Key factors affecting trace element uptake and toxicity in aquatic organisms

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Limits

- Emphasis on aquatic systems, on dissolved metals
- "Metals" = cations
- "Bioavailability" = uptake or toxicity
- Short-term exposures (min → h)
- Laboratory (defined chemistry) → laboratory (presence DOM) → field

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Key Words

- $M(H_20)_n^{z+}$; (H^+, Ca^{2+}); BLM
- $ML^{\pm}$; where L = assimilable ligand
- ML_n^0; i.e., neutral and lipophilic complexes
- pH
- NOM; natural dissolved organic matter

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METAL SPECIATION

B : $(H_2O)_{n-1}M$-OH

hydroxo-complexes

C : M-X

inorganic complexes

X = Cl^-, F^-, SO_4^{2-}, $S_2O_3^{2-}$, HCO_3^-, CO_3^{2-}

A

$M(H_2O)_n^{z+}$

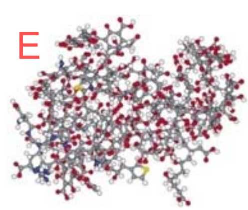
aquo ion

D : M-L

organic complexes (monomeric)

L = amino acids, polycarboxylic acids
+
anthropogenic ligands

E



Humic substances: fulvic and humic acids (polymeric)

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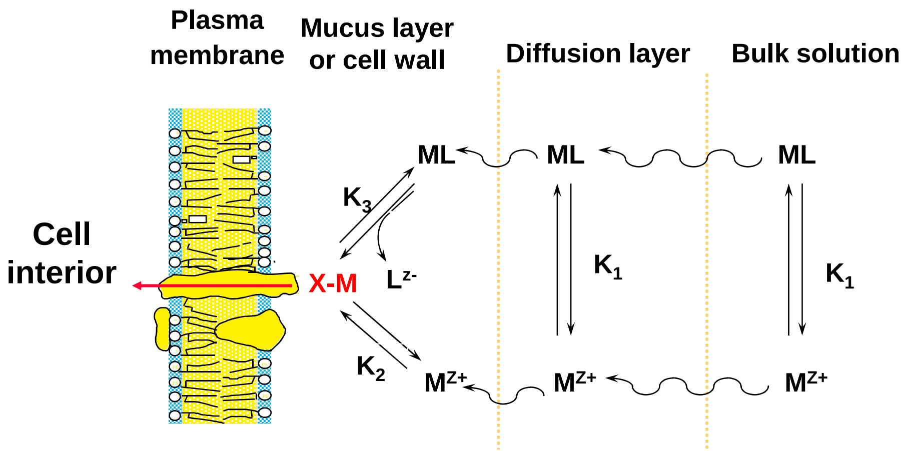
Metal-organism Interactions



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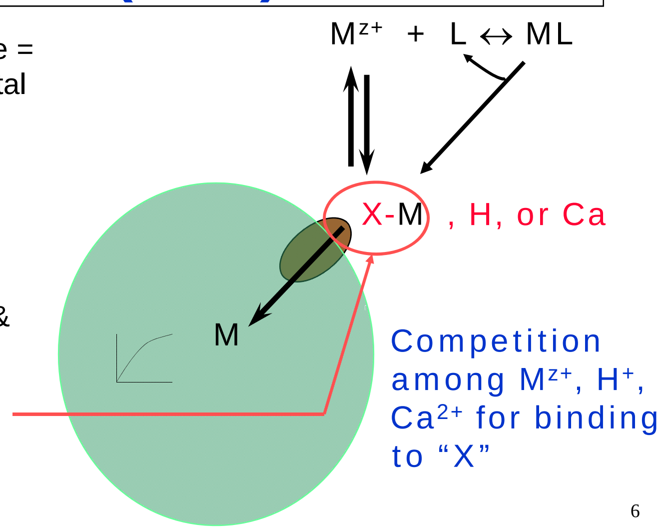
BIOTIC LIGAND MODEL (BLM)

- Plasma membrane = primary site of metal interaction
- Formation surface complex, {M-X-membrane}
- Rapid equilibrium between solution & biological surface
- Response α {M-X-membrane}



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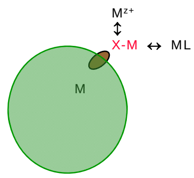

BLM

Examples of cells

- bacteria, algae, micro-invertebrates, macro-invertebrates, fish

Examples of metals

- divalent (Cu >> Cd,Ni,Pb,Zn) > trivalent (Al) > monovalent (Ag)

Examples of ligands

- inorganic (Cl^-; HO^-; SO_4^{2-})
- simple organic (NTA, ETDA, TRIS)

i.e., monomeric, synthetic, non-assimilable ligands, forming hydrophilic metal-ligand complexes

For reviews, see Campbell 1995; Campbell et al. 2002; Niyogi & Wood 2004; Slaveykova & Wilkinson 2005

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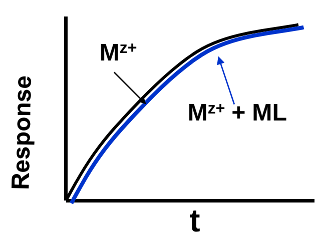
Typical experimental Design #1

- Fix the concentration of the free metal ion
- Vary the total metal concentration, using metal-binding ligands (“metal buffers”)
- Follow the “biological response” (e.g., metal uptake, metal toxicity) as a function of time

The BLM predicts that the biological response should be same, in the presence or absence of a reservoir of complexed metal



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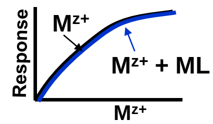
Typical Experimental Design #2



- Fix the exposure time
- Vary the free metal ion concentration in the absence of metal complexing ligands
- Vary the free metal ion concentration in the presence of metal complexing ligands
- Follow biological "response" (e.g., metal uptake, metal toxicity) as a function of [M^{z+}]

BLM predicts same response, regardless of presence or absence of complexed metal

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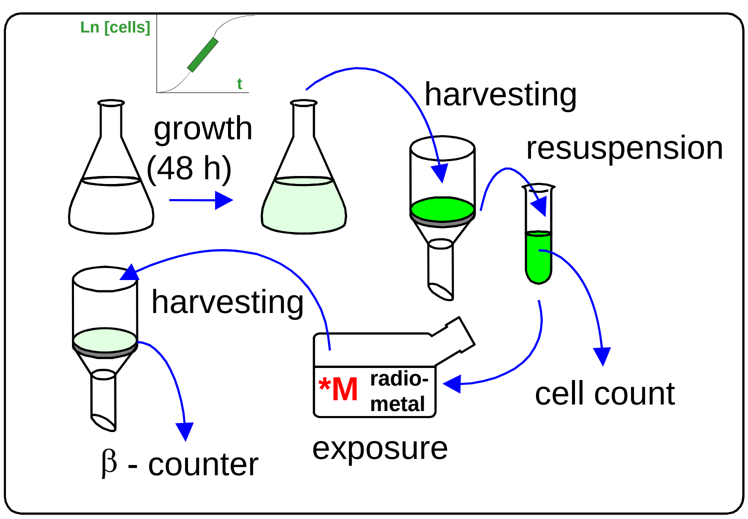
Methodology



