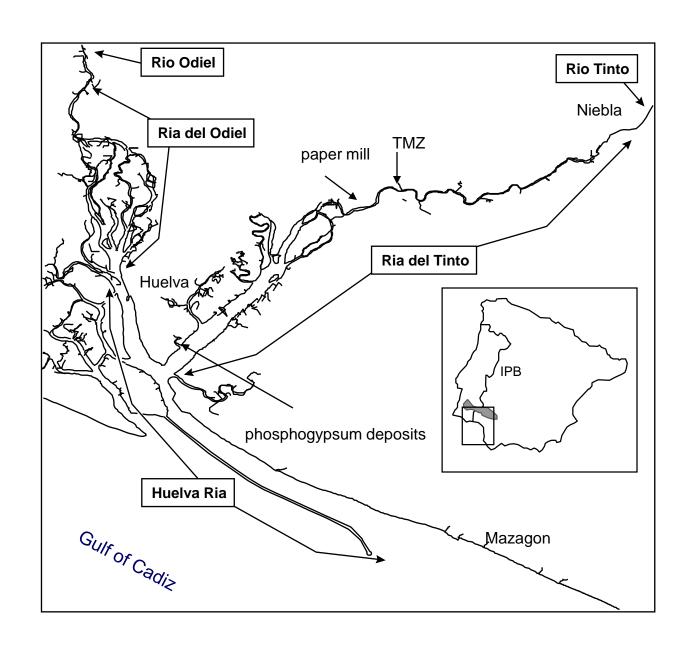
Trace metals, their speciation and toxicity in marine waters

Trace metals, speciation and toxicity

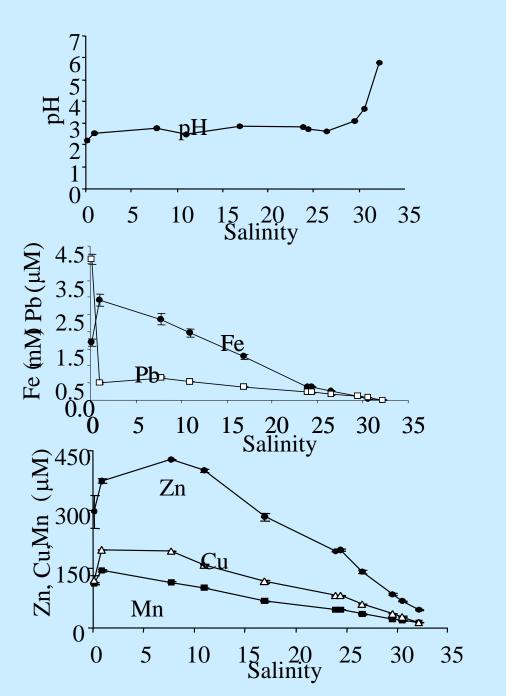
- Trace metals in the marine environment
- Metal pollution in coastal waters
- What is speciation?
- Metal Ligand interactions
 - Inorganic
 - Organic
 - Metal organic complexation results

Trace Metals in marine waters

- Metal lons in natural waters include essential elements
 - Cu, Zn, Fe, Mn, Co
 - Potentially toxic at higher concentrations
- Non-essential elements
 - Hg, Pb, Cd
 - Mostly toxic

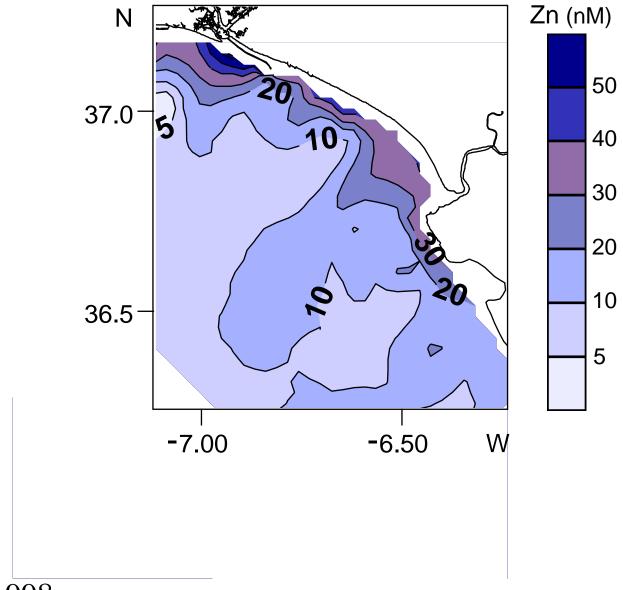






Ria del Tinto

Applied Geochemistry, Braungardt, Achterberg et al. 2003

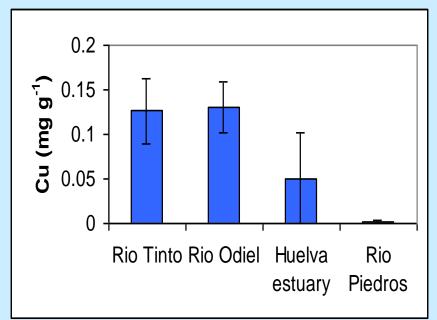


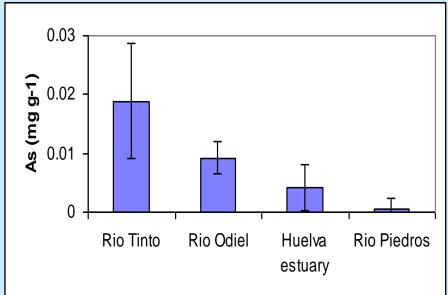
October 1998

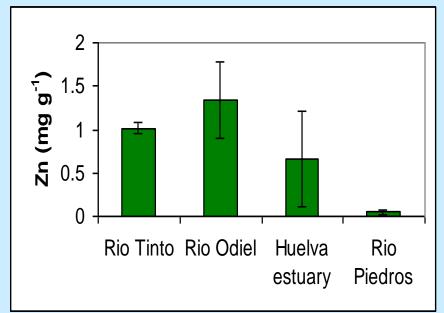
Estuarine discharge into the Gulf of Cadiz

