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Environmental Chemistry

Supplementary Material

Cadmium thiosulfate complexes can be assimilated by a green alga via a sulfate transporter but do not increase Cd toxicity

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Supplementary Material

Supplementary Tables

Table S1: Molar concentrations of cations and anions in the original High Salt Medium (HSM – Macfie, SM et al. (1994)), in the Modified High Salt Medium (MHSM, used in the present work as the algal growth medium), and in the various rinse solutions (MHSM-R1, R2 and R3) used to rinse the algal cells after collection. The pH was maintained at 7 in all these solutions.

Ions[1]	**HSM**	**MHSM**	**MHSM-R1**	**MHSM-R2**	**MHSM-R3**
*NH_4	9.35×10^{-3}	9.37×10^{-4}	9.37×10^{-4}	9.37×10^{-4}	9.37×10^{-4}
*Cl	9.49×10^{-3}	5.98×10^{-6}	–	–	–
*K	2.20×10^{-2}	4.22×10^{-3}	2.24×10^{-3}	2.34×10^{-3}	4.22×10^{-3}
PO_4	1.37×10^{-2}	1.37×10^{-4}	1.37×10^{-4}	1.37×10^{-4}	1.37×10^{-4}
*CO_3	atm[2]	atm[2]	atm[2]	atm[2]	atm[2]
*NO_3	–	5.07×10^{-3}	3.17×10^{-3}	3.43×10^{-3}	5.07×10^{-3}
*SO_4	8.12×10^{-5}	8.12×10^{-5}	8.12×10^{-5}	–	8.12×10^{-5}
*Mg	8.12×10^{-5}	8.12×10^{-5}	8.12×10^{-5}	7.80×10^{-5}	8.12×10^{-5}
*Ca	6.80×10^{-5}	6.80×10^{-5}	6.80×10^{-5}	6.80×10^{-5}	6.80×10^{-5}
*Na	1.02×10^{-4}	1.02×10^{-4}	1.02×10^{-4}	1.02×10^{-4}	1.02×10^{-4}
BO_3	3.01×10^{-6}	3.01×10^{-6}	–	–	–
Mn	2.10×10^{-6}	2.10×10^{-6}	–	–	–
EDTA[3]	8.06×10^{-7}	8.06×10^{-7}	–	–	–
Fe	5.92×10^{-7}	5.92×10^{-7}	–	–	–
MoO_4	3.00×10^{-8}	3.00×10^{-8}	–	–	–
Zn	2.43×10^{-8}	2.43×10^{-8}	–	–	–
Co	1.09×10^{-8}	1.09×10^{-8}	–	–	–
Cu	7.04×10^{-11}	7.04×10^{-11}	–	–	–

[1] Ions marked with an asterisk were present in the simplified exposure media used for the short-term uptake experiments. [2] The carbonate concentration is assumed to be in equilibrium with the atmosphere. [3] Ethylenediaminetetraacetic acid.

MHSM-R1 and MHSM-R2 media were used to rinse the algae before inoculating them into the exposure media used for the short-term uptake experiments (Table 1). The major difference between the two rinse media was the sulfate concentration. In MHSM-R2 rinse medium, which was used prior to exposures in sulfate-free media, the sulfate was replaced with nitrate to obtain a sulfate-free rinse solution. Both media were similar in chemical composition to the exposure media for short-term (< 1 h)

kinetic experiments. The MHSM-R3 rinsing media was used during the kinetic and long-term toxicity (72-h) studies. This medium was similar to the MHSM-1 culture medium, but like the other two rinse media, it did not contain the trace elements contained in the MHSM-1 medium.

Table S2: Exposure conditions for the long-term uptake experiments (72 h) with variable free Cd^{2+} concentrations buffered by EDTA alone (G1 to G6) or by EDTA + thiosulfate (H1 to H6). In addition to the variables shown in this table, the exposure media contained all the MHSM constituents at the concentrations indicated in Table S1, column 3.

