B.3 Use of Earthworms as Bioindicators for ML-ARD

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Bioaccumulation

Bioaccumulation = Bioconcentration + Ingestion

It is the increase in concentration of a substance in organisms relative to the concentration of the substance in the environmental medium (water, soil, air) to which it is exposed.

It has been suggested that the food component is more important than the surrounding medium (bioconcentration) in bioaccumulation of **Hg** and **Se**.

Source: Parametrix Inc. (1995). Persistence, Bioaccumulation and Toxicity of Metals and Metal Compounds. Published by ICME - International Council on Metals & Environment. 92p.

Bioaccumulation

- Once bioaccumulated, a substance can be biomagnified

It is the process by which the tissue concentration of a bioaccumulated substance increases as it passes up the food-chain through at least two levels.

- Biomagnification is a function of the position of each species in the food web

Bioaccumulation

Definitions of **BCF** and **BAF** by Parametrix, Inc. :

- Bioconcentration Factor (BCF) is a unitless value calculated by dividing the steady state tissue concentration of a substance by the steady state environmental concentration, normally water.
- Bioaccumulation Factor (BAF) are a unitless value calculated by dividing the steady state tissue concentration of a substance by the steady state environmental concentration assuming via both water and diet

Source: Parametrix Inc. (1995). Persistence, Bioaccumulation and Toxicity of Metals and Metal Compounds. Published by ICME - International Council on Metals & Environment. 92p.

Metal Detoxification and BAF

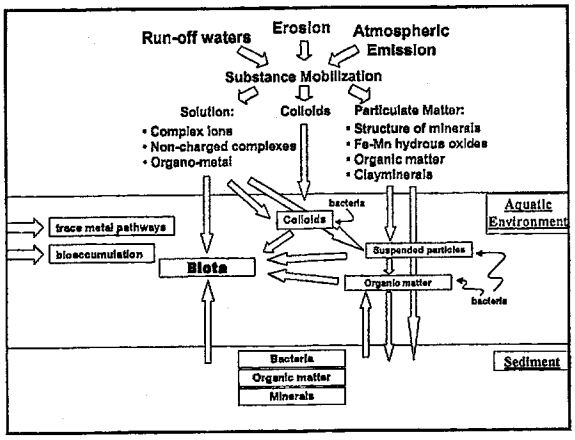
- Organisms have mechanisms to regulate the flow of metals through cells
- European Union: a metal with BAF > 100 is considered bioaccumulative with potential for biomagnification
- Many metals are considered essential and have BAF's greater than 100, however they are still considered dangerous to life according to European legislation
- There is a discrepancy about which BAF value constitutes the threshold for potential biomagnification. The use of such a parameter alone to classify metals as potentially hazardous is inappropriate

Source: Parametrix Inc. (1995). Persistence, Bioaccumulation and Toxicity of Metals and Metal Compounds. Published by ICME - International Council on Metals & Environment. 92p.

Bioaccumulation

- Most metals bioaccumulate to some degree, however the extent of bioaccumulation varies depending on the bioavailability of the metal and the specific organisms being considered, as well as exposure concentrations.

Source: Parametrix Inc. (1995). Persistence, Bioaccumulation and Toxicity of Metals and Metal Compounds. Published by ICME - International Council on Metals & Environment. 92p.



Bioaccumulation

- The purpose of measuring bioaccumulation for a compound is to assess the potential risk for a substance to be bioavailable, i.e. to be absorbed by organisms and cause long-term adverse effects
- There is a number of parameters that influence metal bioavailability (pH, Eh, hardness, alkalinity, particulate matter, organic carbon, temperature, ligands, methylating agents, etc.)

Example: $Cu(OH)_2$ and $CuCl_2$ are more bioavailable than when Cu^{+2} forms complexes with humic substances.

Complexes of Hg with humic and tannic acids are readily bioavailable.

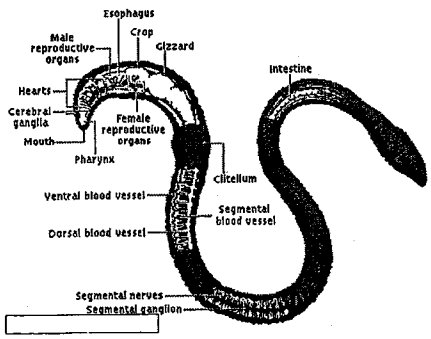
How to Determine Bioavailability ?

