Supplementary Information

Copper Uptake, Intracellular Localization and Speciation in Marine Microalgae Measured by Synchrotron Radiation X-Ray Fluorescence and Absorption Microspectroscopy

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The authors declare no competing financial interest.

Summary: 6 pages, 2 Table, 2 Figures

Table S1. Speciation of Copper (as a fraction) in Test Medium[a] using MINTEQ v3.1

Copper speciation	Fraction (%) of copper species in exposure treatments	
	19 µg/L	**40 µg/L**
Cu^{2+}	1.007	1.885
$CuOH^{+}$	1.571	2.942
$Cu(OH)_{2(aq)}$	0.21	0.394
$CuCl^{+}$	0.275	0.514
$CuCl_{2(aq)}$	0.015	0.028
$CuSO_{4(aq)}$	0.219	0.41
$CuCO_{3(aq)}$	12.398	23.212
$CuHCO_3^{+}$	0.031	0.058
$Cu(CO_3)_2^{-2}$	1.029	1.927
$FA_2Cu_{(aq)}$	18.651	15.422
$FACu^{+}_{(aq)}$	0.028	0.046
$FA_2CuOH_{(aq)}$	64.562	53.149

[a] The medium consisted of natural seawater (filtered, 0.45 µm) + nitrate, phosphate and a measured DOC of 1 mg/L (assumed to be fulvic acid). The MINTEQ v3.1 artificial seawater reference composition was used.

Table S2. Cellular Copper Concentrations[a]

Algae/Treatment	Cellular Cu (femtog/cell)	Volume (μm^3)[b]	Cellular Cu (picog/μm^3)
P. tricornutum, Control	3.21 ± 1.88	50 – 70	46 ± 27 – 64 ± 38
P. tricornutum, 40 µg Cu/L	3.56 ± 2.21	430	8.27 ± 5.15
Tetraselmis sp. Control	15.8 ± 7.6	330 ± 140	12 ± 11.3
Tetraselmis sp. 40 µg Cu/L	3.95 ± 3.33	330 ± 140	48 ± 31
C. closterium, Control	2.55 ± 0.49	NR	NC
C. closterium, 19 µg Cu/L	4.05 ± 1.15	NR	NC
C. closterium, 40 µg Cu/L	10.3 ± 5.5	NR	NC

[a] Studies describing the cellular concentrations of metals in microalgae are commonly report on the basis of per cell or per unit volume or weight, or, carbon content. The cellular copper concentrations in this study were measured using SR-XRF and reported on a per cell basis without further conversion of the units. For the comparison of cellular copper concentrations to that reported in other studies the cellular Cu concentrations have been further converted and reported as: Cu per cellular volume (μm^3) calculated from Cu per cell values (measured by SR-XRF in this study) and the known volume of control and copper exposed cells reported by Levy and co-authors (2008). Errors represent 1 standard deviation.