	Gross Flux Rio Tinto +Rio Odiel	Global Flux	Fraction (%)
Fe (t a-1)	1800	1.40E+06	0.13
Zn (t a-1)	470	5.80E+03	8.1
Cu (t a ⁻¹)	176	1.00E+04	1.8

Applied Geochemistry, Braungardt, Achterberg et al., 2003

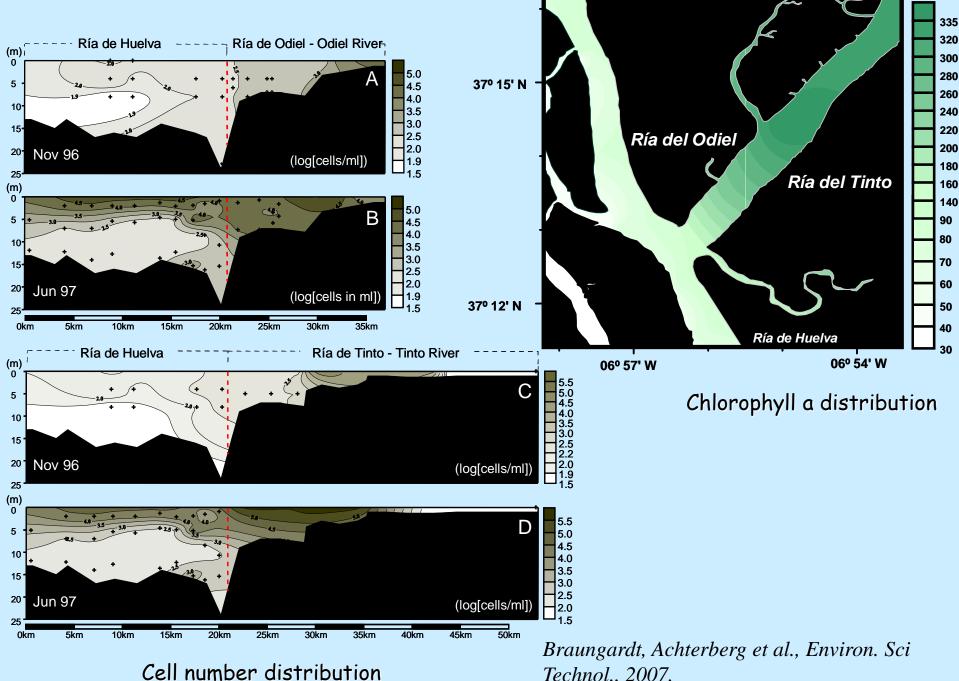






Average Cu, Zn and As concentrations in seaweed *Blindingia marginata*

Braungardt, Achterberg et al., Environ. Sci Technol., 2007.



Technol., 2007.

(μg/l)

Shannon-Wiener diversity index (H).

$$H = -\sum_{i=1}^{N} p_i \ln p_i$$

The proportion of species i relative to the total number of species (p_i) is calculated, and then multiplied by the natural logarithm of this proportion (lnp_i) . The resulting product is summed across species, and multiplied by -1

Highest H values in the Odiel (H=0.78, theoretical maximum H for Odiel 2.64) and Tinto (H=1.1, theoretical maximum H for Tinto 1.79) observed in regions at low to mid salinities (S=3-18), low pH (2.5-3.5) and high metal concentrations.

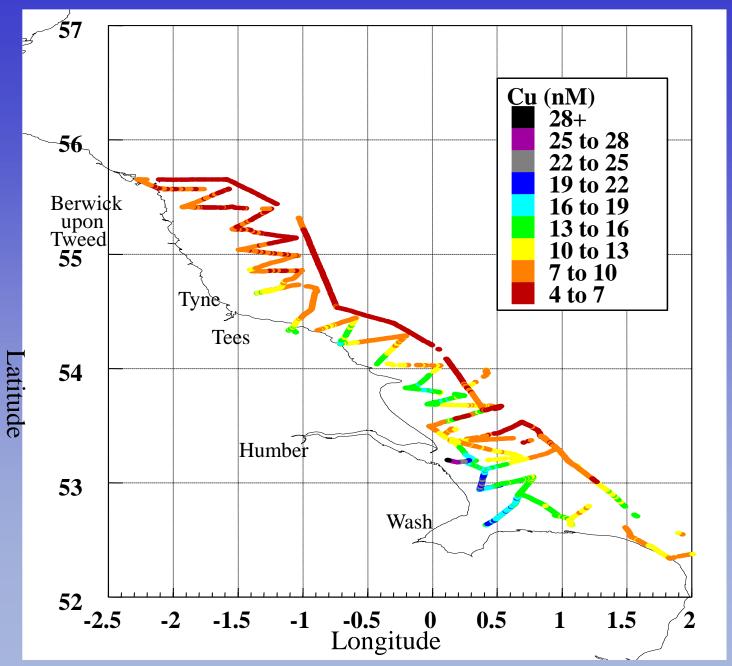
Unicellular *Chlorophyceae* dominant \rightarrow are able to grow in polluted environments.

