Risk e Learning

Metals – Remediation

May 14, 2003

2:00 – 4:00 pm EDT

SUPERFUND
BASIC RESEARCH PROGRAM

Biosurfactants: Applications for Metal Remediation

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SUPERFUND
BASIC RESEARCH PROGRAM

THE UNIVERSITY OF ARIZONA
COLLEGE OF AGRICULTURE & LIFE SCIENCES

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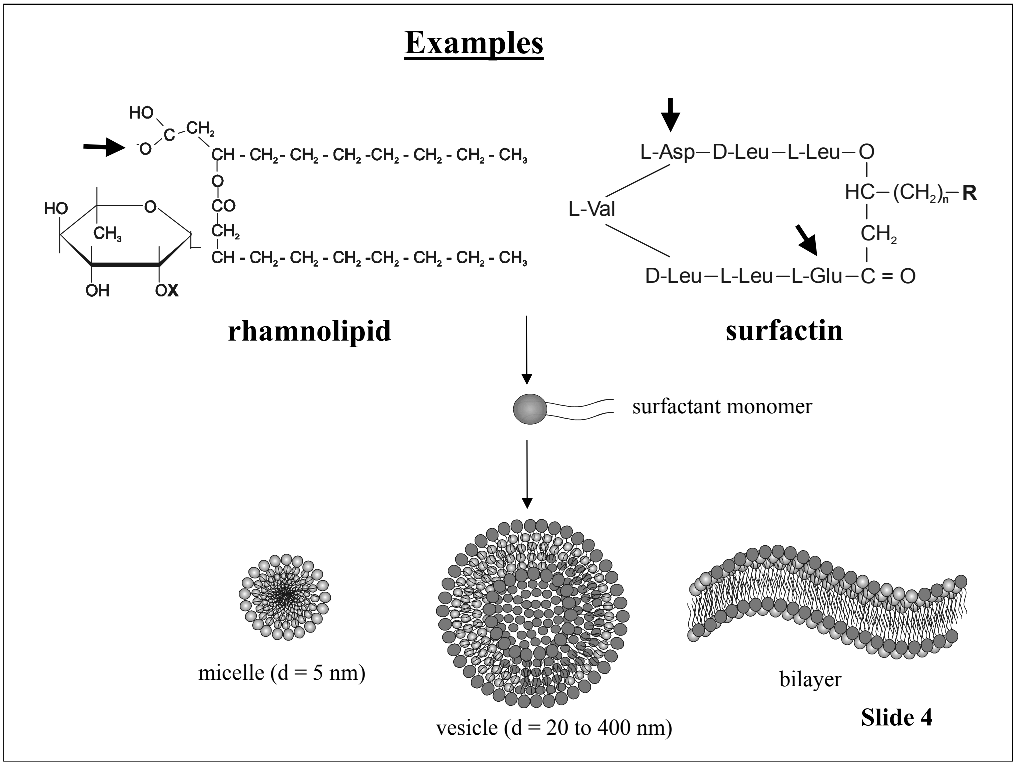
Applications for microbial surfactants

- Production of fine chemicals
- Bioremediation
    - biodegradation of organics
    - biodegradation in the presence of toxic metals
    - removal of organics by flushing
    - removal of metals by flushing
- Biological control
- Antibiotic facilitator

Slide 3

In situations where a metal-contaminated site poses an imminent threat to human or ecological health from metal leaching into the environment, it may be necessary to implement remedial action immediately. An alternative to removal and disposal of the contaminated material is the application of a “facilitated” soil washing or pump and treat action. In this case, these actions are “facilitated” by the addition of metal chelators/complexation agents that increase metal solubility and make metal removal more rapid and complete. This seminar will introduce a class of environmentally compatible metal chelators, biosurfactants.

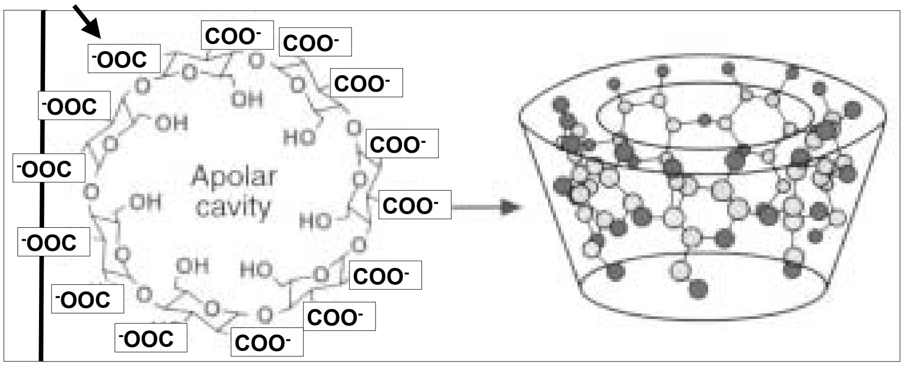


The two largest classes of biosurfactants are glycolipids and lipoproteins. Rhamnolipids are an example of the former class. The structure shown is monorhamnolipid where X = H and dirhamnolipid where X = rhamnose. Surfactin is an example of a lipoprotein, where R is the fatty acid tail. As for any surfactant, these molecules spontaneously aggregate into ordered structures above a concentration called the critical micelle concentration (CMC). The type of structure formed depends on surfactant structure, ionic strength, and pH. Surfactant monomers tend to associate with interfaces and have the ability to lower the surface tension between air and water and the interfacial tension between different liquids or liquids and solids.

The red arrows in this slide show potential complexation sites for cationic metal species. Complexation constants indicate that these molecules bind metals more strongly than would be suggested by the number of carboxyl groups in the structure.

Examples (cont.)

carboxymethyl-beta-cyclodextrin



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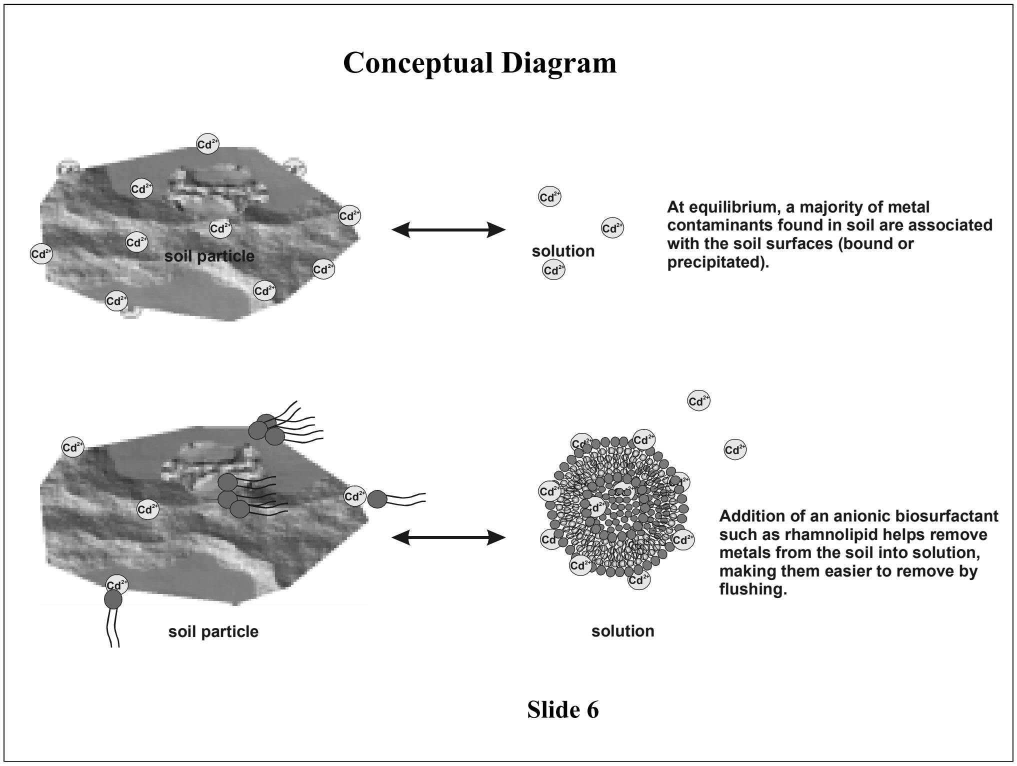
Cyclodextrins are cyclic, nonreducing oligosaccharides produced from the enzymatic degradation of starch by bacteria that contain cyclodextrin glycosyltransferases. These molecules lower surface tension only minimally and do not form typical surfactant aggregates. Cyclodextrins do not bind metals as strongly as rhamnolipid or surfactin but have the advantage of being nonsorbing. All of these surfactants have limited toxicity and are readily biodegradable.



