

SUPPLEMENTARY INFORMATION

Construction of pcs1 mutant

Based on a partial sequence of the *C. reinhardtii* nucleus encoded phytochelatase synthase (NCBI accession number XP_001701021.1), a chloroplast codon optimized sequence (Figure S1) was synthesized de novo by Eurofins Genomics (Ebersberg, Germany) and used as a template in a standard PCR with the primer pair crPCSfor (5'-TATAGGTCTCATATGGGTTTCAAGAAAACCTTCTACAAACGTAAGC-3') and crPCSrev (5'-TATAGGTCTCAGGTAAAGAGCAGCAGCACCAGAACGAACG-3'). The PCR-fragment was utilized to produce and transform a PCS specific expression cassette (Figure S2) into the genome of the *C. reinhardtii* chloroplast as described by Bertalan et al¹. The stable integration of the transgene and the expression of the C-terminal HA-tagged PCS are shown in supplementary Figures S3 and S4, respectively.

1 I. Bertalan, M. C. Munder, C. Weiß, J. Kopf, D. Fischer and U. Johanningmeier, *J. Biotechnol.*, 2015, **195**, 60–66.

Phytochelatase synthase (PCS) from *Chlamydomonas reinhardtii* (partial sequence)

(254 amino acids)

.....GFKKTFYKRKLPSPPAIEFSCPEGRQLFQEALLDGTMTGFFKLMEQFNTQDEPAFCGLASLA
MTLNALSIDPRRTWKGSWRWFHEAMLDCCRPLDAVKEEGITLYQASCLARCNGARVELVPYG
SAGLSLERFRREVEAVCGSGEEHIVVSYSRKAFLQTGDGHFSPIGGYHRGRDLVLVLDVARFKY
PPHWVPLPMLYHGMSYVDKVTGRPRGYMRLASNPLLDVLLTCDVRSAPEDWRPAEAFVRS
GAAAL.....

Alignment of the nucleotide sequence

Core	GGG TTC AAG AAG ACC TTC TAC AAG CGA AAG CTG CCC TCA CCC CCT GCA ATT GAG TTC TCC
Chloro	GGT TTC AAG AAA ACT TTC TAC AAA CGT AAG CTT CCA TCT CCA CCT GCT ATC GAA TTC TCT
	* * * * *
Core	TGC CCC GAA GGC CGG CAG CTG TTC CAA GAG GCG CTC CTG GAC GGC ACC ATG ACC GGC TTC
Chloro	TGT CCA GAA GGT CGT CAA CTT TTC CAA GAA GCT CTT CTT GAC GGT ACT ATG ACT GGT TTC
	* * * * *
Core	TTC AAA CTG ATG GAG CAA TTC AAC ACG CAG GAC GAG CCG GCC TTC TGC GGT CTG GCG TCC
Chloro	TTC AAA CTT ATG GAG CAA TTC AAC ACT CAA GAC GAA CCA GCT TTC TGC GGT CTT GCT TCT
	* * * * *
Core	CTG GCC ATG ACG CTC AAC GCG CTG TCC ATT GAC CCG CGC CGG ACC TGG AAG GGC TCC TGG
Chloro	CTT GCT ATG ACT CTT AAC GCT CTT TCT ATC GAC CCA CGT CGT ACT TGG AAA GGT TCT TGG
	* * * * *
Core	CGC TGG TTC CAC GAG GCC ATG TTG GAC TGC TGC AGG CCG CTG GAC GCT GTG AAG GAG GAG
Chloro	CGT TGG TTC CAC GAA GCT ATG TTA GAC TGC TGC CGT CCA CTT GAC GCT GTT AAA GAG GAA
	* * * * *
Core	GGC ATC ACC CTG TAC CAG GCC TCC TGC CTG GCC CGC TGC AAC GGC GCG CGG GTG GAG CTG
Chloro	GGT ATC ACT CTT TAC CAA GCT TCT TGC CTT GCT CGT TGC AAC GGT GCT CGT GTT GAA CTT
	* * * * *
Core	GTG CCG TAC GGC TCG GCC GGG CTG AGC CTG GAG CGC TTC CGT CGC GAG GTG GAG GCG GTG
Chloro	GTT CCA TAC GGT TCT GCT GGT CTT AGC CTT GAG CGT TTC CGT CGT GAA GTT GAG GCT GTT
	* * * * *
Core	TGC GGC AGC GGC GAG GAA CAC ATC GTG GTG TCC TAC AGC CGC AAG GCA TTC CTG CAG ACG
Chloro	TGC GGT AGC GGT GAG GAA CAC ATC GTT GTT TCT TAC AGC CGT AAA GCT TTC CTT CAA ACT
	* * * * *
Core	GGC GAC GGG CAC TTC AGC CCC ATA GGC GGC TAC CAC CGC GGC CGC GAC CTG GTG CTT GTG
Chloro	GGT GAC GGT CAC TTC TCT CCA ATC GGT GGT TAC CAC CGT GGT CGT GAC CTT GTT CTT GTT
	* * * * *
Core	CTG GAC GTG GCT CGC TTC AAG TAC CCA CCG CAC TGG GTG CCG CTG CCC ATG CTG TAC CAC
Chloro	CTT GAC GTT GCT CGT TTC AAA TAC CCA CCA CAC TGG GTT CCA TTA CCA ATG CTT TAC CAC
	* * * * *
Core	GGC ATG TCG TAC GTG GAC AAG GTG ACG GGC CGC CCG CGC GGC TAC ATG CGG CTG GCC TCC
Chloro	GGT ATG TCT TAC GTT GAC AAA GTT ACT GGT CGT CCA CGT GGT TAC ATG CGT TTA GCT TCT
	* * * * *
Core	AAC CCG CTG CTG GAC AGC GTG CTG CTG ACC TGC GAC GTG CGC AGC GCG CCG GAG GAC TGG
Chloro	AAC CCA TTA CTT GAC TCT GTT CTT TTA ACT TGC GAC GTT CGT TCT GCT CCA GAA GAC TGG
	* * * * *
Core	CGG CCG GCG GAG GCG TTC GTG CGC TCC GGT GCG GCC GCC CTG
Chloro	CGT CCA GCT GAA GCT TTC GTT CGT TCT GGT GCT GCT GCT CTT
	* * * * *

