Supporting Information for ES0615122

Characterizing dissolved Cu and Cd uptake in terms of the biotic ligand and biodynamics using enriched stable isotopes

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7 pages (4 Tables, 4 equations, 1 Figure)

TABLE S1. Ionic composition (nominal), hardness, pH and proportion of free tracer ions in each experimental media as determined by WHAM 6.0

	Deionised	Soft	Moderately hard	Hard
Ca^{2+} (μM)	0	174	349	698
Mg^{2+} (μM)	0	249	499	998
Na^{+} (μM)	0	571	1143	2286
K^{+} (μM)	0	27	54	107
Cl^{-} (μM)	0	27	54	107
SO_4^{-2} (μM)	0	424	848	1695
CO_3^{-2} (μM)	0	571	1143	2286
Hardness (mg $CaCO_3$ l^{-1})	0	40-48	80-100	160-180
pH	6.1	7.6	7.8	7.8
% $^{65}Cu^{2+}$	100[a]	6.88	3.01	1.74
% $^{106}Cd^{2+}$	100[a]	89.9	81.9	73.2

[a]Experimental solutions were assumed free of ligands

TABLE S2. Dissolved tracer concentrations measured before and after 24 h exposure; n=3 for concentrations at time 0 and 24; n=6 for the average (AVG) concentrations. 8 snails were exposed to tracers in 1-L jar (1 jar per treatment)

Media	Treatment	[^{65}Cu] µg l^{-1} (±95% C.I.)			[^{106}Cd] µg l^{-1} (±95% C.I.)		
		T_0	T_{24}	AVG	T_0	T_{24}	AVG
DI	Control	0.003±0.001	0.08±0.02	0.04±0.04	0.001±0.001	0.001±0.001	0.001±0.001
DI	0.1-ppb	0.12±0.003	0.11±0.01	0.11±0.01	0.11±0.001	0.03±0.002	0.07±0.04
DI	1-ppb	1.1±0.01	0.60±0.05	0.88±0.24	1.1±0.004	0.26±0.02	0.7±0.4
DI	10-ppb	11.2±0.3	6.9±0.5	9.1±1.9	11.4±0.3	7.6±0.7	9.5±1.7
DI	100-ppb	111±4	97±1	104±6	103±4	93±1	98±5
DI	300-ppb	320±12	299±21	310±14	299±12	289±20	294±11
SO	0.1-ppb	0.18±0.01	0.27±0.02	0.23±0.04	0.12±0.002	0.09±0.002	0.10±0.01
SO	1-ppb	1.2±0.1	0.83±0.03	1.0±0.2	1.1±0.03	0.56±0.03	0.84±0.25
SO	10-ppb	11.1±0.1	7.8±0.6	9.5±1.5	11.3±0.2	8.5±0.5	9.9±1.3
SO	100-ppb	111±1	91±7	101±9	102±1	94±6	98±4
SO	300-ppb	325±3	288±14	306±18	303±2	284±10	293±9
MO	Control	0.11±0.01	0.13±0.01	0.12±0.01	0.003±0.001	0.004±0.005	0.003±0.002
MO	0.1-ppb	0.19±0.01	0.19±0.01	0.19±0.01	0.11±0.01	0.07±0.01	0.09±0.02
MO	1-ppb	0.83±0.02	0.77±0.01	0.80±0.03	1.1±0.02	0.75±0.01	0.91±0.14
MO	10-ppb	8.6±0.14	7.4±0.02	8.0±0.5	10.8±0.1	9.3±0.1	10.0±0.7
MO	100-ppb	95±1	87±1	91±4	88±1	86±2	87±1
MO	300-ppb	222±2	214±2	218±4	257±2	254±1	256±2
HA	Control	0.25±0.01	0.26±0.01	0.26±0.01	0.002±0.005	0.001±0.001	0.001±3
HA	0.1-ppb	0.34±0.01	0.33±0.01	0.33±0.01	0.11±0.01	0.10±0.01	0.10±0.01
HA	1-ppb	1.0±0.03	0.88±0.01	0.94±0.05	1.1±0.03	0.95±0.01	1.0±0.04
HA	10-ppb	9.1±0.04	7.5±0.1	8.3±0.7	10.5±0.1	10.2±0.1	10.3±0.2
HA	100-ppb	91±1	85±1	88±3	106±1	105±1	106±1
HA	300-ppb	284±1	273±3	279±5	293±1	288±3	291±3