[b] from Levy et al., 2008

NR Not reported

NC Not calculated

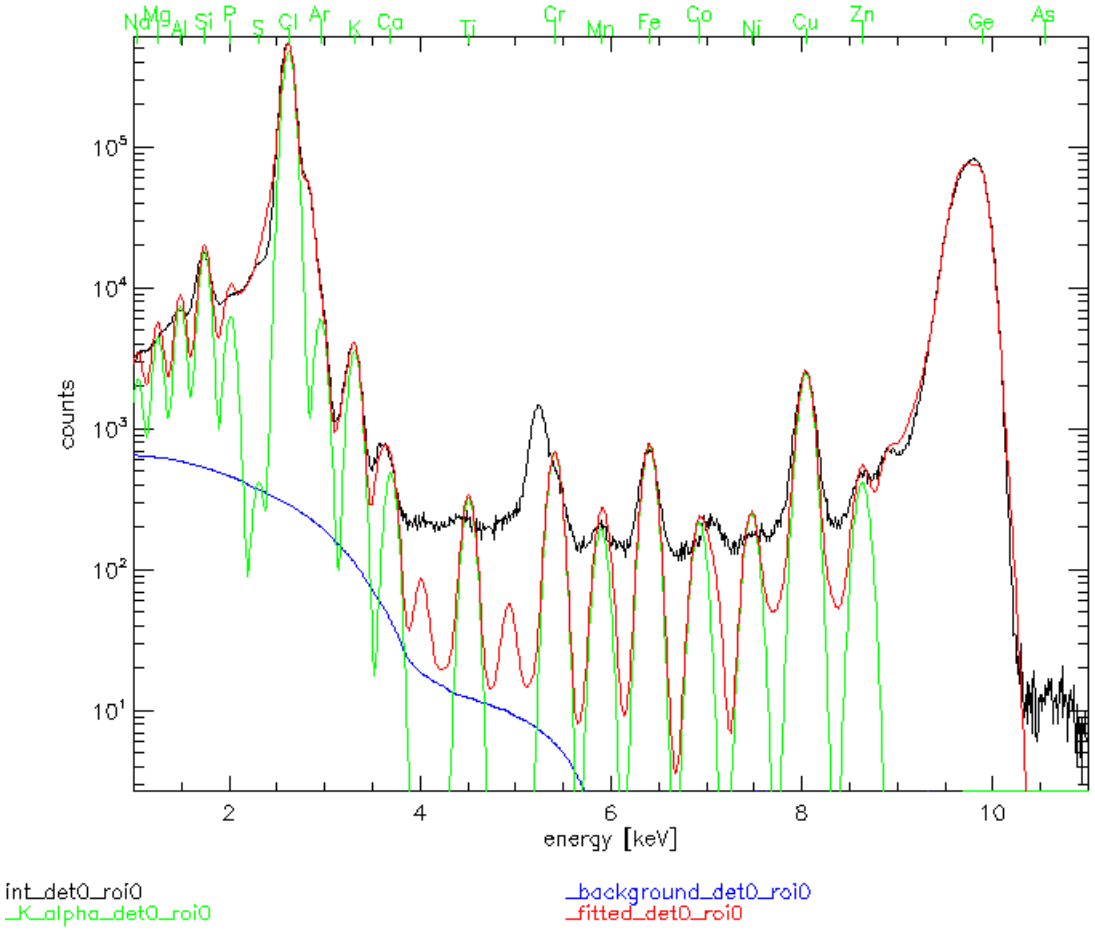
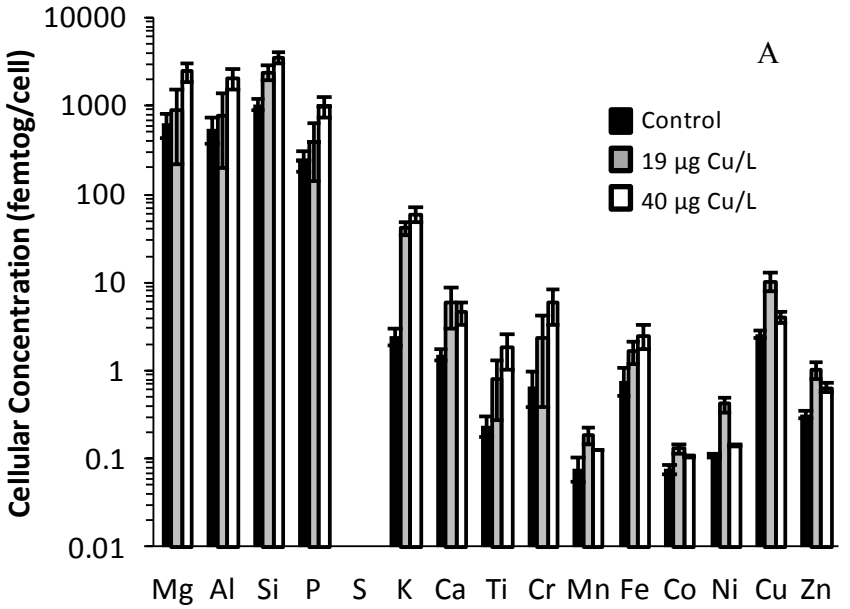
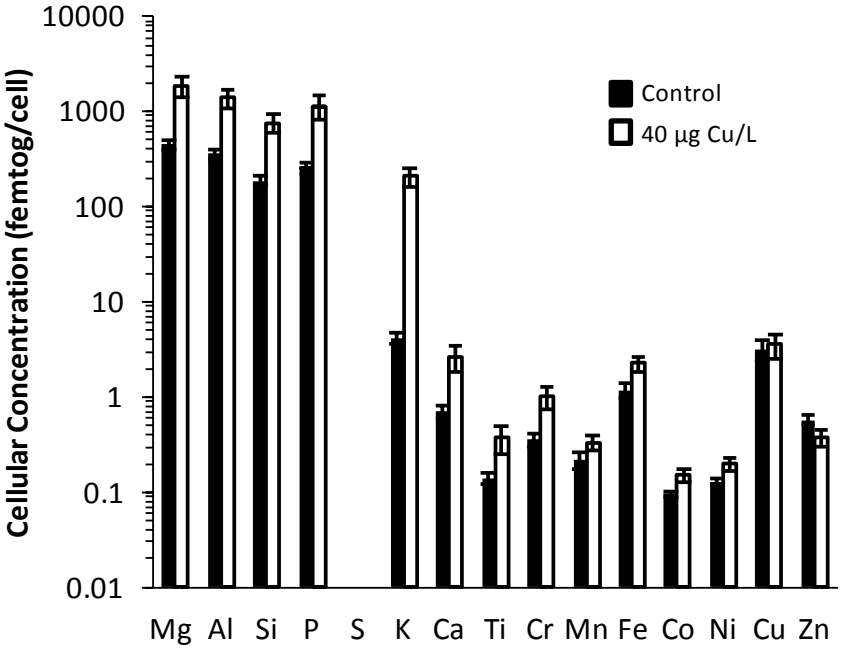