Figure S1: Alignment of the *C. reinhardtii* nucleus encoded PCS sequence (Core) and the *C. reinhardtii* chloroplast codon optimized sequence (Chloro). Substitutions are indicated by an asterisk. The amino acid sequence is shown in the single letter code above the nucleotide sequence.



Figure S2: Gene expression cassette was assembled and inserted into the MCS of pMM2 by Golden Gate cloning as described by Bertalan et al.¹ and used for the expression of the C-terminal HA-tagged PCS in the *C. reinhardtii* chloroplast under control of the 16S promoter, 5'atpA UTR and 3'rbcL UTR.

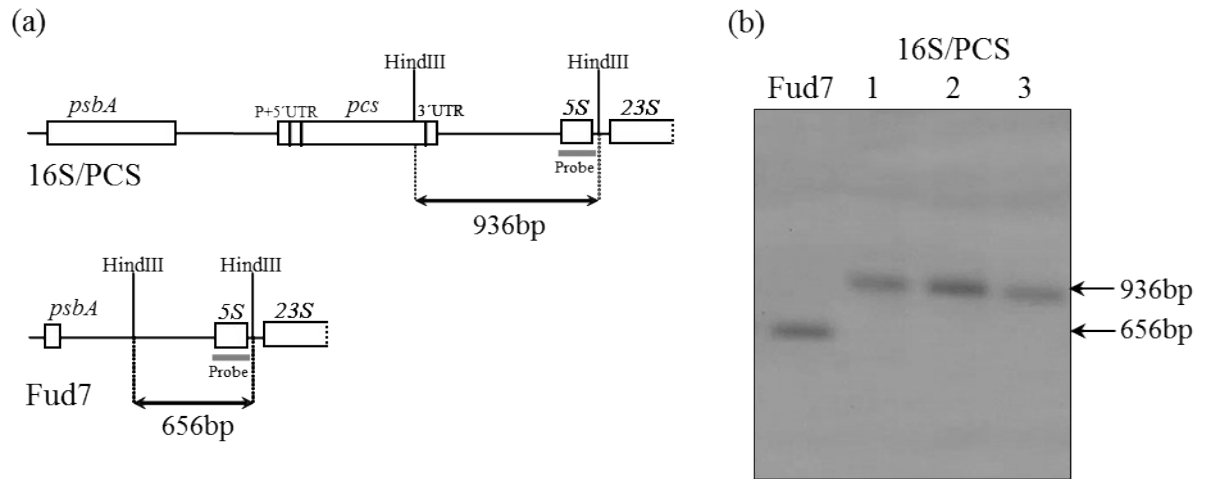


Figure S3: Test of homoplasmy performed by restriction fragment length polymorphism (RFLP)-analysis (Southern blot). (a) Restriction map of the relevant plastome region for the transformation strain and recipient strain Fud7. Indicated are the used 5S rDNA probe and the HindIII restriction sites that produce strain specific DNA-Fragments. (b) Result of the Southern blot analysis. Total cellular DNA from recipient strain Fud7 and three clones (1-3) of the PCS transformant strain was digested with HindIII. Stable integration of the PCS specific transgene in all copies of the chloroplastic genome (homoplasmy) was realized by a 5S rDNA specific probe.

