Risk e Learning

Metals – Bioavailability

April 9, 2003

2:00 – 4:00 pm EDT

SUPERFUND
BASIC RESEARCH PROGRAM

Assessing the Oral Bioavailability of Lead in Soil in Humans

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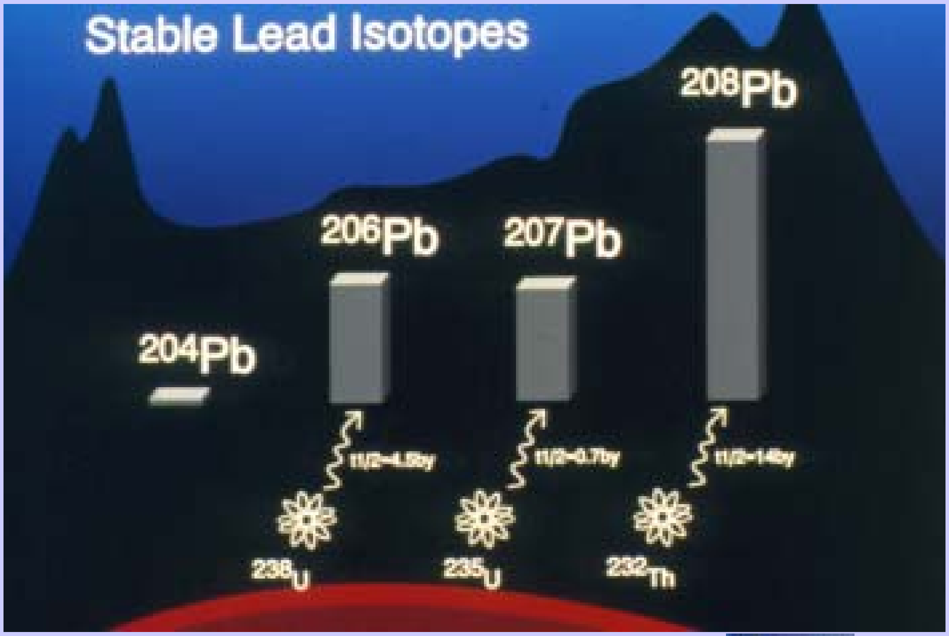
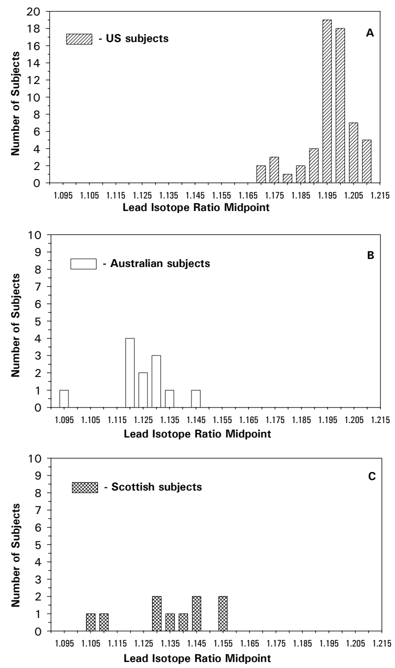
[4]College of Physicians & Surgeons, Columbia University

Assessing the Oral Bioavailability of Lead in Soil in Humans

- Stable isotope dilution
- Previous work on Bunker Hill, ID, soil
- Results from ongoing studies of amended soils (Joplin, MO)


Stable Lead Isotopes
208Pb
206Pb
207Pb
204Pb
t1/2=4.5by
t1/2=0.7by
t1/2=14by
238U
235U
232Th

