Environmental
Toxicology and Chemistry

Original Article

DGT CAN PREDICT THE TOXICITY OF METAL MIXTURES TO TWO MICROALGAE – VALIDATION OF DGT FOR ENVIRONMENTAL MONITORING IN ANTARCTIC MARINE CONDITIONS

Darren J. Koppel, Merrin S. Adams, Catherine K. King, and Dianne F. Jolley

Environ Toxicol Chem., **Accepted Article** • DOI: 10.1002/etc.4399

Original Article **Environmental Toxicology and Chemistry**
DOI 10.1002/etc.4399

DGT CAN PREDICT THE TOXICITY OF METAL MIXTURES TO TWO MICROALGAE – VALIDATION OF DGT FOR ENVIRONMENTAL MONITORING IN ANTARCTIC MARINE CONDITIONS

Darren J. Koppel [a,b*], Merrin S. Adams [b], Catherine K. King [c], and Dianne F. Jolley[a*]

[a] *School of Chemistry, University of Wollongong, Wollongong, NSW, Australia, djk146@uowmail.edu.au*

[b] *CSIRO Land and Water, Lucas Heights, NSW, Australia, merrin.adams@csiro.au*

[c] *Australian Antarctic Division, Kingston, Tasmania, Australia, cath.king@aad.gov.au*

* Corresponding author, Dianne.Jolley@uts.edu.au

HIGHLIGHTS

- Diffusion coefficients for 5 metals were determined under cold marine conditions
- Metal competition on Chelex resin was observed at sub-saturation concentrations
- Deployment times to measure contaminant concentrations in cold waters are given
- DGT and dissolved concentrations predicted similar metal-mixture toxicities

This article includes online-only Supplemental Data.

*Address correspondence to Dianne.Jolley@uts.edu.au or djk146@uowmail.edu.au

DOI: 10.1002/etc.xxxx

This article contains online-only Supplemental Data

Submitted 22 August 2018; Returned for Revisions 13 February 2019; Accepted 15 February 2019

ABSTRACT

Anthropogenic contamination in the Antarctic near-shore marine environment is a challenge for environmental managers because of its isolation, high costs associated with monitoring and remediation activities, and the current lack of Antarctic-specific ecotoxicological data. This study investigated the application of diffusive gradients in thin-films (DGT) with a Chelex-100 binding resin for metal contaminant assessment in Antarctic marine conditions. Diffusion coefficients for cadmium, copper, nickel, lead and zinc, determined at 1 °C, ranged between 2.1 and 2.6 $\times 10^{-6}$ cm^2 s^{-1}, and were up to 32% lower than those derived by theoretical calculations. Competition of metals on the DGT binding resin was observed at sub-saturation concentrations, reducing the effective capacity for metal uptake by approximately 60%. The lability of the dissolved (0.45 µm filterable) cadmium, copper, lead and zinc metal fraction to DGT was generally >90% and unaffected by the presence of the Antarctic marine microalga *Phaeocystis antarctica*. Both DGT and dissolved metal concentrations gave equivalent mixture toxicity predictions in independent action and concentration addition models to *P. antarctica* and *Cryothecomonas armigera*, i.e. predictions using DGT-labile concentrations also showed antagonism to *P. antarctica*, which agrees with previously determined mixture interactivity. The benefits of DGT over traditional sampling techniques (i.e., discrete water sampling) include lower method detection limits, *in situ* assessment, and time-averaged concentrations which capture pulses of contamination typical of the Antarctic near-shore marine environment. This study provides method detection limits and recommended minimum deployment times to guide field deployments in Antarctica. This article is protected by copyright. All rights reserved

KEYWORDS: Multiple stressor, passive sampler, synergism, antagonism, risk assessment, environmental monitoring

INTRODUCTION

Localised areas of the Antarctic near-shore marine environment are contaminated from historical and ongoing anthropogenic activity, particularly near research stations (Tin et al. 2009). The Protocol on Environmental Protection to the Antarctic Treaty System outlines a need for comprehensive environmental protection, including environmental impact assessments and historical waste remediation (ATCM 1991). The Protocol has been in force since 1998, yet very few sites have been remediated (Roura 2004; Stark, Snape, et al. 2006; Errington et al. 2018). This is likely due to the challenges associated with access, difficulties in contaminant monitoring, and the lack of Antarctic-specific ecotoxicology data and remediation guidelines. Simple contaminant monitoring devices are needed to overcome challenges of expensive logistics, limited personnel, and limited access to contaminated sites in Antarctica (Hodgson-Johnston et al. 2017; Raymond and Snape 2017).

Monitoring strategies in Antarctic marine environments typically investigate the presence of metals by directly measuring concentrations in water (e.g. total or dissolved) or collected organisms (Palmer et al. 2006). These techniques provide useful information but do not necessarily inform environmental risk from metals. For example, the Antarctic near-shore marine environment is characterised by strong seasonality with ice melt, ice-berg scour, and algal blooms in summer that can lead to temporal variability in contaminant concentrations (Fryirs et al. 2013). Thus, a water sample taken at a specific timepoint may not account for potential pulse(s) of contamination (e.g. due to terrestrial ice melt or sea-ice scour of sediments), or factors known to modify metal toxicity such as metal speciation (e.g. binding to organic carbon, and water chemistry) (ANZECC/ARMCANZ 2000; Batley et al. 2014). Measuring metal concentrations in collected organisms will demonstrate the presence of a contaminant, but not necessarily that there is a toxic effect (e.g. metals can be stored in non-metabolically available forms (Rainbow et al. 2015)). This approach also requires the collection and destructive analysis of organisms, which is not desirable for Antarctic organisms because of their long developmental times and limited geographic distribution (Chapman and Riddle 2005; Griffiths 2010).

DGT has been used as an alternative, simple, and inexpensive contaminant monitoring tool, which measures the fraction of metals most likely to cause toxicity to organisms (Amato et al. 2014; Amato et al. 2015; Vannuci-Silva et al. 2017; Li et al. 2018 Dec 11). DGT has good potential to overcome the challenges associated with environmental management in the Antarctic marine environment because: (i) DGT preconcentrates analytes to a binding layer allowing for very low detection limits, which are required for the typically low contaminant concentrations present in Antarctic marine systems; (ii) DGT with a Chelex binding resin can simultaneously accumulate mixtures of cationic metals; (iii) it measures a time-averaged concentration over the deployment period which can capture pulse events (Frache et al. 2001; Stark, Johnstone, et al. 2006), and; (iv) studies are increasingly showing that DGT-labile metal concentrations is the fraction that best predicts toxicity to biota (Degryse et al. 2003; Degryse et al. 2009; Simpson et al. 2012; Amato et al. 2015; Amato et al. 2016). Despite this, DGT measurements are yet to be coupled to existing ecotoxicological data to predict metal-toxicity in Antarctic marine environments.

Coupling DGT-labile metal concentrations and single-metal toxicity data in mixture modelling may provide a simple method to predict the risk of contaminants in the Antarctic marine environment. This construct is meaningful only under the assumption that the metal fraction measured by DGT (i.e. free metal ions and metal complexes that dissociate in the DGT) is a close approximation of the biologically available fraction of metals measured in toxicity tests used to generate toxicity data. Recent research has generated single-contaminant toxicity data for a range of Antarctic organisms (King and Riddle 2001; Runcie and Riddle 2007; Hill et al. 2009; Gissi et al. 2015; Marcus Zamora et al. 2015; Holan et al. 2016; Koppel et al. 2017). However, it is not yet clear how DGT-labile metal concentrations relate to dissolved metal concentrations in exposure solutions typical of Antarctic ecotoxicological studies, or if they can be coupled with ecotoxicology data to predict toxicity from metal mixtures.

This study aims to: (i) assess the performance of DGT at temperatures reflective of Antarctic marine environments by validating their performance in laboratory exposures to the common contaminants cadmium, copper, nickel, lead, and zinc. This included determining metal diffusion coefficients, limits of detection (based on extractions of DGT binding resins from unexposed devices), minimum deployment times required to reach limits of detection at environmentally relevant exposure concentrations, method detection limits (i.e. minimum concentration in the exposure solution necessary to exceed limits of detection as a function of deployment time), and metal competition interactions; (ii) investigate the effects of the mucogenic Antarctic microalgae, *Phaeocystis antarctica*, on the uptake of metals by DGT devices co-deployed with microalgae ; and (iii) evaluate the suitability of using DGT-labile metal concentrations to predict metal-mixture toxicity to the Antarctic marine algae *P. antarctica* and *Cryothecomonas armigera* using previously determined single-metal toxicity data.

