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Supplementary material

When are metal complexes bioavailable?

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Table S1. Representative examples of some exceptions to the classic models: passive diffusion by lipophilic complexes

DDC, diethyldithiocarbamate; NTA, nitrilotriacetic acid; XANT, ethylxanthate

	Organisms	Metal (M)	Ligands (M)	Medium	Exposure time	Endpoints	Metal complex	Comments	Ref.
Cladoceran	*Daphnia magna*	[Cd] 64 nM	DDC	Natural freshwater; pH 7.5	2 days	Cd bioaccumulation	Cd–DDC	DDC-enhanced Cd bioaccumulation	[1]
Marine diatom	*Nitzschia closterium*	[Cu] 79 nM	Phenanthroline-type (2,9-dmp); 8-hydroxyquinoline; l-(2-pyridylazo)-2-naphthol; 1-(2-thiazolylazo)-2-naphthol	Seawater; pH 8.2	3 days	Growth rate; photosynthesis	Cu–L	Toxicity index is higher for hydrophobic complexes than hydrophilic complexes	[2]
Marine diatom	*Thalassiosira weissflogii*	[Cu] 15 nM, [Cd] 9 nM, [Pb] 5 nM	Sulfoxine; oxine; DDC	UV seawater; pH 8	5 h	Metal uptake	$Cu(Ox)_2^0$; $Cu(DDC)_2^0$; $Cd(DDC)_2^0$; $Pb(DDC)_2^0$	$M(DDC)_2^0$ uptake is much higher than M′ and $(MEDTA)^{2-}$	[3]
Marine diatom	*Thalassiosira weissflogii*	[Cu] 5 nM, [Zn] 3.4 µM, [Pb] 5 nM	Dithiocarbamates: Ziram 3.3 µM; Maneb 2 µM	UV seawater; pH 8	5 h	Metal uptake	Cu–L	Zn, Cu and Pb content in cell was enhanced in the presence of Ziram and Maneb	[4]
Marine algae	*Synechococcus* clone DC2, *Amphidinium carterae*, *Chrysochromulina polylepis*, *Ditylum brightwelli*, *Prorocentrum micans*	[Cu] 10 and 400 nM	Oxine; NTA	Natural seawater; pH	2.5 h	Cellular metal content	Cu–oxine	Cellular Cu content is significantly higher in the presence of Cu–oxine than Cu–NTA	[5]
Freshwater alga	*Pseudokirchneriella subcapitata*	[Cd] 0.38 nM	[DDC] 1 µM	Synthetic freshwater; pH ~5.5–7.0	<40 min	Metal uptake rate	$[Cd(DDC)_2]^0$	The uptake of lipophilic metal complex is pH-dependent	[6]

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	Organisms	Metal (M)	Ligands (M)	Medium	Exposure time	Endpoints	Metal complex	Comments	Ref.
Freshwater alga	*Pseudokirchneriella subcapitata; Chlamydomonas reinhardtii; Chlorella fusca*	[Cd] 0.38 nM	[DDC] 1 µM; XANT, 100 µM	Synthetic freshwater; pH 5.5 and 7.0	30 min	Metal uptake rate	$[Cd(DDC)_2]^0$; $[Cd(XANT)_2]^0$	The uptake of lipophilic metal complex is pH-dependent	[7]
Marine diatom	*Thalassiosira weissflogii*	[Hg] ~0–10 nM	[Cl] ~10 µM–0.32 M	Synthetic seawater; pH ~4–8	4 h	Metal uptake rate; growth rate	$[HgCl_2]^0$; $[CH_3HgCl]^0$	Hg bioaccumulation is dependent on $[HgCl_2]$	[8]

Table S2. Representative examples of some exceptions to the classic models: metal uptake through ligand transport system