The lower region of the Tinto estuary with enhanced nutrient, chlorophyll and phytoplankton cell concentrations, showed a very low diversity (H< 0.0015)

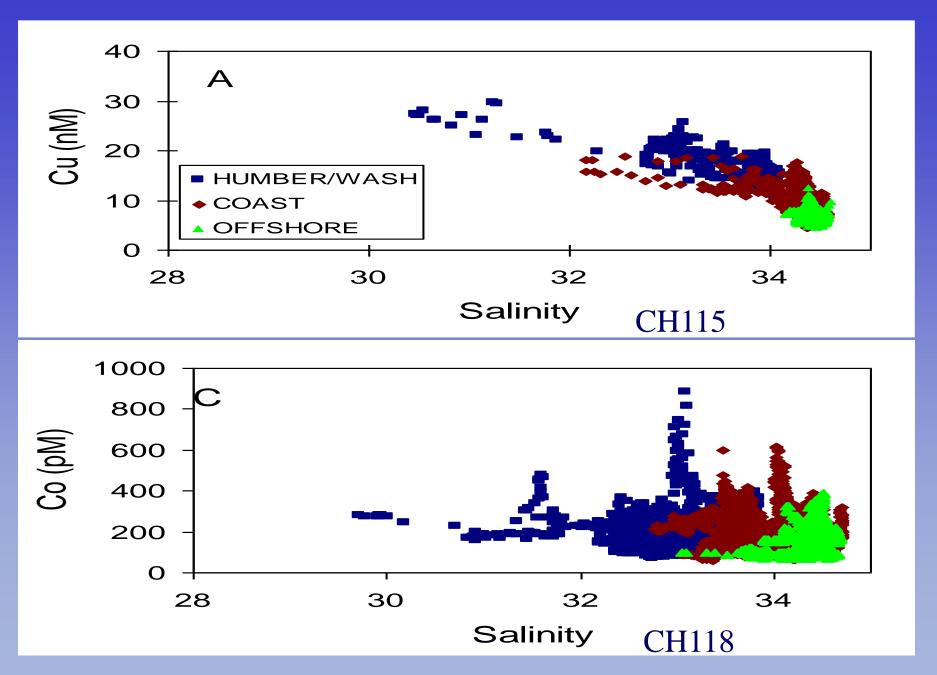
Huelva estuary the diversity of plankton species and their abundance increased with increasing salinity and diminishing metal concentrations.

Huelva estuary: highest Shannon-Wiener diversity index (H = 0.22 at salinity 35.0; theoretical maximum H for Huelva 3.37) \rightarrow strong influence of metal pollution on phytoplankton community structure, with unicellular *Chlorophyceae* dominating.

Trace metals in Western North Sea



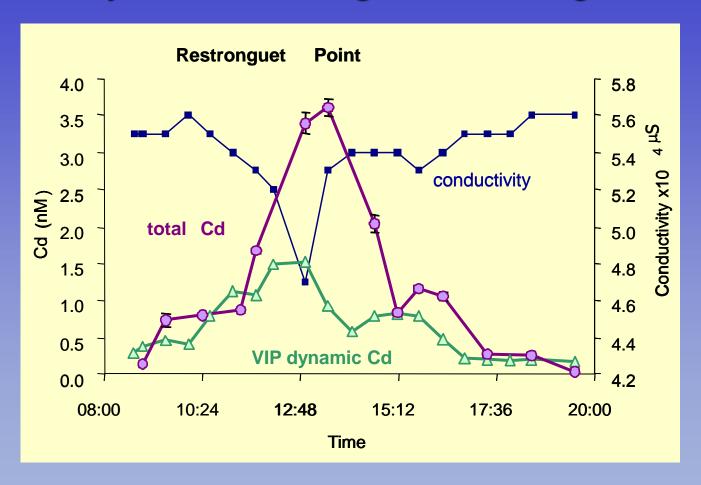
Continental Shelf Research, Achterberg et al. 2003



Continental Shelf Research, Achterberg et al. 2003



Tidal Cycle Monitoring at Restronguet Point

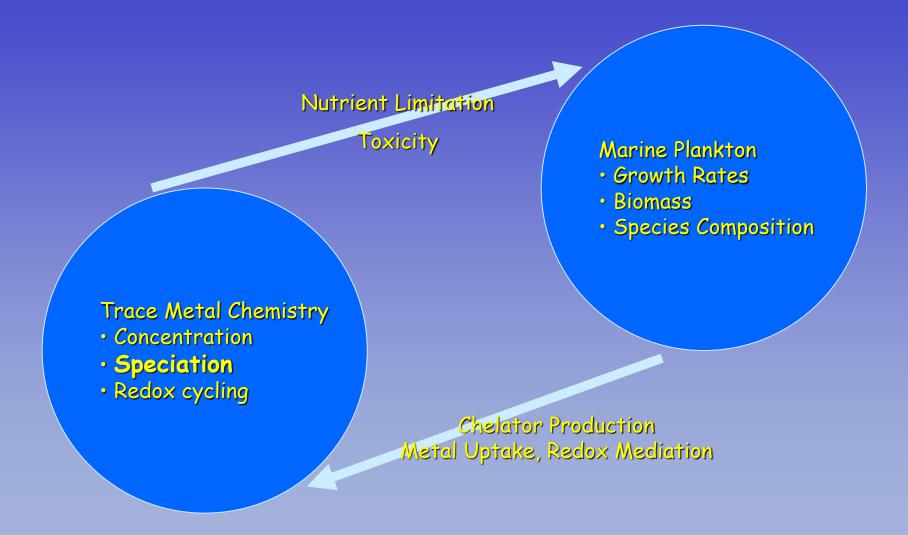


- highest Cd concentrations at low water
- mobile Cd proportion lowest at high water

Metal-organism interactions

https://www.youtube.com/watch?v=1prVpdyq3fg

Influence of Trace Metals - Phytoplankton



Trace Metal Speciation

- Trace metals exist in seawater in a variety of chemical species
- Speciation strongly influences their availability to phytoplankton and their biogeochemical cycling
 - Free aquo ions (Cu²⁺, Mn²⁺, Fe²⁺/Fe³⁺, Zn²⁺)
 - Complexes with inorganic ligands
 - Complexes with organic ligands
 - Adsorbed on particulate (or colloidal) phases
 - In particulate (or colloidal) phases