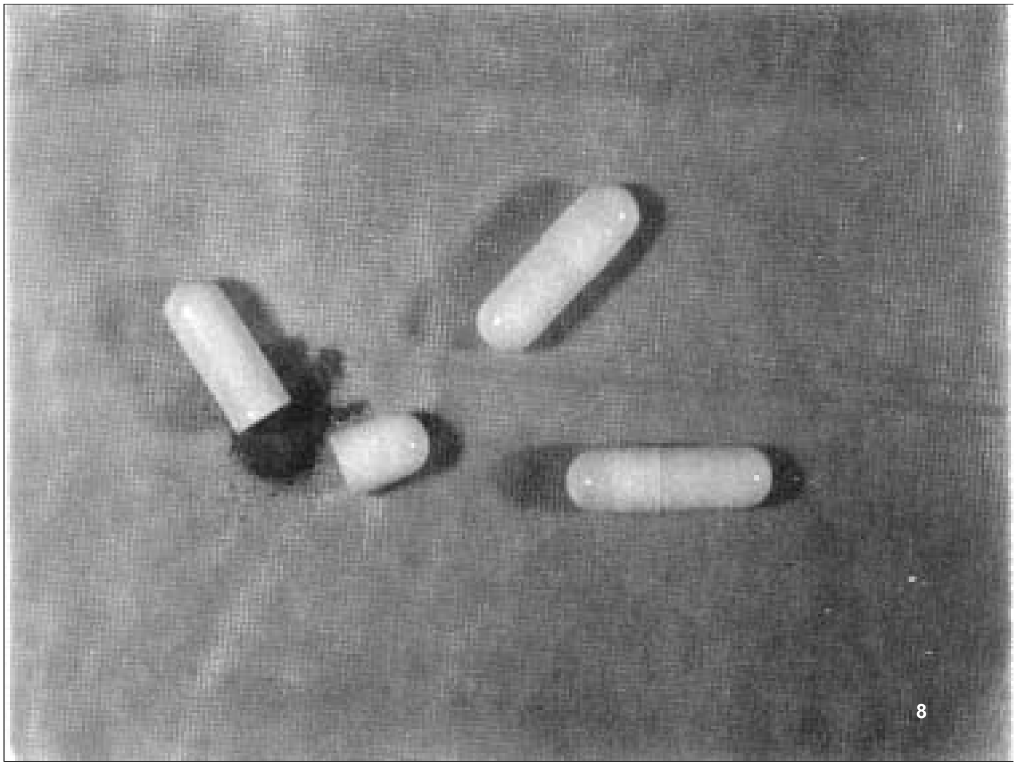

- US subjects
A
Number of Subjects
Lead Isotope Ratio Midpoint
1.095 1.105 1.115 1.125 1.135 1.145 1.155 1.165 1.175 1.185 1.195 1.205 1.215
- Australian subjects
B
Number of Subjects
Lead Isotope Ratio Midpoint
1.095 1.105 1.115 1.125 1.135 1.145 1.155 1.165 1.175 1.185 1.195 1.205 1.215
- Scottish subjects
C
Number of Subjects
Lead Isotope Ratio Midpoint
1.095 1.105 1.115 1.125 1.135 1.145 1.155 1.165 1.175 1.185 1.195 1.205 1.215

Lead Isotope Ratios of Select Sites

Location	Superfund Site	$^{206}Pb/^{207}Pb$
Kellogg, ID	Bunker Hill	1.057
Butte, MT	Silver Bow Creek	1.148
Leadville, CO	California Gulch	1.170
Buffalo, NY	Bern Metal	1.205
Triumph, ID	Triumph Mine	1.253
Tar Creek, OK	Tar Creek	1.350
Joplin, MO	Oronogo-Duenweg Mining Belt	1.378