	G_1	G_2	G_3	G_4	G_5	G_6
Cd_T (μM)	12.1	12.9	13.6	14.3	15.0	15.4
Cd^{2+} (nM)	2.82	12.7	42.9	176	592	895
EDTA (μM)	15	15	15	15	15	15
	H_1	H_2	H_3	H_4	H_5	H_6
Cd_T (μM)	12.2	13.1	14.1	16.0	19.6	22.2
Cd^{2+} (nM)	3.17	15.4	61.5	293	806	1150
EDTA (μM)	15	15	15	15	15	15
$S_2O_3^{2-}$ (mM)	1	1	1	1	1	1

Table S3: Exposure conditions for the long-term experiments following the dissolved Cd concentration and the free Cd^{2+} concentration (IET measurements) over a 72-h algal growth period. In addition to the variables shown in this table, the exposure media contained all the MHSM constituents at the concentrations indicated in Table S1, column 3.

	I1	I2	J1	J2
Cd_T (μM)	12.1	15.4	12.2	22.2
Cd^{2+} (nM)	2.82	895	3.17	1150
EDTA (μM)	15	15	15	15
$S_2O_3^{2-}$ (mM)	–	–	1	1

Table S4: Exposure conditions for the long-term algal growth experiments with increasing concentrations of Cd^{2+} in the presence of EDTA alone. In addition to the variables shown in this table, the exposure media contained all the MHSM constituents at the concentrations indicated in Table S1, column 3.

Media[(1)]	$[Cd]_T$ (μM)	$[Cd^{2+}]$ (nM)	Cd^{2+}/Cd_T (%)	[EDTA] (μM)	$[S_2O_3^{2-}]$ (mM)
L_1	–	–	–	–	–
L_2	–	–	–	15	–
L_3	12.1	2.82	0.02	15	–
L_4	12.9	12.7	0.10	15	–
L_5	13.6	42.9	0.32	15	–
L_6	14.3	176	1.2	15	–
L_7	15.0	592	3.9	15	–
L_8	15.4	895	5.8	15	–

(1) L_1 is the standard growth medium (MHSM), which serves as the reference. L_2 is the MHSM medium with no Cd but with added EDTA, to test for any effect of EDTA. L_3 to L_8 correspond to G_1 to G_6 in Table S2.

Table S5: Exposure conditions for the long-term algal growth experiments with increasing concentrations of Cd^{2+} in the presence of both EDTA and thiosulfate. In addition to the variables shown in this table, the exposure media contained all the MHSM constituents at the concentrations indicated in Table S1, column 3.

Media[(1)]	$[Cd]_T$ (μM)	$[Cd^{2+}]$ (nM)	$[CdS_2O_3]$ (nM)	$[Cd(S_2O_3)_2^{2-}]$ (nM)	$[Cd_2(S_2O_3)_2]$ (nM)	Cd^{2+}/Cd_T (%)	[EDTA] (μM)	$[S_2O_3^{2-}]$ (mM)
M_1	–	–	–	–	–	–	–	–
M_2	–	–	–	–	–	–	15	1
M_3	12.2	3.17	12.1	2.8	0.004	0.03	15	1
M_4	13.1	15.4	59	13.7	0.1	0.1	15	1
M_5	14.1	61.5	235	4.7	1.60	0.4	15	1
M_6	16.0	293	1120	260	36.2	1.8	15	1
M_7	19.6	806	3070	710	272	4.1	15	1
M_8	22.2	1150	4370	1009	552	5.2	15	1

(1) M_1 is the standard growth medium (MHSM), which serves as the reference. M_2 is the MHSM medium with no Cd but with added EDTA and thiosulfate, to test for any effect of these added ligands. M_3 to M_8 correspond to H_1 to H_6 in Table S2.

Table S6: Formation constants used for the binding of Cd^{2+} and H^{+} to thiosulfate, NTA and EDTA in the MINEQL+ calculations (I = 0).