METHODS

General laboratory techniques and reagents

All plastic containers and equipment in contact with the test solutions were washed with nitric acid (10% v/v HNO_3) for ≥24 h, followed by extensive rinsing with ultrapure water (Milli-Q®, 18MΩ.cm^{-1}; Merck Millipore). Metal stock solutions were prepared from copper (II) sulfate;

cadmium (II) sulfate octahydrate, lead chloride, nickel sulfate hexahydrate, and zinc sulfate (all analytical grade or higher), acidified to 0.1% v/v HCl.

Seawater

Seawater was collected from Cronulla, New South Wales, Australia (34°04'13.4"S 151°09'24.5"E), in clean high-density polyethylene containers. Collected seawater was immediately filtered to 0.45 µm with a filter cartridge and stored at 4 °C in the dark. Salinity, pH, and dissolved oxygen saturation were measured with instruments calibrated as per the manufacturer's instruction. Metal concentrations in the collected seawater were below detection limits (see below). Seawater dissolved organic carbon concentrations from this source are typically 1.4 ± 0.3 mg $C.L^{-1}$ (APHA 5310 B) (Gissi et al. 2015).

Preparation of DGT devices and binding resin extraction

DGT pistons with a Chelex binding layer and agarose-crosslinked polyacrylamide diffusive gel were prepared following the procedures recommended by DGT Research (Lancaster, UK) as outlined in Davison (2016). The binding resin was a 0.4 mm thick Chelex-100 (Bio-Rad, mesh 200-400) impregnated polyacrylamide gel. During the binding resin synthesis, gravitational settling resulted in the Chelex-100 beads concentrating towards the bottom of the gel. This concentrated side was placed towards the window of the DGT, in contact with the 0.8 mm thick diffusive gel. A 0.13 mm thick, 0.45 µm pore size polyethersulfone filter paper was placed on top of the diffusive layer. The three layers were sandwiched on the piston base by a housing with a 2 cm diameter window. Prior to deployment, assembled pistons were conditioned for 24 h in a 0.12 M NaCl solution (Suprapur, Merck Millipore). Prepared DGT were stored moist in low-density polyethylene bags at 4°C.

Following deployment, DGT devices were disassembled and the binding resin placed in 1 mL of 1 M HNO_3 (Suprapur grade, Merck Millipore) for ≥12 h on an orbital shaker. The eluants were diluted to a final concentration of 0.2% HNO_3 before metal analysis.

Metal analysis

Metal concentrations were determined by inductively coupled plasma - atomic emission spectrometry (ICP-AES, Varian 730-ES) using matrix-matched calibration standards (i.e. ultrapure water for DGT eluants or seawater for treatment solution subsamples). A multi-element standard (QCS27; Analytical West Inc.) was used for seawater matrices to correct for measurement suppression over time. Metal detection limits of the ICP-AES were generally 3, 17, 27, 16, and 26 nM for cadmium, copper, nickel, lead and zinc, respectively.

Subsamples of the exposure solutions were taken when DGT were retrieved, and at the start and end of the algal bioassays. Subsamples were filtered to 0.45 µm and acidified to 0.2% HNO_3 before analysis.

DGT performance in cold marine waters

DGT performance, aim (i), was assessed with laboratory deployments of DGT to three different metal mixtures in cold marine conditions. DGT were then retrieved periodically over 36 days. Two mixtures used a ratio of five metals, based on measured concentrations at a historically contaminated bay in East Antarctica: 2 Cd : 8 Cu: 5 Ni : 1 Pb : 69 Zn (multiple of 1, in nM) (Stark, Johnstone, et al. 2006). The ratio was multiplied by 10 and 500 to give two treatments termed the Low and High Environmental mixture, respectively. The third treatment was an Equimolar mixture of five metals each at a nominal concentration of 10 µM. The average of all dissolved metal concentrations from each treatment are given in Table 1 (a-c).

DGT (n = 48 per treatment) were deployed in 20 L of 0.45 µm filtered seawater supplemented with metal solutions described above (Table 1 a-c). Treatments were equilibrated at 1 ± 1 °C in a temperature controlled environmental chamber for ≥72 h before DGT were deployed. A temperature of 1 °C was used as a compromise between the temperature of the near-shore Antarctic marine environment, which ranges from -2 to 1°C, and to ensure that the seawater would not freeze during the experiment.

A poly(methyl methacrylate) (Perspex) cube with 2.5 cm diameter holes was used to hold DGT pistons for the duration of their deployments and the solutions were agitated by magnetic stirrers or by an orbital shaker (rotating at approximately 60 RPM with an orbital diameter of 2.5 cm) for the duration of the exposure. DGT devices were retrieved periodically over 36 days, rinsed with ultrapure water and stored moist at 4 °C in clean plastic bags until they were disassembled and eluted. No changes to the integrity of the diffusive or binding gels were observed following deployment.

Diffusion coefficients. DGT binding resins were eluted and the mass of metal accumulated (M_i, nmol) was calculated using Equation 1.

Equation 1 $$M_i = \frac{C_e\,(V_e + V_{gel})}{f_e}$$

Where C_e is the concentration of the eluent, V_e and V_{gel} are the volumes of the eluent and gel, respectively, and f_e is the elution factor, which was 0.8 for all metals (Zhang and Davison 1995). The diffusion coefficients for each metal was calculated for 1 ± 1 °C using Equation 2.

Equation 2 $$D_i = \frac{M_i\,\Delta g}{t\,C_b\,A}$$

Where D_i is the diffusion coefficient of metal *i* (in units of x10^{-6} $cm^2.s^{-1}$ at 1°C), M is mass (nmol) accumulated over time t (s) over the linear uptake range, Δg is the thickness of the overall diffusion layer (0.093 cm, the combined thickness of the diffusive gel and filter membrane, assuming negligible water diffusion layer thickness), C_b (µM) is the time averaged dissolved metal concentration for the linear uptake range, and A (3.14 cm^2) is the surface area of the exposed window of the DGT piston (Zhang and Davison 1995). The linear range of metal accumulation was determined by the R^2 values of a linear model fit to each metal for each mixture against time. The slope of the longest deployment time that gave $R^2 \geq 0.95$ was selected to calculate the diffusion coefficient.

The DGT-labile metal concentration (C_{DGT}, µM) was then determined by Equation 3 (Zhang and Davison 1995), assuming a steady-state flux of metals to the binding resin over the deployment time.

Equation 3 $$C_{DGT} = \frac{M_i\, \Delta g}{D_i\, t\, A}$$

Limit of detection and time to limit of detection. The LOD (nmol) was determined as three times the standard deviation of M_i from unexposed blank DGT devices (≥3 DGT per experiment) analysed by ICP-AES (see Metal analysis section). The time to LOD was calculated as the minimum DGT deployment time needed to reach the LOD in cold marine water for a given cold marine metal concentration. Using Equation 3, C_{DGT} was fixed at theoretical metal concentrations (C_b, Table 1), which included treatments from aim (i) as well as example concentrations to help guide minimum field deployment times, including: the equivalent of the Australian and New Zealand marine water quality guideline value for 99% and 95% species protection levels (ANZECC/ARMCANZ 2000), the environmental ratio (see above), and near-shore marine water concentrations from Marion Cove, King George Island on the West Antarctic Peninsula (Kim et al. 2015).

Method detection limits. Method detection limits (MDL, µM) were calculated as the minimum metal concentrations in cold marine water required for a DGT to accumulate metal to its LOD for a given deployment time. The MDL was calculated using Equation 3 at various time points (1 – 30 d) where M_i was the average LOD from all DGT blanks.

Competition effects. Competition effects were defined as a deviation from a C_{DGT}/C_b ratio of 1 ± 0.2 for a metal, occurring where $\sum M_i$ showed linear accumulation. This was also observed for some metals in the residuals of a linear model fit of the M_i over time (Supplementary information S1).

Investigating the influence of P. antarctica *to DGT metal uptake*

DGT were co-deployed with *P. antarctica* to investigate how a mucogenic microalgae affects the DGT-lability of metals, aim (ii). DGT were deployed with and without the addition of *P. antarctica* ($1\text{-}3 \times 10^3$ cells.mL^{-1}) in treatments of the environmental ratio at multiples of 10, 20, 40, and 60 (equal to a molar total of 1, 2, 3 and 5 µM, respectively) and an equitoxic mixture (equal to 15 µM), where each metal was present at the concentration which reduced *P. antarctica* population growth rate by 10% (i.e. their EC10) based on single metal exposure (Gissi et al. 2015).