NTA, nitrilotriacetic acid

Organisms		Metal (M)	Ligands (M)	Medium	Exposure time	Endpoints	Comments	Ref.
Freshwater alga	*Pseudokirchneriella subcapitata*	$[Cd^{2+}]$ ~9 nM–0.87 μM; $[Zn^{2+}]$ ~23 nM–2.04 μM	Citrate 100 μM; NTA 100 μM	Synthetic freshwater; pH 7.3	72 h for toxicity; 6 min for uptake	Growth rate; cell density; metal uptake; ligand uptake	Cd was accidentally transported with citrate through citrate transporter at the rate of one fourth that of citrate	[9]
Freshwater alga	*Chlamydomonas reinhardtii*	$[Ag^{+}]$ 10 and 114 nM	$[Cl^{-}]$ 0.05 and 4 mM; $[(S_2O_3)^{2-}]$ 114 nM; $[(SO_4)^{2-}]$ 81 μM	Synthetic freshwater; pH 7.0	≤1 h	Metal uptake	Ag is internalised through $S_2O_3^{2-}/SO_4^{2-}$ transport system	[10]
Freshwater alga	*Chlamydomonas reinhardtii*; *Pseudo-kirchneriella subcapitata*	[Ag] ~10–104 nM	$[(S_2O_3)^{2-}]$ ~11.4–114 nM	Synthetic freshwater; pH 7.0	4 days	Growth rate; uptake rate	Ag is internalised through $S_2O_3^{2-}$ transport system	[11]
Bacterium	*Arthrobacter* sp. *JM018*	[Zn] ~0–250 μM	$[PO_4^{3-}]$; [glycerol-3-phosphate] 33 μM	Synthetic freshwater; pH 6, 7 and 8	150 h	Growth rate	$ZnHPO_4^0(aq)$ is taken up through Pit inorganic phosphate transport system	[12]

Table S3. Representative examples of some exceptions to the classic models: ternary complex formation

DOM, dissolved organic matter; EM, electrophoretic mobility; SRHA, Suwannee River humic acid; SRFA, Suwannee River fulvic acid; AA, alginic acid; PGA, polygalacturonic acid; XAN, xanthan; SUC, succinoglycan; MES, 2-(*N*-morpholino)ethanesulfonic acid; GSH, glutathione; PC2, phytochelatin-2; His, histidine; DFB, desferrioxamine-B; IDA, iminodiacetic acid

	Organisms	Metal (M)	Ligands (M)	Medium	Exposure time	Endpoints	Ternary complex	Comments	Ref.
Juvenile fish	*Salmo salar*	[Al] ~5–8 µM; inorganic [Al] ~0.8–4 µM	[F^-] 1.5 µM	Synthetic freshwater pH ~4.5–4.9	7 days	Al uptake in gill	<F-Al-R>	Mortality increased from 50 to 100 % at ~4.2–9.4 µM [AlF_x] (pH 4.5)	[13]
Gill cells of large-mouth bass	*Micropterus salmoides*	[Al] ~0–50 µM	[F^-] ~0–10 µM; DOM ~0–10 mg L^{-1}	Synthetic freshwater pH 4.5		EM	<F-Al-R>; <Al-DOM-gill cell> or <DOM-Al-gill cell>	At constant [Al] 10 µM, increasing [F] did not increase EM to the extent predicted by [Al^{3+}]	[14]
Green alga	*Chlorella kesslerii*	[Pb] ~0.5 µM–0.1 mM	10 mg L^{-1} each for the SRHA, the SRFA, AA, PGA, XAN, SUC	Synthetic freshwater MES; pH 6	≤1 h	Pb uptake	<LPb–R> and <PbL–R>	<LPb–R> contributes to uptake when $K_s/K_{SPbL}K_{PbL}$[L] << 1	[15]
Green alga	*Chlorella kesslerii*	[Cd^{2+}], [Cu^{2+}] and [Pb^{2+}] ~10^{-9}–10^{-5} M	Citrate 5 mM; humic acid 10 mg L^{-1}	Synthetic freshwater MES; pH 6	35 min	Cumulative mortality; Al uptake in gill;	<Pb-HA-Cell>	95 to 57 % Pb in cell is from <Pb–HA–cell> when [Pb^{2+}] ranging from nanomolar to micromolar levels	[16]
Green alga	*Chlorella kesslerii*	1 µM for Cd, Cu and Pb	Citrate 50 µM to 0.3 mM; humic acid 10 mg L^{-1}	Synthetic freshwater MES; pH 6	1 h	Metal uptake	<HA-Pb-R>	The contribution of ternary complex is dependent on the concentration of ternary complex	[17]
Algae diatom; coccolitho phore	*Thalassiosira weissflogii*; *Emiliania huxleyi*	[Zn] ~0–10 µM	Cysteine ~0.5–40 µM; GSH 22 µM, PC2 1 µM, His 40 µM, DFB 8 µM	Filtered Gulf Stream seawater; pH ~7.9–8.2	2-4 h	Zn uptake rate	<Cys-Zn-R>	Metal complex with 1 : 1 ratio is the substrate for ternary complex formation	[18]
Green alga	*Chlamydomonas reinhardtii*	[Sc] ~0.2 nM–2 µM	[OH] ~0.3 nM–0.8 µM	Synthetic freshwater, pH ~4.5–7.9	≤1 h	Sc internalisation flux	<OH-Sc-R>	The ternary complex contributed to Sc uptake at pH > 6.5	[19]