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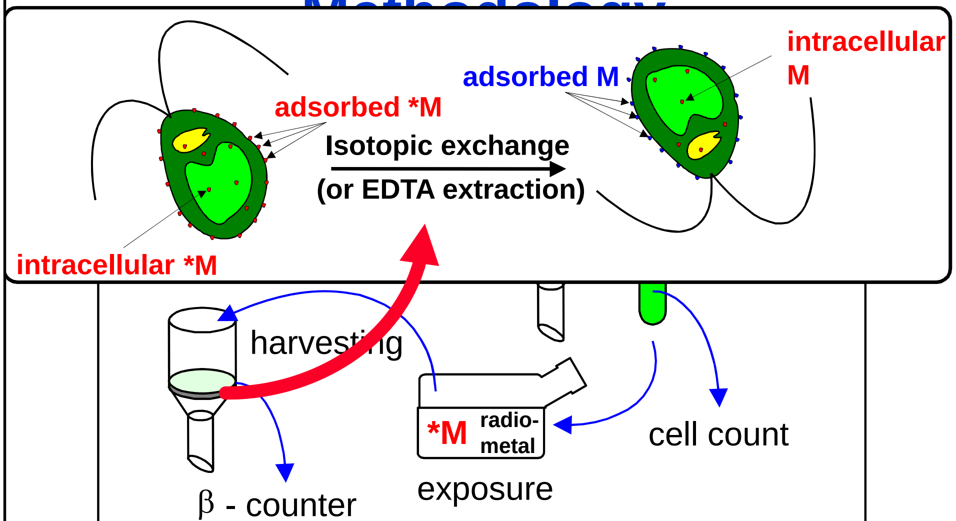
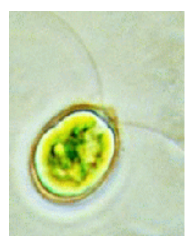
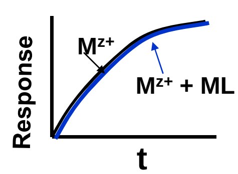
Methodology



BLM – Known exceptions (1a) – assimilable ligands

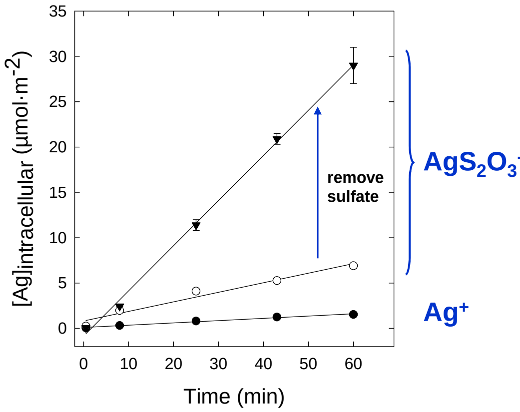
- **metal = ^{110m}Ag**
- **alga = *Chlamydomonas reinhardtii***
- **ligand = thiosulfate**
- **response = bioaccumulation**
  - **[Ag^+] = 10 nM, constant**
  - **[$S_2O_3^{2-}$] = 110 nM**





Effect of thiosulfate on the uptake of silver by *Chlamydomonas reinhardtii*

- Free [Ag^+] = 10 nM, similar in all three media
- Silver uptake greatly increased in the presence of thiosulfate (114 nM)



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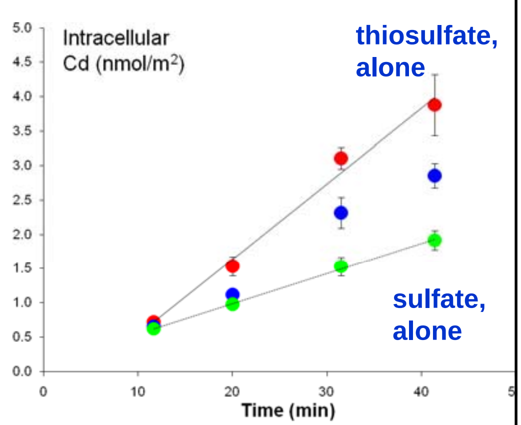
BLM – Known exceptions (1b)

Assimilable ligands

- **metal = ^{109}Cd**
- **alga = *Chlamydomonas reinhardtii***
- **ligand = thiosulfate**
- **response = bioaccumulation**
  - **[Cd^{2+}] = 2.8 nM, constant**
  - **[$S_2O_3^{2-}$] = 1 mM**



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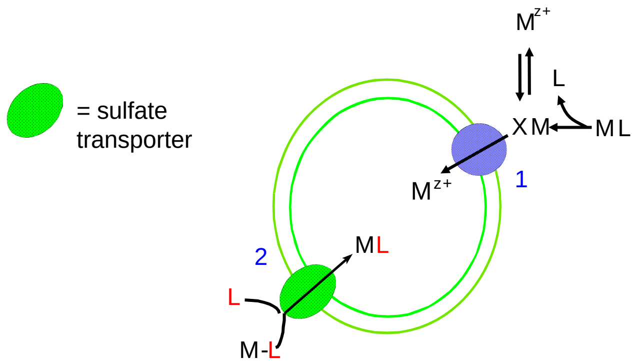
Metal uptake mechanisms



1. Facilitated transport of M^{z+}

2. Facilitated transport of L, or of M-L

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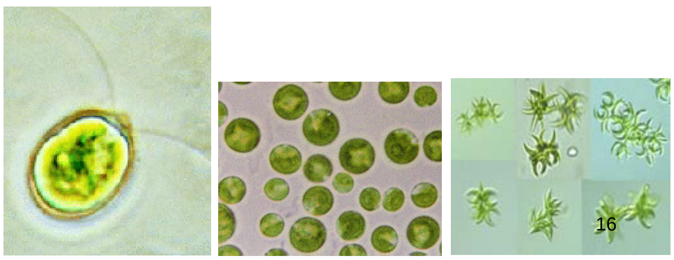
BLM – Known exceptions (2) – lipophilic complexes