A conceptual diagram of biosurfactant-facilitated removal of metals from soil.

Rhamnolipid complexation of various metals

Metal	Stability constant (Log K)	Molar Ratio c
Al^{3+}	10.3	2.48
Cu^{2+}	9.27	2.31
Pb^{2+}	8.58	2.37
Cd^{2+}	6.89	1.91
Zn^{2+}	5.62	1.58
Fe^{3+}	5.16	1.20
Hg^{2+}	4.49	1.21
Ca^{2+}	4.10	1.32
Co^{2+}	3.58	1.03
Ni^{2+}	3.53	0.93
Mn^{2+}	2.85	0.90
Mg^{2+}	2.66	0.84
K^{+}	0.96	0.57

Cyclodextrin 3.7

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Ochoa-Loza et al., 2000

Note that the complexation constants for metals of concern such as lead and cadmium are much higher than for common soil cations such as calcium, magnesium, and potassium.

Rhamnolipid has a much higher stability constant for cadmium (6.89) than cyclodextrin (3.7), but this advantage is offset since rhamnolipids sorb to soil components.

Environmental Compatibility vs. Strength of Metal Complexation

	Stability Constants		
Organic ligand	**Cd**	**Pb**	**Remarks**
DTPA	19.00	18.66	toxic, p. carcinogen
EDTA	16.36	17.88	limited biod., toxic
NTA	9.78	11.34	class II carcinogen
Rhamnolipid	6.89	8.58	environ. compatible
Oxalic acid	2.75	4.00	environ. compatible
Citric acid	2.73	4.08	environ. compatible
SDS	1.95	N.D.	toxic
Acetic acid	1.56	2.15	environ. compatible

Maier and Soberon-Chavez, 2000

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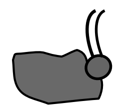
Among the most effective soil-washing agents investigated are strong acids and chelating agents. However, use of these agents can be both lethal to soil microflora and destructive to soil physical and chemical structure due to mineral dissolution.

Anticipated problems in application

- interference by naturally occurring metals Ca^{2+} K^{+} Mg^{2+}

- interference by naturally occurring organic ligands Humic acids Fulvic acids

- interference from sorption



- metal aging

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Rhamnolipid and Fulvic Acid Complexation with Metals

Ligand	Cu	Pb	Cd	Zn	Fe	Hg	Co	Ni
Rhamnolipid	9.27	8.58	6.89	5.62	5.16	4.49	3.58	3.53
SFA	8.69[5]	6.13[5] 10.1[3]	-	3.7[6]	6.1[4]	5.1[2]	3.69[5] 4.2[6]	4.2[4] 3.2[1]
WFA	5.67[7]	-	-	-	-	-	-	-

SFA = soil derived fulvic acid
WFA = water derived fulvic acid

[1] Adhikari and Hazra (1972)
[2] Cheam and Gamble (1974)
[3] Saar and Weber (1980)
[4] Schnitzer and Hansen (1970)
[5] Schnitzer and Skinner (1966, 1967)
[6] Schnitzer and Khan (1972)
[7] Schuman and Cromer (1979)

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Soil properties that impact rhamnolipid sorption

Clays:
illite > kaolinite > Ca-montmorillinite

Metal oxides:
hematite (Fe_2O_3) > MnO_2 > gibbsite ($AlOH_3$)

Organic matter:
humic acid

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Rhamnolipid-enhanced removal of cadmium from soil

Soil Type	Cd loaded (mg/Kg)	Removal by Electrolyte (%)	Removal by Rhamnolipid (%)	Total Cd Removed (%)
Vinton	592	23	54	80
Vinton	658	36	41	79
Vinton*	765	26	50	78
Vinton**	736	---	96	102

* aged one month
** no electrolyte pretreatment

Slide 12 Torrens et al., 1998

A series of column experiments conducted under saturated flow conditions suggest that freshly contaminated soils which contain only soluble, exchangeable, and organic matter bound metals are readily treatable.

Metal removal efficiency from freshly contaminated soils ranges from 50 to 100% depending on the soil type.

Aged soils are more problematic.

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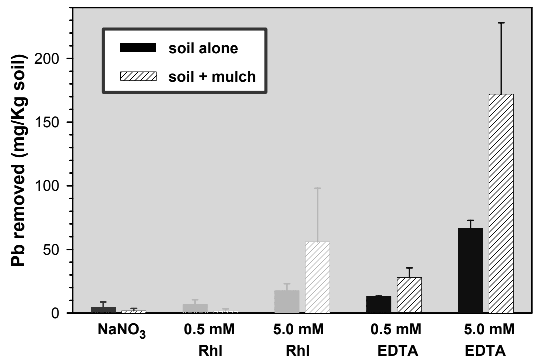
Removal of Aged Soil-Bound Metals – 3 examples

Post Office Site (2000 mg Pb/Kg soil)



Contaminant metals present	TCLP
Pb: 2000 mg/Kg	Pb: 12.5 mg/L
Cu: 21,000 mg/Kg	Zn: 26.7 mg/L
Zn: 2,500 mg/Kg	Cu: 33.3 mg/L
Fe: 150,000 mg/Kg	

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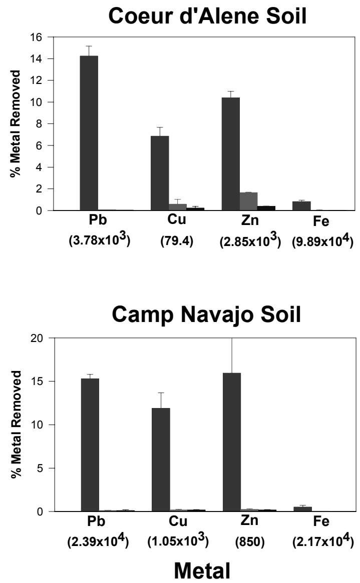
Three historically contaminated soils were studied in a series of batch experiments. The first material is a 30-year old mine tailing waste dumped near a Post Office site in Tucson, AZ. A 5 mM treatment of EDTA removed approximately 9% of the Pb with an extraction ratio of 5.9 mmol EDTA to 1 mmol Pb. A 5 mM treatment with rhamnolipid removed approximately 3% of the lead with an extraction ratio of 17 mmol rhamnolipid to 1 mmol Pb.