Clinical Protocol

- Screening and physical exam
- Obtain informed consent
- Three day admission
- Subject dosed at 250 μg Pb/70 kg body wt
- Collect blood and urine samples
- Standardized meals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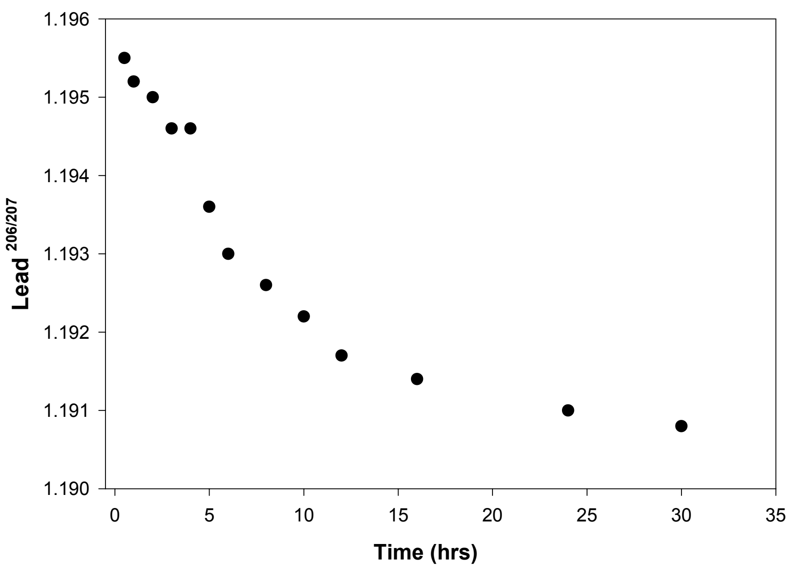
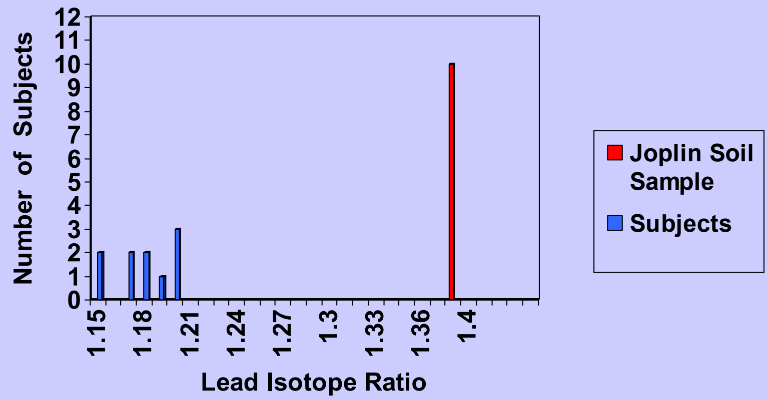

1.196
1.195
1.194
1.193
1.192
1.191
1.190
Lead 206/207
0
5
10
15
20
25
30
35
Time (hrs)

Results of Previous Studies from Bunker Hill Soil

Group (n=6)	Age (yrs)	Weight (kg)	Pb Dose (ug)	Soil Dose (mg)	Bioavailability (%)
Fasted	28	59.7	213	72.9	26.2 (18.0-35.6)
Fed	28	67.9	242	82.9	2.52 (0.2-5.2)

Lead Isotope Ratio Distributions



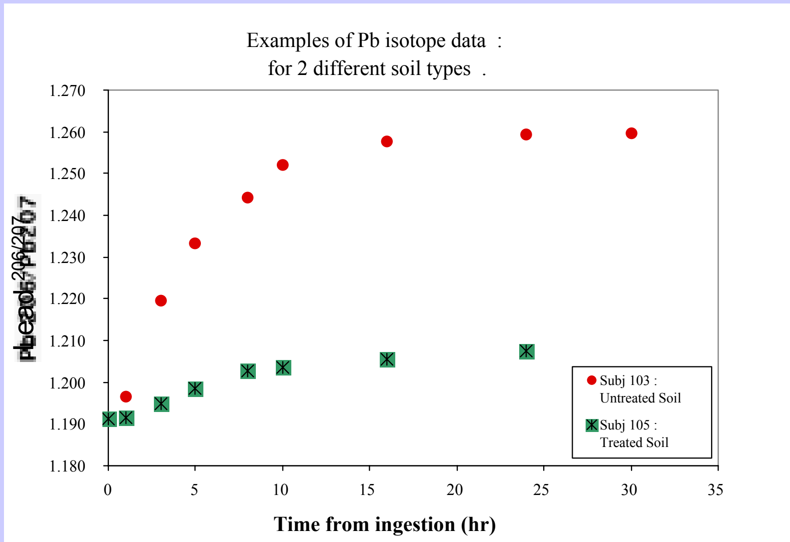
Soil Homogeneity

Sample	Lead (ppm)	Weight (mg)	$^{206}Pb/^{207}Pb$
Untreated	5,200	75.5	1.3816
“		69.3	1.3826
“		62.2	1.3817
Phosphate-Amended*	4,240	66.8	1.3790
“		65.9	1.3784
“		66.2	1.3796

* 1% Phosphate, field–treated aged 18 months

Subject Demographics

Subject	Soil Type	Age	Race/Ethnicity	Gender	Weight (kg)	Pb Dose (ug)	Soil Dose (mg)
1	Non-amend	30	African Amer	F	60	239.2	46
2	Amended	37	Caucasian	F	53.6	195.5	46.1
3	Non-amend	23	Caucasian	M	66	257.4	49.5
4	Amended	25	Caucasian	M	89.5	324.4	76.5
5	Amended	40	African Amer	M	73.5	262.5	61.9
6	Non-amend	35	Asian	F	60.5	215.8	41.5
7	Amended	41	African Amer	F	81	296.4	69.9
8	Non-amend	34	Middle Eastern	M	79	282.4	54.3
9	Amended	20	Caucasian	M	62.7	224.7	53
10	Non-amend	23	Caucasian	F	59	210.6	40.5