Complex	Log K	Complex	Log K
CdS_2O_3	3.920	$HEDTA^{3-}$	10.948
$Cd(S_2O_3)_2$	6.300	$H_2(EDTA)^{2-}$	17.221
$Cd(S_2O_3)_3^{4-}$	6.400	$H_3(EDTA)^{-}$	20.360
$Cd(S_2O_3)_4^{6-}$	8.200	H_4EDTA	23.480
$Cd_2(S_2O_3)_2$	12.300	H_5EDTA^{+}	25.000
$CdEDTA^{2-}$	18.260	$HNTA^{2-}$	10.334
$CdNTA^{-}$	11.100	H_2NTA^{-}	13.274
$Cd(NTA)_2^{4-}$	15.050	H_3NTA	14.900
$HS_2O_3^{-}$	1.600	H_4NTA^{+}	15.900
$H_2S_2O_3$	2.200		

Table S7: Comparison between $[Cd^{2+}]$ measured by the ion exchange technique (IET) and the $[Cd^{2+}]$ calculated with MINEQL 5 for the experiment (72 h) without thiosulfate. [EDTA] = 15 µM. The $[Cd^{2+}]$ measured value is the average of two measurements performed at t = 0.

Media[(1)]	$[Cd]_T$	$[Cd^{2+}]_{calculated}$	$[Cd^{2+}]_{measured}$	SD
L_3	1.21×10^{-5} M	2.82×10^{-9} M	1.4×10^{-8} M	0.1×10^{-8} M
L_4	1.29×10^{-5} M	1.27×10^{-8} M	2.8×10^{-8} M	0.2×10^{-8} M
L_5	1.36×10^{-5} M	4.29×10^{-8} M	8.3×10^{-8} M	0.4×10^{-8} M
L_6	1.43×10^{-5} M	1.76×10^{-7} M	2.97×10^{-7} M	0.02×10^{-7} M
L_7	1.50×10^{-5} M	5.92×10^{-7} M	7.3×10^{-7} M	0.2×10^{-7} M
L_8	1.54×10^{-5} M	8.95×10^{-7} M	9.84×10^{-7} M	0.04×10^{-7} M

[(1)] The media in this column correspond to those in Table S4.

Table S8: Comparison between $[Cd^{2+}]$ measured by the ion exchange technique (IET) and the $[Cd^{2+}]$ calculated with MINEQL 5 for the experiment (72 h) with thiosulfate (1 mM) and [EDTA] = 15 μM. The $[Cd^{2+}]$ measured value is the average of two measurements performed at t = 0.

Media[(1)]	$[Cd]_T$	$[Cd^{2+}]_{calculated}$	$[Cd^{2+}]_{measured}$	SD
M_3	1.22×10^{-5} M	3.17×10^{-9} M	1.3×10^{-8} M	0.2×10^{-8} M
M_4	1.31×10^{-5} M	1.54×10^{-8} M	2.65×10^{-8} M	0.01×10^{-8} M
M_5	1.41×10^{-5} M	6.15×10^{-8} M	1.35×10^{-7} M	0.01×10^{-7} M
M_6	1.60×10^{-5} M	2.93×10^{-7} M	3.2×10^{-7} M	0.1×10^{-7} M
M_7	1.96×10^{-5} M	8.06×10^{-7} M	8.10×10^{-7} M	0.02×10^{-7} M
M_8	2.22×10^{-5} M	11.5×10^{-7} M	10.6×10^{-7} M	0.5×10^{-7} M

[(1)] The media in this column correspond to those in Table S5.

Consideration of the possibility that cadmium accumulation by *Chlamydomonas reinhardtii* is limited by the rate of diffusion of labile Cd species from the bulk solution to the algal surface.