- **Algae (*C. reinhardtii, Chlorella fusca, Pseudokirchneriella subcapitata*)**
- **Cd**
- **Organic ligand (DDC or xanthate)**



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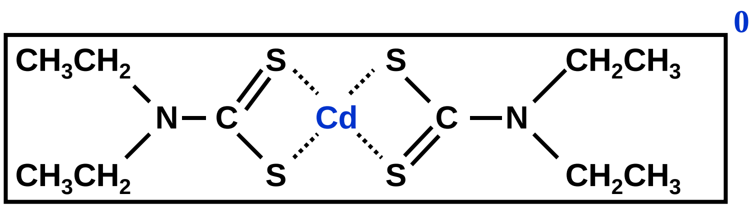
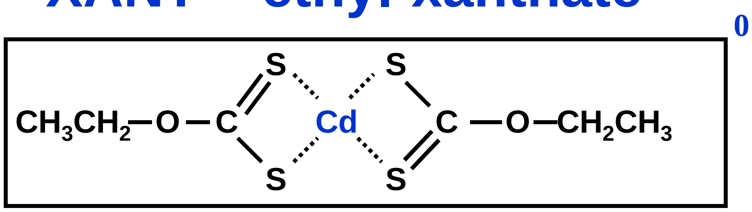
DDC = diethyl-dithiocarbamate



XANT = ethyl-xanthate



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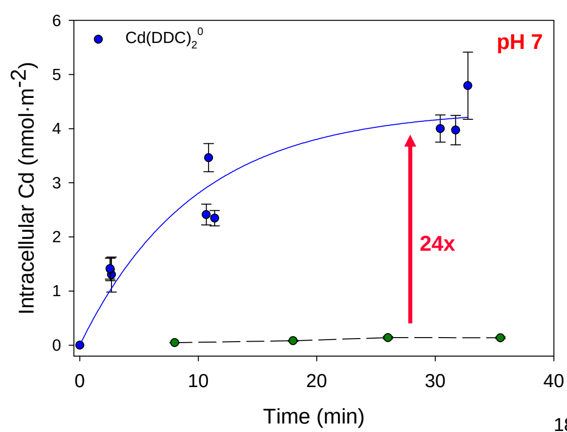
Cd uptake by *C. reinhardtii*
(presence / absence DDC)

Cd uptake greatly accelerated in the presence of DDC



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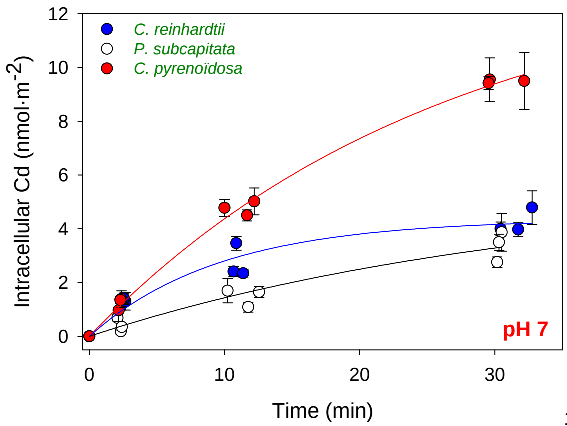
Cd uptake by all three algae
(presence / absence DDC)

Cd uptake accelerated in the presence of DDC <u>for the three algae</u>



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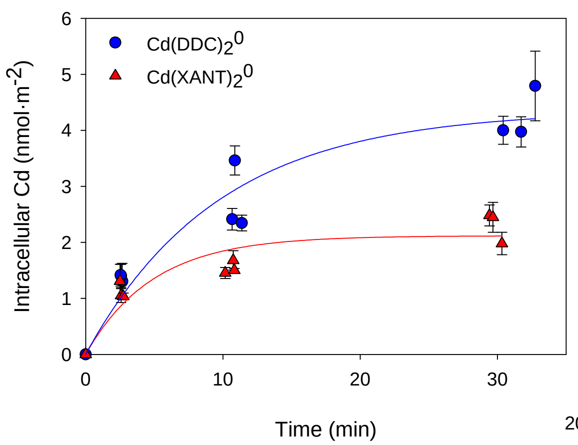
Uptake of CdL_2^0 complexes by *C. reinhardtii*
(comparison DDC and xanthate)

Cd uptake accelerated in both cases…

i.e., general phenomenon



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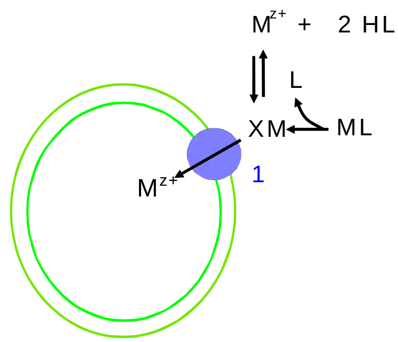
Metal uptake mechanisms



1. Facilitated transport of M^{z+}

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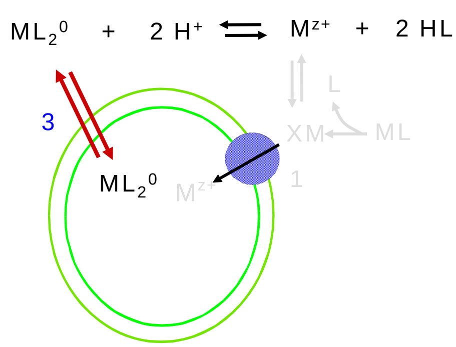
Metal uptake mechanisms



1. Facilitated transport of M^{z+}

3. Passive diffusion of ML_2^0

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BLM – Known exceptions (2) lipophilic complexes – cont.

Other examples of enhanced uptake of lipophilic metal complexes :

- $HgCl_2^0$; (but not $Hg(OH)_2^0$, $HgOHCl^0$)
- CH_3HgCl

Note: It is not sufficient simply to have a neutral complex (charge = 0); the complex must also be lipophilic.