Examples of Pb isotope data :
for 2 different soil types .
1.270
1.260
1.250
1.240
1.230
1.220
1.210
1.200
1.190
1.180
0
5
10
15
20
25
30
35
Time from ingestion (hr)
Subj 103 : Untreated Soil
Subj 105 : Treated Soil

Subject Change in Blood Lead

Subject #	Soil Type	BPb Start	BPb End	Change	Mean
101	Non-Amended	4.19	5.91	1.72	
103	“	1.30	2.22	0.92	
106	“	1.49	3.32	1.83	
108	“	2.06	2.50	0.44	
110	“	1.11	2.20	1.09	**1.20**
102	Amended	1.84	2.32	0.48	
104	“	3.62	4.05	0.43	
105	“	4.88	5.78	0.90	
107	“	2.95	3.82	0.87	
109	“	2.27	2.60	0.33	**0.60**

Preliminary Results

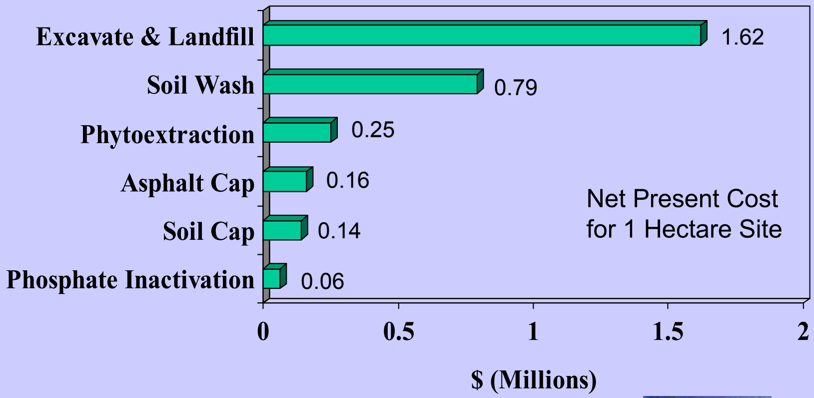
Group	Age (yrs)	Weight (kg)	Pb Dose (ug)	Soil Dose (mg)	Bioavailability (%, Absolute)
Untreated	29.0	64.9	241.1	46.4	**35.8** (14.6-54.2)
Amended	32.6	72.1	260.7	61.5	**15.3** (8.0-26.2)

Comparative Results: Animal, In vitro & Human

	Animal*	**In vitro** (pH 2.3)**	**Human**
% Reduction in Bioavailability	38	38	57

* Casteel et. al., ** Ruby et. al.

Relative Costs:
Remedies for Metal-Contaminated Soils



* Source: EPA Technical Innovation Office

Manuscripts in Progress

- Graziano, JH, LoIacono, NJ, Chillrud, SN, Ross, J, Blum, C. Oral bioavailability of lead in phosphate-amended and non-amended soils from a Missouri smelter site.

- Chillrud, SN, Hemming, G, Wallace, S and Ross, J. Determination of lead isotopes in blood with separation by iron hydroxide co-precipitation and analysis by multi-collector ICP-MS, In preparation for Journal of Analytical Atomic Spectroscopy

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BASIC RESEARCH PROGRAM

Determination of Metal Speciation in Aquatic Ecosystems Using Equilibrium Gel Samplers

Martha Chang | Jen Galvin

Sarah Griscom | Chris Lewis

Dave Senn | Jim Shine

Crista Trapp

Harvard School of Public Health - Dept. Environmental Health

NIEHS Superfund Basic Research Program

Outline:

1. Importance of Metal Speciation

2. Gel Flux Samplers

3. 'Gellyfish' Equilibrium Gel Sampler
   - As a metal speciation tool
   - As a bio-mimic