Coeur d'Alene Soil- mining
Soluble, Exchangeable,
Oxide-bound and residual

Camp Navajo Soil – army depot
Soluble, Exchangeable,
Carbonate-bound and residual

Soil Washing Agents

- **10mM Rhamnolipid**
- **50mM $Ca(NO_3)_2$**
- **10mM KNO_3**

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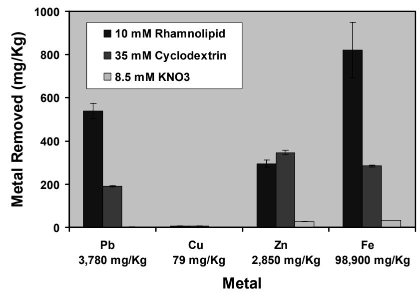
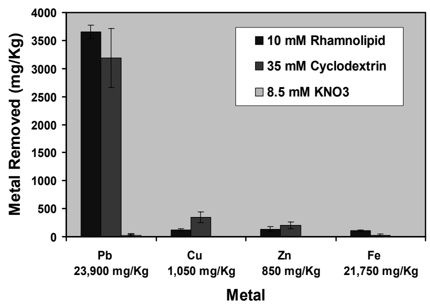
Neilson et al., 2003

The second and third soils are from Coeur d'Alene, Idaho an area that has been subjected to extensive mining and Camp Navajo, Arizona, an abandoned army depot site where the lead comes primarily from lead-based paint and lubricating oils. Coeur d'Alene soil is a sandy loam from a floodplain and is frequently subject to saturated conditions and as a result contains a large amount of amorphous iron oxides. The effective chelant to metal molar ratios in the above graphs were 3.2:1 for Coeur d'Alene and 1.4:1 for Camp Navajo. Results indicated that continued washing would remove further metal at the same extraction efficiency.

A comparison of Rhamnolipid and Cyclodextrin



<u>Coeur d'Alene Soil</u>
Soluble, Exchangeable,
Oxide-bound and residual



<u>Camp Navajo Soil</u>
Soluble, Exchangeable,
Carbonate-bound and residual

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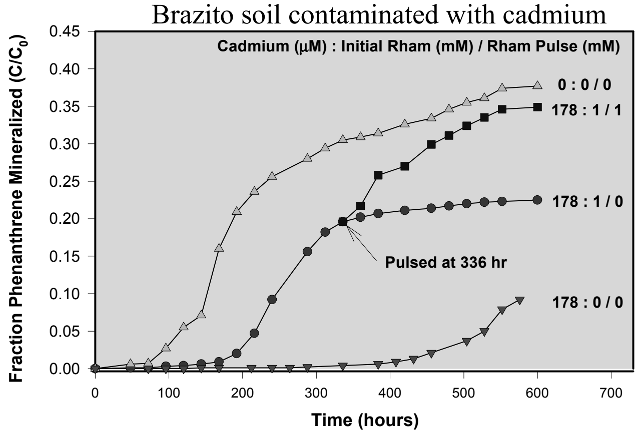
Neilson et al., 2003

Rhamnolipid is more effective than cyclodextrin in the Coeur d'Alene Soil, because it has a higher complexation constant with Fe and the lead is largely associated with amorphous iron hydroxides. These amorphous iron hydroxides are typically found in floodplain sediments such as are found in Coeur d'Alene. The two agents work equally effectively in the Navajo soil for the soluble and exchangeable fractions, but both are less effective against the carbonate-bound and residual metal.

Biosurfactant application to facilitate biodegradation in co-contaminated sites

Rhamnolipid-facilitated biodegradation of phenanthrene in Brazito soil contaminated with cadmium



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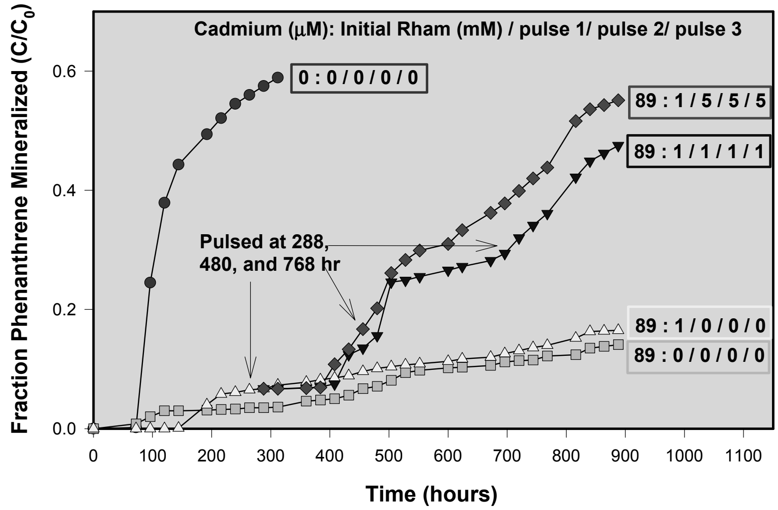
17Maslin and Maier, 2000

Rhamnolipid-facilitated biodegradation of phenanthrene in Gila soil contaminated with cadmium



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Conclusions

- Biosurfactants are an example of an environmentally compatible agent with potential for remediation of metals.
- Likely we can improve on performance by looking at other natural products including:

  other biosurfactants
  siderophores
  metallothioneins

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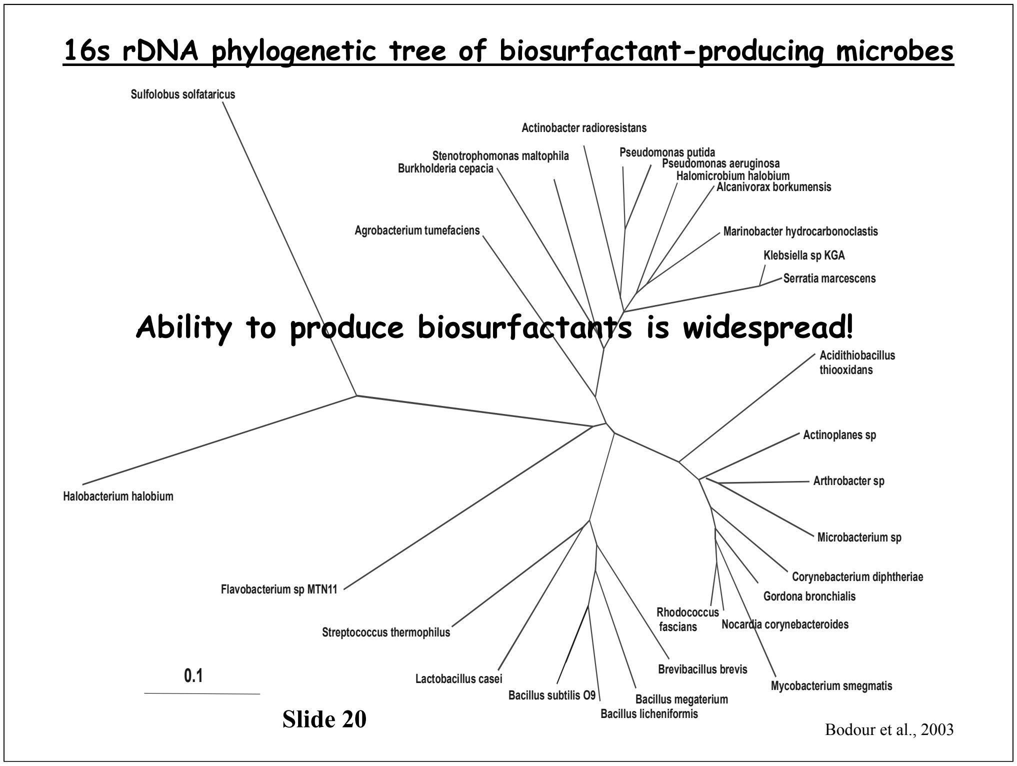
The two biosurfactants discussed here are environmentally compatible (low toxicity, biodegradable) and commercially available. There are advantages/disadvantages of each surfactant. Rhamnolipid is a stronger metal chelator and is easily recycled. Cyclodextrin is nonsorbing. The information available concerning each of these surfactants allows good predictive capabilities for success in application of these surfactants at the field scale. These surfactants are ready for field trials.