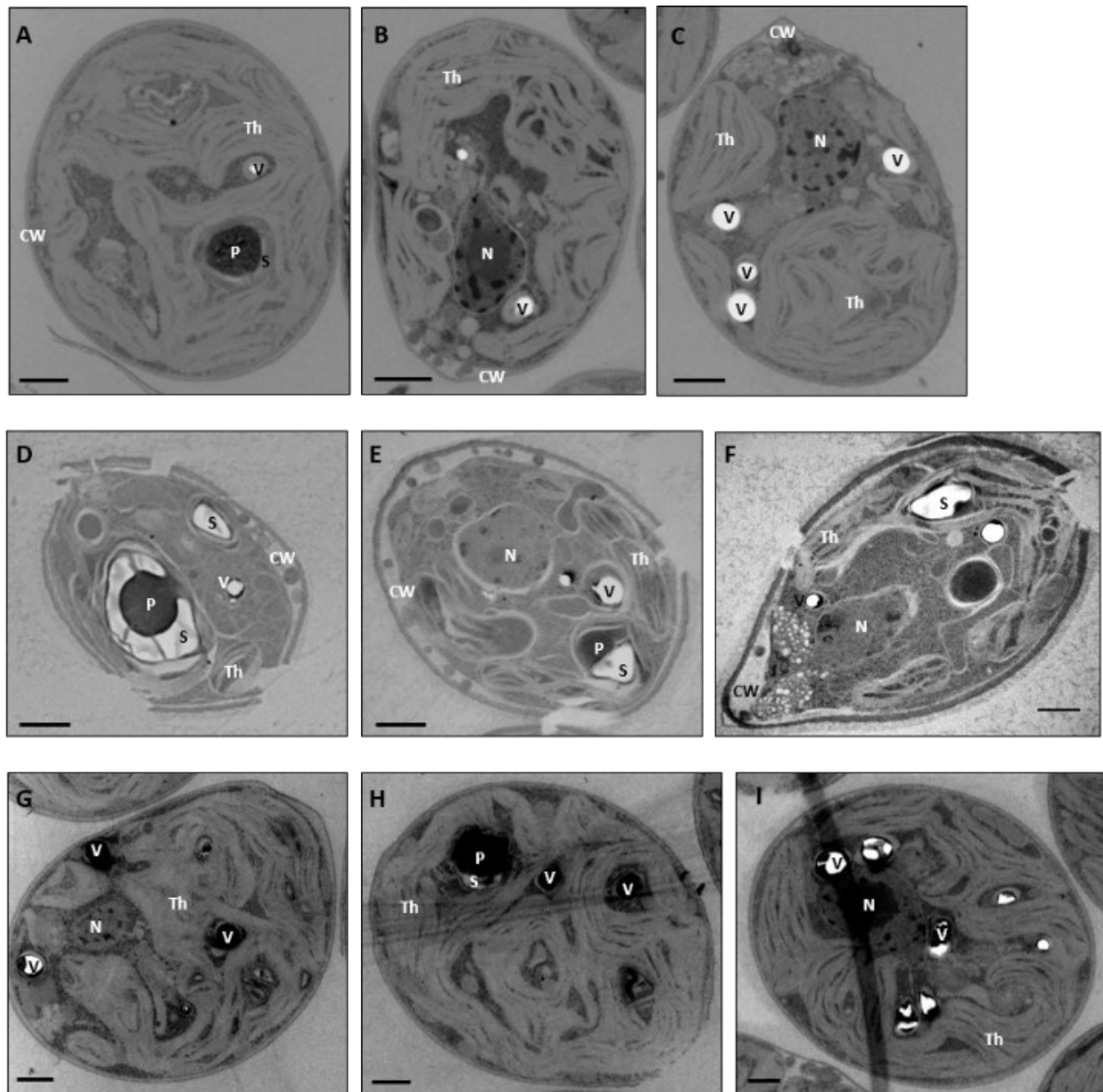


Figure S4: Electron micrographs of wt (A, B, C), *pcs1* (D, E, F) and *cw15* (G, H, I) strains cultivated in control conditions. Scale bar = 1 μm. Abbreviations: N, nucleus; P, pyrenoid; S, starch; Th, thylakoid; V, vacuole.