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	Organisms	Metal (M)	Ligands (M)	Medium	Exposure time	Endpoints	Ternary complex	Comments	Ref.
Wheat cultivar	*Triticum aestivum* L.	[Zn^{2+}] ~5 nM–20 µM	Citrate 0.45 and 1.5 mM; L-histidine 0.98 and 1.3 mM	Synthetic freshwater, pH 7.2	3 h	Zn influx	<histidine–Zn–R>	Zn–histidine formed ternary complex with transporters	[20]
Green alga	*Chlamydomonas reinhardtii*	[Tm] ~1 nM–100 µM	Citric acid, malic acid, NTA, diglycolic acid, malonic acid and IDA	Synthetic freshwater MES, pH 6	1 h	Tm internalisation flux	<L–Tm–R>	The contribution of ternary complex to metal uptake is dependent on the concentration of dominant metal species	[21]

Table S4. Representative examples of some exceptions to the classic models: contribution from the dissociation of labile complex

HEPES, *N*-(2-hydroxyethyl)piperazine-*N′*-(2-ethanesulfonic acid); EDDA, *N*,*N*-ethylenediaminediacetate; HEDTA: *N*-(2-hydroxyethyl)ethylenediamine-*N*,*N′*,*N′*-triacetate; EGTA: ethylene-bis-(oxyethylenenitrilo)-tetraacetate; CDTA: trans-1,2-cyclohexyldiamine-*N*,*N*,*N′*,*N′*-tetraacetate; MES, 2-(*N*-morpholino)ethanesulfonic acid