The ability to produce biosurfactants is exhibited by many eubacteria as well as some archaea. Biosurfactant production is genus and sometimes species specific.

Physical-chemical properties of surfactants vary greatly resulting in different potential applications.

- **This is true for different types of biosurfactants**
- **It is also true within a biosurfactant type.**

ATCC 9027	IGB83	158
m-Rhl	m-Rhl + d-Rhl	Rhl-methyl esters

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For example, the only species that produces rhamnolipid is *Pseudomonas aeruginosa*. A number of different types of rhamnolipids are produced. The majority of species produce a mixture of mono- and dirhamnolipid, but some, such as *P. aeruginosa* ATCC 9027, produce only monorhamonlipid, and *P. aeruginosa* strain 158 produces a nonionic methylester form of rhamnolipid.

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Green Engineering

Harry R. Compton

Environmental Engineer

U.S. EPA - ERT

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Mine Sites

- Lack of vegetation result of:
  - __ Fertility
  - __ Soil physical properties
  - __ Acidity
  - __ Metal toxicities
  - __ Salts

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Goals of remediation

- Reduce bioavailability of contaminant in place
- Rebuild soil or build new soil
- Restore soil function
    * Sustain plant growth
    * Sustain soil fertility
- Establish native plant ecosystem

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Why use wastes?

- Different wastes can be used to remedy a number of factors that may potentially contribute to a soil's inability to support a vegetative cover.
  - -- pH
  - -- soil fertility
  - -- soil physical properties, and
  - -- potentially toxic concentrations of trace metals

- By combining different materials together, and applying to the soils in-place, soil problems can be corrected.
  - -- lower costs
  - -- recycling wastes

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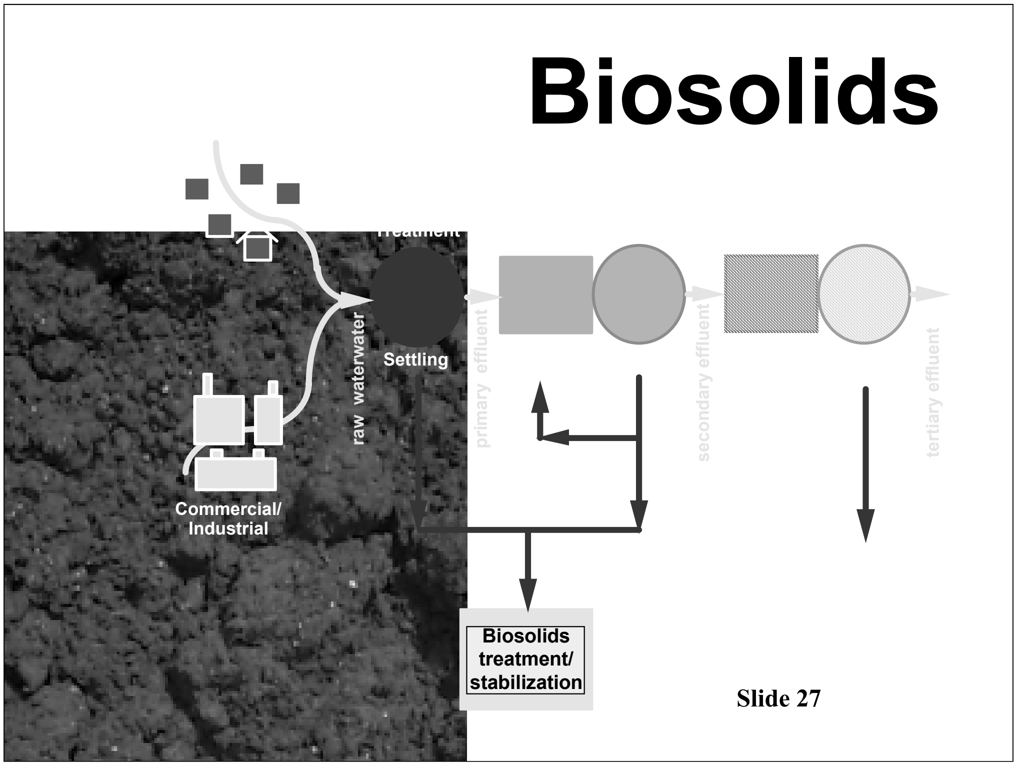
Biosolids
Treatment
raw waterwater
Settling
primary effluent
secondary effluent
tertiary effluent
Commercial/
Industrial
Biosolids
treatment/
stabilization
Slide 27

Metals in Biosolids

- Regulatory limit

(pollutant concentration limits)

- Cadmium
  - 39 mg kg
- Lead
  - 300 mg kg
- Copper
  - 1500 mg kg
- Zinc
  - 2800 mg kg

- National Means

(1990 national sewage sludge survey)

- Cadmium
  - 7 mg kg
- Lead
  - 134 mg kg
- Copper
  - 741 mg kg
- Zinc
  - 1202 mg kg

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Scientific basis of treatments

- Biosolids/compost add:
  *** nutrients**
  *** organic matter**
  *** metal complexing ability**
- Wood ash/waste lime add:
  *** pH adjustment**
  *** adhesive properties**
  *** nutrients**
- Wood waste/other C-rich residuals:
  *** limits N availability**
  *** adds bulk**
  *** physical soil benefits**

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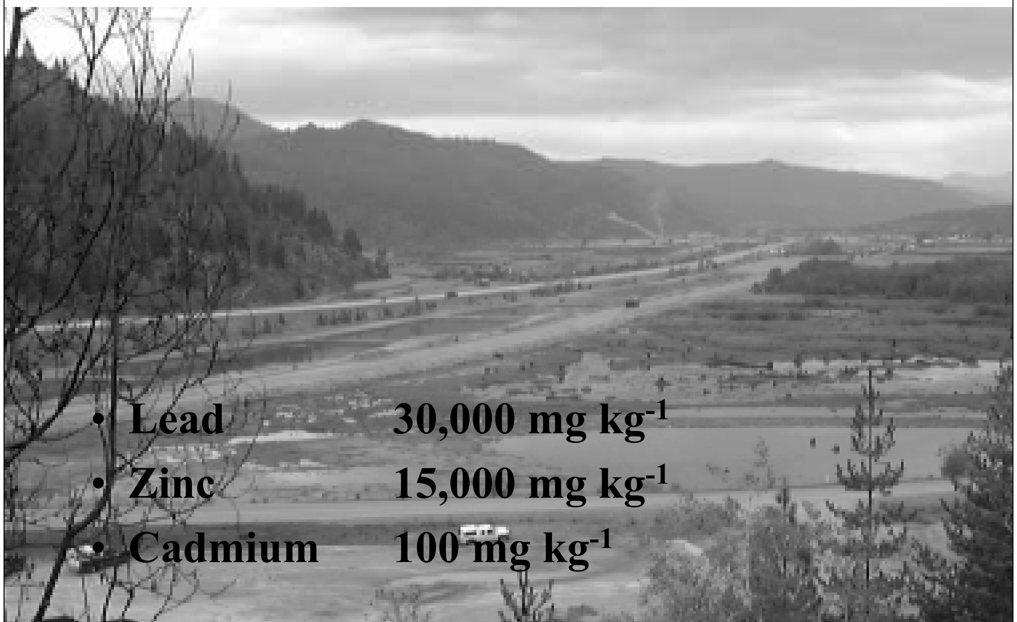
Bunker Hill - wetland restoration