We calculated the uptake flux of Cd and compared it to the predicted maximum flux of free Cd^{2+} across the boundary layer. To do so, we used the maximum uptake rate (i.e., Figure 1, plot with thiosulfate but no sulfate), expressed as pmol $Cd \cdot cm^{-2} \cdot s^{-1}$ (1.51×10^{-4} pmol $Cd \cdot cm^{-2} \cdot s^{-1}$), and then compared this rate with the diffusional flux of Cd across the unstirred boundary layer, assuming a boundary layer thickness of 8×10^{-4} cm. This flux through the unstirred boundary layer (J_{Cd}, expressed in units of pmol $\cdot cm^{-2} \cdot s^{-1}$) is described by the following equation (Crank, J, 1956),

$$J = \frac{4\pi D\left(\frac{r_c r_d}{r_d - r_c}\right)\left(C_b - C_s\right)}{A}$$

where r_c is the radius of the cell (cm), r_d is the radius of the cell ($2 \cdot 10^{-4}$ cm) plus the thickness of the unstirred boundary layer (estimated to be 8×10^{-4} cm; thus, $r_d = 1 \times 10^{-3}$ cm), D is the diffusion coefficient of Cd^{2+} (0.7×10^{-5} $cm^2 \cdot s^{-1}$; Kariuki, S and Dewald, HD (1996)), A is the algal surface area ($4\pi r_c^2 = 5.03 \times 10^{-7}$ cm^2), and finally, C_b and C_s are the free cadmium concentrations in the bulk solution (3.0 pmol $\cdot cm^{-3}$) and at the surface (0 pmol $\cdot cm^{-3}$), respectively. The algal uptake flux was 1.51×10^{-4} pmol Cd $cm^{-2} s^{-1}$ whereas the diffusional flux was 1.31×10^{-1} pmol $\cdot cm^{-2} s^{-1}$.

Description of Ion Exchange Technique used to determine the free Cd^{2+} concentration in the long-term algal growth media (Fortin, C and Campbell, PGC, 1998)

In the presence of an ion-exchange resin (in the sodium form), an equilibrium can be established with a trace metal M^{z+} (eq. 1). This reaction can be described by the equilibrium constant K^c_{IE} (eq. 2),

$$M^{z+} + zRNa \xleftrightarrow{K^c_{IE}} R_zM + zNa^+ \qquad (1)$$

$$K^c_{IE} = \frac{[R_zM][Na^+]^z}{[M^{z+}][RNa]^z} \qquad (2)$$

where R = resin, RNa = resin binding sites occupied by Na^+, R_zM = resin binding sites occupied by metal M^{z+}, and z = number of binding sites involved in retaining metal M. In the presence of a sufficient amount of strong electrolyte, such as $NaNO_3$, concentrations of sodium in solution, $[Na^+]$, and on the resin, [RNa], will not be significantly affected by the exchange of metal ions. Trace conditions are fulfilled when the metal M occupies less than 1% of the total resin sites ($[RNa^+] >> [R_zM]$). At fixed ionic strength and pH, equation (2) can be rearranged to yield a distribution coefficient $\lambda_{o,i,pH}$ (expressed in $L \cdot g^{-1}$):

$$\lambda_{o,i,pH} = K^c_{IE}\frac{[RNa]^z}{[Na^+]^z} = \frac{[R_zM]}{[M^{z+}]} \qquad (3)$$

The metal bound to the resin (R_zM) can be measured experimentally by eluting the resin with a volume V of strong acid. According to the quantity of resin used (m_r) and the concentration of metal measured in the eluate, $[R_zM]$ can be calculated by equation (4):

$$[R_zM] = \frac{[M_{Eluate}] * V}{m_r} \qquad (4)$$

Combining and rearranging equations (3) and (4) gives the relationship between the concentration of metal bound to the resin and the free-metal ion concentration in solution:

$$[M^{z+}] = \frac{[M_{Eluate}] * V}{\lambda_{o,i,pH} * m_r} \qquad (5)$$

Once a distribution coefficient specific to the metal of interest has been determined, for the relevant concentration and nature of the electrolyte and for the pH of solution, one can easily calculate $[M^{z+}]$.