P. antarctica was sourced and cultured as described by Gissi et al. (Gissi et al. 2015). Test solutions were 160 mL of 0.45 µm filtered seawater in 400 mL tall glass silanised beakers at 1 ± 1 °C supplemented with 0.15 mg PO_4^{3-}.L^{-1}, 1.5 mg NO_3^{-}.L^{-1}, and metal stock solutions to give concentrations equivalent to their nominated mixture. Three replicates of each treatment were tested. DGT were deployed to beakers 3 days after test commencement, maintained on an orbital shaker, and retrieved after 1-7 days, depending on the metal solution.

The ratio of C_{DGT} / C_b was determined for each solution and significant differences between deployments with and without *P. antarctica* for each metal were determined by an analysis of variance (ANOVA) after testing for normality and homogeneity of variance of the ratios.

Using DGT-labile metal concentrations to predict metal mixture toxicity

The ability of DGT-labile concentrations to predict metal mixture toxicity to the algae *P. antarctica* and *C. armigera* was investigated using the Independent Action (IA) and Concentration Addition (CA) metal mixture models (Berenbaum 1985; Jonker et al. 2005). These are additive models that synthesise single-metal toxicities into 'expected' toxicities for mixtures. Single-metal toxicity data was used to parameterise the models, using previously determined EC10 and slope parameters from 3-parameter log-logistic model fits (Gissi et al. 2015; Koppel et al. 2017).

C_{DGT} measurements from aim (ii) were used in IA and CA mixture models using the approach outlined in Koppel et al. (Koppel et al. 2018) adapted from (Hochmuth et al. 2014). For CA toxic units were based on individual metal's EC10s, calculated by dividing the measured dissolved concentration of each metal in the mixture by its corresponding EC10 value calculated from single-metal treatments (Gissi et al. 2015; Koppel et al. 2017). The program R was used for all statistical analysis (R Core Team 2016) using the tidyverse package for graphing and data analysis (Wickham 2009).

IA and CA mixture toxicity predictions using DGT-labile concentrations from the multiples of the environmental ratio were compared to previously determined observed toxicity and predicted toxicity which used dissolved-metal concentrations (Koppel et al. 2018).

RESULTS

DGT performance in cold marine waters. Diffusion coefficients were determined to be ($x10^{-6}$ cm^2 s^{-1}): Cd 2.4 ± 0.4, Cu 2.1 ± 0.5, Ni 2.2 ± 0.1, Pb 2.6 ± 0.2, and Zn 2.1 ± 0.2 as mean ± standard deviation from deployments in the High Environmental, Low Environmental, and Equimolar solutions, (Table 2). Only the linear-portion of the gradient of metal uptake over time was used in the calculations (see deviations from uptake in Fig. 1 and residuals of linear model fits in Supplementary information S1). The R^2 values of the linear models at the different time points are given in Supplementary information S2. The parameters used to calculate diffusion coefficients (Equation 2) for each metal mixture are given in Supplementary information S3.

The LOD and time to LOD for DGT at 1 °C in marine waters are given in Table 1. The dissolved metal concentrations in the High Environmental and Equimolar solutions remained stable throughout the experiment (Supplementary information S3); however, metal concentrations in the Low Environmental treatment decreased by 20-50% over the duration of DGT deployments (Supplementary information S3), possibly due to adsorption to the container wall or metal precipitation. This decrease was accounted for by using the time-average dissolved metal concentration for each DGT deployment period.

The minimum deployment time to exceed the LOD for all metals tested was <1 d for the High Environmental and Equimolar mixtures, 17 d for the Low Environmental mixture, 4 and 15 d for waters with concentrations equivalent to the Australian and New Zealand water quality guideline values for 95 and 99% species protection (ANZECC/ARMCANZ 2000), respectively, 43 d for the environmental ratio, and 332 d for background concentrations in the Antarctic near-shore marine environment. However, times varied for individual metals and are given in Table 1.

The method detection limits (MDL) for deployments of various times ranged from 165 nM for nickel in a 1-d deployment to 0.3 pM for cadmium in a 30-d deployment (Table 3). There is a linear relationship between the MDL and 1/t (Equation 3) which can be used to calculate the MDL for other deployment times.

Metal uptake and competition effects. The ratio of DGT-labile metal (C_{DGT}) to dissolved metal (C_b) concentrations in the solution for all metals in all mixtures was generally 1 ± 0.2 (Fig. 2). The Low Environmental mixture was the most variable of the mixtures, with a C_{DGT}/C_b ratio for copper and nickel of approximately 0.8, and for cadmium between 1.2 and 1.5, particularly at early time points (of <400 h, Fig. 2). Given the low metal concentrations in this treatment, this is most likely due to the increased variability in ICP-AES measurements near the detection limits for those metals.

Metal competition was observed in the Equimolar and High Environmental mixtures, where cadmium lost quantitative uptake (Fig. 1 b, c). Cadmium lost linearity (defined as $R^2 < 0.95$ of the linear model fit) after 16 d in the Equimolar solution and 23 d in the High Environmental mixture, where the resin had accumulated ∑5.4 ± 0.3 µmol of metals (at 486 h). Cadmium displacement from the resin was observed in the High Environmental mixture where a maximum of 71 ± 8 nmol at 23 d decreased to 35 ± 10 nmol Cd at 30 d. This suggests active substitution of cadmium by other metals which retained steady-state uptake kinetics. This was less clear for cadmium in the equimolar mixture, where concentrations were more variable where the resin had accumulated ∑4.8 ± 0.7 µmol of metals at 486 h (Fig. 1). Zinc appeared to reach saturation in both the Equimolar and High Environmental mixtures at around 30 d (Fig. 1), but the experiment ended before competition affected the linearity of zinc uptake (Supplementary information S1).

Investigating the influence of P. antarctica *to the DGT metal uptake*

No difference was observed between the C_{DGT}/C_b ratio in deployments in the presence and absence of *P. antarctica* for Cd (1.0 ± 0.2 / 1.0 ± 0.2), Cu (1.0 ± 0.4 / 0.9 ± 0.2), Pb (0.9 ± 0.1 / 1.0 ± 0.2), and Zn (0.94 ± 0.04 / 0.9 ± 0.1) (Fig. 3), across all treatments (Table 4). However, the C_{DGT}/C_b ratio for nickel was significantly lower in deployments in the presence of *P. antarctica* than in the absence (0.75 ± 0.11 / 0.86 ± 0.14, $p = 0.03$). The ratios from individual treatments and metals are given in Supplementary information S4.

Using DGT-labile metal concentrations to predict metal mixture toxicity

Both independent action and concentration addition mixture models gave similar toxicity predictions to *P. antarctica* and *C. armigera* microalgae. The models were good predictors of metal mixture toxicity to *C. armigera* within the concentration range tested, but overestimated toxicity to *P. antarctica* (Fig. 4).

DISCUSSION

DGT performance in cold marine waters

DGT was shown to be a sensitive measure of metals in seawater, with LODs generally in the low nmol to pmol range (Table 1). Our results suggest that DGT is suitable for the environmental monitoring of metal contamination in cold marine waters (Table 1). For example, a deployment period (i.e. time to LOD) of >4 or 15 d would be needed to measure seawater metal concentrations equivalent to the Australian and New Zealand marine water quality guidelines values for 95 and 99% species protection, respectively (ANZECC/ARMCANZ 2000). With the detection limits of an ICP-AES, DGT would not be suitable for the measurement of trace metals in cold seawater because of the long deployment times >332 d needed. However, DGT could be practical if using ultra-trace metal clean laboratory techniques and sensitive analytical instrumentation, such as inductively couple plasma mass spectroscopy.

The diffusion coefficients derived in this study are the first to be determined in conditions (i.e. temperature, salinity, and pH) reflective of the near-shore Antarctic marine environment. They are up to 32% lower than those previously determined at similar temperatures (Table 2) (Larner et al. 2006; Buffle et al. 2007; DGT Research 2018). These reported diffusion coefficients decrease in increasingly complicated media (e.g. theoretical > estuarine > seawater), possibly due to an increased likelihood of collisions, electrostatic repulsion, changes in solution viscosity, or the presence of metal complexes (Buffle et al. 2007). The size of a metal complex will change diffusion rates across the DGT diffusive layer (Zhang and Davison 2000). This can be influenced by different pH's, with a lower pH increasing the likelihood of a metal existing as a free ion (Byrne et al. 1988), subsequently increasing the diffusion rate. Based on the results of this study, using previously reported diffusion coefficients could underestimate C_{DGT} calculations by as much as 32%.