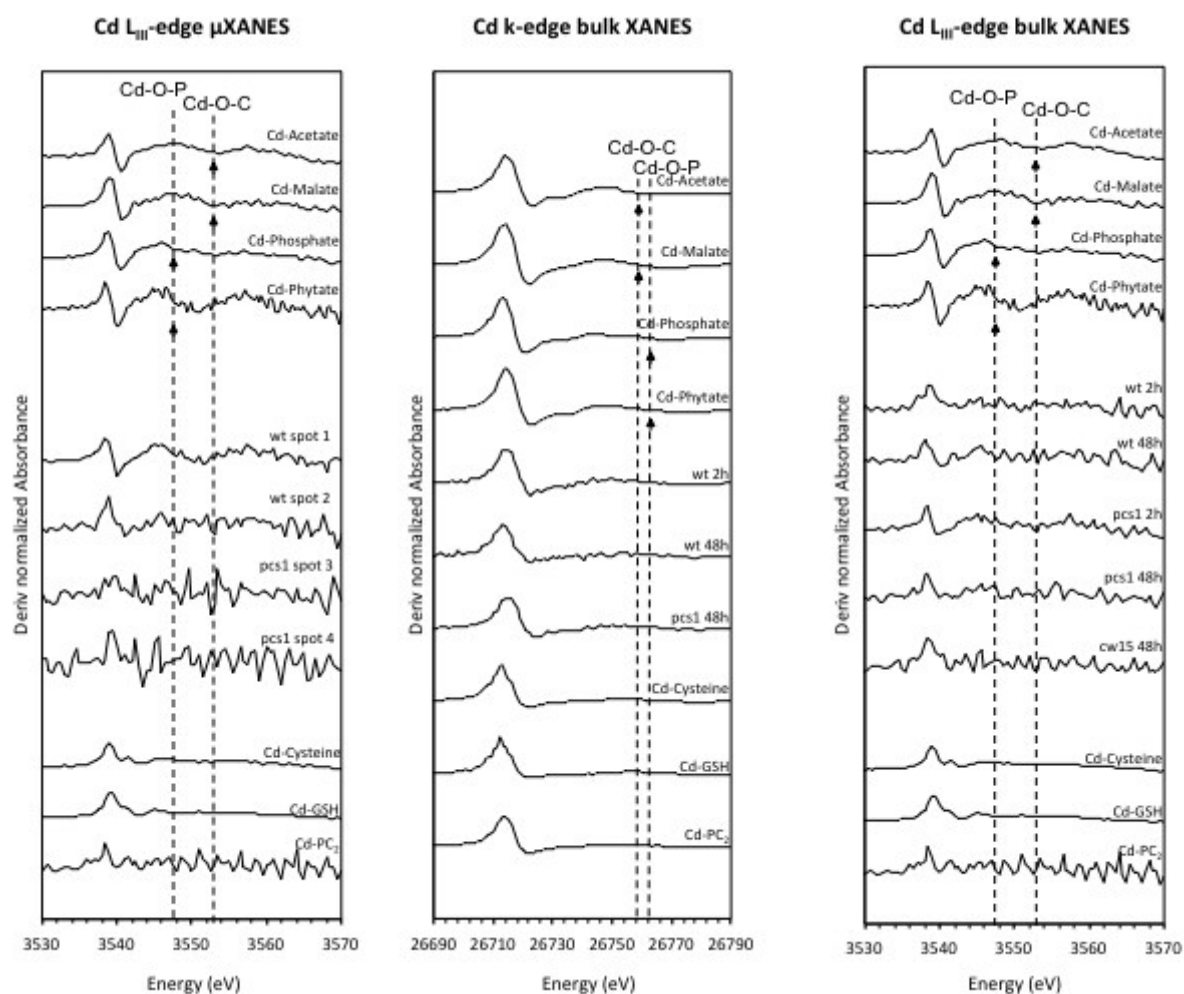


Figure S5: Derivatives of Cd references and algal spectra for Cd L_{III} and K-edges. Arrows and dotted lines display the positions of the arrows displayed in Figure 5 and 6. Distinction between Cd-O-C and Cd-O-P is clear on the derivatives of Cd L_{III}-edge spectra whereas it is more ambiguous on the Cd K-edge.

Table S1: Ionic activity product (IAP) and saturation indexes for the TAP medium contaminated with 70 μM CdCl_2 calculated using VMINTEQ software.

Mineral	log IAP	Sat. index
Antlerite $\text{Cu}_3(\text{OH})_4\text{SO}_4$	-3.537	-12.325
Atacamite $\text{Cu}_2\text{Cl}(\text{OH})_3$	0.214	-7.177
Bianchite $\text{ZnSO}_4 \cdot 6\text{H}_2\text{O}$	-9.114	-7.349
Brochantite $\text{Cu}_4\text{SO}_4(\text{OH})_6$	1.144	-14.078
Brucite $\text{Mg}(\text{OH})_2$	10.242	-6.858
$\text{Ca}_3(\text{PO}_4)_2$ (am1)	-29.396	-3.896
$\text{Ca}_3(\text{PO}_4)_2$ (am2)	-29.396	-1.146
$\text{Ca}_3(\text{PO}_4)_2$ (beta)	-29.396	-0.476
$\text{Ca}_4\text{H}(\text{PO}_4)_3 \cdot 3\text{H}_2\text{O}(\text{s})$	-49.183	-1.233
$\text{CaHPO}_4(\text{s})$	-19.787	-0.512
$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}(\text{s})$	-19.787	-0.792
$\text{CaMoO}_4(\text{s})$	-9.337	-1.387
$\text{Cd}(\text{BO}_2)_2(\text{s})$	0.841	-8.999
$\text{Cd}(\text{OH})_2(\text{s})$	8.318	-5.326
$\text{Cd}_3(\text{OH})_4\text{SO}_4(\text{s})$	7.372	-15.188
$\text{Cd}_3(\text{PO}_4)_2(\text{s})$	-34.973	-2.373
$\text{Cd}_3\text{OH}_2(\text{SO}_4)_2(\text{s})$	-10.209	-16.919
$\text{Cd}_4(\text{OH})_6\text{SO}_4(\text{s})$	15.69	-12.71
$\text{CdCl}_2(\text{s})$	-9.98	-9.321
$\text{CdCl}_2 \cdot 1\text{H}_2\text{O}(\text{s})$	-9.98	-8.286