- Geochemical methods (such as sequential or selective chemical extraction) provide indirect hints about the metal-bearing minerals and consequently their availability to be incorporated into organisms.
- Many studies attempted to find correlations between environmental factors and metal accumulation.
- Frequently these environmental variables do not show direct correlation with bioaccumualtion due to large number of internal correlations between them and cumulative effects.

How to Determine Bioavailability ?

- Most Bioassays involves establishment of lethal dose (LD50). Sub-lethal concentrations are usually not investigated.
- Aquatic and terrestrial organisms - such as fish, birds, plants and larger mammals - have been used extensively and *effectively* around the world to characterize metals bioavailability
- A *simple* methodology could provide a *quick* and *inexpensive* assessment of metals bioavailability within a given media.

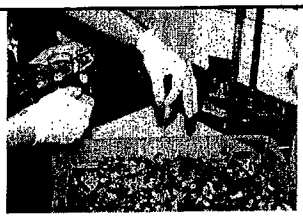
Earthworm Structure



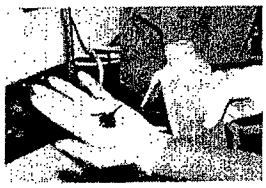
Earthworms as Bio-indicators

SUITABILITY:

- **Ingest large quantities of soil**
- **full contact with the substrate they consume**
- **constitute a food source for many organisms**
- **widely distributed, easily bred and relatively immobile**
- **accumulate a wide range of inorganic and organic compounds**
- ***Eisenia foetida* species commonly used in lab experiments, easily accessible, and is recognized as a bioindicator by several international organizations including:**
  - **European Economic Community**
  - **US EPA (Method 100.3)**
  - **Organization for Economic Cooperation and Development**
  - **ASTM (E-1676-95)**
  - **US Engineer Corp.**



Culture



Washing

Earthworms as Bio-indicators

The idea

Jars

Worms + Tailings or Solutions (effluents)

Exposure for a number of days

Earthworms as Bio-indicators

Methodology

Basic Questions:

- How many days should we run the tests to reach steady-state?
- What will the worms eat during the test?
- What is the best weight for the worms to survive the test?
- What are the main problems in handling worms?
- What are the temperature and humidity conditions for a high surviving rate?

Earthworms as Bio-indicators

Methodology

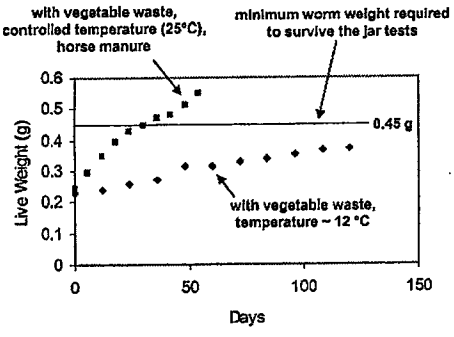
- All methodologies were conceived to determine bioaccumulation of substances in contaminated soils not in mining tailings.
- ASTM E1676-95 determines 28 days
- EPA 600 388 029 determines 14 or 28 days
- Edwards and Goats (1982) utilized 14 days
- 28 or 14 days ???? It's a matter of reaching the steady-state of bioaccumulation

Source: Rhett, Simmer and Lee (1988). Eisenia Foetida used as a Biomonitoring Tolt to Predict the Potential Bioaccumulation of Contaminants from Contaminated Dredged Material. Earthworms in Waste and Environmental Management. p.321-328. SPB Academic Publishing.

Earthworms as Bio-indicators

Methodology

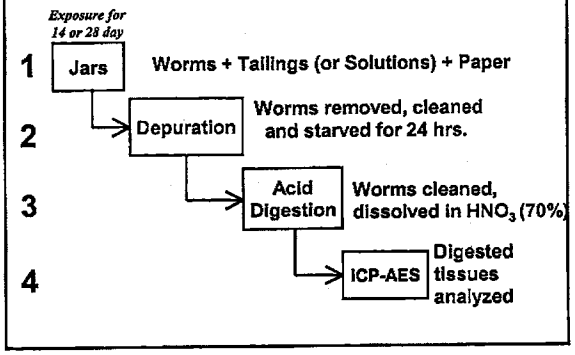
- In our initial tests, we mixed : shredded paper + worms + tailings + water
- However, worms were not surviving 28-day test
- Change the type of paper did not work
- Blend the paper with more water, neither
- Additional food source, neither
- The main causes for death were: stress and worm size (< 0.45g/worm = worms do not survive the jar tests.)
- When worms are farmed in horse manure, they gain wight very fast.