The Chelex resin does not act as a perfect metal sink at sub-saturation concentrations. This is an underreported finding, but in agreement with previous studies (Degryse et al. 2003; Jiménez-Piedrahita et al. 2017). A Chelex-100 resin has a capacity for divalent cations of 10-15 µmol per DGT piston (Tankéré-Muller et al. 2012; Bio-Rad laboratories 2013). In our study, competition effects were observed after approximately 20 days in the Equimolar and High Environmental mixtures where the mass of the five metals on the binding resin were 4.8 ± 0.7 and 5.4 ± 0.3 µmol, respectively. This highlights significant competition effects at a total molar concentration between 30-50% of the theoretical resin capacity. The competition observed agreed with the metal's selectivity for the Chelex resin (normalised to zinc, at a circumneutral pH): Cu^{2+} (126) >> Ni^{2+} (4.4) > Pb^{2+} (3.9) > Zn^{2+} (1) > Co^{2+} (0.6) > Cd^{2+} (0.4) > Fe^{2+} (0.1) > Mn^{2+} (0.02) (Bio-Rad laboratories 2013), i.e. of the metal analytes in the mixture, cadmium and zinc have the lowest selectivity for Chelex resin and were the first to lose linearity of their uptake. Cadmium was clearly outcompeted in the High Environmental mixture, but in the Equimolar mixture, this was less clear with some DGT showing expected cadmium accumulation while others showed lower accumulation after 20 d (Fig. 1).

The relative metal concentrations, as well as their selectivity, can influence competition effects. Tankéré-Muller et al. (2012) found that Mg^{2+} and Ca^{2+} saturate a Chelex-100 binding resin when deployed in seawater for longer than 2 h, suggesting that metal binding occurs by active exchange of metals with differing selectivities on a saturated resin. However, when Fe^{2+} was present in high concentrations, both Mn^{2+} and Cd^{2+} were displaced, despite Cd^{2+} having a greater selectivity than Fe^{2+}. This may be caused by a reduction in binding affinity for metals to the Chelex-100 resin from electrostatic screening of the functional groups in high ionic strengths (Jiménez-Piedrahita et al. 2017). These confounding factors may explain the variability in the cadmium measurements in the equimolar mixture which had broadly equivalent concentrations of metals, compared to the high environment mixture where cadmium was at a much lower concentration compared to copper and zinc.

Investigating the influence of P. antarctica *to the DGT metal uptake*

All metals had C_{DGT}/C_b ratios >0.9, except for nickel which was 0.75 in the presence of *P. antarctica* and 0.86 in its absence (average of all treatments, Fig. 3, Supplementary information S4). Ratios <1 are commonly observed in studies with complex matrices. For example, DGT measurements of seawater overlying sediments had C_{DGT}/C_b ratios of 0.8 for zinc and 0.25 for copper were previously reported, possibly from dissolved organic carbon (DOC) or particle adsorption (Amato et al. 2015). For this reason, the addition of *P. antarctica* was expected to reduce the C_{DGT}/C_b ratio. *P. antarctica* is known to produce DOC as a mechanism to chelate micronutrients like iron and zinc in marine waters (Alderkamp et al. 2007; Thuróczy et al. 2012). Other micronutrients may also be bound in this process, such as nickel which is an essential metal for urease - an enzyme most marine phytoplankton use to hydrolyse urea to NH_3 and CO_2 (Dupont et al. 2010). While a significant reduction in the C_{DGT}/C_b ratio was observed for nickel, it was not for other micronutrients like zinc or copper. The reduction in nickel DGT concentrations could be a Type I error, given the sample sizes of 15 and 12 for deployments in the presence and absence of *P. antarctica*. The ratio could also be affected by uncertainty in the measurement of nickel (C_b) which was present at low concentrations in multiples of the environmental ratio and has a relatively high instrument detection limit. This is most likely because of the metals tested, copper has a higher binding affinity for DOC and should have a lower ratio in the presence of *P. antarctica*, compared to nickel (Smith et al. 2002).

Overall, the high ratios >0.9 for most metals suggest that dissolved metals in 0.45 µm filtered, low DOC (1.4 ± 0.3 mg C L^{-1}), seawater mediums are DGT-labile. This gives confidence in relating C_{DGT} to previously determined ecotoxicology data, which is typically derived from dissolved metal concentrations from laboratory exposures.

Using DGT-labile metal concentrations to predict metal mixture toxicity

DGT-labile metal concentrations and dissolved metal concentrations provided similar predictions of metal-mixture toxicity to *P. antarctica* and *C. armigera* and also highlighted metal-mixture interactivity (Fig 4). *P. antarctica* is known to exhibit significant antagonistic mixture interactions in response to increasing multiples of the environmental ratio (Koppel et al. 2018). The cause of the antagonism is not known but may be due to extracellular mucous production reducing the bioavailability of metals like copper and cadmium (Oleinikova et al. 2018). *C.*

armigera may also exhibit synergistic interactions at high effect concentrations. This study did not test a concentration range where this was expected to have been observed.

Practical considerations of using DGT in cold marine environments

The Antarctic near-shore marine environment has variable metal fluxes, DOC concentrations, and concentrations of inorganic adsorbents like iron or aluminium hydroxides. In such a complex environment, DGT allows for the simultaneous uptake of common metal contaminants, with ultra-trace detection limits, time-averaged over the deployment period (Jolley et al. 2016).

Water quality guideline values specific for the Antarctic marine ecosystem have not yet been developed, so minimum deployment times to reach DGT LODs are suggested based on seawater concentrations equivalent to the Australian and New Zealand marine water quality guideline values for 99 and 95% species protection (ANZECC/ARMCANZ 2000). At these concentrations a recommended minimum deployment time of at least 15 d is needed (Table 1). Alternatively, MDLs at various deployment times are given in Table 3. As per Equation 3, increasing deployment time will decrease MDL. It should be noted that these values will depend on the instrument sensitivity, cleanliness, and LODs achieved by each laboratory.

The risk of metal competition on the Chelex resin is low for Antarctic marine deployments, given the low metal concentrations in contaminated marine areas (Larner et al. 2006; Stark, Johnstone, et al. 2006), and low diffusion coefficients (Table 2). However, where the molar total of metal-analytes with different selectivities exceeds approximately $\sum 4$ µmol, care should be taken in the interpretation of the results.

The application of DGT outlined in this study differs from the limited capacity in which DGT has already been used for environmental monitoring in the Antarctic environment (Larner et al. 2006; Stark, Johnstone, et al. 2006). These studies assessed the presence of contaminants rather than concentrations relating to the risk they pose to marine organisms. DGT measures a biologically available fraction of metals in solution which is the fraction most likely to cause toxicity to organisms (Simpson et al. 2012; Amato et al. 2014; Amato et al. 2016). This study has shown that DGT-labile metal fractions in seawater can be used to predict the toxicity of metal contaminants to two Antarctic marine algae, where single-metal toxicity exists – at least as well as dissolved metal concentrations.

This study validates the use of DGT in Antarctic marine environments and provides guidance around their deployment. These results should be confirmed in an *in-situ* field study where factors such as biofouling or interference from native fauna can be investigated.

CONCLUSION

DGT were shown to be a sensitive tool to measure metals in cold marine waters, where the dynamic environment, limited accessibility, and costly logistics necessitates new monitoring techniques. This study provides empirical diffusion coefficients for five common metal contaminants, method detection limits, minimum deployment times for environmental monitoring, and highlights that metal competition at sub-saturation concentrations needs to be considered where the molar total of metals accumulated to the Chelex resin exceeds approximately ∑4 µmol.

DGT-labile concentrations are suitable to use in mixture toxicity modelling. However, DGT-labile concentrations are no better than dissolved metal concentrations at predicting biological mixture toxicity interactions (i.e. synergism or antagonism). DGT is well positioned to be used in the environmental risk assessment of metal contaminants in cold marine environments, particularly with the ongoing generation of ecotoxicological data towards the derivation of Antarctic-specific environmental quality guidelines.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

ACKNOWLEDGEMENT

Funding and support for the present study was provided by the Australian government through Australian Antarctic Science Grant (AAS 4326), an Australian Government Research Training Program Scholarship for DK, and by CSIRO Land and Water. We thank E. Amato (University of Antwerp) and G. Bately (CSIRO) and the anonymous journal reviewers for comments which improved the manuscript. Data generated from this study are available from the Australian Antarctic Data Centre http://dx.doi.org/10.4225/15/5746938EC8C8B.