$\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}(\text{s})$	-9.98	-8.067
$\text{CdMoO}_4(\text{s})$	-11.196	2.954
$\text{CdOHCl}(\text{s})$	-0.831	-4.368
$\text{CdSO}_4(\text{s})$	-9.264	-9.091
$\text{CdSO}_4 \cdot 1\text{H}_2\text{O}(\text{s})$	-9.264	-7.537
$\text{CdSO}_4 \cdot 2.67\text{H}_2\text{O}(\text{s})$	-9.264	-7.391
Chalcanthite $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	-12.9	-10.26
$\text{Co}(\text{BO}_2)_2(\text{s})$	-0.186	-27.256
$\text{Co}(\text{OH})_2$ (am)	7.292	-5.802
$\text{Co}(\text{OH})_2$ (c)	7.292	-4.998
$\text{Co}_3(\text{PO}_4)_2(\text{s})$	-38.051	-3.363
$\text{CoCl}_2(\text{s})$	-11.006	-19.273
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}(\text{s})$	-11.006	-13.542
$\text{CoHPO}_4(\text{s})$	-22.671	-3.611
$\text{CoMoO}_4(\text{s})$	-12.222	-4.461
$\text{CoO}(\text{s})$	7.292	-6.295
$\text{CoSO}_4(\text{s})$	-10.29	-13.092
$\text{CoSO}_4 \cdot 6\text{H}_2\text{O}(\text{s})$	-10.29	-7.817
$\text{Cu}(\text{OH})_2(\text{s})$	4.681	-4.609
$\text{Cu}_3(\text{PO}_4)_2(\text{s})$	-45.883	-9.033
$\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}(\text{s})$	-45.883	-10.763
$\text{CuMoO}_4(\text{s})$	-14.833	-1.756
$\text{CuOCuSO}_4(\text{s})$	-8.219	-18.522
$\text{CuSO}_4(\text{s})$	-12.9	-15.84
Epsomite $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	-7.34	-5.213
$\text{Fe}(\text{OH})_2$ (am)	8.676	-4.814
$\text{Fe}(\text{OH})_2$ (c)	8.676	-4.214
$\text{FeMoO}_4(\text{s})$	-10.838	-0.747
Goslarite $\text{ZnSO}_4 \cdot 7(\text{H}_2\text{O})$	-9.114	-7.102
Gypsum $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	-7.405	-2.795