Supplementary Figures

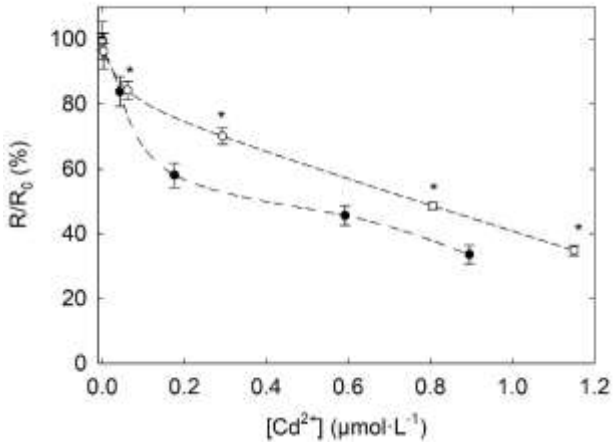

Figure S1: Relative yields of *Chlamydomonas reinhardtii* after 72 h as a function of the calculated initial free Cd^{2+} concentration in media buffered with EDTA alone (•) or with EDTA + thiosulfate (o). Series A. EC50 = 0.73 ± 0.11 µM Cd^{2+} in medium with EDTA + thiosulfate and 0.41 ± 0.09 µM Cd^{2+} in medium with EDTA alone. The asterisks indicate exposures that resulted in significantly reduced yields (P < 0.5). The error bars correspond to the standard deviation (N = 3).

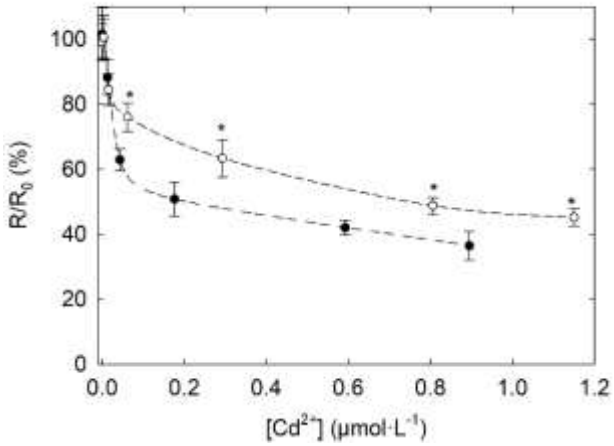

Figure S2: Relative yields of *Chlamydomonas reinhardtii* after 72 h as a function of the calculated initial free Cd^{2+} concentration in media buffered with EDTA alone (•) or with EDTA + thiosulfate (o). Series B. EC50 = 0.74 ± 0.19 µM Cd^{2+} in medium with EDTA + thiosulfate and 0.25 ± 0.11 µM Cd^{2+} in medium with EDTA alone. The asterisks indicate exposures that resulted in significantly reduced yields (P < 0.5). The error bars correspond to the standard deviation (N = 3).

References cited in the Supplementary Information

Crank, J 1956. *The Mathematics of Diffusion,* Oxford, UK, Clarendon Press.

Fortin, C & Campbell, PGC 1998. An ion-exchange technique for free-metal ion measurements (Cd^{2+}, Zn^{2+}): applications to complex aqueous media. *International Journal of Environmental Analytical Chemistry,* **72,** 173-194. 10.1080/03067319808035889

Kariuki, S & Dewald, HD 1996. Evaluation fo diffusion coefficients of metallic ions in aqueous solutions. *Electroanalysis,* **8,** 307-313.

Macfie, SM, Tarmohamed, Y & Welbourn, PM 1994. Effects of cadmium, cobalt, copper, and nickel on growth of the green alga *Chlamydomonas reinhardtii*: The influences of the cell wall and pH. *Archives of Environmental Contamination and Toxicology,* **27,** 454-458. 10.1351/pac200577040739