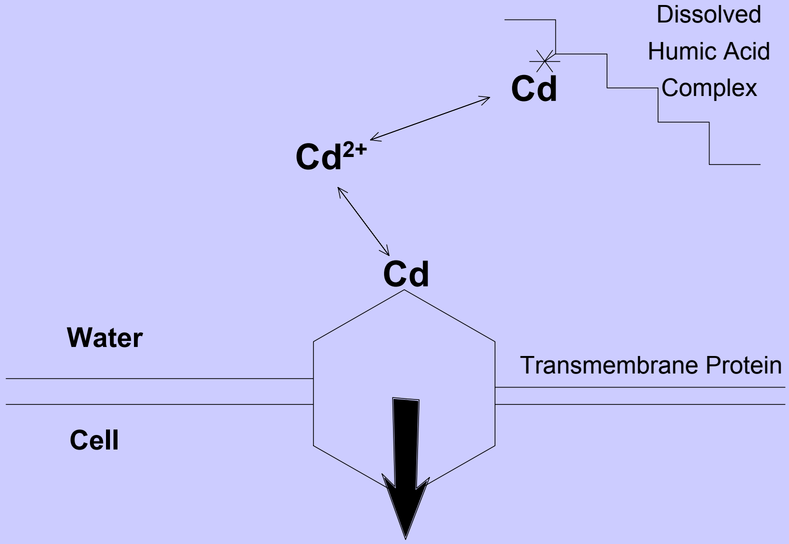
4. Conclusions

Knowledge of the Free Metal Ion Concentration is Important:

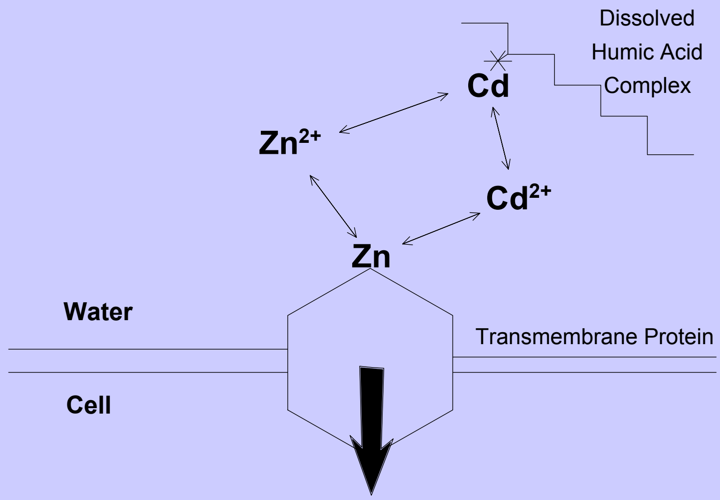
- Key determinant describing partitioning amongst different phases:
    - DOC bound
    - POC bound (settling particles)
    - Biological Uptake

-Thus a determinant describing:
    - Transport
    - Fate
    - Effects

Biotic Ligand Model:



Need to Understand Competitive Interactions:



-Interactions can be antagonistic, protective, or neutral

-Need simultaneous data for multiple metals

Current Metal Speciation Techniques:

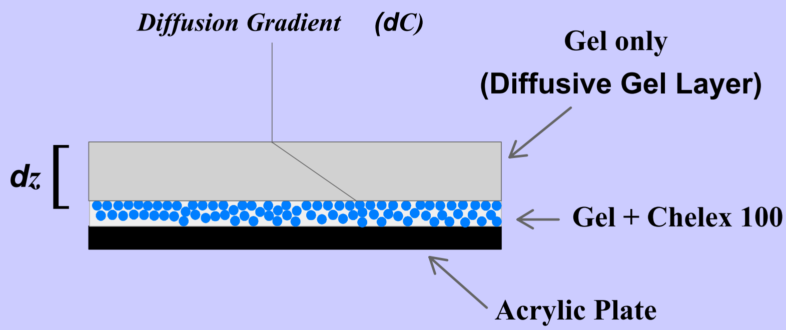
- Indirect
  - titrations to characterize ligands
  - labor Intensive

- Methodological Uncertainties
  - titration window
  - L_1 & L_2 ligands ??