Mineral	log IAP	Sat. index
$\text{H}_2\text{MoO}_4(\text{s})$	-19.514	-6.638
Halite NaCl	-5.788	-7.338
Hydroxyapatite $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$	-39.006	5.327
$\text{K}_2\text{MoO}_4(\text{s})$	-11.076	-14.338
$\text{KCl}(\text{s})$	-4.93	-5.83
Langite $\text{Cu}_4(\text{SO}_4)(\text{OH})_6 \cdot 2\text{H}_2\text{O}$	1.144	-16.345
Lime CaO	10.177	-22.522
Melantherite Cu_2OCl_2	-13.616	-19.873
Melantherite $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	-8.905	-6.696
$\text{Mg}(\text{OH})_2$ (active)	10.242	-8.552
$\text{Mg}_2(\text{OH})_3\text{Cl} \cdot 4\text{H}_2\text{O}(\text{s})$	11.335	-14.665
$\text{Mg}_3(\text{PO}_4)_2(\text{s})$	-29.201	-5.921
$\text{MgHPO}_4 \cdot 3\text{H}_2\text{O}(\text{s})$	-19.721	-1.546
$\text{MgMoO}_4(\text{s})$	-9.272	-7.422
Mirabilite $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$	-10.86	-9.746
$\text{Mn}_3(\text{PO}_4)_2(\text{s})$	-33.15	-9.323
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}(\text{s})$	-9.372	-12.087
$\text{MnHPO}_4(\text{s})$	-21.038	4.362
$\text{MnSO}_4(\text{s})$	-8.656	-11.239
$\text{MoO}_3(\text{s})$	-19.514	-11.514
$\text{Na}_2\text{Mo}_2\text{O}_7(\text{s})$	-32.307	-15.71
$\text{Na}_2\text{MoO}_4(\text{s})$	-12.793	-14.283
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}(\text{s})$	-12.793	-14.017
Periclase MgO	10.242	-11.342
Portlandite $\text{Ca}(\text{OH})_2$	10.177	-12.527
Powellite CaMoO_4	-9.337	-1.417
Pyrochroite $\text{Mn}(\text{OH})_2$	8.925	-6.269
Struvite $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$	-14.914	-1.654
Tenorite(am) CuO	4.681	-3.809
Tenorite (c) CuO	4.681	-2.959
Thenardite Na_2SO_4	-10.86	-11.182
Vivianite $(\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O})$	-33.898	3.862
Zincite ZnO	8.468	-2.762
Zincosite ZnSO_4	-9.114	-13.043
$\text{Zn}(\text{BO}_2)_2(\text{s})$	0.991	-7.299
$\text{Zn}(\text{OH})_2$ (am)	8.468	-4.006
$\text{Zn}(\text{OH})_2$ (beta)	8.468	-3.286
$\text{Zn}(\text{OH})_2$ (delta)	8.468	-3.376
$\text{Zn}(\text{OH})_2$ (epsilon)	8.468	-3.066
$\text{Zn}(\text{OH})_2$ (gamma)	8.468	-3.266
$\text{Zn}_2(\text{OH})_2\text{SO}_4(\text{s})$	-0.646	-8.146
$\text{Zn}_2(\text{OH})_3\text{Cl}(\text{s})$	7.787	-7.404
$\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}(\text{s})$	-34.523	0.897
$\text{Zn}_3\text{O}(\text{SO}_4)_2(\text{s})$	-9.759	-28.673
$\text{Zn}_4(\text{OH})_6\text{SO}_4(\text{s})$	16.29	-12.11
$\text{Zn}_5(\text{OH})_8\text{Cl}_2(\text{s})$	24.042	-14.458
$\text{ZnCl}_2(\text{s})$	-9.83	-16.88
$\text{ZnMoO}_4(\text{s})$	-11.046	-0.921
$\text{ZnSO}_4 \cdot 1\text{H}_2\text{O}(\text{s})$	-9.114	-8.476