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Key Words

- **$M(H_20)_n^{z+}$; (H^+, Ca^{2+}); BLM**
- **$ML^{\pm}$; where L = assimilable ligand**
- **ML_n^0; c.-à-d., neutral and lipophilic complexes**
- **pH**
- **NOM; natural dissolved organic matter**

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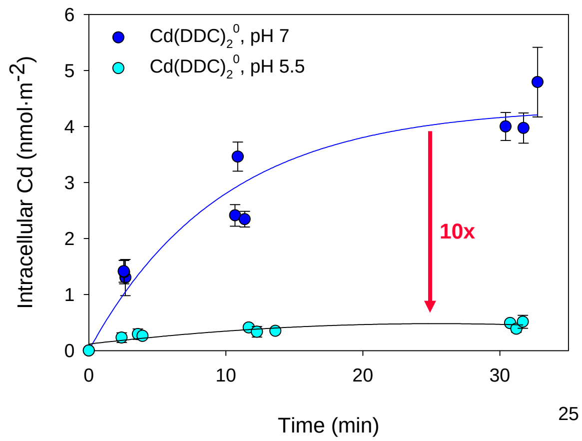
Uptake of $Cd(DDC)_2^0$ by *C. reinhardtii* (effect of pH)

Uptake inhibited as pH decreases

Unexpected effect



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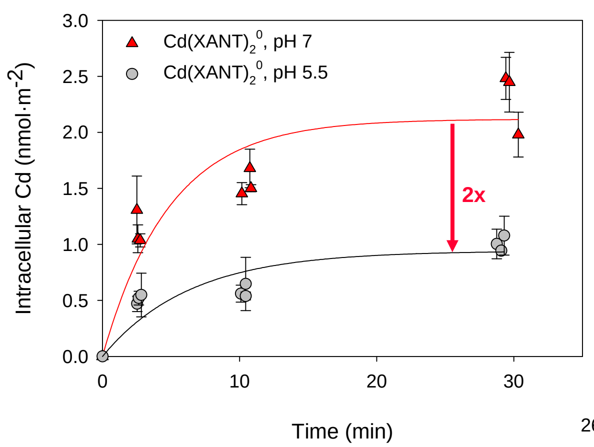
Uptake of $Cd(XANT)_2^0$ by *C. reinhardtii* (effect of pH)

Uptake inhibited at low pH for $Cd(XANT)_2^0$ too

General phenomenon



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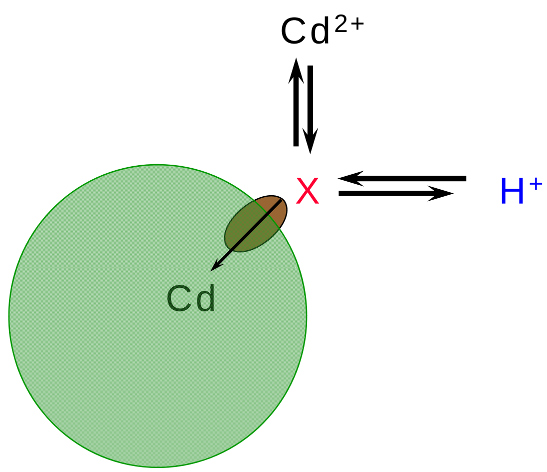
Effect of pH

The protective effect of the H^+-ion is well recognized in the case of the uptake of Cd^{2+}…

Normally explained in terms of a competition between M^{z+} & H^+



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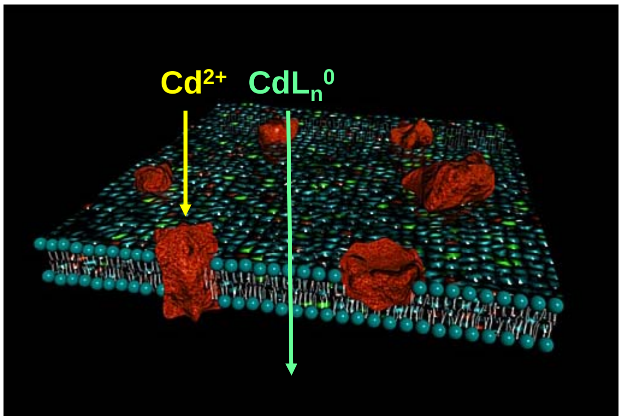
Effect of pH

The protective effect of the H^+-ion is well recognized in the case of the uptake of Cd^{2+}…

but without precedent in the case of neutral complexes



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Key Words

- $M(H_20)_n^{z+}$; (H^+, Ca^{2+}); BLM
- $ML^{\pm}$; where L = assimilable ligand
- ML_n^0; i.e., neutral and lipophilic complexes
- pH
- **NOM; natural dissolved organic matter**

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Question: But what about metal behaviour in the presence of humic and fulvic acids?

- **all natural waters contain humic substances (*HS*) …**
- **<u>but</u> the data presented to this point in support of the BLM has been derived from experiments run in <u>absence</u> of *HS***

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PROPERTIES OF « *HS* »

PROPERTY	BIOGEOCHEMICAL ROLE
• acidic functional groups	● contribution to the acidity of natural waters; metal complexation
• electron donating groups present	● (photo)reduction of metals
• chromophores, π electrons present	● photosensitized oxidation
• hydrophobic domains present	● adsorption/complexation of organic molecules
• co-existence of hydrophilic and hydrophobic domains	● accumulation at interfaces: <air - water> and <water - particle>

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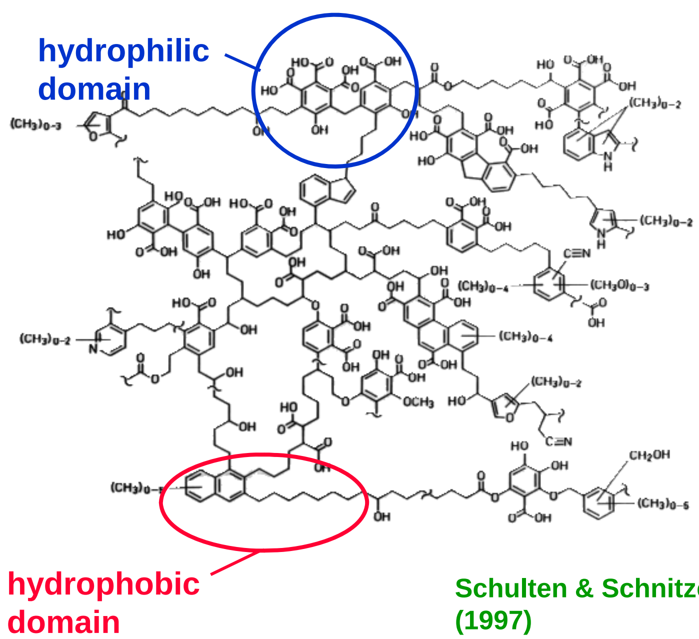
Representative Structure of Natural Humic Substances



Schulten & Schnitzer (1997)

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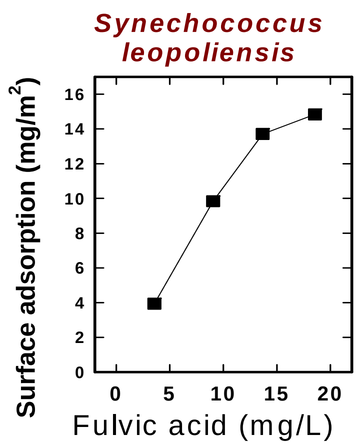
ADSORPTION OF *HS* AT ALGAL SURFACES: concentration dependence