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Waterfowl:

- Use Lateral Lakes wetlands as feeding, nesting area
- Dive for roots and tubers
- 20% of diet is sediment
- Acute Pb poisoning
- 100 sq mile area is Pb 'enriched'

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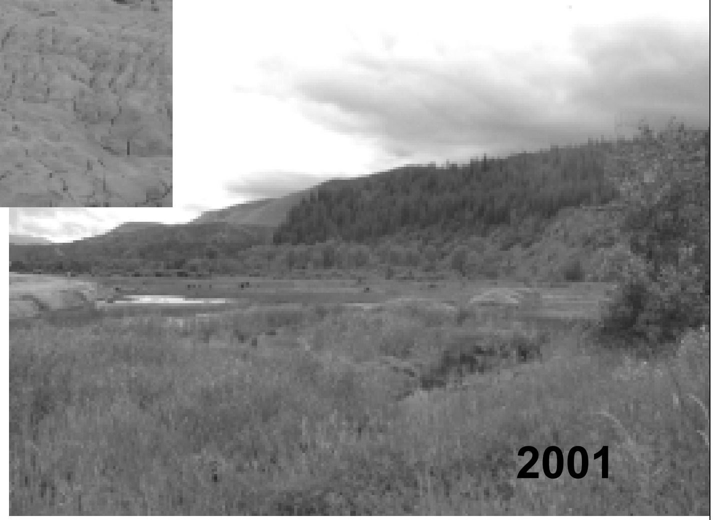
Coeur d'Alene Wetlands 1998- 2001



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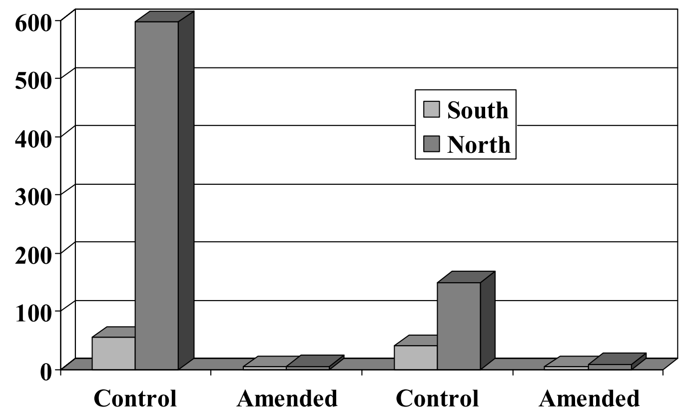
Wetland - Plant lead
(mg kg^{-1})



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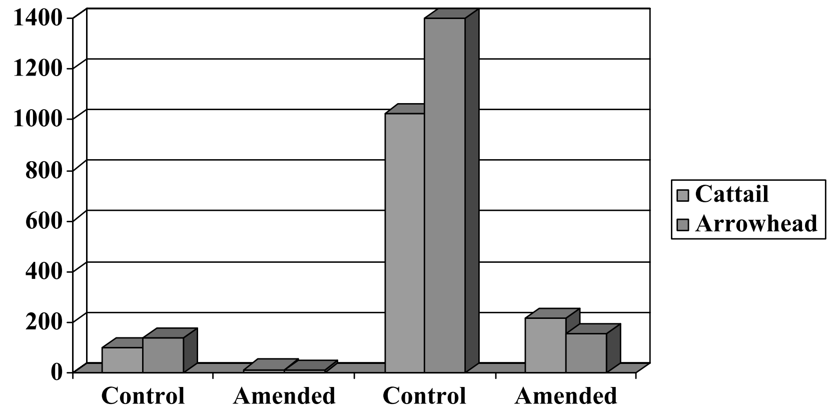
Other metals



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Pb Speciation

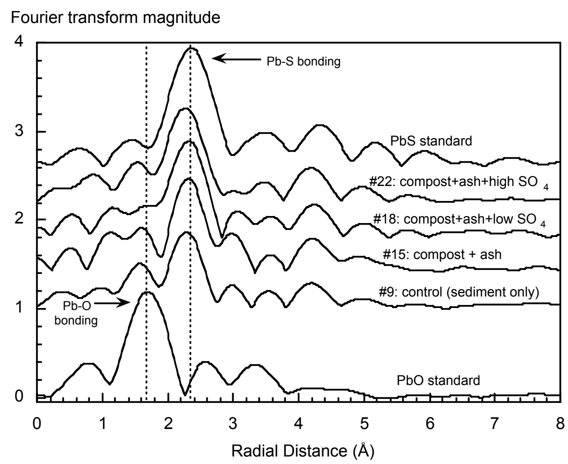

Fig. 1. Radial structure functions (RSFs) derived from Fourier transformation of lead L_{III}-EXAFS data (w=3) for contaminated sediment subjected to various treatments.
The Pb-S bonding apparent for all sediment samples indicates a dominance of Pb-sulfide.