Trace Metal Speciation

- Inorganic ligands and metal ions
- Free metal ion form small fraction in seawater
- Metal complexed with inorganic ligands
 - Hydroxides (OH)
 - $-CO_3^{2-}$
 - CI-
 - SO₄²-
 - HS⁻ in anoxic conditions
- These were thought as the main complexing ligands based on thermodynamic equilibria

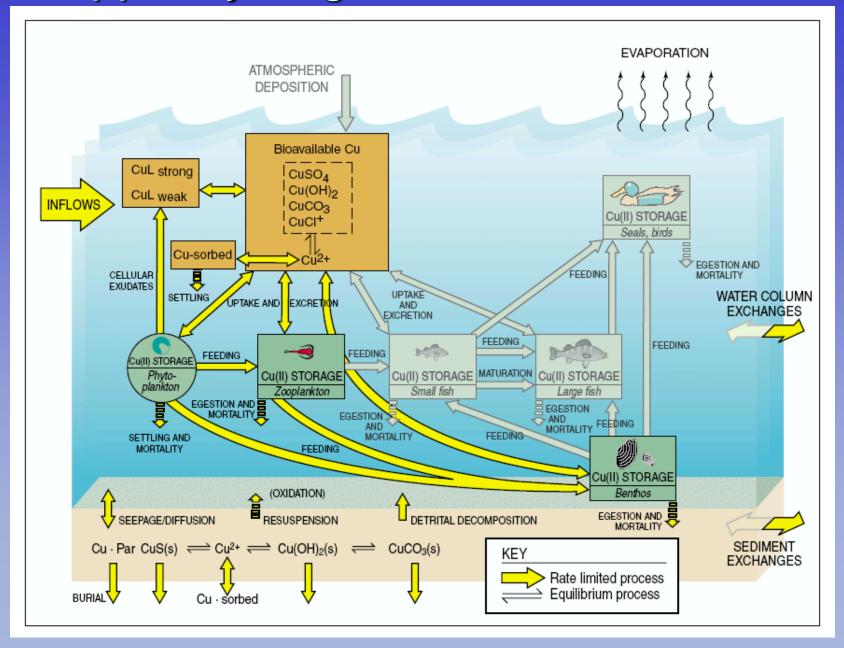
Trace Metal Speciation

- Various schemes were drawn up relating metals to free and inorganic complexes
- Mainly free metals based on total concentration measurements and thermodynamics

Cation	Free	ОН	CI	SO ₄	CO ₃
Mn ²⁺	72	0	21	5	2
Fe ²⁺	69	2	13	5	10
Zn ²⁺	66	5	16	5	9
Cu ²⁺	5	8	2	1	85
Cd ²⁺	4	0	96	0	0

- From late 1980's onwards
- New analytical techniques ASV/CSV
- Large proportion of metal in seawater is complexed with (unknown) organic ligands
- □ ~ 99%
- □ Fe(III), Fe(II)? Zn, Cu
- And other metals Co, Cd are also organically complexed

Copper cycling in the water column



Zn

- Bruland K. W. (1989) Complexation of zinc by natural organic ligands in the central North Pacific.

 Limnology and Oceanography **34**(2), 269-285.
- Donat J. R. and Bruland K. W. (1990) A Comparison of 2 Voltammetric Techniques for Determining Zinc Speciation in Northeast Pacific-Ocean Waters.
 Marine Chemistry 28(4), 301-323.
- >98% was bound in strong complexes by zinc specific ligands at low concentrations (1.2 nM)
- □ Total Zn = 0.3 nM Zn' = 0.008 nM
- \square Zn²⁺ 10^{-12.7}M ~ 0.2 pM

