SUPPORTING INFORMATION:

DGT-INDUCED COPPER FLUX PREDICTS BIOACCUMULATION AND TOXICITY TO BIVALVES IN SEDIMENTS WITH VARYING PROPERTIES

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S1 Methodology:

S1.1 Preparation of diffusive gradients in thin films (DGT) probes

Plastic DGT assemblies with open windows of 1.8 × 15 cm and overall dimensions of 24 × 4 × 0.5 cm were purchased from DGT Research (Lancaster, UK). All glass and plasticware for DGT probes preparation were cleaned by soaking in detergent (commercial detergent diluted in tap water) for 24 h, then in 10% (v/v) HNO_3 (70%, AR grade, Ajax Finechem Pty Ltd) for 24 h and rinsed thoroughly with MQ water. All glass and plasticware for DGT probes analysis were cleaned by soaking in 10% (v/v) HNO_3 for 24 h and rinsed thoroughly with MQ water.

DGT gels were prepared from a stock solution comprising of 15% (w/v) acrylamide (40% acrylamide solution, Electrophoresis Purity Reagent, Bio-Rad Laboratories) and 0.3% (w/v) DGT cross-linker (2% aqueous solution DGT Cross Linker, DGT Research, Lancaster, U.K.). The gel chemical polymerization for diffusive gels (0.50 mm-thick) was initiated by adding 75 µL of 10% (w/v) freshly made APS (98+% ammonium persulfate, for analysis ACS, Acros Organics) to 10 mL of stock solution and catalysed by adding 25 µL of TEMED (99% N,N,N,N-tetramethylethylenediamine, Molecular Biology tested, Sigma). The solution was stirred for 3½ minutes, then immediately cast between a pair of glass plates separated by a 0.5 mm plastic spacer. The gel solution was left to polymerize for 1 h at 45ºC.

Wet Chelex resin was prepared by mixing 2 g of dry Chelex resin with 10 mL of MQ water, then allowing the resin to settle and withdrawing the overlying water with a pipette. Chelex gels (0.25 mm-thick) were prepared by adding 5 mL of stock solution to 2 g of wet chelating resin, then 25 µL of initiator (10% (w/v) freshly made APS) and 7.5 µL of catalyst (TEMED). The solution was mixed for 3 minutes, then immediately cast between a pair of glass plates separated by a 0.25 mm plastic spacer. The solution was then left to polymerize for 1 h at 45ºC.

The resulting diffusive and Chelex gels were removed from the glass plates and hydrated in Milli-Q water for 24 h, replenishing the water three times to remove all unreacted chemicals. Diffusive gels were stored in

0.01 M $NaNO_3$ (AR grade, Chem Supply) at room temperature and Chelex gels were stored in Milli-Q water in a refrigerator at 4ºC until use for probe construction. Gels were cut with a Teflon coated razor blade using a plastic rectangular strip of the desired dimensions in order to fit in the DGT device. Acid-cleaned 0.45 µm filter membranes were cut and stored in MQ water. Probes were assembled by laying a wet filter membrane on the base, overlaying a Chelex gel layer, then a diffusive gel layer and finally another wet filter membrane on the DGT backing plates and closing the devices with the front window plates. Care was taken to ensure no air bubbles were trapped within the layers. The DGT units were kept in sealed clean plastic bags containing few drops of MQ water to avoid gel drying and stored in a refrigerator until deployment.

S1.1 ***DGT probes deployment, retrieval and analysis***

To prevent the introduction of oxygen into the sediments during the deployment, DGT probes were deoxygenated for 24 h prior to deployment by immersing them in a 0.05 M NaCl (>95.5%, Sigma) solution saturated with nitrogen gas (continually bubbling to remove the dissolved oxygen). DGT devices were immediately gently inserted into the test beakers to a depth of 4 cm with care to ensure a good contact between the sediment and the DGT membrane. After 24 h of deployment, DGT probes were gently removed from the beakers and both sediment and overlying water levels were noted. Devices were immediately rinsed with MQ water to remove all remaining sediment particles. Probes were put in clean plastic bags and kept in a cool room (4ºC) until disassembly.