- **alga = *Synechococcus leopoliensis***
- **Suwannee River fulvic acid**
- **pH 5 constant, [FA] variable**



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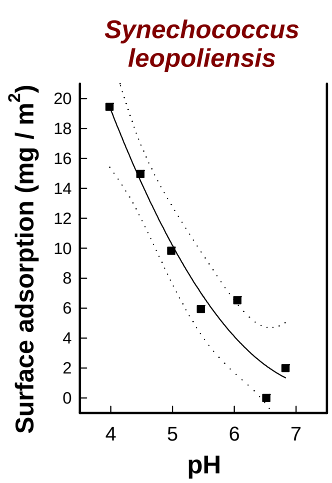
ADSORPTION OF DOM AT ALGAL SURFACES: pH dependence

- **alga = *Synechococcus leopoliensis***
- **Suwannee River fulvic acid**
- **[FA] = 10 mg /L, pH variable**



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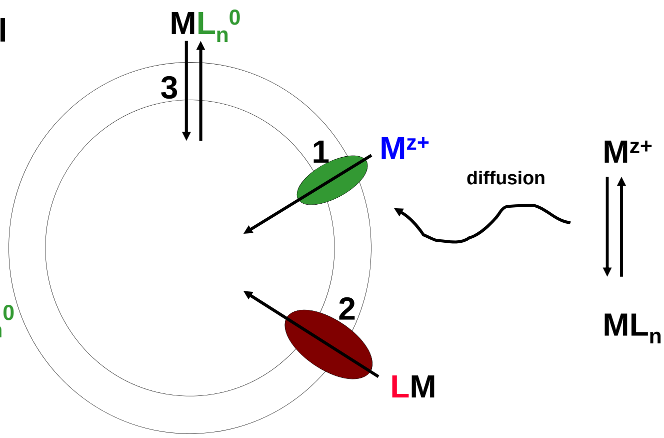
Metal Organism Interactions

Diffusive control

1. **Facilitated transport M^{z+}**
2. **Facilitated transport L-M**
3. **Passive transport ML_n^0**



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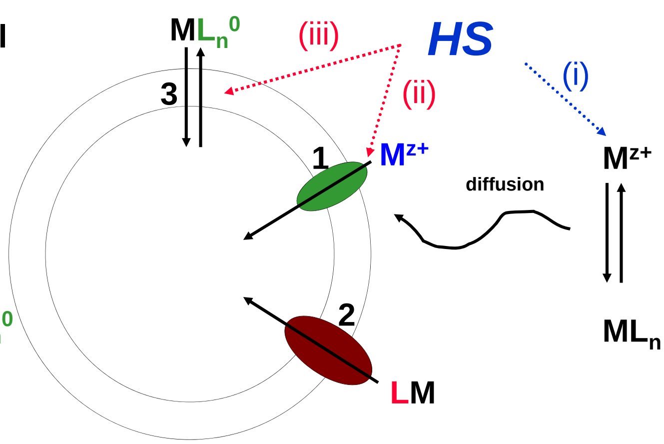

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QUESTION TO EXPLORE

Can we simply assume that the only role of *HS* is to complex the metal in solution

(mechanism (i), as the BLM implicitly assumes),

or do the direct interactions of *HS* at the organism surface affect metal bioavailability?

(mechanisms (ii) and (iii))

Will consider two cases: lipophilic ML_n^0 and hydrophilic $ML_n^{\pm}$.

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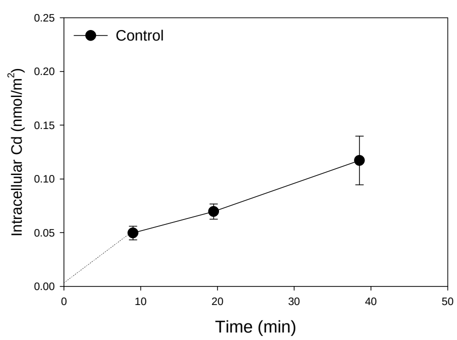
Effect of *HS* on uptake of lipophilic ML_n^0

- **metal = Cd**
- **ligand = diethyldithio-carbamate (DDC)**
- **alga = *Pseudokirchneriella subcapitata***
- **response = uptake (8-40 min)**
- **Suwannee River humic acid**
- **at pH 5**



Boullemant et al. 2004

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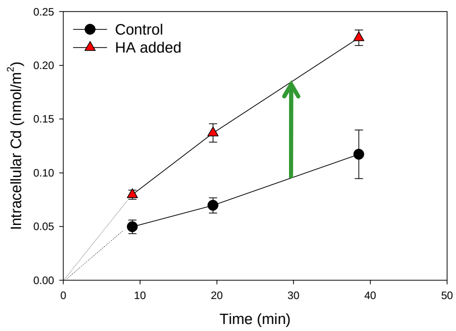
Effect of *HS* on uptake of lipophilic ML_n^0

- metal = Cd
- ligand = diethyldithio-carbamate (DDC)
- alga = *Pseudokirchneriella subcapitata*
- response = uptake (8-40 min)
- Suwannee River humic acid
- at pH 5, *HS* increased uptake rate



Boullemant et al. 2004

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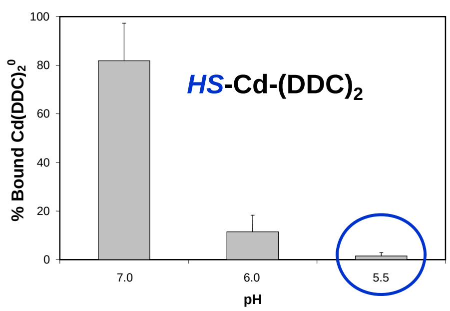
Unexpected complication

- *HS* bind to lipophilic metal complexes in solution
- binding at pH 7 >> at pH 5.5
- formation of ternary complex, *HS*-Cd-$(DDC)_2$?