TABLE S3. Some metal-binding characteristics from fish-gill studies[a]

Species	Metal	log K_{gill}	B_{max} (nmol g^{-1})	Hardness	Reference
Rainbow trout[b]	Cu	8.4	1.88	soft	*35*
Rainbow trout[b]	Cu	9.9	3.63	hard	*35*
Fathead minnow[c]	Cu	7.4	30	Very soft	*14*
Fathead minnow[c]	Cd	8.6	2	Very soft	*14*
Rainbow trout[b]	Cu	7.9	1.85	soft	*17*
Rainbow trout[b]	Cu	9.2	0.59	hard	*17*
Rainbow trout[b]	Cu	7.50	30	Very soft	*23*
Brook trout[d]	Cu	7.25	60	Very soft	*23*
Rainbow trout[b]	Cd	7.6	1.78	Soft	*19*
Rainbow trout[b]	Cd	7.3	1.78	Hard	*19*

[a]Exposure time is 3 h for all studies, except in *23* where fish were exposed for 24 h
[b]*Oncorhynchus mykiss*
[c]*Pimephales promelas*
[d]*Salvelinus fontinalis*

TABLE S4. Some differences between the BLM and the Biodynamic model

BLM	Biodynamic model
Model parameters are determined upon high exposure concentrations (often not realistic environmentally), i.e., saturation kinetics is required to infer B_{max} and *log K*	Parameters determined upon low exposure concentrations
Provides a mechanistic understanding of the interaction of metals with biological surfaces	Determines the outcome (influx rate) of metal uptake mechanisms
Takes into account metal speciation and competing ions	Metal speciation empirically taken into account
Does not consider physiological loss rates Does not consider dietary exposures	Unifies dissolved uptake, dietary uptake and loss
In practice biological parameters (*log K*, B_{max}) are often inferred from correlation with toxicity data.	Biodynamic parameters determined empirically
Relates toxicity to the fraction of physiological active sites impacted by reactive metal species	Consistent with the concept that toxicity occurs when metal influx rates exceed rates of loss and detoxification, the model suggests that dissolved toxicity might be predicted from k_u, but correlations with toxicity have not been directly investigated

Calculation of accumulated ^{106}Cd concentrations. The relative abundance of ^{106}Cd is determined using the signal intensities of each Cd isotope in the standards used to calibrate the ICP-MS. For example,

$$p^{106} = \text{Intensity}_{\text{Standard}} \frac{^{106}\text{Cd}}{^{106}\text{Cd} + ^{108}\text{Cd} + ^{110}\text{Cd} + ^{111}\text{Cd} + ^{112}\text{Cd} + ^{113}\text{Cd} + ^{114}\text{Cd}} \quad (1)$$

Concentrations of ^{106}Cd in the experimental organisms ($[^{106}\text{Cd}]_{\hat{e}}$) are calculated as the product of p^{106} and the total Cd concentrations inferred by the ICP-MS software from tracer intensity ($[T^{106}\text{Cd}]$):

$$[^{106}\text{Cd}]_{\hat{e}} = p^{106} \times [T^{106}\text{Cd}] \quad (2)$$

Total Cd concentrations inferred from the intensity of the most abundant isotope are then used to derive the original load of tracer ($[^{106}\text{Cd}]^0_{\hat{e}}$) that occurred in each sample in the absence of a spike, e.g.,

$$[^{106}\text{Cd}]^0_{\hat{e}} = p^{106} \times [T^{114}\text{Cd}] \quad (3)$$

Consequently, net ^{106}Cd uptake ($\Delta[^{106}\text{Cd}]_{\hat{e}}$) is $[^{106}\text{C}]_{\hat{e}}$ as derived from the total experimental Cd inferred from ^{106}Cd signal (equation 2) minus the pre-existing load of ^{106}Cd ($[^{106}\text{Cd}]^0_{\hat{e}}$ from equation 3,

$$\Delta[^{106}\text{Cd}]_{\hat{e}} = [^{106}\text{Cd}]_{\hat{e}} - [^{106}\text{Cd}]^0_{\hat{e}} \quad (4)$$