DGT devices were disassembled (within 10 days of retrieval) and resin gel slices cut using a Teflon coated blade to obtain the desired vertical profile: two 2-cm slices in the overlying water, a one 1-cm slice at the sediment-water interface (SWI) and one 2-cm slice in the sediment. Some of the slices from below the SWI had sediment particles adhered to them which were removed by washing with MQ water. Each slice was weighed and put into a 5-mL vial, then eluted with 1 mL of 1 M HNO_3 for 16-24 h before analysis by ICP-MS. Undeployed DGT probes were analysed as handling blanks and their copper concentrations were less than 5% of the lowest measured concentration.

Table S1. Lethal copper concentrations for the bivalve, *Tellina deltoidalis*.

	TR-Cu, mg/kg (Sand)	**TR-Cu, mg/kg (Silty-sand)**	**TR-Cu, mg/kg (Silt)**
LC50 (95% CL)	130 (110-156)	355 (244-518)	530 (---)
LC20 (95% CL)	102 (78-136)	223 (120-415)	490 (---)
LC10 (95% CL)	88 (63-132)	170 (67-414)	460 (---)
	AE-Cu, mg/kg (Sand)	**AE-Cu, mg/kg (Silty-sand)**	**AE-Cu, mg/kg (Silt)**
LC50 (95% CL)	75 (61-91)	178 (93-339)	350 (---)
LC20 (95% CL)	54 (39-77)	87 (35-224)	300 (---)
LC10 (95% CL)	44 (29-74)	57 (16-217)	280 (---)
	OW-Cu, µg/L (All treatments)	**Tissue-Cu, mg/kg (All treatments)**	**DGT-Cu, ug Cu/m^2/h (All treatments)**
LC50 (95% CL)	27 (25-30)	1000 (770-1300)	31 (24-42)
LC20 (95% CL)	21 (17-27)	650 (380-1100)	19 (11-36)
LC10 (95% CL)	18 (13-26)	500 (230-1100)	15 (6-35)

LC50 (95% CL) = concentration causing 50% lethality (measured after 30 days).

LC20 and LC10 represent 20% and 10% effect concentrations, respectively.
95% CL = 95% confidence limit (--- = not possible to calculate 95% CL).

TR-Cu = total recoverable copper concentration (aqua regia, mg/kg).

AE-Cu = dilute acid-extractable copper concentration (1-M HCl, mg/kg)

OW-Cu = overlying water copper concentrations (30-day time averaged concentration, µg/L)

Tissue-Cu = copper concentrations after 30 days in surviving bivalves (mg/kg, dry weight)

DGT-Cu = Peak DGT-induced Cu flux at the sediment-water interface (ug Cu/m^2/h)

Effects thresholds for TR-Cu and AE-Cu were calculated separately for sand, silty-sand and silt treatments

Effects thresholds for OW-Cu, Tissue-Cu and DGT-Cu were calculated using the combined data from treatments

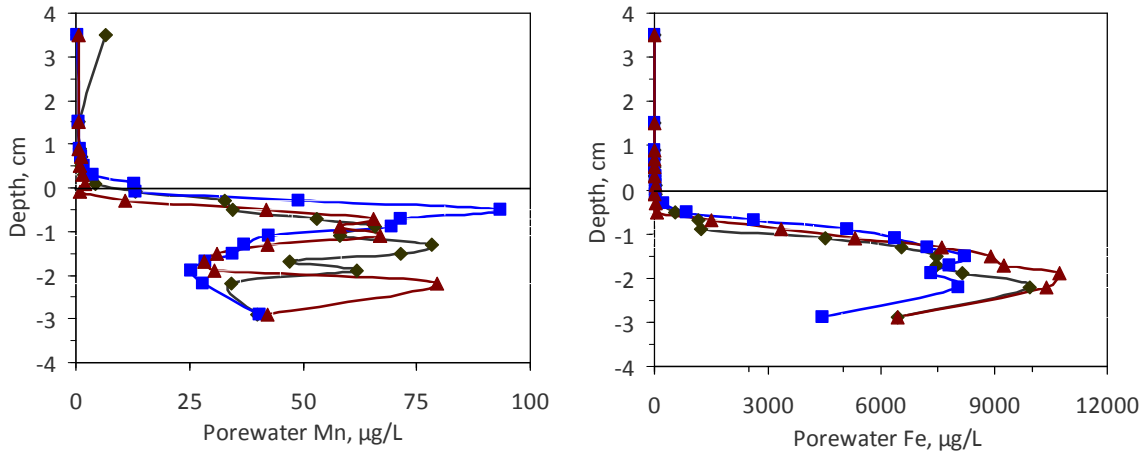

Figure S1. Porewater Mn and Fe concentrations within silty-sand that had been spiked with a copper mineral phase (unpublished results), confirming that the expected porewater Fe and Mn profiles develop following sediment disturbance and 1 month equilibration following spiking