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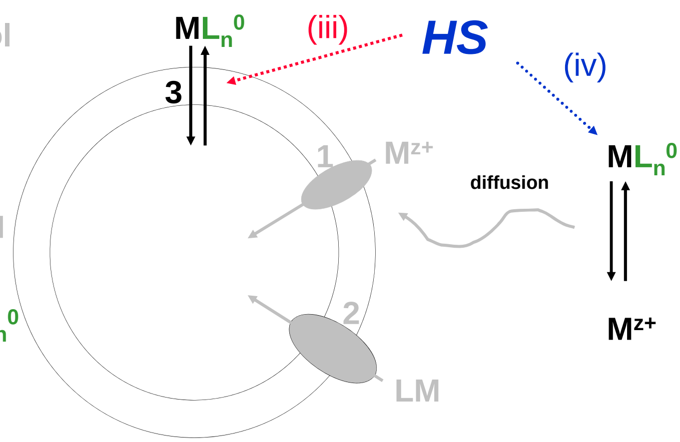
Summary – lipophilic metal complexes

Diffusive control

1. Facilitated transport M^{z+}
2. Facilitated transport L-M
3. **Passive transport ML_n^0**



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QUESTION TO EXPLORE

Can we simply assume that the only role of *HS* is to complex the metal in solution

(mechanism (i), as the BLM implicitly assumes),

or do the direct interactions of *HS* at the organism surface affect metal bioavailability?

Will consider <u>two cases</u>: lipophilic ML_n^0 and hydrophilic $ML_n^{\pm}$.

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Examples

Start with simple (?) unicellular organisms (algae)

- metals : Cu, Cd, Ag, Pb
- defined metal speciation
- biological response = metal uptake and/or growth inhibition

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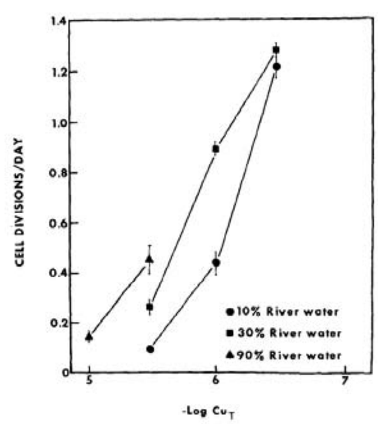
Sunda & Lewis (1978)

- metal = Cu
- alga = *Monochrysis lutheri*
- response = growth
- Cu^{2+} ise
- varied [*HS*] by diluting organic-rich river water



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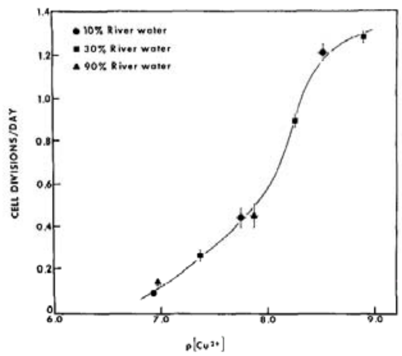

i.e., BLM-type response

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Vigneault & Campbell (2005)

- metal = Cd
- alga = *Chlamydomonas reinhardtii* or *Pseudokirchneriella subcapitata*
- response = short-term uptake
- Cd^{2+} eqm ion exch
- Suwannee River *HS*, pH 5-7

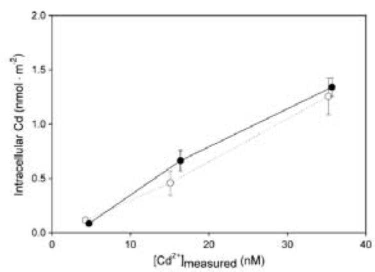

FIG. 1. Uptake of Cd by *Chlamydomonas reinhardtii* at pH 5 in the presence of NTA (open circles) or Suwannee River fulvic acid (filled circles; mean ± SD, $n = 3$). [NTA], 100 nM; [SRFA], 5.4 mg C·L^{-1}. Exposure duration, 15 min.

i.e., BLM-type response

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Vigneault & Campbell (2005)

- metal = Cd
- alga = *Chlamydomonas reinhardtii* or *Pseudokirchneriella subcapitata*
- response = short-term uptake
- Cd^{2+} eqm ion exch
- Suwannee River *HS*, pH 5-7

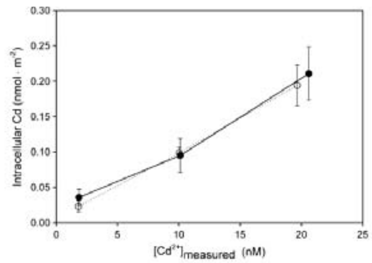


FIG. 2. Uptake of Cd by *Pseudokirchneriella subcapitata* at pH 5 in the presence of NTA (open circles) or Suwannee River humic acid (filled circles; mean ± SD, $n = 3$). [NTA], 100 nM; [SRHA], 5.4 mg C · L^{-1}. Exposure duration, 20 min.

i.e., BLM-type response

Vigneault & Campbell (2005)

- metal = Cd
- alga = *Chlamydomonas reinhardtii* or *Pseudokirchneriella subcapitata*
- response = short-term uptake
- Cd^{2+} eqm ion exch
- Suwannee River *HS*, pH 5-7

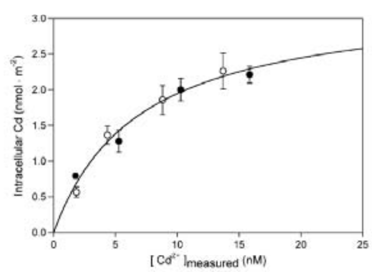


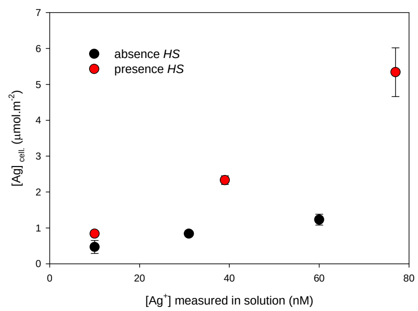
FIG. 3. Uptake of Cd by *Pseudokirchneriella subcapitata* at pH 7 in the presence of NTA (open circles) or Suwannee River fulvic acid (filled circles; mean ± SD, $n = 3$). [NTA], 100 nM; [SRFA], 5.4 mg C · L^{-1}. Exposure duration, 20 min. The line represents predicted Cd uptake (J_{int} × exposure duration) based on the Michaelis-Menten flux equation for $J_{max} = 3.29$ nmol · m^{-2} · s^{-1} and $K_m = 6.9$ nM.

i.e., BLM-type response

Porcher (MSc 2004)

- metal = Ag
- alga = ***Chlamydomonas reinhardtii*** **or** ***Pseudokirchneriella subcapitata***
- response = short-term uptake
- Ag^+ eqm ion exch
- Suwannee River *HS*, pH 7