Oversaturated phases are indicated in red.

Table S2: Total and percentage of dissolved and precipitated phases (A), and distribution of dissolved species (B) in the TAP medium contaminated with 70 μM CdCl_2 calculated using VMINTEQ software.

A)

Component	Total dissolved (μM)	% Dissolved	Total precipitated (μM)	% Precipitated
Acetate-1	17400.00	100.00	0.00	0.00
Ca+2	32.75	9.63	307.25	90.37
Cd+2	63.81	91.16	6.19	8.84
Cl-1	8220.00	100.00	0.00	0.00
Co+2	6.80	100.00	0.00	0.00
Cu+2	6.30	100.00	0.00	0.00
EDTA-4	134.00	100.00	0.00	0.00
Fe+2	1.05	5.85	16.85	94.16
H+1	19412.00	100.00	0.00	0.00
H3BO3	184.00	100.00	0.00	0.00
K+1	1940.00	100.00	0.00	0.00
Mg+2	409.99	100.00	0.00	0.00
Mn+2	0.00	0.01	25.60	100.00
MoO4-2	0.01	0.19	6.19	99.81
Na+1	270.00	100.00	0.00	0.00
NH4+1	7480.00	100.00	0.00	0.00
PO4-3	776.23	77.62	223.77	22.38
SO4-2	509.99	100.00	0.00	0.00
Tris-1	20000.00	100.00	0.00	0.00
Zn+2	72.62	94.93	3.88	5.07

B)

Component	% of total dissolved concentration	Species name
Cd+2	3.254	Cd+2
	0.639	Cd-Tris+1
	1.444	CdCl+
	0.037	CdCl2 (aq)
	0.115	CdSO4 (aq)
	0.043	CdNH3+2
	1.929	CdHPO4 (aq)
	89.661	CdEDTA-2
	0.013	CdHEDTA-
	2.641	Cd-Acetate+
NH4+1	0.215	Cd-(Acetate)2 (aq)
	99.225	NH4+1
	0.286	NH4SO4-
K+1	0.488	NH3 (aq)
	98.614	K+1
	0.304	KCl (aq)
	0.188	KSO4-
	0.155	KHPO4-
	0.056	KH2PO4 (aq)
	0.683	K-Acetate (aq)