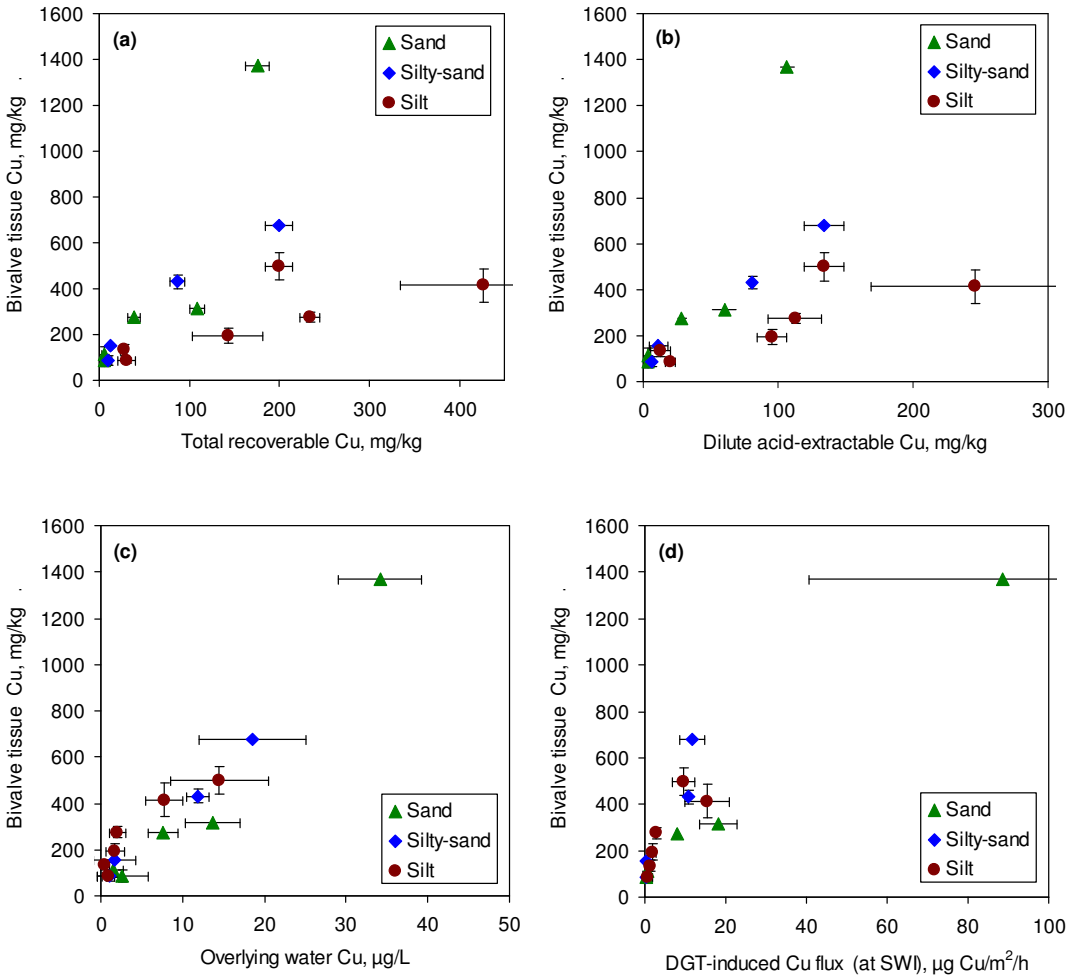

Figure S2. Relationships between copper bioaccumulation by bivalve, *Tellina deltoidalis*, and different copper exposures: (a) total recoverable copper (TR-Cu), (b) dilute acid-extractable copper (AE-Cu), (c) dissolved copper in overlying water (OW-Cu, time averaged) and (d) peak DGT-induced Cu flux at the sediment-water interface (DGT-Cu). Data presented for three sediment types for the different Cu-spike concentrations (mean ±SD, n=2).