C. reinhardtii



i.e., <u>non</u> BLM-type response

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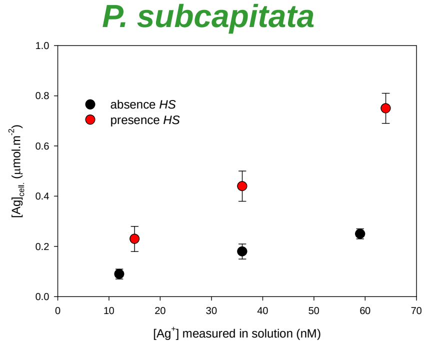
Porcher (MSc 2004)

- metal = Ag
- alga = ***Chlamydomonas reinhardtii*** **or** ***Pseudokirchneriella subcapitata***
- response = short-term uptake
- Ag^+ eqm ion exch
- Suwannee River *HS*, pH 7

P. subcapitata



i.e., <u>non</u> BLM-type response

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Slaveykova et al. (2003)

- metal = Pb
- alga = *Chlorella kesslerii*
- response = short-term uptake
- Pb^{2+} ise
- Suwannee River *HS*, pH 6

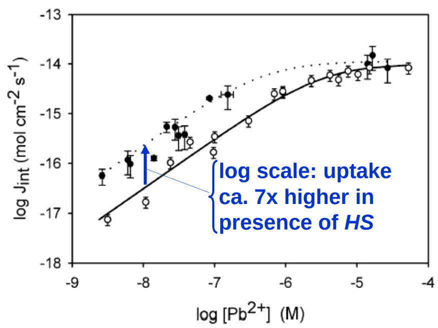

FIGURE 1. Dependence of the Pb internalization fluxes on the free lead ion concentration, $[Pb^{2+}]$, in the presence of 20 mg L^{-1} SRFA

i.e., <u>non</u> BLM-type response

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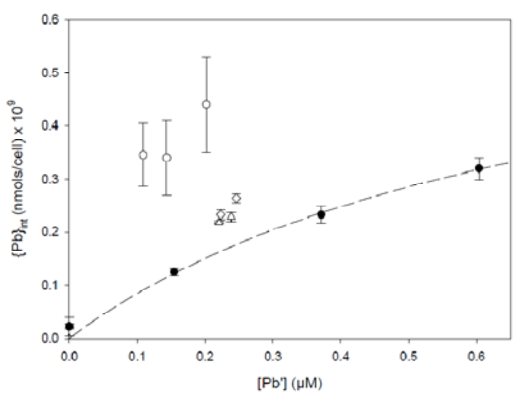
Sánchez-Marín (PhD 2010)

- metal = Pb
- alga = *Isochrysis galbana* (naked)
- response = short-term uptake
- ASW; asv labile Pb
- HA, FA, SR *DOM*, pH 8



Filled circles = no DOM; Open circles = Aldrich HA; triangles = fulvic acid; diamonds = Suwannee River DOM

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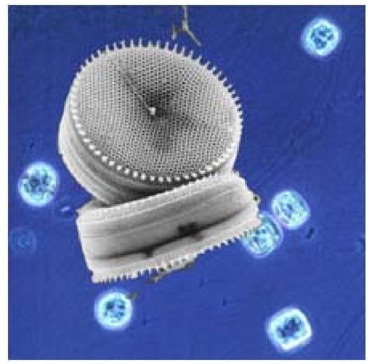
Sánchez-Marín et al. (2010)

- **metal = Pb**
- **alga = *Thalassiosira weissflogii***
- **response = short-term uptake**
- **ASW; asv labile Pb**
- **Aldrich *HA*, pH 8 (open circles)**



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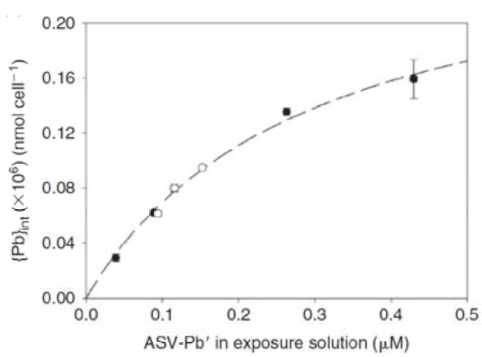
Sánchez-Marín et al. (2010)

- **metal = Pb**
- **alga = *Thalassiosira weissflogii***
- **response = short-term uptake**
- **ASW; asv labile Pb**
- **Aldrich *HA*, pH 8 (open circles)**



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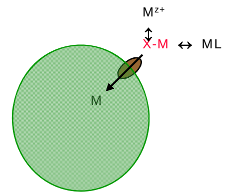
Possible mechanisms for greater-than-predicted metal bioavailability in the presence of *HS*



- **rate-limiting step = diffusion in boundary layer, not metal internalization**
- **adsorbed *HS* increase cell surface charge → increased [M^{z+}] in boundary layer → increased [-X-M] (effect M^{+2} >> M^{+1})**
- ***HS* interact with cell membrane (intercalation?) → affect membrane permeability, fluidity, … and transporters?**

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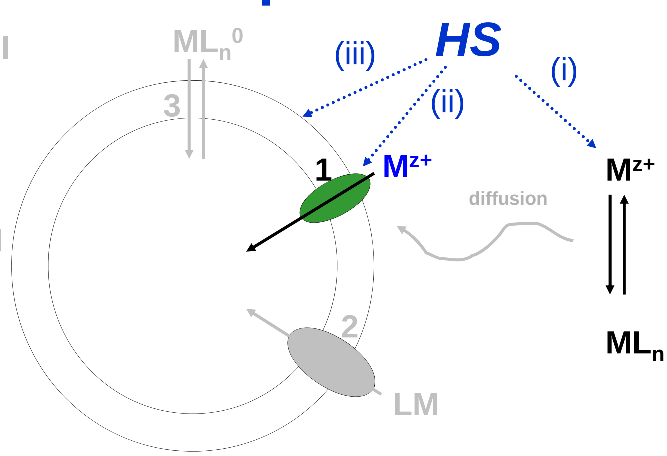
Summary – hydrophilic metal complexes

Diffusive control

1. Facilitated transport M^{z+}

2. Facilitated transport L-M

3. Passive transport ML_n^0



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What about organisms other than algae?