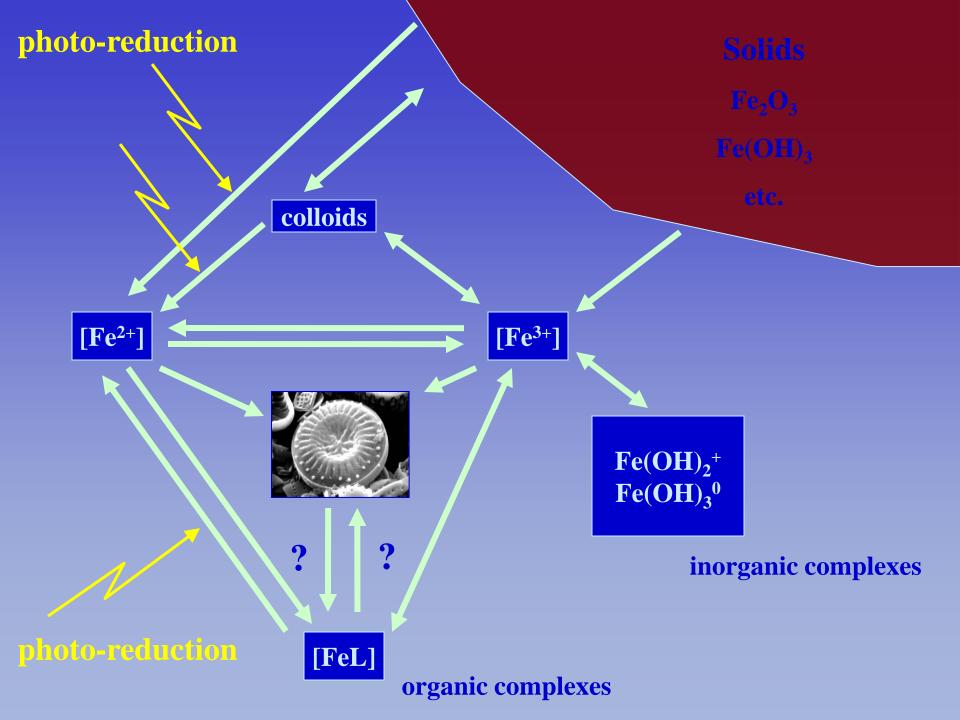
Cu

- Coale K. H., and Bruland, K. W. (1988) Copper complexation in the Northeast Pacific. *Limnology and Oceanography* **33**(5), 1084-1101.
- Bruland K. W., Rue E. L., Donat J. R., Skrabal S. A., and Moffett J. W. (2000) Intercomparison of voltammetric techniques to determine the chemical speciation of dissolved copper in a coastal seawater sample. *Analytica Chimica Acta* 405(1-2), 99-113.
- Donat J. R., Lao K. A., and Bruland K. W. (1994) Speciation of Dissolved Copper and Nickel in South San- Francisco Bay a Multimethod Approach. *Analytica Chimica Acta* 284(3), 547-571.
- >98% was bound in strong complexes by copper specific ligands at low concentrations (1.8 nM)
- \Box Cu²⁺ = 1.4 x 10⁻¹⁴ M ~ 0.01 pM

- Fe
- Extremely important micronutrient
- Fe(II) and Fe(III)
- Fe(III) ~ 99.97% Organically complexed
 - Rue E. L. and Bruland K. W. (1995) Complexation of Iron(III) by Natural Organic-Ligands in the Central North Pacific as Determined by a New Competitive Ligand Equilibration Adsorptive Cathodic Stripping Voltammetric Method. *Marine Chemistry* 50(1-4), 117-138.
 - Wu J. and Luther III G. W. (1995) Complexation of Fe(III) by natural organic ligands in the Northwest Atlantic Ocean by a competitive ligand equilibration method and a kinetic approach. *Marine Chemistry* 50(1-4), 159-177.
 - Van Den Berg C. M. G. (1995) Evidence for organic complexation of iron in seawater. *Marine Chemistry* 50(1-4), 139-157.
- □ Example 0.24 nM Total Only 0.05 nM in HNLC regions
- □ 0.207 nM as L₁ 86.2%
- $0.033 \text{ nM as L}_2 13.8\%$
- □ Fe' 0.00007 nM (0.07 pM) 0.03%

Trace Metal Speciation Open Ocean Trace Metal Concentrations

Cation	Dissolved	% Org Comp	Free (Mn+)
Manganese	0.2 to 1 nM	N.S.	10 ⁻¹⁰ to 10 ^{-9.3}
Iron	0.02 – 0.4 nM	> 99%	~ 10 ⁻¹³ to 10 ⁻²³
Cobalt	0.007 – 0.02 nM	~ 90%	~ 10 ⁻¹⁵
Copper	0.5 – 1 nM	> 99%	~ 10 ⁻¹³
Zinc	0.1 – 0.3 nM	> 98%	~ 10 ⁻¹²
Cadmium	0.002 – 0.2 nM	~ 80%	~ 10 ^{-13.5}





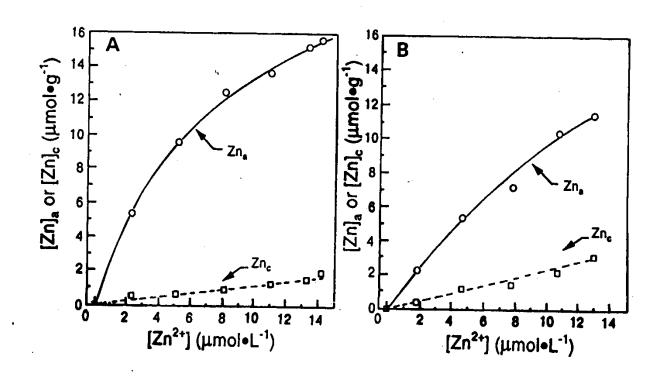


Figure 4. Variation in adsorbed Zn (\bigcirc) and cellular Zn (\square) in two green algae as a function of the free-Zn concentration, [Zn²⁺], for (A) Chlamydomonas variabilis and (B) Scenedesmus subspicatus. Incubation for 10 min in the presence of a fixed total Zn concentration ([Zn]_T = 15.3 μ M) and different EDTA concentrations. [Modified from B. 's, S. S. et al., J Phycol., 18, 521 (1982)]

Adsorbed $Zn \rightarrow Langmuir$ Uptake \rightarrow linear relationship with Zn^{2+} concentrations

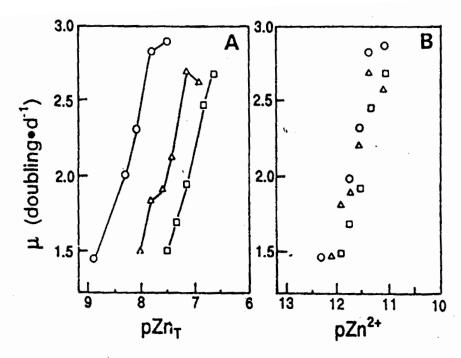


Figure 2. Variation in the growth rate of the marine diatom, Thalassiosira weissflogii, as a function of (A) total Zn, $[Zn]_T$, or (B) free-Zn concentration, $[Zn^{2+}]$. Three different EDTA concentrations were used: 10^{-5} M (O); $10^{-4.3}$ M (\triangle), and 10^{-4} M (\square). [Modified from Anderson, M. A, et al., Nature, 276, 70 (1978)]