Component	% of total dissolved concentration	Species name
Mg+2	76.772	Mg+2
	0.397	Mg-Tris+1
	1.42	MgCl+
	2.115	MgSO4 (aq)
	5.663	MgHPO4 (aq)
	13.628	Mg-Acetate+
Cl-1	99.832	Cl-1
	0.011	CdCl+
	0.071	MgCl+
	0.072	KCl (aq)
SO4-2	93.102	SO4-2
	0.02	ZnSO4 (aq)
	0.014	CdSO4 (aq)
	1.7	MgSO4 (aq)
	0.177	CaSO4 (aq)
	0.077	NaSO4-
	0.714	KSO4-
	4.191	NH4SO4-

Component	% of total dissolved concentration	Species name
Na+1	98.246	Na+1
	0.05	Na-Tris (aq)
	0.303	NaCl (aq)
	0.145	NaSO4-
	0.24	NaHPO4-
	0.055	NaH2PO4 (aq)
	0.962	Na-Acetate (aq)
EDTA-4	49.28	ZnEDTA-2
	42.698	CdEDTA-2
	3.083	CuEDTA-2
	4.837	CoEDTA-2
	0.084	FeEDTA-2
Tris-1	8.896	Tris-1
	0.023	Cu-(Tris)4-2
	0.012	Cu-(Tris)3-1
	91.047	H-Tris (aq)
Acetate-1	99.051	Acetate-1
	0.489	H-Acetate (aq)
	0.321	Mg-Acetate+
	0.022	Ca-Acetate+
	0.015	Na-Acetate (aq)
	0.076	K-Acetate (aq)
H3BO3	98.696	H3BO3
	0.635	H3BO3-Acetate-
	0.664	H2BO3-
Co+2	3.013	Co+2
	0.439	Co-Tris+1
	0.091	CoSO4 (aq)
	0.012	Co(NH3)+2
	0.4	CoHPO4 (aq)
	95.311	CoEDTA-2
	0.017	CoHEDTA-
	0.705	Co-Acetate+
Cu+2	18.632	Cu-(Tris)4-2
	13.075	Cu-(Tris)3-1
	2.366	Cu-(Tris)2 (aq)
	0.295	Cu-Tris+1
	0.015	CuHPO4 (aq)
	65.566	CuEDTA-2
	0.014	CuHEDTA-
	0.015	Cu-Acetate+
MoO4-2	89.949	MoO4-2
	0.102	HMoO4-
	9.672	MgMoO4(aq)
	0.277	CaMoO4(aq)

Component	% of total dissolved concentration	Species name
Mn+2	59.691	Mn+2
	0.277	MnCl+
	1.607	MnSO4 (aq)
	0.015	MnNH3+2
	18.568	MnHPO4 (aq)
	5.201	MnEDTA-2
	14.627	Mn-Acetate+
PO4-3	47.245	HPO4-2
	48.637	H2PO4-
	0.03	FeHPO4 (aq)
	2.991	MgHPO4 (aq)
	0.179	CaHPO4 (aq)
	0.016	CaH2PO4+
	0.083	NaHPO4-
	0.388	KHPO4-
	0.159	CdHPO4 (aq)
	0.094	ZnHPO4 (aq)
	0.139	KH2PO4 (aq)
	0.019	NaH2PO4 (aq)
Fe+2	47.926	Fe+2
	0.125	FeOH+
	0.14	FeCl+
	1.781	FeSO4 (aq)
	0.045	FeNH3+2
	5.133	FeH2PO4+
	22.306	FeHPO4 (aq)
	10.733	FeEDTA-2
	0.067	FeOHEDTA-3
	11.744	Fe-Acetate+
Ca+2	79.452	Ca+2
	0.366	Ca-Tris+1
	0.927	CaCl+
	2.755	CaSO4 (aq)
	4.246	CaHPO4 (aq)
	0.131	CaPO4-
	0.38	CaH2PO4+
	11.731	Ca-Acetate+
Zn+2	4.155	Zn+2
	2.097	Zn-Tris+1
	0.027	ZnOH+
	0.056	ZnCl+
	0.138	ZnSO4 (aq)
	0.025	ZnNH3+2
	1.003	ZnHPO4 (aq)
	90.93	ZnEDTA-2
	0.016	ZnHEDTA-
	1.506	Zn-Acetate+
	0.043	Zn-(Acetate)2 (aq)