Data accessibility—This article has earned an Open Data/Materials badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at [https://data.aad.gov.au/metadata/records/AAS_4326_DGTvalidation]. Learn more about the Open Practices badges from the Center for Open Science: https://osf.io/tvyxz/wiki..

REFERENCES

Alderkamp AC, Buma AGJ, van Rijssel M. 2007. The carbohydrates of *Phaeocystis* and their degradation in the microbial food web. In: van Leeuwe MA, Stefels J, Belviso S, Lancelot C, Verity PG, Gieskes WWC, editors. *Phaeocystis*, major link in the biogeochemical cycling of climate-relevant elements. Springer Netherlands. p. 99–118.

Amato ED, Simpson SL, Belzunce-Segarra MJ, Jarolimek CV, Jolley DF. 2015. Metal fluxes from porewaters and labile sediment phases for predicting metal exposure and bioaccumulation in benthic invertebrates. Environ Sci Technol. 49(24):14204–14212. doi:10.1021/acs.est.5b03655.

Amato ED, Simpson SL, Jarolimek CV, Jolley DF. 2014. Diffusive gradients in thin films technique provide robust prediction of metal bioavailability and toxicity in estuarine sediments. Environ Sci Technol. 48(8):4485–4494. doi:10.1021/es404850f.

Amato ED, Simpson SL, Remaili TM, Spadaro DA, Jarolimek CV, Jolley DF. 2016. Assessing the effects of bioturbation on metal bioavailability in contaminated sediments by diffusive gradients in thin films (DGT). Environ Sci Technol. 50(6):3055–3064. doi:10.1021/acs.est.5b04995.

ANZECC/ARMCANZ. 2000. Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand. [accessed 2018 Feb 26]. http://www.agriculture.gov.au:80/water/quality/guidelines.

ATCM. 1991. The Protocol on Environmental Protection to the Antarctic Treaty. Antarctic Treaty Consultative Meeting.

Batley GE, van Dam RA, Warne MS, Chapman JC, Fox DR, Hickey CW, Stauber JL. 2014. Technical rationale for changes to the method for deriving Australian and New Zealand water quality guidelines values for toxicants. CSIRO Land and Water: Commonwealth Scientific and Industrial Research Organisation.

Berenbaum MC. 1985. The expected effect of a combination of agents: the general solution. J Theor Biol. 114(3):413–431. doi:10.1016/S0022-5193(85)80176-4.

Bio-Rad laboratories. 2013. Chelex® 100 and Chelex 20 Chelating Ion Exchange Resin Instruction Manual.

Buffle J, Zhang Z, Startchev K. 2007. Metal flux and dynamic speciation at (bio)interfaces. Part I: Critical evaluation and compilation of physicochemical parameters for complexes with simple ligands and fulvic/humic substances. Environ Sci Technol. 41(22):7609–7620. doi:10.1021/es070702p.

Byrne RH, Kump LR, Cantrell KJ. 1988. Inorganic Marine Chemistry The influence of temperature and pH on trace metal speciation in seawater. Mar Chem. 25(2):163–181. doi:10.1016/0304-4203(88)90062-X.

Chapman PM, Riddle MJ. 2005. Toxic effects of contaminants in polar marine environments. Environ Sci Technol. 39(9):200A-207A.

Davison W. 2016. Diffusive Gradients in Thin-Films for Environmental Measurements. Lancaster, UK: Cambridge University Press (Cambridge Environmental Chemistry Series).

Degryse F, Smolders E, Oliver I, Zhang H. 2003. Relating soil solution Zn concentration to diffusive gradients in thin films measurements in contaminated soils. Environ Sci Technol. 37(17):3958–3965. doi:10.1021/es034075p.

Degryse F, Smolders E, Zhang H, Davison W. 2009. Predicting availability of mineral elements to plants with the DGT technique: a review of experimental data and interpretation by modelling. Environ Chem. 6(3):198–218. doi:10.1071/EN09010.

DGT Research. 2018. Diffusion Coefficients. DGT Res. [accessed 2018 Jul 13]. https://www.dgtresearch.com/diffusion-coefficients/.

Dupont CL, Buck KN, Palenik B, Barbeau K. 2010. Nickel utilization in phytoplankton assemblages from contrasting oceanic regimes. Deep-Sea Res Part Oceanogr Res Pap. 57(4):553–566. doi:10.1016/j.dsr.2009.12.014.

Errington I, King CK, Wilkins D, Spedding T, Hose GC. 2018. Ecosystem effects and the management of petroleum-contaminated soils on subantarctic islands. Chemosphere. 194:200–210. doi:10.1016/j.chemosphere.2017.11.157.

Frache R, Abelmoschi ML, Grotti M, Ianni C, Magi E, Soggia F, Capodaglio G, Turetta C, Barbante C. 2001. Effects of ice melting on Cu, Cd and Pb profiles in Ross Sea waters (Antarctica). Int J Environ Anal Chem. 79(4):301–313. doi:https://doi.org/10.1080/03067310108044391.

Fryirs KA, Snape I, Babicka N. 2013. The type and spatial distribution of past waste at the abandoned Wilkes Station, East Antarctica. Polar Rec. 49(4):328–347. doi:10.1017/S0032247412000721.

Gissi F, Adams MS, King CK, Jolley DF. 2015. A robust bioassay to assess the toxicity of metals to the Antarctic marine microalga *Phaeocyctis antarctica*. Environ Toxicol Chem. 34(7):1578–1587. doi:10.1002/etc.2949.

Griffiths HJ. 2010. Antarctic marine biodiversity - what do we know about the distribution of life in the southern ocean? PLoS ONE. 5(8):e11683. doi:10.1371/journal.pone.0011683.

Hill NA, King CK, Perrett LA, Johnston EL. 2009. Contaminated suspended sediments toxic to an Antarctic filter feeder: Aqueous- and particulate-phase effects. Environ Toxicol Chem. 28(2):409–417.

Hochmuth JD, Asselman J, De Schamphelaere KAC. 2014. Are interactive effects of harmful algal blooms and copper pollution a concern for water quality management? Water Res. 60:41–53. doi:10.1016/j.watres.2014.03.041.

Hodgson-Johnston I, Jackson A, Jabour J, Press A. 2017. Cleaning up after human activity in Antarctica: legal obligations and remediation realities. Restor Ecol. 25(1):135–139.

Holan JR, King CK, Davis AR. 2016. Sensitivity of six subantarctic marine invertebrates to common metal contaminants. Environ Toxicol Chem. 35(9):2245–2251. doi:10.1002/etc.3382.

Jiménez-Piedrahita M, Altier A, Cecilia J, Puy J, Galceran J, Rey-Castro C, Zhang H, Davison W. 2017. Extending the use of diffusive gradients in thin films (DGT) to solutions where competition, saturation, and kinetic effects are not negligible. Anal Chem. 89(12):6567–6574. doi:10.1021/acs.analchem.7b00704.

Jolley DF, Mason S, Gao Y, Zhang H. 2016. Practicalities of Working with DGT. In: Diffusive Gradients in Thin-Films for Environmental Measurements. Cambridge: Cambridge University Press. (Cambridge Environmental Chemistry Series). p. 263–290.

Jonker MJ, Svendsen C, Bedaux JJM, Bongers M, Kammenga JE. 2005. Significance testing of synergistic/antagonistic, dose level-dependent, or dose ratio-dependent effects in mixture dose-response analysis. Environ Toxicol Chem. 24(10):2701–2713. doi:10.1897/04-431R.1.

Kim I, Kim G, Jung Choy E. 2015. The significant inputs of trace elements and rare earth elements from melting glaciers in Antarctic coastal waters. Polar Res. 34(1):24289. doi:10.3402/polar.v34.24289.

King CK, Riddle MJ. 2001. Effects of metal contaminants on the development of the common Antarctic sea urchin *Sterechinus neumayeri* and comparisons of sensitivity with tropical and temperate echinoids. Mar Ecol Prog Ser. 215:143–154.

Koppel DJ, Adams MS, King CK, Jolley DF. 2018. Chronic toxicity of an environmentally relevant and equitoxic ratio of five metals to two Antarctic marine microalgae shows complex mixture interactivity. Environ Pollut. 242:1319–1330. doi:10.1016/j.envpol.2018.07.110.

Koppel DJ, Gissi F, Adams MS, King CK, Jolley DF. 2017. Chronic toxicity of five metals to the polar marine microalga *Cryothecomonas armigera* – Application of a new bioassay. Environ Pollut. 228:211–221. doi:10.1016/j.envpol.2017.05.034.