Growth limitation

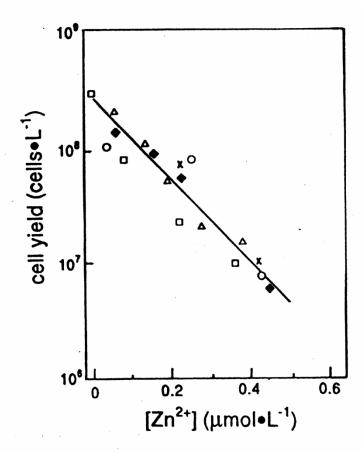
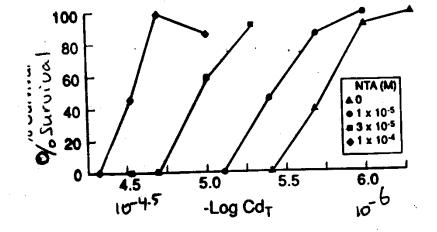
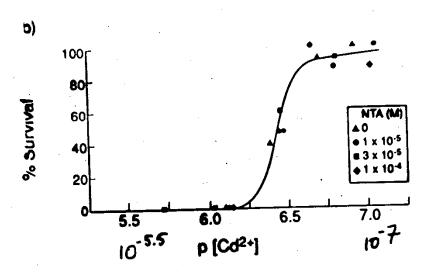


Figure 3. Yield of the blue-green alga, *Microcystis aeruginosa*, after 5 days' growth as function of the free-Zn concentration $[Zn^{2+}]$. Various different ligands were used adjust $[Zn^{2+}]$: EDTA (\Box) , NTA (\triangle) , ODS (\aleph) , CMOS (\bigcirc) , control (\clubsuit) . [Modifie from Allen, H. E. et al., Environ. Sci. Technol., 14, 441 (1980).]

Toxic effects





ig. 15-11 Effects of strong complexation on metal ion oxicity. (a) Increasing concentration of NTA, a strong nulti-dentate complexing agent, decreases the toxicity of Id to grass shrimp. All systems have equal concentrations if total Cd. (b) When the results are replotted showing survival as a function of Cd²⁺ concentration, the data for all concentrations of NTA collapse to a single curve. Adapted rom Sunda et al. (1978) with the permission of the American Chemical Society.

FIAM Free ion activity model

 For many metals: free ions rather than total metal concentration are responsible for biological effects

- Francois Morel
- Campbell in Tessier and Turner (1995)

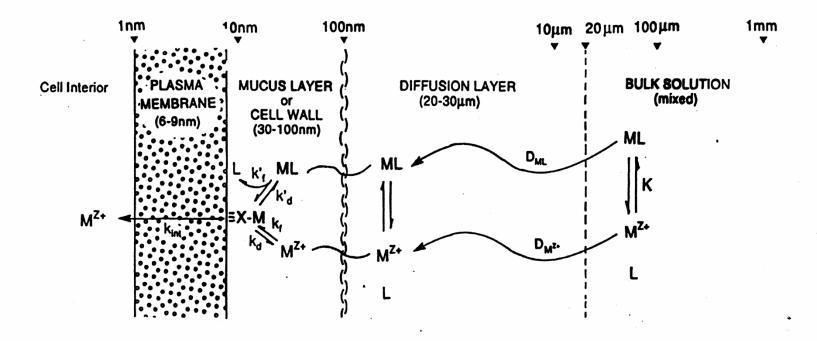


Figure 1. Conceptual model of metal-organism interactions. M^{z+} = free-metal ion; ML = metal complex in solution; M-X-membrane = surface metal complex; k_f , k'_f = rate constants for formation of the surface complex; k_d , k'_d = rate constants for dissociation of the surface complex; k_{int} = rate constant for 'internalization' or transport of the metal across the biological membrane. Charges on ligand not shown for simplicity. [Modified from Tessier, A. et al., in Chemical and Biological Regulation of Aquatic Systems (Boca Raton: Lewis, 1994) Chap. 6]

From Tessier and Turner 1995

- Metal-Organism interactions
- Step 1: Transport of the metal from solution to the biological surface (advection, diffusion)
- Step 2: Diffusion of metal through protective outer layer
- Organisms have a protective polysaccharide or glycoprotein layer (cell wall for m.o. and higher plants (lignin, cellulose); mucus for animals)
- -negative charge (oxygen containing functional groups; non-chelating)
- Step 3 Sorption/surface complexation of the metal at passive binding sites within the protective layer, or at sites on the outer surface of the cell membrane
- Cell membranes are composed of lipids (fats and waxes) and proteins
- Lipids: phospholipids, cholesterol, glycolipids
- Most common phospholipid is phosphatic acid --- > hydrophobic and hydrophilic end → bilayer formed with non-polar groups in interior region
- Function of proteins:
- maintain overall structural integrity of membrane
- act as enzymes (ATPase at mitochondrial membrane)
- act as carrier for ions and other molecules across membrane

- Transport through cell membrane:
- Simple diffusion (oxygen, water..) through protein-lined channels
- Facilitated diffusion (from higher to lower concentration, but substance is first complexed by carrier molecule (probably protein) (glucose transport)
- Active transport: against concentration gradient → cost energy Na, K
 ATPase → Na, K pump
- Step 4: Uptake of metal (transport across membrane) → consequent biological effects on metabolism, photosynthesis are important
- Interaction of metals with biota:
- 3 levels of concern:
- A. metal speciation in outside environment
- B. metal interaction with biological membrane separating outside-inside
- □ C. metal partitioning in organism and its biological effects

Metals in solution bind to cell membrane

- if not on active site then the sorption/complexation follows the Langmuir isotherm
- If on active site the complexation and uptake follows linear relation ship with free metal activity
- This could be a physiologically active site at cell surface (fish gill) --- > direct response
- or X corresponds to transport site that allows M to traverse cell membrane (subsequent biological effects)
- or X could be transport site normally used by essential micronutrient.
- Binding at cell surface by M would inhibit the supply of the essential element and induce nutrient deficiency (e.g. Mn/Cu and Fe/Cd phytoplankton)