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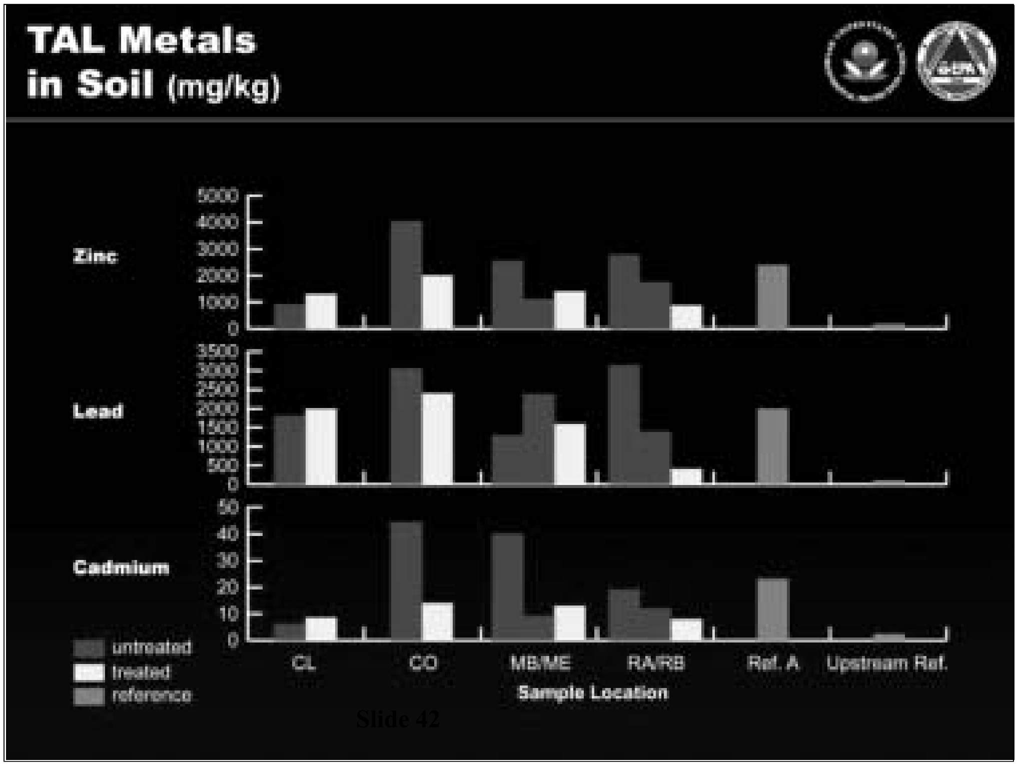
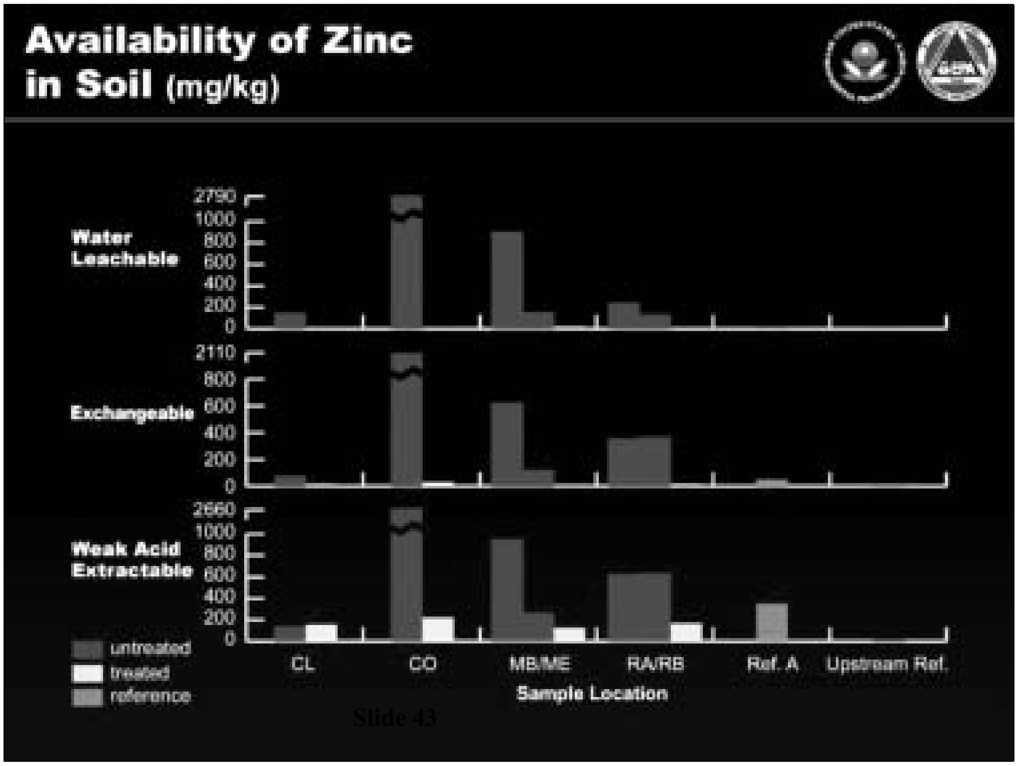


TAL Metals
in Soil (mg/kg)
Zinc
Lead
Cadmium
5000
4000
3000
2000
1000
0
3500
3000
2500
2000
1500
1000
500
0
50
40
30
20
10
0
untreated
treated
reference
CL
CO
MB/ME
RA/RB
Ref. A
Upstream Ref.
Sample Location
Slide 42


Availability of Zinc
in Soil (mg/kg)
Water
Leachable
2790
1000
800
600
400
200
0
Exchangeable
2110
800
600
400
200
0
Weak Acid
Extractable
2660
1000
800
600
400
200
0
untreated
treated
reference
CL
CO
MB/ME
RA/RB
Ref. A
Upstream Ref.
Sample Location