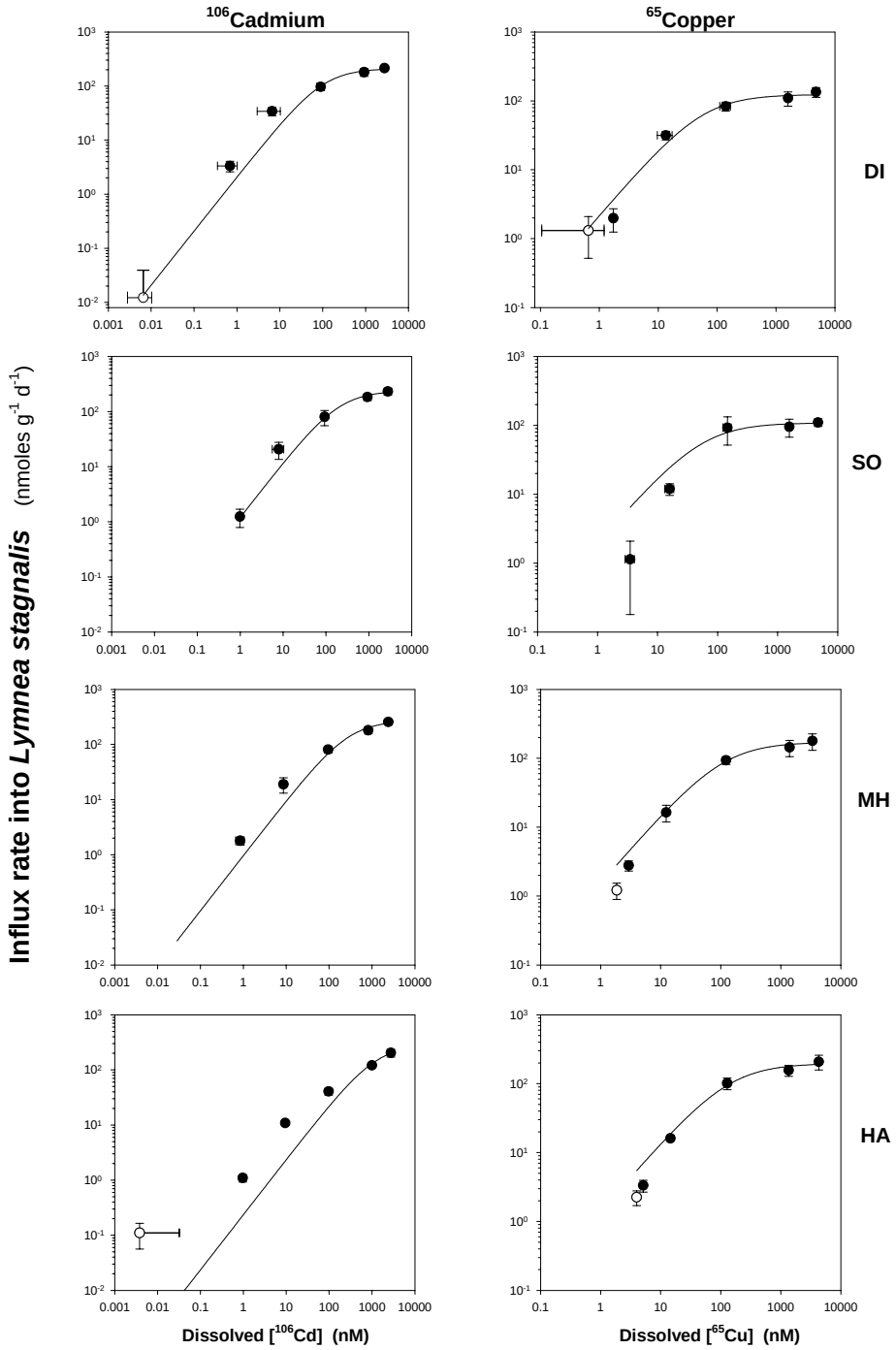

FIGURE S1. Metal uptake rates (nmoles g^{-1} d^{-1}) in *L. stagnalis* at different water hardness. ^{106}Cd and ^{65}Cu concentrations (soft tissue) were determined in snails exposed for 24 h to waterborne metals at concentrations ranging from 0.004 to 2778 nM for ^{106}Cd and from 0.7 to 4770 nM for ^{65}Cu in deionised (DI) and synthetic water (SO, MH, HA). Each symbol represents mean metal concentrations of 8 individuals and 6 water samples (± 95% confidence interval). Open circles are for controls; Solid circles are for the experimental snails. Curves represent nonlinear regression fits to Michaelis-Menten equation.