Worm Growth Control

Earthworms as Bio-indicators

Proposed Methodology



Tailing from a gold mine in B.C.

	Al	As	Cd	Pb	Sb	Tl	Zn
ppm	5000	531	87.1	2212	136	10.6	18940

Metal concentration (ppm) in worms after 28 days

	Al	As	Cd	Pb	Sb	Tl	Zn
Jar	9.76	8.87	3.61	144.2	0.228	2.84	47.8
BAF	0.003	0.03	0.08	0.12	0.003	0.01	0.005

Bioaccumulation Factor (BAF) = $\frac{\text{Metal in Worm Tissue}}{\text{Metal in Substrate}}$

The BAF is not indicating bioaccumulation

NPR = NP/AP of this tailing = 0.5

Tailing from a gold mine in B.C.

Sample	Hg in the tailing (ppm)	Hg in worms (ppm)
Jar A	no tailings	0.035
Jar B	19.2	0.071
BAF		0.007

Contaminated soil from an chlor-alkali plant in B.C.

Sample	Hg in the soil (ppm)	Hg in worms (ppm)
Jar A	no tailings	0.042
Jar B	25,000	263
BAF		0.02

The BAF is not indicating bioaccumulation

Tailing from a Pb-Zn mine in Mexico

	As	Cd	Cu	Ni	Pb	Zn
ppm	212	69	41.5	5.5	1129	13431

Metal concentration (ppm) in worms after 28 days (in triplicate)

	As	Cd	Cu	Ni	Pb	Zn
28 days (ppm)	18	7.5	2.3	0.24	34	47
BAF	0.1	0	0	0.02	0.03	0

NPR = NP/AP of this tailing = 0.41

The BAF is not indicating bioaccumulation

Criticism

- Even with very high Hg concentration in worms (263 ppm), in the case of the contaminated soil from a chlor-alkali plant, the BAF is not indicating bioaccumulation.
- The main reason is:

Bioaccumulation Factor (BAF) = $\frac{\text{Metal in Worm Tissue}}{\text{Metal in Substrate}}$

When the <u>substrate</u> is extremely contaminated or rich in metals, the BAF does not reflect the metal incorporation into the organism

Tailing from a Cu-Mo mine in B.C.

	As	Cd	Cu	Ni	Pb	Zn
ppm	2	0.55	337	10.5	5.5	89

Metal concentration (ppm) in worms after 14 and 28 days (in triplicate)

	As	Cd	Cu	Ni	Pb	Zn
14 days (ppm)	5.7	0.45	35	0.17	0.13	19
BAF	0.8	0.7	0.28	0.01	0	0.15
28 days (ppm)	15	0.6	25	0.16	0.01	26
BAF	4.4	0.6	0.1	0.01	0	0.2

NPR = NP/AP of this tailing = 3.1

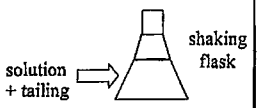
The BAF is indicating bioaccumulation

Criticism

- As this tailing has low concentrations of metals, then the BAF is showing high accumulation of metals.
- It seems that we should find other ways to interpret the results.
- In addition, there is no indication that ADVERSE EFFECTS are occurring.
- Then, we have extracted metals from the tailing (simulating ARD conditions) and give the solutions to worms to observe bioaccumulation when metals are in a more bioavailable form.

Tailing from a Cu-Mo mine in B.C.

- **We mixed tailings with different solutions:**
- **Distilled water**
- **H_2O_2 (5% vol)**
- **H_2O_2 (10% vol)**
- **Sulphuric acid (0.02M)**
- **Solid:liquid ratio = 1:1; 2:1; 3:1; 4:1**
- **Liquid volume constant = 100 ml**
- **Time of shaking 24 hours**
- **Temperature = 22°C**



Bioassay with Worms with solutions obtained from tailings of a Cu-Mo mine in BC

Average	97.2%	16.1%	11.8%

Tailing from a Cu-Mo mine in B.C.