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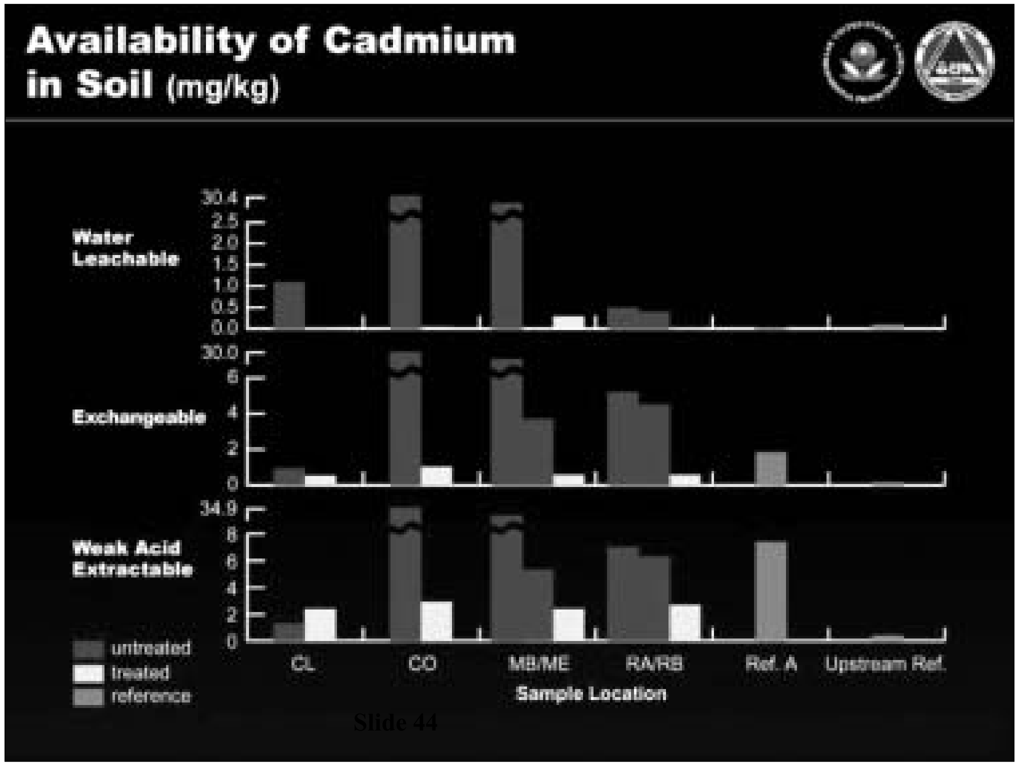
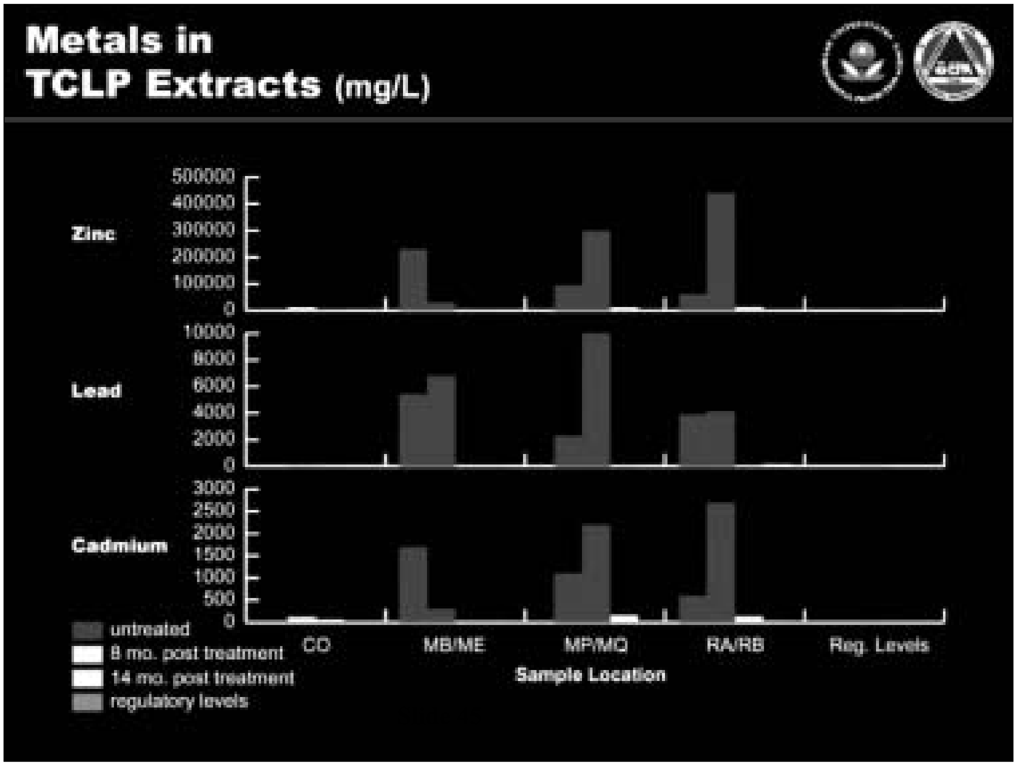
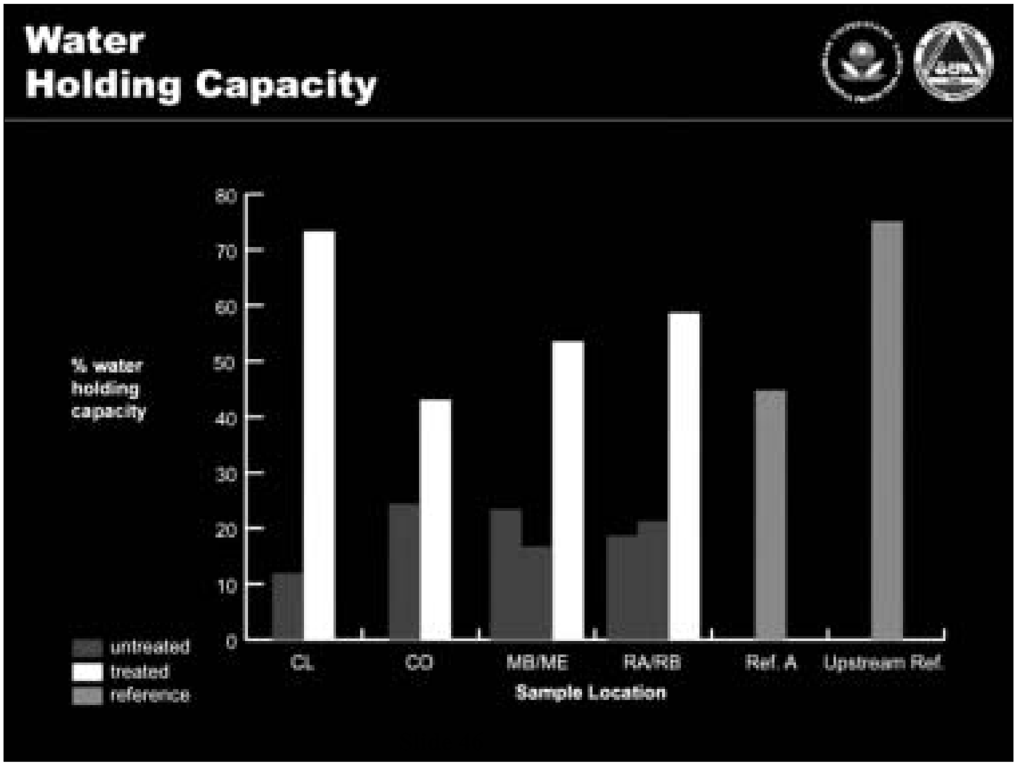
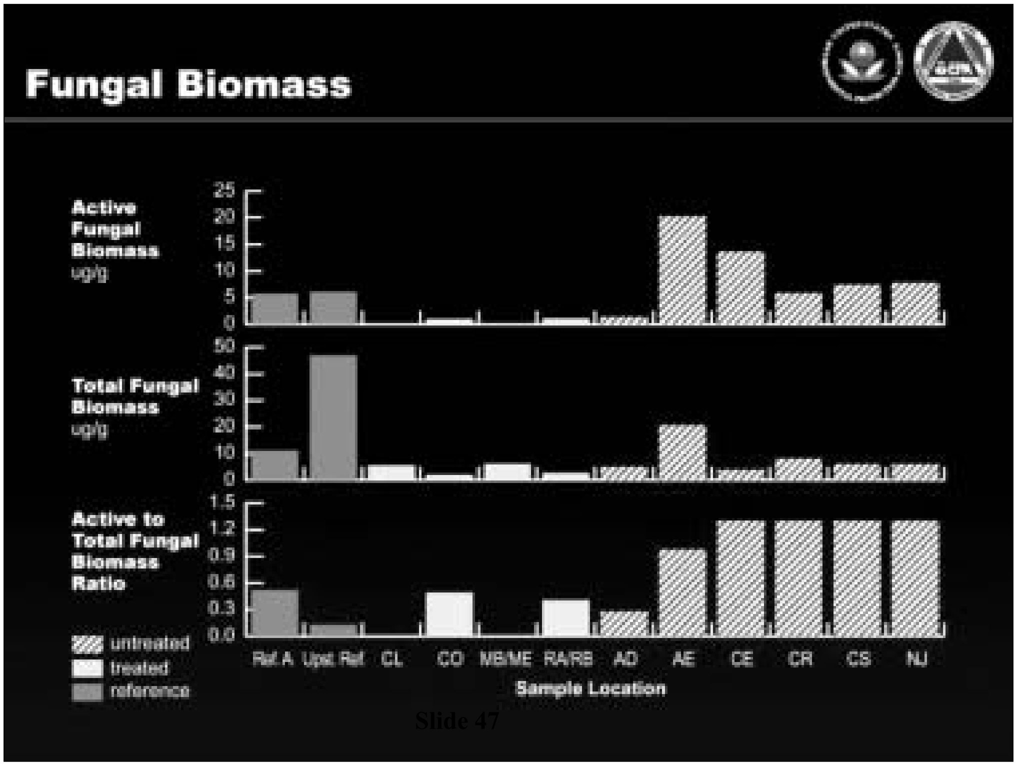


Availability of Cadmium in Soil (mg/kg)
Water Leachable
Exchangeable
Weak Acid Extractable
30.4
2.5
2.0
1.5
1.0
0.5
0.0
30.0
6
4
2
0
34.9
8
6
4
2
0
untreated
treated
reference
CL
CO
MB/ME
RA/RB
Ref. A
Upstream Ref.
Sample Location
Slide 44


Metals in
TCLP Extracts (mg/L)
Zinc
500000
400000
300000
200000
100000
0
Lead
10000
8000
6000
4000
2000
0
Cadmium
3000
2500
2000
1500
1000
500
0
untreated
8 mo. post treatment
14 mo. post treatment
regulatory levels
CO
MB/ME
MP/MQ
RA/RB
Reg. Levels
Sample Location


Water
Holding Capacity
% water holding capacity
80
70
60
50
40
30
20
10
0
untreated
treated
reference
CL
CO
MB/ME
RA/RB
Ref. A
Upstream Ref.
Sample Location


Fungal Biomass
Active Fungal Biomass ug/g
Total Fungal Biomass ug/g
Active to Total Fungal Biomass Ratio
untreated
treated
reference
Ref. A
Upst. Ref
CL
CO
MB/ME
RA/RB
AD
AE
CE
CR
CS
NJ
Sample Location