- One metal per titration

Gel Flux Samplers:

- Diffusion Gradient Thin-film (DGT) Samplers
- Zhang & Davison. 1995. Anal. Chem. 67:3391-3400



Assuming C=0 at Chelex/Gel interface:

a) Determine a flux rate of metals to the Chelex 100

b) F = -D dC/dz solved for C, the external concentration

Strengths and Weaknesses of Flux Samplers:

(-) Measures a flux, not a concentration

(-) Unclear what metal species is measured

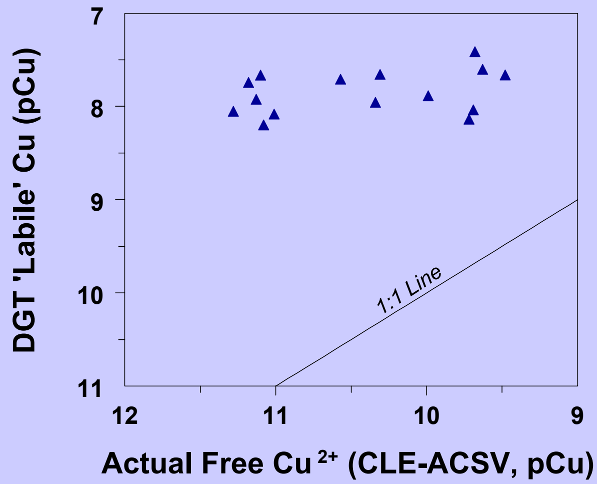
(+) Flux of individual metals independent of the underlying complex mixture

(-) Flux of individual metals independent of the underlying complex mixture

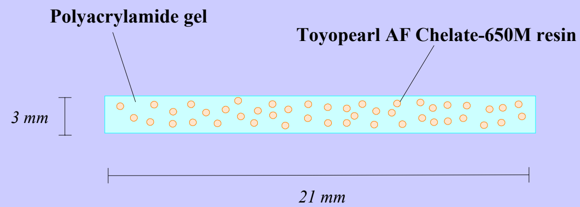
- less suitable as a biotic ligand analog?

Twiss and Moffett (2002)

- “Impacted Sites”



Equilibrium Sampler (Gellyfish) : Design Criteria



Toyopearl Resin (Tosohaas, Inc.):

- Mean bead size: 65 µm
- exchange capacity: 35 µeq/ml

Gel Crosslinker: DGT Crosslinker (DGT Research, Ltd.)

Final Resin Concentration Inside Gel:

- 3×10^{-4} eq/L
- Designed for less than 5% depletion of metal from surrounding 2 L solution

Strengths and Weaknesses of Gellyfish Sampler:

(+) Is in equilibrium with a concentration, not a flux

(+) Clearer what metal species is measured (free metal ion)

(-) Accumulation of individual metals related to the underlying complex mixture

- possible correction procedures?

(+) Accumulation of individual metals related to the underlying complex mixture

- more suitable as a biotic ligand analog?

General Differences

Flux Sampler = Empirical

- What you see is what you get
- Is what you get what you want to see?

Gellyfish = Mechanistic

- What you see is the free metal ion concentration
- BUT… you must be able to account for all possible confounding factors

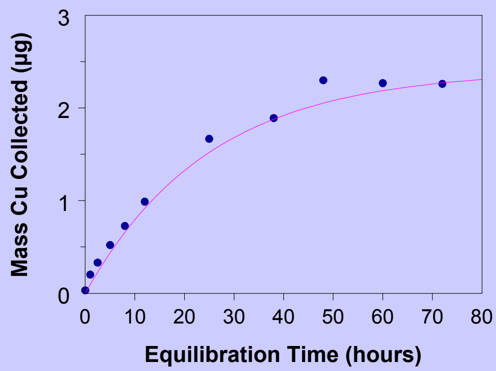
The 'Gellyfish':

Equilibration, Back Extraction, and Analysis:

-Experiments conducted with artificial seawater

- AQUIL salts recipe (no nutrients)
  - Metal speciation controlled with EDTA
- Equilibration
  - Gellyfish suspended in 2L sample with teflon string for appropriate equilibration period
- After Equilibration:
  - Gellyfish removed from test solution, rinsed in DI water
  - Gellyfish placed in 10 ml 10% HNO_3
  - After 24 - 48 hrs, back extract analyzed for Cu
  - ICP-MS (Perkin Elmer ELAN 6100 DRC)

Equilibration Times:

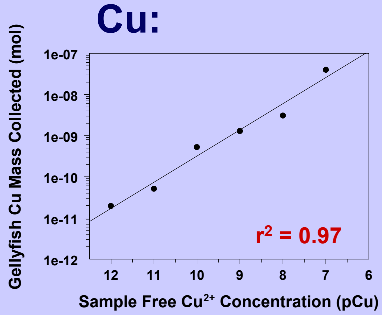


Range of t_{90} equilibration times: 15 - 30 hours

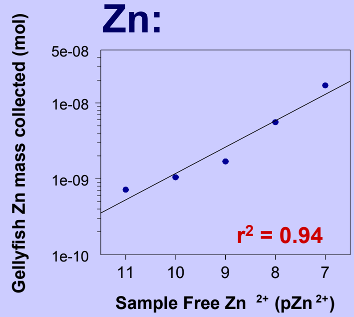
Coefficient of Variation for Repeated Measures: ~ 5%

Calibration with Free Metal Ions:

Cu:



Zn:



Salinity = 20 ppt (pH = 7.9 - 8.1)

Total Copper:

1.5×10^{-6} M (nominal pCu = 7, 8, 9, 10)

1.5×10^{-7} M (nominal pCu = 11, 12)

Salinity = 20 ppt (pH = 7.8 - 8.0)

Total Zinc:

5×10^{-7} M (nominal pCu = 7, 8, 9)

5×10^{-8} M (nominal pCu = 10, 11)

Correction Procedure for Complex Mixtures:

-$K'_{Cu\text{-}Toso}$ = [CuToso]/[Cu^{2+}]*[$Toso_{free}$] independent of other solution components

-Requirement: Semi-conservative solution component

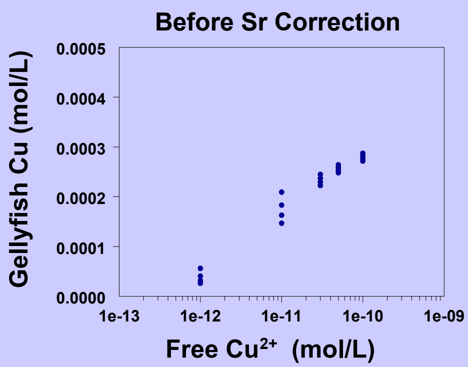
- Conservative behavior in water ($X_{total} = X_{free}$)
- Reacts with Tosohaas resin

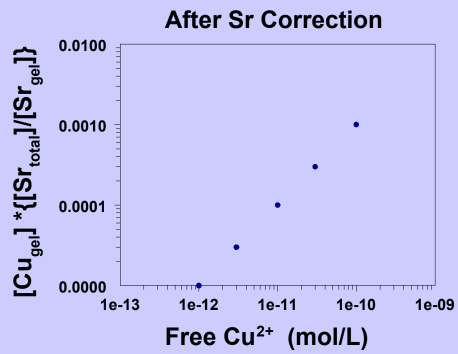
- Candidate: **Sr**
- Assumption: Sr_{total} = free Sr^{2+}
  - therefore: $K'_{Sr\text{-}Toso}$ = [SrToso]/[Sr_{total}]*[$Toso_{free}$]
  - solve above equation for [$Toso_{free}$]
  - substitute into original equation

  - **Result: $Cu^{2+} = K^* * [Cu_{gel}] *\{[Sr_{total}]/[Sr_{gel}]\}$**
    where $K^* = K'_{Cu}/K'_{Sr}$

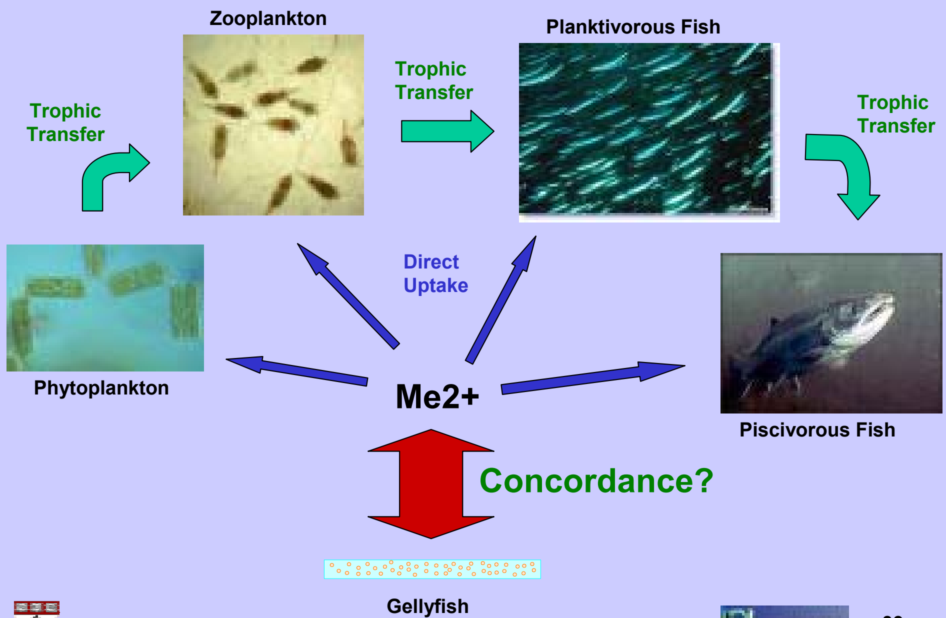
Model Test of Sr Correction Procedure:

- 5 free Cu ion levels: pCu range: 10 - 12
- 4 salinity levels : 10, 15, 20, 25 o/oo (= 20 total treatments)
- No Correction: Cu_{gel} at fixed pCu varies w/ salinity
- Correction Applied: Data collapse to a single line

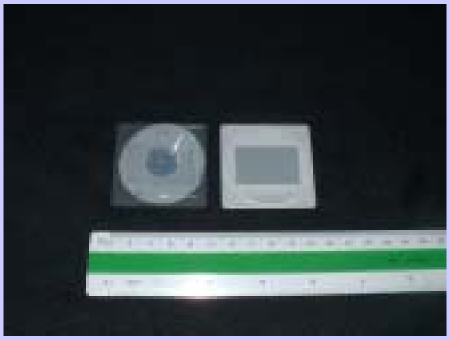




Alternate Use: Bio-mimic:



The ‘Gellyfish’: Field Deployment Apparatus



Gellyfish placed in a piece of LDPE sheet with a hole punched through it, sandwiched by polycarbonate filters, and held togehter with snap-together slide holders.

Slides mounted in a plastic basket for field deployment

Initial Experiments: Calibrate with Biomonitoring Organisms

A) Water:

Gellyfish

Mytilus edulis

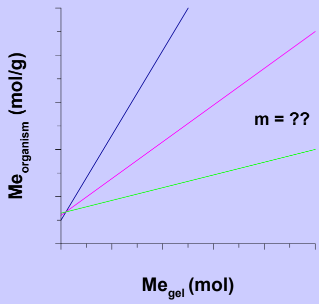
Field Study

B) Sediment:

Gellyfish

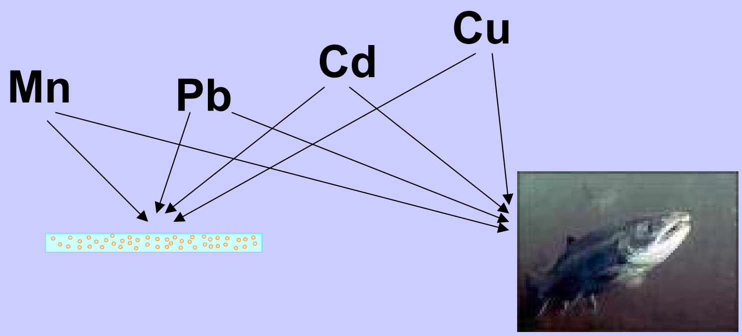
Neries Virens

Lab Study



Theoretical Basis:

- Competetive Metal Interactions governing metal uptake by Gellyfish = interactions governing uptake by biological organisms



Potential Advantages as Monitoring Tool:

1) Gellyfish Don't Die (can they be eaten?)

2) Gellyfish don't undergo gametogenesis

3) Gellyfish are all the same (good or bad?)

4) Equilibration time can be controlled

- Surface area/volume ratio
- Can select a desired integration period

Current/ Near Future Experiments:

- **Effect of complex mixtures on Metal uptake**
  - **Proof of Sr correction concept**
- **Use with other metals (Pb, Cd)**
  - **similar analytical windows for different metals?**
- **Calibrate with uptake into aquatic organisms**
  - select desired equilibration time?

Conclusions:

- Gel Flux (DGT) Samplers
  - are the strengths weaknesses?
- 'Gellyfish' Sampler
  - Equilibrium based
  - Are the 'weaknesses' strengths?
  - Proof of concept for Cu, Zn promising
- Potential Biotic Ligand Analog?
  - Supporting tool for speciation-based WQ approaches?
- Allow new types of experiments in Metal Speciation Research?
  - ligand specificity studies

Thank You

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Links to Additional Resources