Figure S1. Fluorescence spectrum for a single *C. closterium* cell (copper exposed) measured by SR-XRF. Fluorescence data are shown as: fitted model (red), Kα peaks (green), integrated (black) and background (blue). The elements corresponding to the Kα peaks are listed at the top of the plot. For each element, integration of the fitted model peak and comparison to the corresponding peak from the standards (NBS-1832 and NBS-1833, National Bureau of Standards, Gaithersburg, MD, USA) were used to calculate the cellular concentrations.


A
Control
19 µg Cu/L
40 µg Cu/L
Cellular Concentration (femtog/cell)
10000
1000
100
10
1
0.1
0.01
Mg Al Si P S K Ca Ti Cr Mn Fe Co Ni Cu Zn


Control
40 µg Cu/L
Cellular Concentration (femtog/cell)
10000
1000
100
10
1
0.1
0.01
Mg Al Si P S K Ca Ti Cr Mn Fe Co Ni Cu Zn

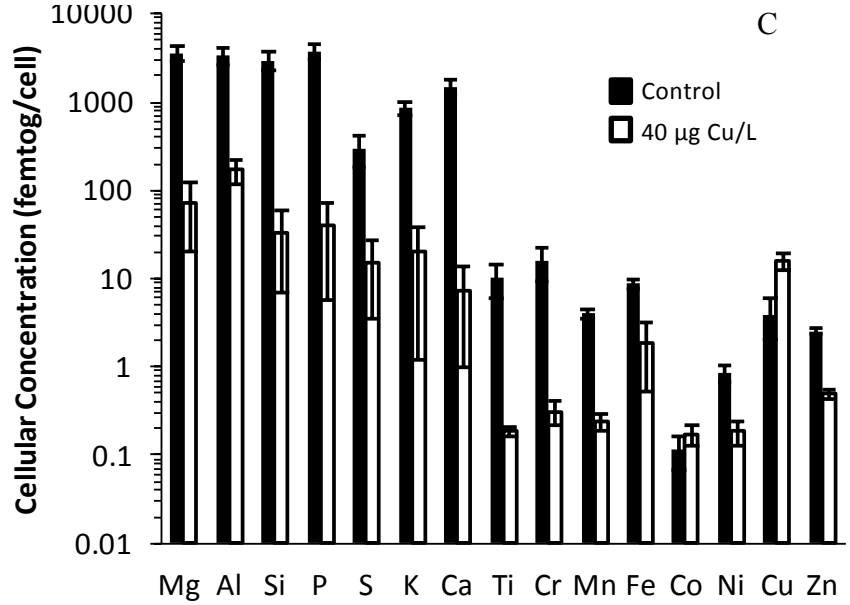

C
Control
40 µg Cu/L
Cellular Concentration (femtog/cell)
10000
1000
100
10
1
0.1
0.01
Mg Al Si P S K Ca Ti Cr Mn Fe Co Ni Cu Zn

Figure S2. Intracellular concentrations of elements in whole cells of A) *C. closterium*, B) *P. tricornutum* and C) *Tetraselmis* sp. after 72 h exposure to copper (0, 19 and 40 µg/L) measured by SR-XRF. Error bars represent the standard error. Cellular concentrations derived for S for the two diatoms (*C. closterium* and *Tetraselmis* sp.) were unreliable and not presented.