC3	Mar-15	1	unfiltered	7.7	2.20	21.1	6.1	13.7	0.21	1.5E-04	2.2E-06	1.5E-04
HC3	Mar-15	2	unfiltered	7.7	2.20	56.7	21.6	13.8	0.24	4.8E-05	7.0E-07	4.9E-05
HC4	Mar-15	1	unfiltered	7.6	6.46	32.2	10.9	14.0	0.24	1.7E-04	2.8E-06	1.7E-04
HC4	Mar-15	2	unfiltered	7.6	6.46	15.8	3.4	14.5	0.22	1.2E-04	2.0E-06	1.2E-04
HC5	Mar-15	1	unfiltered	7.7	1.89	55.0	12.7	13.8	0.16	4.0E-05	5.6E-07	4.1E-05
HC5	Mar-15	2	unfiltered	7.7	1.89	50.7	12.4	13.5	0.16	8.4E-05	1.2E-06	8.5E-05
NB3	Mar-15	1	unfiltered	7.8	48.50	66.7	6.7	15.4	0.23	5.5E-05	1.0E-06	5.6E-05
NB3	Mar-15	2	unfiltered	7.8	48.50	69.6	4.9	15.1	0.14	1.0E-04	1.9E-06	1.0E-04

Supplementary Figure

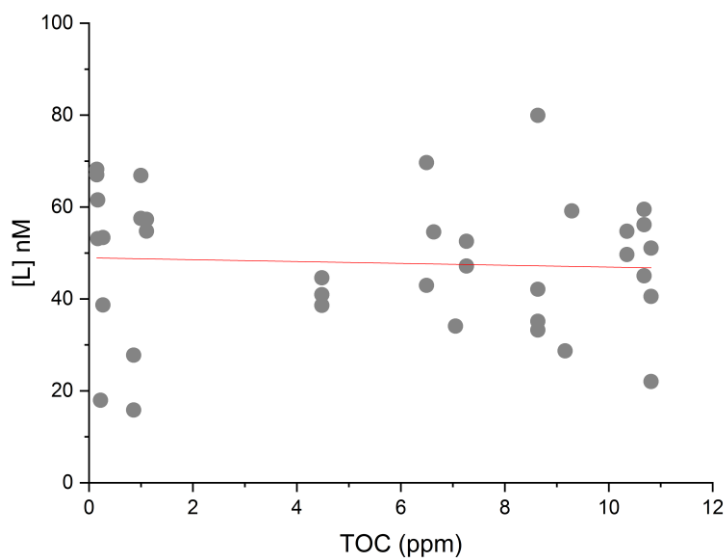


Figure S-1 Scatter plot and linear regression of total organic carbon (TOC) concentration vs. Cu-binding ligand concentration. Data show no correlation.