Bioconcentration Factor in worms after 14 days exposed to solutions from shaking flasks

	As	Cd	Cu	Ni	Pb	Se	Zn
Water	1.1	10.7	0.3	0.9	0.3	0.7	4.4
H2O2 (5%)	0	7.6	0.1	0.6	0	0	7.1
H2O2 (10%)	0	2.6	0.1	0.4	0.2	0	1.7
H2SO4 (0.02M)	1.4	13.6	1.4	0.8	1.9	0.4	20.6

S:L = 4:1

The BAF is indicating bioaccumulation

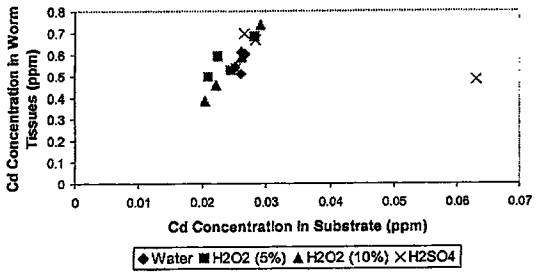
Tailing from a Cu-Mo mine in B.C.

Bioconcentration Factor in worms exposed for 14 days to H2O2 solutions from shaking flasks

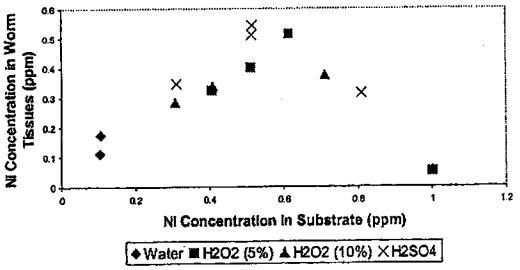
S:L	As	Cd	Cu	Ni	Se	Zn
1:1	2.4	9.3	0.4	0.7	0.8	7.3
2:1	4.4	6.9	0	0.3	0	5.1
3:1	0.04	11	0.2	0.6	0.3	9.7
4:1	0	8.6	0.5	0.7	0.2	8.5

The BAF is indicating bioaccumulation

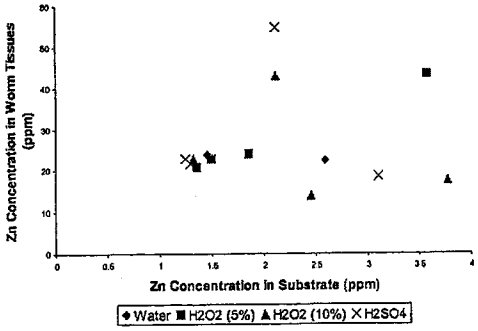
Tailing from a Cu-Mo mine in B.C.



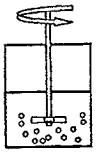
Tailing from a Cu-Mo mine in B.C.



Tailing from a Cu-Mo mine in B.C.



Worms Exposed to Mercury Solution



- 3 g of metallic Hg
- 300 ml, 0.005 M tannic acid
- 60°C
- stirring for 20 hours
- initial pH = 3.7
- final pH = 3.4
- pH adjusted with NaOH = 6

Hg Solution → 80 ml, 696 ppb Hg

Jar Contents: 10 g Worms, 175 g Silica, 25 g Paper } Substrate

Preliminary Results

Hg in the Substrate	278 ppb
Hg in Worm Tissue	861 ppb

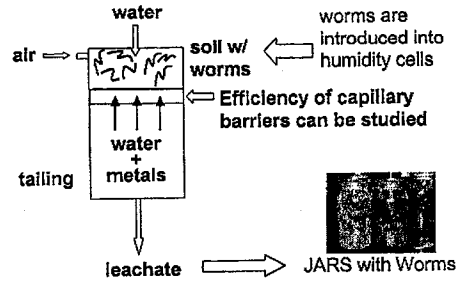
Bioconcentration Factor (BAF) = 3

Methylmercury in worm tissue = 32.2 ppb

Preliminary results suggest earthworms may bioaccumulate Hg-tannate and convert it into methylmercury. Tannate is a major soluble substance of darkwater rivers.

Suggestions for Different Configurations of Tests

For example:



Conclusion

- There is potential for using earthworms as indicators of bioavailability of metals from tailings, waste rocks and liquid effluents.
- BAF is not a useful parameter for evaluating bioavailability and hazardous impact.
- Earthworm bioassays are very useful for comparative studies on reclamation techniques.
- Other parameters such as mobility, discoloration and weight loss are probably better parameter to indicate adverse effects.
- Definitely, more studies are needed to establish a robust methodology.