Larner BL, Seen AJ, Snape I. 2006. Evaluation of diffusive gradients in thin film (DGT) samplers for measuring contaminants in the Antarctic marine environment. Chemosphere. 65(5):811–820. doi:https://doi.org/10.1016/j.chemosphere.2006.03.028.

Li C, Ding S, Yang L, Wang Y, Ren M, Chen M, Fan X, Lichtfouse E. 2018 Dec 11. Diffusive gradients in thin films: devices, materials and applications. Environ Chem Lett. doi:10.1007/s10311-018-00839-9. [accessed 2019 Feb 11]. https://doi.org/10.1007/s10311-018-00839-9.

Marcus Zamora L, King CK, Payne SJ, Virtue P. 2015. Sensitivity and response time of three common Antarctic marine copepods to metal exposure. Chemosphere. 120:267–272. doi:10.1016/j.chemosphere.2014.07.051.

Oleinikova OV, Shirokova LS, Drozdova OY, Lapitskiy SA, Pokrovsky OS. 2018. Low biodegradability of dissolved organic matter and trace metals from subarctic waters. Sci Total Environ. 618:174–187. doi:10.1016/j.scitotenv.2017.10.340.

Palmer AS, Snape I, Stark JS, Johnstone GJ, Townsend AT. 2006. Baseline metal concentrations in *Paramoera walkeri* from East Antarctica. Mar Pollut Bull. 52(11):1441–1449. doi:10.1016/j.marpolbul.2006.04.012.

R Core Team. 2016. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

Rainbow PS, Liu F, Wang WX. 2015. Metal accumulation and toxicity: the critical accumulated concentration of metabolically available zinc in an oyster model. Aquat Toxicol Amst Neth. 162:102–108. doi:10.1016/j.aquatox.2015.03.007.

Raymond TC, Snape I. 2017. Using triage for environmental remediation in Antarctica. Restor Ecol. 25(1):129–134. doi:10.1111/rec.12383.

Roura R. 2004. Monitoring and remediation of hydrocarbon contamination at the former site of Greenpeace's World Park Base, Cape Evans, Ross Island, Antarctica. Polar Rec. 40(1):51–67. doi:10.1017/S0032247403003292.

Runcie JW, Riddle MJ. 2007. Assessing the toxic effects of dissolved copper on the Antarctic macroalga *Desmarestia menziesii* using chlorophyll fluorescence imaging in ecotoxicological tests. Toxicol Environ Chem. 89(4):641–653.

Simpson SL, Yverneau H, Cremazy A, Jarolimek CV, Price HL, Jolley DF. 2012. DGT-induced copper flux predicts bioaccumulation and toxicity to bivalves in sediments with varying properties. Environ Sci Technol. 46(16):9038–9046. doi:10.1021/es301225d.

Smith DS, Bell RA, Kramer JR. 2002. Metal speciation in natural waters with emphasis on reduced sulfur groups as strong metal binding sites. Comp Biochem Physiol - C Toxicol Pharmacol. 133(1–2):65–74. doi:10.1016/S1532-0456(02)00108-4.

Stark JS, Johnstone GJ, Palmer AS, Snape I, Larner BL, Riddle MJ. 2006. Monitoring the remediation of a near shore waste disposal site in Antarctica using the amphipod *Paramoera walkeri* and diffusive gradients in thin films (DGTs). Mar Pollut Bull. 52(12):1595–1610.

Stark JS, Snape I, Riddle MJ. 2006. Abandoned Antarctic waste disposal sites: Monitoring remediation outcomes and limitations at Casey Station. Ecol Manag Restor. 7(1):21–31. doi:10.1111/j.1442-8903.2006.00243.x.

Tankéré-Muller S, Davison W, Zhang H. 2012. Effect of competitive cation binding on the measurement of Mn in marine waters and sediments by diffusive gradients in thin films. Anal Chim Acta. 716:138–144. doi:10.1016/j.aca.2011.12.028.

Thuróczy CE, Alderkamp AC, Laan P, Gerringa LJA, Mills MM, Van Dijken GL, De Baar HJW, Arrigo KR. 2012. Key role of organic complexation of iron in sustaining phytoplankton blooms in the Pine Island and Amundsen Polynyas (Southern Ocean). Deep-Sea Res Part II Top Stud Oceanogr. 71–76:49–60.

Tin T, Fleming ZL, Hughes KG, Ainley DG, Convey P, Moreno CA, Pfeiffer S, Scott J, Snape I. 2009. Impacts of local human activities on the Antarctic environment. Antarct Sci. 21(01):3–33. doi:10.1017/S0954102009001722.

Vannuci-Silva M, de Souza JM, de Oliveira FF, de Araújo MAG, Francioni E, Eismann CE, Kiang CH, Govone JS, Belzunce-Segarra MJ, Menegário AA. 2017. Bioavailability of Metals at a Southeastern Brazilian Coastal Area of High Environmental Concern Under Anthropic Influence: Evaluation Using Transplanted Bivalves (*Nodipecten nodosus*) and the DGT Technique. Water Air Soil Pollut. 228(6). doi:10.1007/s11270-017-3387-4.

Wickham H. 2009. ggplot2: Elegant Graphics for Data Analysis. New York: Springer-Verlag (Use R!). [accessed 2018 Sep 3]. //www.springer.com/gp/book/9780387981413.

Zhang H, Davison W. 1995. Performance characteristics of diffusion gradients in thin films for the in situ measurement of trace metals in aqueous solution. Anal Chem. 67(19):3391–3400.

Zhang H, Davison W. 2000. Direct In Situ Measurements of Labile Inorganic and Organically Bound Metal Species in Synthetic Solutions and Natural Waters Using Diffusive Gradients in Thin Films. Anal Chem. 72(18):4447–4457. doi:10.1021/ac0004097.

Graphical abstract. DGT-measured metal concentrations are used to predict the toxicity of metal mixtures to two Antarctic marine microalgae.

Figure 1. Mass of analyte (nmol) accumulated over time (h) during uptake experiments in the (a) Equimolar, (b) High Environmental, and (c) Low Environmental mixtures. Note the different y-axis scales for (b) and (c) where copper, nickel, and zinc (left) were present at higher concentrations than cadmium and lead (right). Concentrations of lead were below detection limit in the Low Environment mixture.

Figure 2. The ratio of DGT measured to dissolved (0.45 µm filterable) metal concentration (C_{DGT}/C_b) for the Equimolar, High Environmental, and Low Environmental metal mixtures. A ratio of 1, solid horizontal line, indicates equivalent concentrations measured. The dotted line above and below the solid line represent a 20% deviation from 1.

Figure 3. The relationship between DGT-labile (C_{DGT}) and total dissolved metal concentrations (C_b) for deployments in the equitoxic and multiples of the environmental ratio treatments in the presence (purple) and absence of *Phaeocystis antarctica* (grey). Points falling on the 1:1 line suggest that the C_{DGT} fraction is representative of the dissolved metals (C_b) in cold marine conditions.

Figure 4. Predicted toxicity using DGT-labile metal concentrations (C_{DGT}) to *Phaeocystis antarctica* (a) and *Cryothecomonas armigera* (b). Predictions from C_{DGT} by Concentration Addition (CA, blue squares) and Independent Action (IA, orange diamonds) models are overlayed against previously determined toxicity predictions using dissolved metal concentrations (clear squares and diamonds, respectively) and observed toxicity (black circles) from multiples of the environmental ratio (Koppel et al. 2018). Toxicity as population growth rate inhibition is plotted against the sum of toxic units based on EC10 concentrations (TU_{EC10}).