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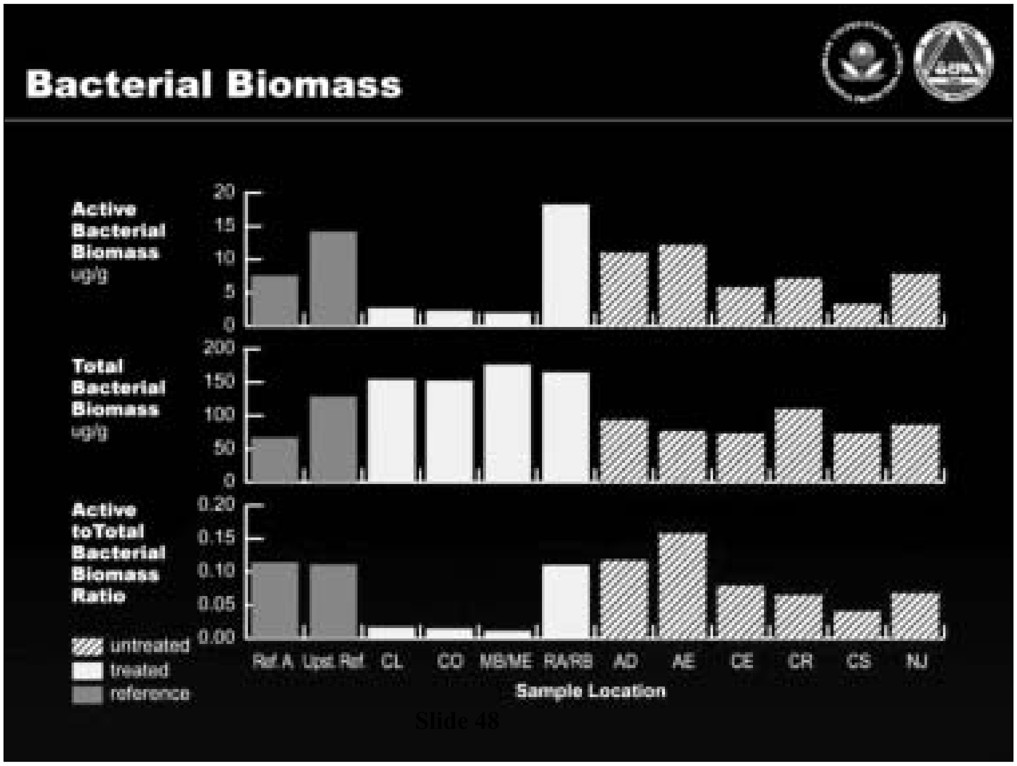
Bacterial Biomass
Active Bacterial Biomass ug/g
Total Bacterial Biomass ug/g
Active toTotal Bacterial Biomass Ratio
untreated
treated
reference
20
15
10
5
0
200
150
100
50
0
0.20
0.15
0.10
0.05
0.00
Ref. A
Upst. Ref.
CL
CO
MB/ME
RA/RB
AD
AE
CE
CR
CS
NJ
Sample Location

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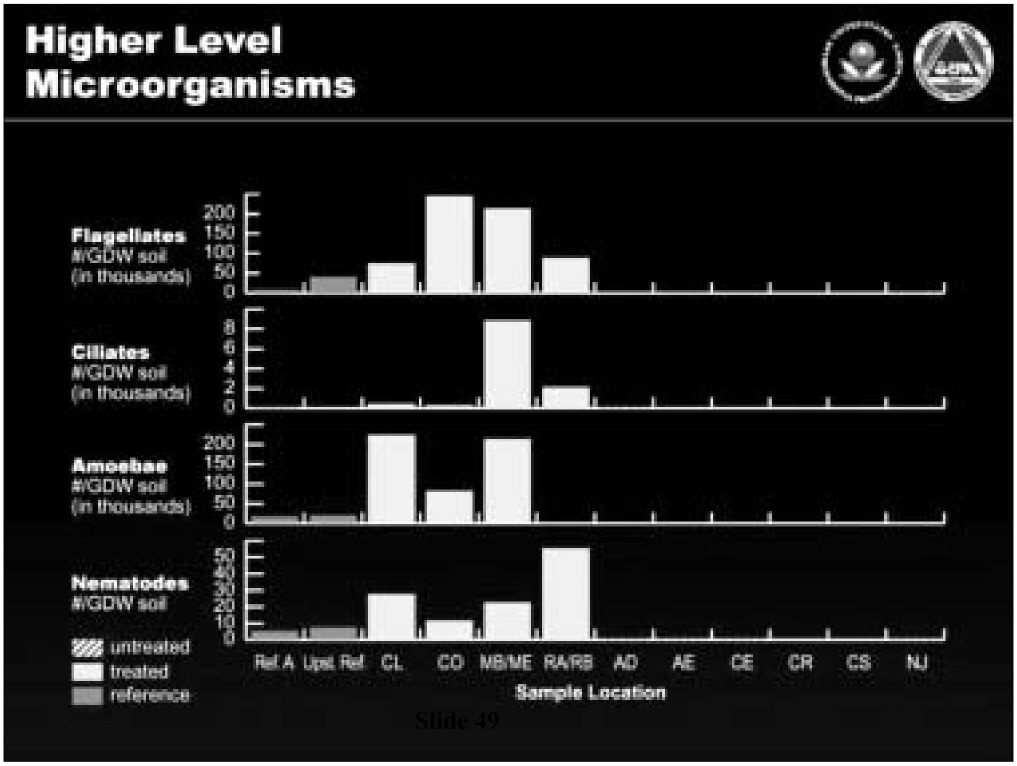
Higher Level Microorganisms
Flagellates
#/GDW soil
(in thousands)
Ciliates
#/GDW soil
(in thousands)
Amoebae
#/GDW soil
(in thousands)
Nematodes
#/GDW soil
untreated
treated
reference
Ref. A
Upst. Ref.
CL
CO
MB/ME
RA/RB
AD
AE
CE
CR
CS
NJ
Sample Location
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Ryegrass *(Lolium perenne)* Assays - Germination

Sample	Untreated (%)	Treated (%)
CL	0	85.7
CO	0	71.4*
MB/ME	-	100.0
RA/RB	-	90.5
Ref. A	-	95.2
Upst. Ref.	-	90.5
Lab Con.	85.7	95.2

* significantly < reference samples and/or control sample

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Earthworm *(Eisenia foetida)* Assays - Survivorship & Biomass/Organism

Sample	Untreated		Treated	
	Survival (%)	Biomass (mg)	Survival (%)	Biomass (mg)
CL	0	NA	100.0	329.3
CO	0	NA	98.9	323.0
MB/ME	0/0	NA	90.0	372.0
RA/RB	0/0	NA	10.0*	280.3
Ref. A	-	-	98.7	244.0
Upst. Ref.	-	-	96.7	196.0
Lab Con.	100	not measured	100.0	258.6

* significantly < reference samples and/or control sample

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Summary of Desorption

- Biosolids significantly increased hysteresis
  - For a site hystersis was related to rate of application
- The effect of biosolids application on hystersis was also apparent on the inorganic fraction of the samples
- Removal of organic carbon and the Fe/Mn fraction from the samples removed the difference caused by biosolids

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Summary Adsorption/Desorption

- **Biosolids increased the soils ability to adsorb and retain Cd**
- **These changes are apparent in the inorganic fraction of the samples**
- **Removal of organic carbon and the Fe/Mn fraction from the samples removes the difference caused by biosolids addition**
- **Thus the Fe/Mn fraction of the biosolids is an important component of the change in adsorption/desorption**

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Slide 55

Thank You

After viewing the links to additional resources, please complete our online feedback form.

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