- Key Assumptions underlying FIAM
- 1. plasma membrane is primary site for metal interaction with biota
- 2. metal-membrane interactions are surface complexation reaction, forming M-X-cell (metals being hydrophilic)
- □ 3. metal transport in solution, formation of M-X-cell is rapid (equilibrium established) → faster than biological response
- 4. biological response (uptake/nutrition/toxicity) is dependent on concentration of M-X-cell surface complex
- 5. In the range of metal concentration of toxicological interest, the concentration of free sites (-X) remains virtually constant and {M-X-cell} follows those [Mz+] in solution
- 6. Metals do not induce changes to plasma membrane during exposure time

The use of phytoplankton as markers for trace metal pollution

Phytochelatins and glutathione

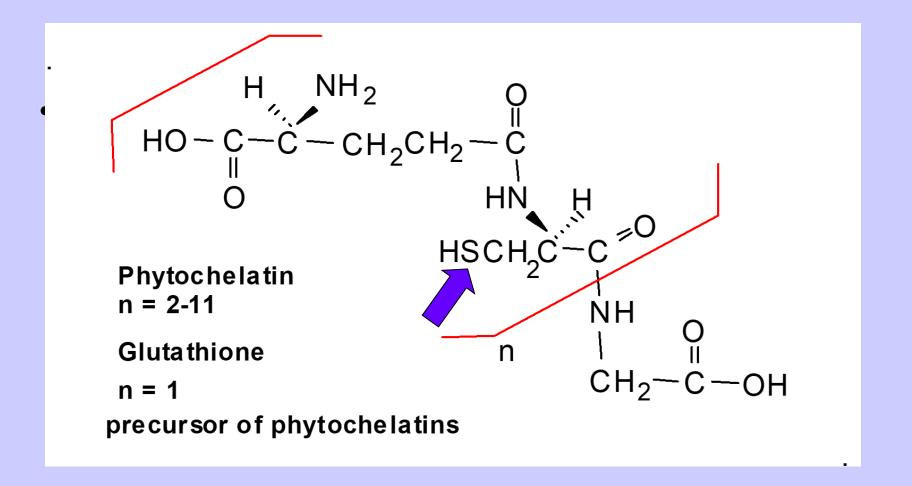
Glutathione and phytochelatins are metal-binding peptides:

produced by eukaryotic organisms

⇒marine phytoplankton

Glutathione (GSH):

naturally occurring compound the major thiol (R-SH) in animals, plants, phytoplankton, bacteria



SH groups → efficient metal binding sites

metal detoxification mechanism

regulation of intracellular metal concentrations homeostasis

Phytochelatins

Used as biomarkers to quantify trace metal stress in plants

- -intra-cellular metal-binding peptides produced by plants and algae to chelate heavy metals
- Synthesised by the enzyme Phytochelatin synthase from glutathion.
- The enzyme needs a metal for activity.
- Phytochelatins have been measured in marine phytoplankton cultured in conditions ranging from low to growth inhibiting free ion concentrations.
- PC concentrations increase with increasing metals, well before other physiological parameters (e.g. growth rate) are affected.
- In small harbors in New England, PC concentrations varied systematically with free Cu concentrations (sewage discharges, antifouling boat paints). High in-shore, low off-shore.

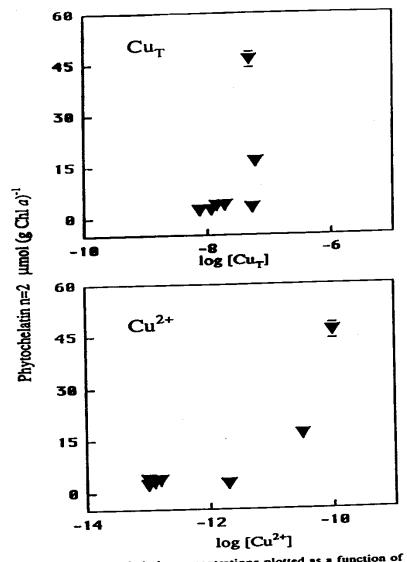
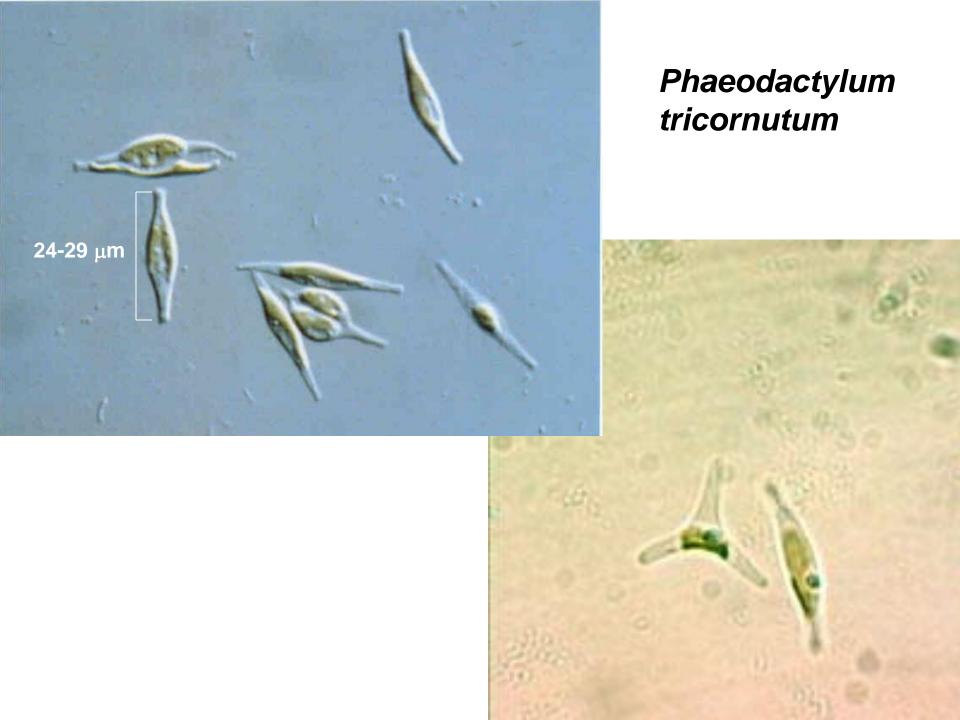
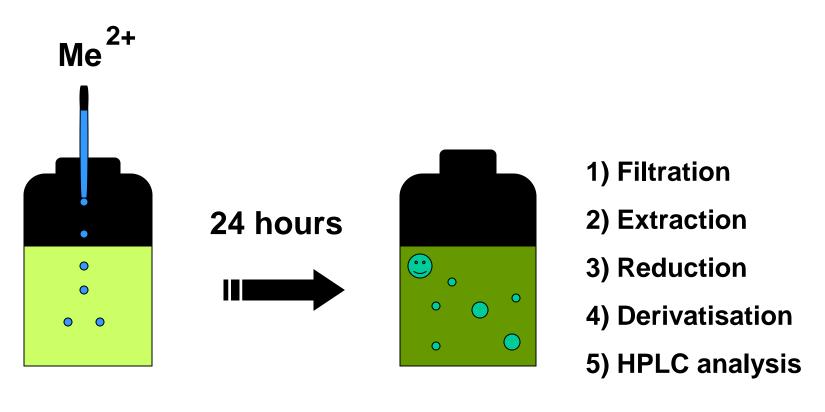