Table 1. Average dissolved metal concentrations for the DGT deployment period (C_b, µM), limit of detection (LOD, nmol), and time to limit of detection (h) for DGT deployments in 1 ± 1 °C seawater for (a) High Environmental, (b) Low Environmental, (c) Equimolar mixtures, (d) meals in seawater at water quality guideline values for 95% protection (ANZECC/ARMCANZ 2000), (e) seawater at water quality guideline values for 99% protection (ANZECC/ARMCANZ 2000), (f) environmental ratio, and (g) reported concentrations from the Antarctic near-shore environment.

a. High Environmental	**Cd**	**Cu**	**Ni**	**Pb**	**Zn**
C_b	0.51 ± 0.02	5.1 ± 0.2	2.4 ± 0.1	0.48 ± 0.02	33.1 ± 0.9
LOD	0.01	0.6	0.7	0.1	0.6
Time to LOD	0.4	0.3	1.7	1.3	0.05
b. Low Environmental					
C_b	0.007 ± 0.002	0.1 ± 0.01	0.05 ± 0.02	0.0015 ± 0.0003	0.57 ±0.07
LOD	0.1	0.3	2	0.1	0.4
Time to LOD	29	16	80	395	3
c. Equimolar					
C_b	10.4 ± 0.7	7.8 ± 0.4	9.7 ± 0.8	3.0 ± 0.2	8.9 ± 0.6
LOD	0.1	0.3	2	0.1	0.4
Time to LOD	0.02	0.2	0.4	0.2	0.2
d. Water quality guideline values for 95% species protection (ANZECC/ARMCANZ 2000)					
C_b[a]	0.05	0.02	1.2	0.02	0.2
LOD[b]	0.1	0.4	1.1	0.2	0.4
Time to LOD	4	79	3	29	8
e. Water quality guideline values for 99% species protection (ANZECC/ARMCANZ 2000)					
C_b[a]	0.006	0.005	0.12	0.01	0.1
LOD[b]	0.1	0.3	2	0.1	0.4
Time to LOD	31	341	33	57	16
f. Environmental ratio (Larner et al. 2006)					
C_b[a]	0.009	0.002	0.005	0.001	0.07
LOD[b]	0.1	0.4	1.1	0.2	0.4
Time to LOD	22	1024	774	419	25
g. Reported concentrations from the Antarctic near-shore environment (Kim et al. 2015)					
C_b[a]	0.00003	0.0006	0.0006	0.0001	0.002
LOD[b]	0.1	0.4	1.1	0.2	0.4
Time to LOD	6975	2647	7002	7963	1107

[a] = Concentration from literature used to calculate time to LOD

[b] = Calculated as the average of DGT blanks from all experiments

Table 2. Comparison of diffusion coefficients (D_i) from various studies. Empirically determined diffusion coefficients from this study are the mean and standard deviation of the three treatments.

	Temp (°C)	Salinity (‰)	pH	Diffusion coefficients (x10^{-6} cm^2.s^{-1})					Reference
				Cd	Cu	Ni	Pb	Zn	
Seawater	1 ± 1	34	7.9 ± 0.1	2.4 ± 0.4	2.1 ± 0.5	2.2 ± 0.1	2.6 ± 0.2	2.1 ± 0.2	This study
Estuarine	-1	20	6.7	3.0	2.6	ND	3.6	2.9	(Larner et al. 2006)
Theoretical	1	NA	NA	2.8	2.9	2.7	3.8	2.8	(Buffle et al. 2007; 2018)

Table 3. Method detection limits (MDL, nM) at various deployment times.

Metal	LOD (nmol)	MDL (nM) for deployment times (d)				
		1	2	7	15	30
Cd	0.06	8.0	4.0	1.1	0.5	0.3
Cu	0.40	67	34	10	4.5	2.2
Ni	1.08	165	82	24	11	5.5
Pb	0.19	25	13	3.6	1.7	0.8
Zn	0.44	72	36	10	4.8	2.4

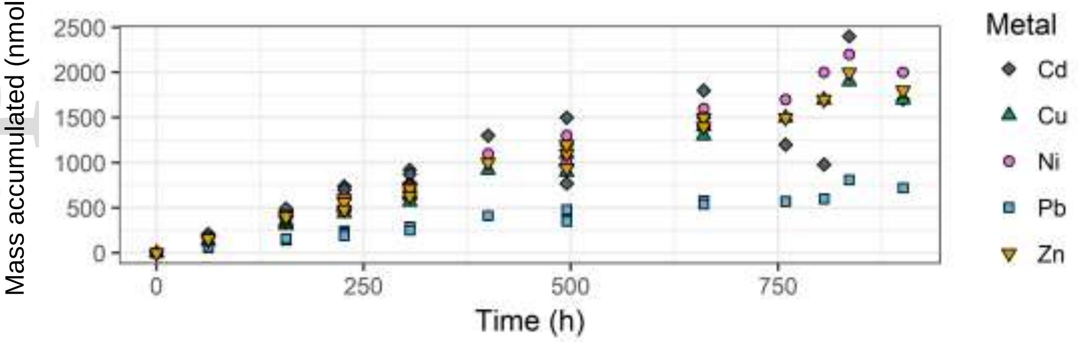
Table 4. Dissolved metal concentrations (C_b, µM) for the equitoxic mixture and multiples of the environmental ratio for DGT deployments in the presence (+) or absence (-) of *P. antarctica*. No 60- treatment was tested.

Metal	Equitoxic -	Equitoxic +	Multiples of the environmental ratio						
			10 -	**10 +**	**20 -**	**20 +**	**40 -**	**40+**	**60+**
Cd	1.7	1.7	0.01	0.01	0.02	0.02	0.04	0.04	0.06
Cu	0.11	0.08	0.20	0.16	0.31	0.29	0.50	0.52	0.77
Ni	8.7	8.9	0.06	0.07	0.11	0.11	0.20	0.23	0.33
Pb	0.54	0.44	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Zn	3.4	3.4	0.72	0.69	1.4	1.4	2.7	2.7	4.3

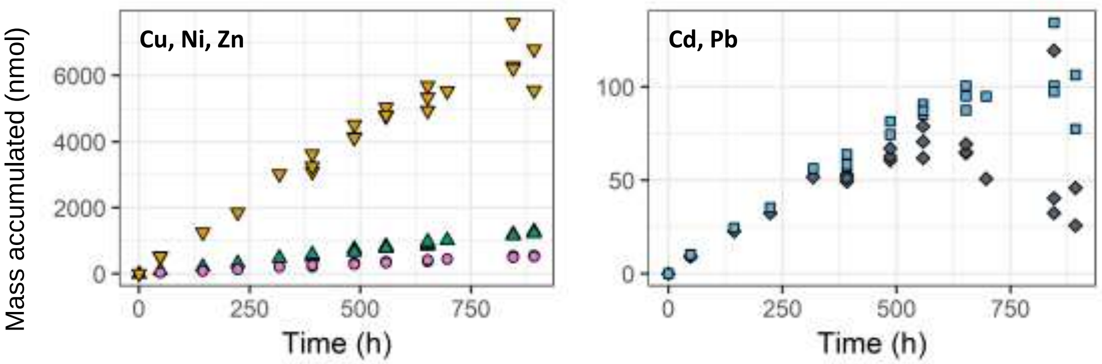
BDL = Below Detection Limit (<0.009 µM Pb)