	Organisms	Metal (M)	Ligands (M)	Medium	Exposure time	Endpoints	Metal conc. for observed diffusion limitation	Lability of complex	Comments	Ref.
Algae	*Chlamydomonas reinhardtii*	$[Ag^+]$ ~1–25 nM	[Cl] ~5 µM–50 mM	Synthetic freshwater; pH 7	≤1 h	Ag uptake	Limited diffusion at $[Ag^+] \leq 10$ nM	$AgCl^0$, $(AgCl_2)^-$	Fast Ag uptake by algae	[22]
	Chlamydomonas reinhardtii	[Pb] ~0.01–0.75 µM; [Cu] ~0.015–0.5 µM	Dissolved inorganic carbon ~0–4 mM	Synthetic freshwater; pH 7	35 min	Uptake flux	Limited diffusion at $[Pb^{2+}] \leq 0.1$ µM	$PbCO_3$ labile	Fast Pb uptake by algae	[23]
	Chlorella kesslerii	[Zn] 10 pM and 1 nM for culture; ~4 pM–1 mM for exposure	NTA (5–400 µM); Tiron (50–500 µM)	Synthetic freshwater (HEPES); pH 7	25 min	Uptake flux	Limited diffusion at $[Zn^{2+}] \leq 20$ pM for algae grown at 10 pM Zn	ZnNTA inert	Increased Zn uptake caused by more binding sites for algae in Zn deficient media	[24]
	Periphyton	$[Cu^{2+}]$ ~$10^{-16.5}$–$10^{-14.5}$ M;	NTA	Natural freshwater; pH 8.4	2 days	Cu and Zn uptake	Limited diffusion at $[Cu^{2+}] \leq 1.9 \times 10^{-15}$ M	Cu complexes weaker than CuNTA are labile	Cu content is in proportion to weakly complexed Cu	[25]
Macro-alga	*Ceramium tenuicorne*	$[Cu^{2+}]$ ~10^{-15}–10^{-12} M	Nordic fulvic acid ~2–4 mg L^{-1}; salicylaldoxime (SA)	Synthetic seawater; pH 8	7 days	EC50 and Cu uptake	Limited diffusion at $[Cu^{2+}]$ ~0.1–0.8 pM	Weakly bound Cu is labile	The diffusion flux of weakly bound Cu is comparable with Cu uptake flux in the presence of fulvic acid	[26]
Plant	Spinach *Spinacia oleracea*	[Cd] ~1 nM–100 µM; $[Cd^{2+}]$ 1nM	EDDA (100 µM); NTA (100–3000 µM); HEDTA (3.0–280 µM); EDTA (100 µM); EGTA (5.9–500 µM); CDTA (1.4–138 µM)	Synthetic freshwater (MES); pH 6	4 h	Cd uptake	Limited diffusion at $[Cd^{2+}]$ 1 nM (uptake increased in stirred medium)	Fully labile: Cd–EDDA and Cd–NTA; inert: Cd–EGTA and Cd–CDTA	At constant $[Cd^{2+}]$ 1 nM, Cd uptake is dependent on the lability of Cd complexes	[27]

	Organisms	Metal (M)	Ligands (M)	Medium	Exposure time	Endpoints	Metal conc. for observed diffusion limitation	Lability of complex	Comments	Ref.
	Spinach *Spinacia oleracea* L.; Tomato *Lycopersicon esculentum* L.	[Cu] 2 and 5 µM; [Zn] 1, 10 and 100 µM;	NTA 1.1 and 1.9 mM; HEDTA ~83–183 µM; EDTA 45 and 110 µM; CDTA ~129–229 µM Chelex resin	Synthetic freshwater; pH 6	18 days	Cu and Zn bioaccumulation	Limited diffusion at $[Zn^{2+}] \leq 10$ nM	CuNTA > CuHEDTA > CuEDTA > CuCDTA	Enhanced uptake by Zn–L is related to dissociation rate constant of metal complex	[28]
	Durum wheat *Triticum turgidum*	$[Cu^{2+}]$ ~0.29 pM–5.5 mM	NTA	Synthetic freshwater; pH 6	2 h	Cd bioaccumulation	Limited diffusion at $[Cu^{2+}]$ ~0.1–1 nM; $[Cd^{2+}] \leq$ 0.1 nM	Not mentioned	Cu–NTA enhanced Cu uptake 6-fold for $[Cu^{2+}]$ ~0.1 nM –0.1 µM	[29]
	Wheat *Triticum aestivum*	$[Zn^{2+}]$ 15 nM	NTA; EDTA	Synthetic freshwater (MES); pH 6	8 h	Zn uptake in root	Limited diffusion at $[Zn^{2+}] \leq 0.1$ µM	Zn–NTA labile; Zn–EDTA inert	The contribution from Zn labile complex is related to dissociation rate constant	[30]
Bivalve	Mussel *Mytilus edulis*	$[Zn^{2+}]$ ~0.01 nM–6 µM	Citrate; NTA; EDTA; Glycine	Synthetic seawater; pH 6	24 h	Zn uptake in gills	Limited diffusion at $[Zn^{2+}] \leq 0.1$ µM	Zn–Gly labile; Zn–Cit labile; Zn–NTA inert; Zn–EDTA inert		[31]

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