Fig. 1. Phytochelatin concentrations plotted as a function of total Cu (top) and free Cu concentrations (bottom). Samples were collected in September 1993 from various harbors in southeastern New England. Error bars represent the average of measurements made on two separate samples taken from the same bottle; for most of the data points the error bars are within the size of the symbol.



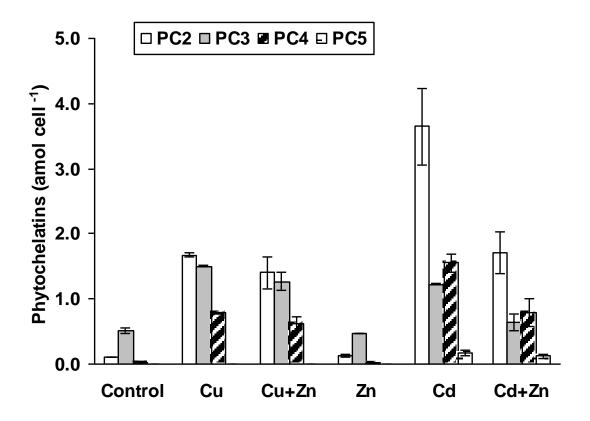
Short-term metal exposure experiment using *Phaeodactylum tricornutum*



- Control
- Addition of 10 μM Cd (5.0 μM Cd²⁺ - MINEQL)

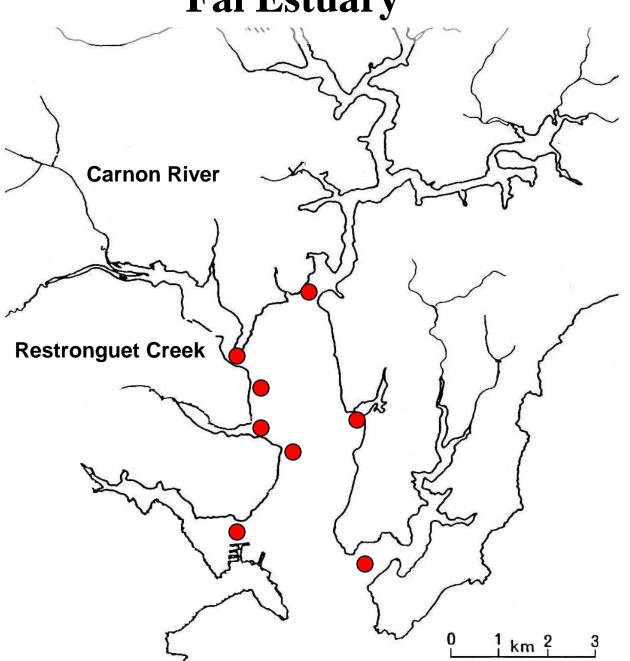
Glutathione = PC1

PC2, PC3, PC4, PC5

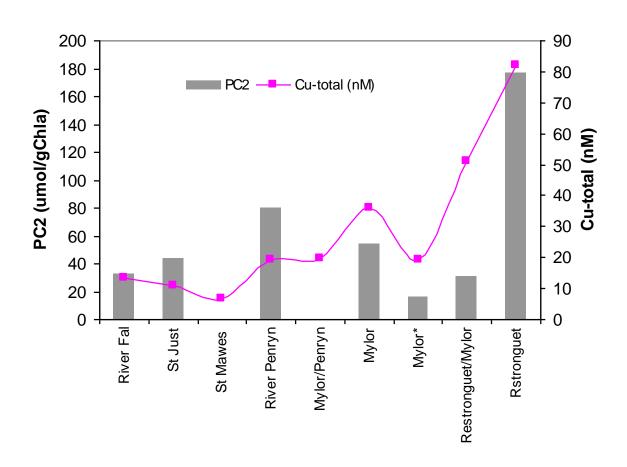


Phytochelatins produced by *Phaedactylum tricornutum* under metal exposure. Kawakami, Achterberg et al., 2006

Fal Estuary



Metal-binding peptides in the particulate phase in the Fal Estuary



Metal-binding peptides in the particulate phase of natural waters

	μmol (g Chl <i>a</i>) ⁻¹	
	Phytochelatin2	Glutathione
Fal Estuary	2 – 36	99 – 273
Polluted harbours (USA)	10 – 50	-
Galveston Bay (USA)	Up to 6.3	10 – 40
Southern California Bight (USA)	-	100 – 250

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- Turner, D. R. and Hunter, K. A. (2001). The Biogeochemistry of Iron in Seawater. John Wiley and Sons.