Figure 1

(a) Equimolar



(b) High Environmental



(c) Low Environmental

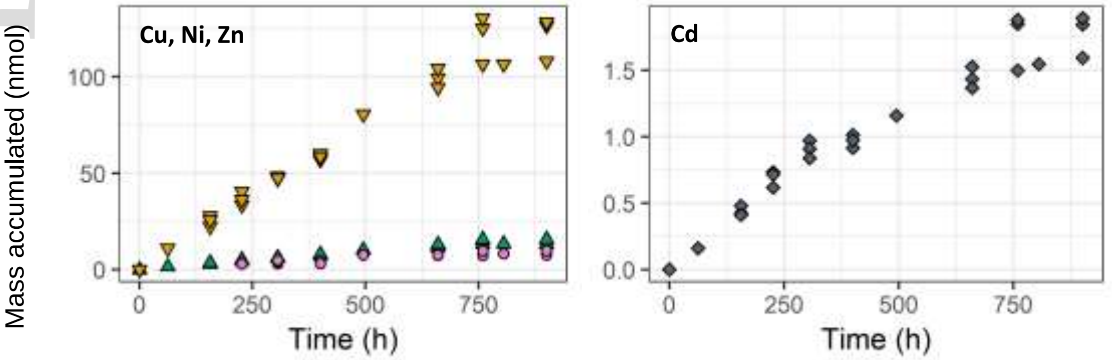


Figure 2

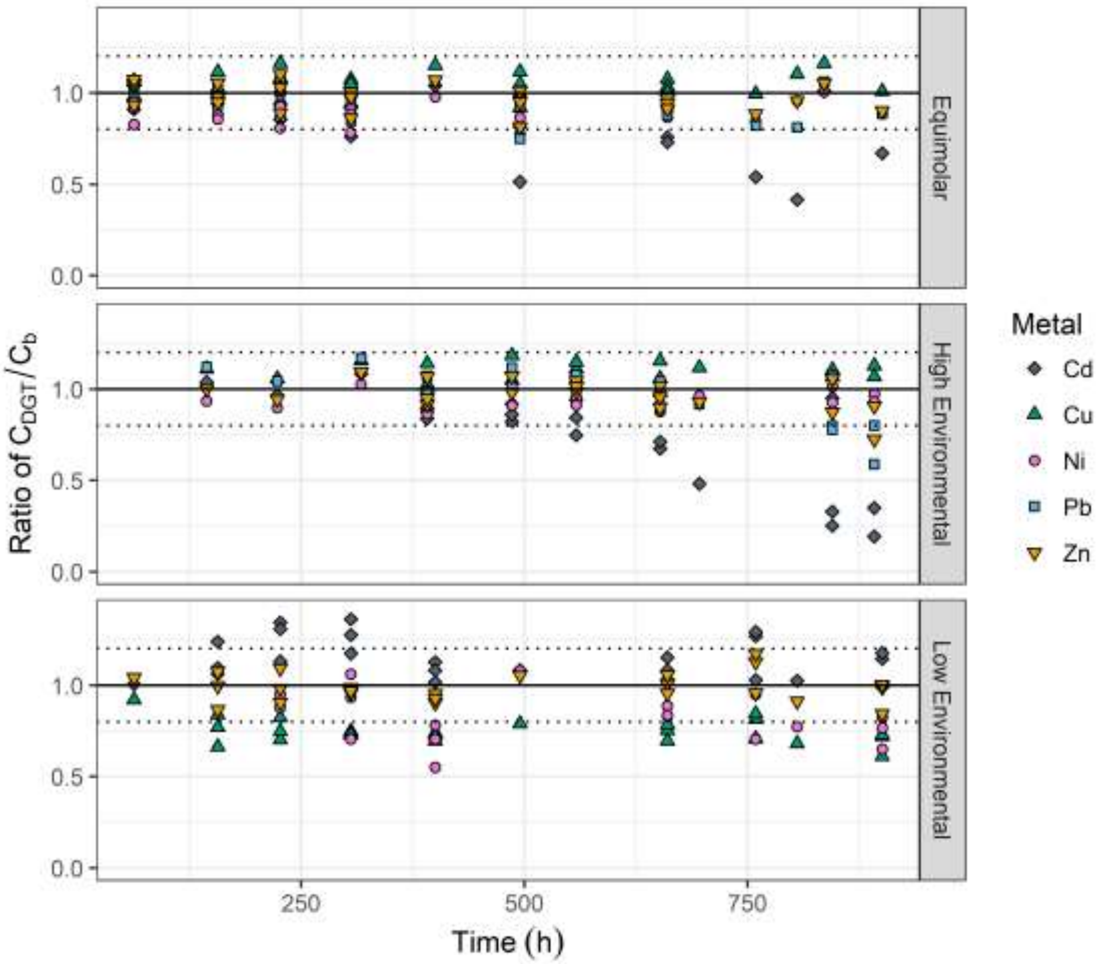

Equimolar
High Environmental
Low Environmental
Ratio of C_{DGT}/C_b
0.0
0.5
1.0
250
500
750
Time (h)
Metal
Cd
Cu
Ni
Pb
Zn

Figure 3

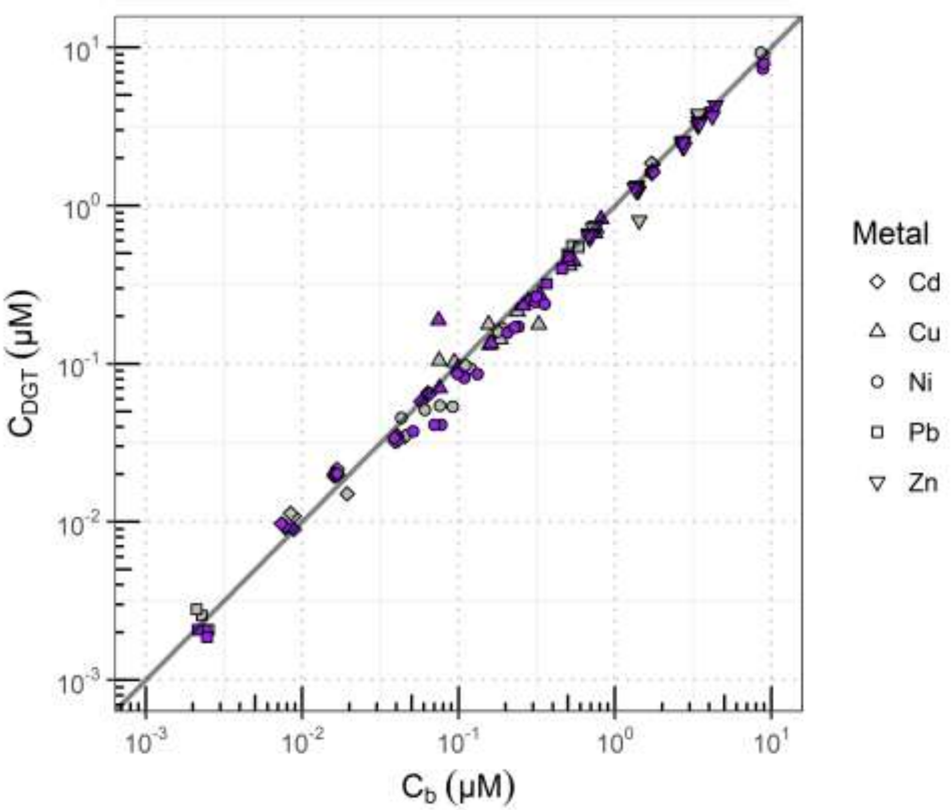
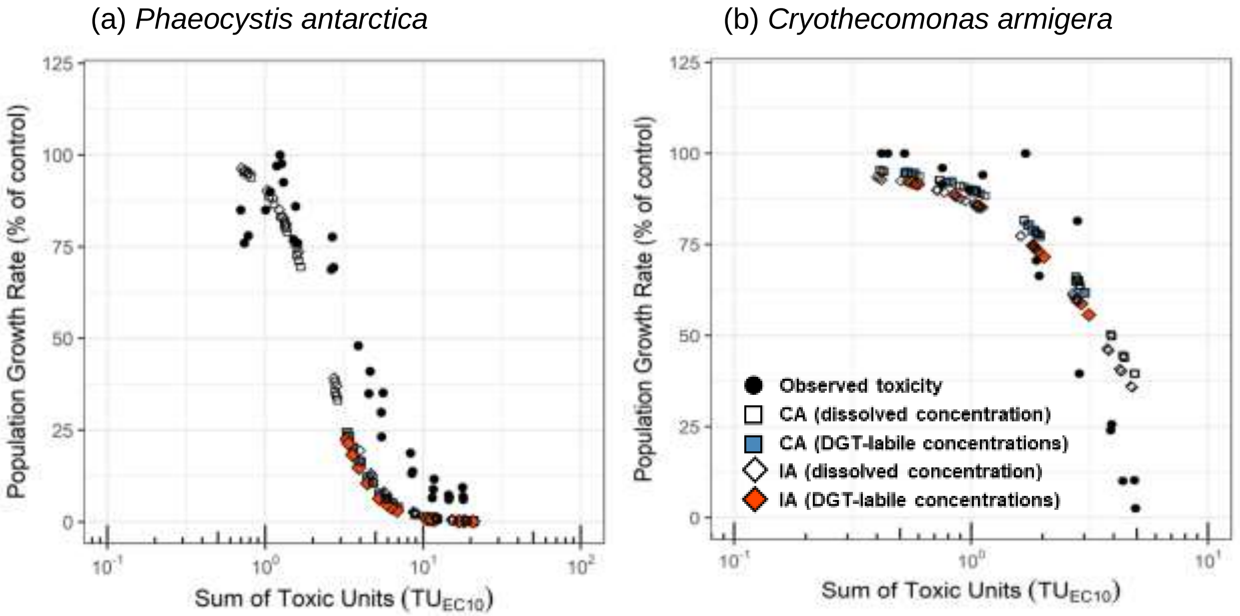

10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
C_{DGT} (μM)
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
C_b (μM)
Metal
◇ Cd
△ Cu
○ Ni
□ Pb
▽ Zn

Figure 4


(a) *Phaeocystis antarctica*
(b) *Cryothecomonas armigera*
Population Growth Rate (% of control)
125
100
75
50
25
0
Sum of Toxic Units (TU_{EC10})
10^{-1}
10^{0}
10^{1}
10^{2}
● Observed toxicity
□ CA (dissolved concentration)
■ CA (DGT-labile concentrations)
◇ IA (dissolved concentration)
◆ IA (DGT-labile concentrations)