- **Fewer experiments than for algae (<20), picture even less clear**
  - **follow BLM predictions: Cd (2) Cu (6) Ni (1)**
  - **greater-than-expected bioavailability: Cd (1) Cu (4) Pb (2)**
  - **less-than-expected bioavailability: Al (2), Cu (1)**

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Van Genniken et al. 2001

- **metal = Cd**
- **organism = juvenile *Cyprinus carpio* (common carp)**
- **response = 3-h uptake**
- **Cd^{2+} (CLE/SE)**
- **pH 8**
- ***HS* = Aldrich HA**

i.e., BLM-type response

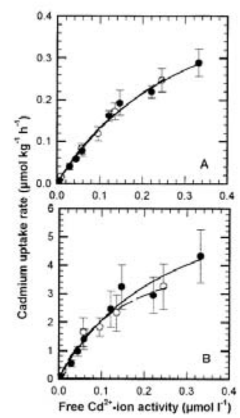

Fig. 3. Rate of Cd uptake in (a) total carp and (b) carp gills as a function of the free Cd^{2+} ion activity in the water of exposure. The exposure media contained varying amounts of total Cd in the absence (●) or presence (○) of 6.5 mg C l^{-1} commercial DOC. Data points represent means with standard deviations of four to five replicates (pH 8.0, $t = 25$°C). The curves through the data points were obtained by fitting a Michaelis–Menten model to the Cd uptake data.

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Erickson et al. 1996

- metal = Cu
- organism = larval fathead minnow
- response = 96-h LC_{50}
- Cu^{2+} (ISE)
- *HS* = Aldrich HA
- toxicity in presence of *HS*, greater-than expected

i.e., non-BLM type response

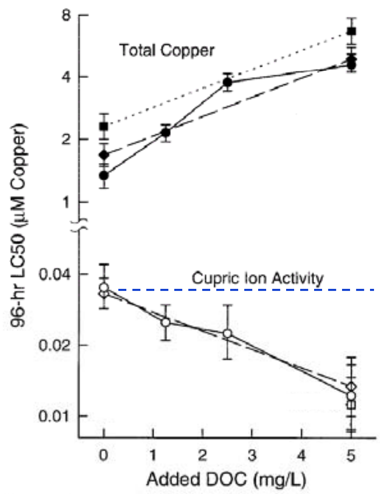
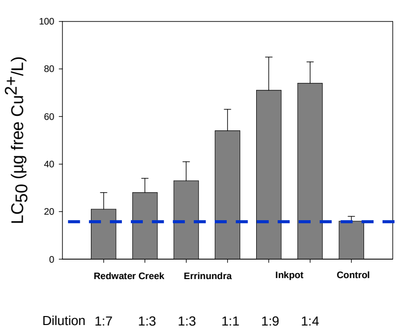


Fig. 4. Effect of added humic acid on copper toxicity (96-h LC50 ± 95% CL) expressed on the basis of both total copper and cupric ion.

Daly et al. 1990

- metal = Cu
- organism = freshwater shrimp
- response = 96-h LC_{50}
- Cu^{2+} (ISE)
- *HS* = diluted natural waters
- toxicity in presence of *HS*, less-than-expected

Copper toxicity to Australian freshwater shrimp (*Paratya australiensis*)



i.e., non-BLM type response

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Main ideas

- **In the <u>absence</u> of natural organic matter (fulvic, humic acids), provided that**
  - you consider possible competition with H^+, Ca^{2+}, Mg^{2+}
  - you take into account the role of assimilable ligands
  - you are dealing with short-term exposures

  i.e., "BLM usually works"

- **In the <u>presence</u> of natural organic matter… the *HS* may**
  1. complex metal cations, M^{z+} } solution phenomena
  2. bind lipophilic metal complexes, ML_n^0 } solution phenomena
  3. increase membrane permeability } cell surface phenomena
  4. affect membrane transport(ers) } cell surface phenomena

Only #1 is considered by present BLM

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Summary Table: Influence of DOM on trace element bioavailability

You <u>aren't</u> supposed to be able to read this table…!

but your help in completing it would be most appreciated.

Table: Influence of natural dissolved organic matter (DOM) on trace metal bioavailability.

A – BLM behaviour, metal as available as expected

Species	Metal	Response	Reference
1. *Monochrysis lutheri* (euryhaline alga)	Cu	growth inhibition	Sunda & Lewis (1978)
2. *Selenastrum capricornutum* (freshwater alga)	Cd	metal uptake	Vigneault & Campbell (2005)
3. *Chlorella kesslerii* (freshwater alga)	Cd, Cu	metal uptake	Lamelas & Slaveykova (2007)
4. *Daphnia magna* (freshwater cladoceran)	Cu	mortality	Meador (1991)
5. *Xenopus laevis* (embryo) (freshwater amphibian)	Cu	larval development	Buchwalter *et al.* (1996)
6. *Cyprinis carpio* (common carp)	Cu	metal uptake (gill and whole fish)	Van Ginneken *et al.* (2001)
7. Marine bacterium (Gram negative)	Cu	glucose uptake	Sunda & Gillespie (1979)
8. *Mytilus edulis* (marine bivalve)	Cu	metal uptake (gill)	Lorenzo *et al.* (2005)
9. *Paracentrotus lividus* (sea urchin)	Cu	toxicity (embryo-larval growth)	Lorenzo *et al.* (2006)

B – non-BLM behaviour, metal <u>more</u> available than expected

Species	Metal	Response	Reference
1. *Selenastrum capricorntum* (freshwater alga)	Cd	$^{14}CO_2$ uptake	Laegreid *et al.* (1983)
2. *Chlorella kesslerii* (freshwater alga)	Pb	metal uptake	Slaveykova *et al.* (2003); Lamelas & Slaveykova (2007)
3. *Simocephalus serrulatus* (freshwater cladoceran)	Cu	mortality; metal uptake	Giesy *et al.* (1983)
4. *Dreissena polymorpha* (zebra mussel)	Cd	metal uptake (whole body)	Voets *et al.* (2004)
5. *Daphnia magna* (freshwater cladoceran)	Cu	immobilization	Borgmann & Charlton (1984)
6. *Pimephales promelas* (larval fathead minnow)	Cu	mortality	Erickson *et al.* (1996)
7. *Mytilus edulis* (marine bivalve)	Cu	metal uptake (whole body)	Lorenzo *et al.* (2005)
8. *Mytilus edulis* (excised gill) (marine bivalve)	Pb	metal uptake	Sanchez-Marin *et al.* (2007)
9. *Paracentrotus lividus* (sea urchin)	Pb	toxicity (embryo-larval growth)	Sanchez-Marin *et al.* (2007)

C – non-BLM behaviour, metal <u>less</u> available than expected

1. *Chlorella pyrenoidosa* (freshwater alga)	Al	growth inhibition	Parent *et al.* (1996)
2. *Paratya australiensis* (freshwater shrimp)	Cu	mortality	Daly *et al.* (1990)
3. *Salmo salar* (juvenile Atlantic salmon)	Al	mortality	Roy & Campbell (1997)

(copies available)

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