**Cu transport and complexation by the marine diatom *Phaeodactylum tricornutum*: implications for trace metal complexation kinetics in the surface ocean**

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**SUPPLEMENTARY MATERIAL**

**Figure S1.** Example of a Cu titration curve in seawater containing Cu-binding ligands excreted by *P. tricornutum* grown in the control media and measured using CLE-CSV.

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**Figure S2.** Percentage of total Cu in solution (added 4.43•10-9 mol L-1 + initial [Cu] of 4.4•10-10 mol L-1) that was adsorbed onto *P. tricornutum* as a function of time, at the natural ESTOC seawater pHF of 8.01 ± 0.05, and a cell density of 48·103 cells ml-1.



**Table S1.** Dissolved trace metalconcentrations at the ESTOC site, measured and consensus values (nM) for the reference materials (SAFE S and D2). Measurements were carried out within 12 months after collection on a SF-HR-ICP-MS Element XR instrument (Thermo Fisher, Bremen, Germany) coupled with an online seaFAST system (Elemental ScientificTM), at Pôle Spectrométrie Océan (IFREMER, France).

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|   | **ESTOC** | **SAFE S** | **SAFE D2** |
|  |   | Measured | Consensus | Measured | Consensus |
| **Mn** | 0.860±0.019 | 0.760±0.070 | 0.790±0.060 | 0.340±0.070 | 0.350±0.050 |
| **Fe** | 0.668±0.018 | 0.160±0.010 | 0.090±0.010 | 0.960±0.080 | 0.930±0.020 |
| **Co** | 0.030±0.001 | 0.004±0.002 | 0.005±0.001 |  | 0.046±0.003 |
| **Cu** | 0.420±0.008 | 0.430±0.020 | 0.520±0.050 | 2.116±0.040 | 2.280±0.150 |
| **Zn**  | 0.420±0.005 |  | 0.069±0.010 | 7.634±0.086 | 7.430±0.250 |
| **Ni** | 1.423±0.025 | 2.269±0.039 | 2.28±0.090 | 8.769±0.025 | 8.630±0.250 |

**Table S2.** Exponential absolute growth rate (µ, d-1), doublings per day (*k*, dd-1) and the surface area (µm2) for the two experimental conditions COMEDIUM and CyMEDIUM. Growth rate data in the f/2 media used for culture maintenance in the Spanish Bank of Algae in Gran Canaria is also included. (NM, not measured). \*values are statistically different (p < 0.05) from the Control. n = 11 and 10 for COMEDIUM and CyMEDIUM, respectively.

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| --- | --- | --- | --- |
| **Rate** | **COMEDIUM**  | **CyMEDIUM** | ***f/2* media** |
| **µ (d-1)** | 0.94±0.44 | 0.97±0.30 | 1.72±0.028 |
| ***k* (dd-1)** | 1.35±0.64 | 1.40±0.44 | 2.48±0.04 |
| **µm2** | 47.24±9.23 | 35.27±5.39\* | NM |

**Table S3.** Summary of kinetic parameters of the Cu-transport system in *P. tricornutum* (this study), *T. oceanica* (CCMP1003) and *T. pseudonana* (CCMP1335; Guo et al. 2010). Vmax is the maximum Cu uptake rates, and Km (nM [Cu]total or pM [Cu']) is the half-saturation constants of the Cu uptake system. [Cu] total is the total Cu concentration (nM) and [Cu´] is the dissolved inorganic Cu concentration (nM). In the case of *P. tricornutum*, the Km for total Cu was converted to Cu’ considering the presence of L1 and L2 released by the algae (Table 3). For *T. oceanica* and *T. pseudonana*, the Cu’ values used were those in Table 5 in Guo et al. 2010.

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| --- | --- | --- | --- |
|   | **Low affinity** |  | **High affinity** |
|  | Vmax (10-21 mol Cu·µm-2·h-1) | Km (nM [Cu]total) | Km (pM [Cu']) |  | Vmax(10-21 mol Cu·µm-2·h-1) | Km(nM [Cu]total) | Km(pM [Cu']) |
| ***P. tricornutum*** | 19.3±10.6 | 2636±2156 | 1.37±1.12 |  | 1.48±0.60 | 154±130 | 0.08±0.07 |
| ***T. oceanica*** | 238±63 | 6336±2253 | 3.33±1.18 |  | 1.3±1.1 | 7.3±32 | 0.08±0.35 |
| ***T. pseudonana*** | 63±4.9 | 1117±525 | 0.59±0.28 |   | 4.8±7.8 | 52±129 | 0.64±1.6 |

**Table S4.** Rate constants, oceanic concentrations and physiological parameters for trace metals transport in *T. weissflogii*, as in Hudson and Morel 1993, with revised and updated data for Cd and Cu.

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Metal** | ***k*f (M-1 s-1)** | **[M]T (nM)** | **[M'] (M)** | **pM** | **Q0.9 (mol cell-1)** | **[L']min (mol cell-1)** | ***k*limin (s-1)** |
| **Mn** | 3.00x107 | 1 | 1.00 x10-9 | 9.2 | 8.00 x10-17 | 4.04 x10-20 | 3.00 x10-2 |
| **Zn** | 4.00x107 | 0.2 | 4.00 x10-12 | 11.7 | 2.00 x10-17 | 1.90 x10-18 | 1.60 x10-4 |
| **Ni** | 1.00x105 | 2 | 2.00 x10-9 | 9 | 2.00 x10-17 | 1.52 x10-18 | 2.00 x10-4 |
| **Fe** | 2.00x106 | 0.06 | 6.00 x10-11 | 20.2 | 8.00 x10-17 | 1.01 x10-17 | 1.20 x10-4 |
| **Co** | 4.00x106 | 0.02 | 2.00 x10-11 | 10.9 |  |  |  |
| **Cd** | 4.00x108 | 0.002 | 6.00 x10-13 | 13.7 | 6.40 x10-18 (c) | 4.04 x10-19 (d) | 2.40 x10-4 (d) |
| **Cu** | 1.10x107 (a) | 0.6 | 2.00 x10-12 | 12.7 | 2.00 x10-18 (b) | 1.38 x10-18 (d) | 2.20 x10-5 (d) |
|  |  |  |  |  | 8.00 x10-18 (b) | 7.95 x10-18 (d) | 2.20 x10-5 (d) |

Note: This is a replica of Table 1 in Hudson and Morel (1993), with new values for Cd and Cu.

1. *k*f was calculated in this study.
2. The added Q0.9 for Cu are for *T.weissflogii* from Annett et al. (2008)
3. The added Q0.9 for Cd is from Ho et al. (2003), assuming a Cd quota of 8.9 µmol Lcell volume-1 and that *T.weissflogii* cell volume is 731 fL cell-1.
4. [L']min (mol cell-1) and *k*limin (s-1) were calculated as indicated in Hudson and Morel (1993)

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