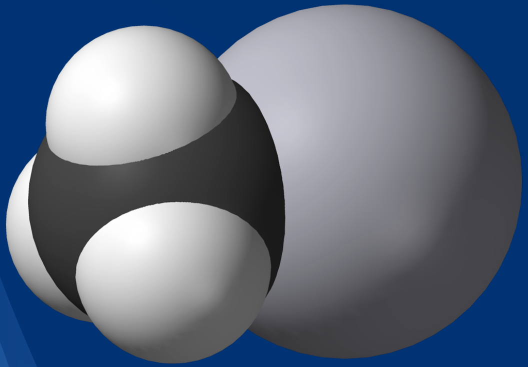
Mercury Compounds in Aquatic Systems and Their Relevance to Coal Production



Ryan Wicks 14 October 2014

Presentation Overview

- Background
  - Types and toxicity
  - Why Hg compounds are an emerging concern: bioaccumulation in seafood and rising ocean levels*
  - My specific interest: leachate containing Hg compounds
- Transformation by organisms: formation potential of organic and inorganic forms
  - Solubility and Transport
- Applications to forming models and solutions

Organic and Inorganic Forms

Types and Toxicity

Hg^0, Hg^{2+}: 0.002 µg/L ~ 2 ppb (water); kidney, renal, ocular damage, developmental damage

$(CH_3\text{-}Hg)^+$: ~1 ppm (food); severe neurological damage, developmental damage **acutely toxic**

Organic Mercury in Ocean Ecosystems

- Relatively high concentration in fish via “biomagnification”**
- Rising levels of mercury in ocean systems - anthropogenic origins, coal burning is a major source**
- 2004: U.S. FDA issue warning that pregnant women and children should restrict their consumption of certain kinds of fish: Shark, Swordfish, King Mackerel, Tilefish*

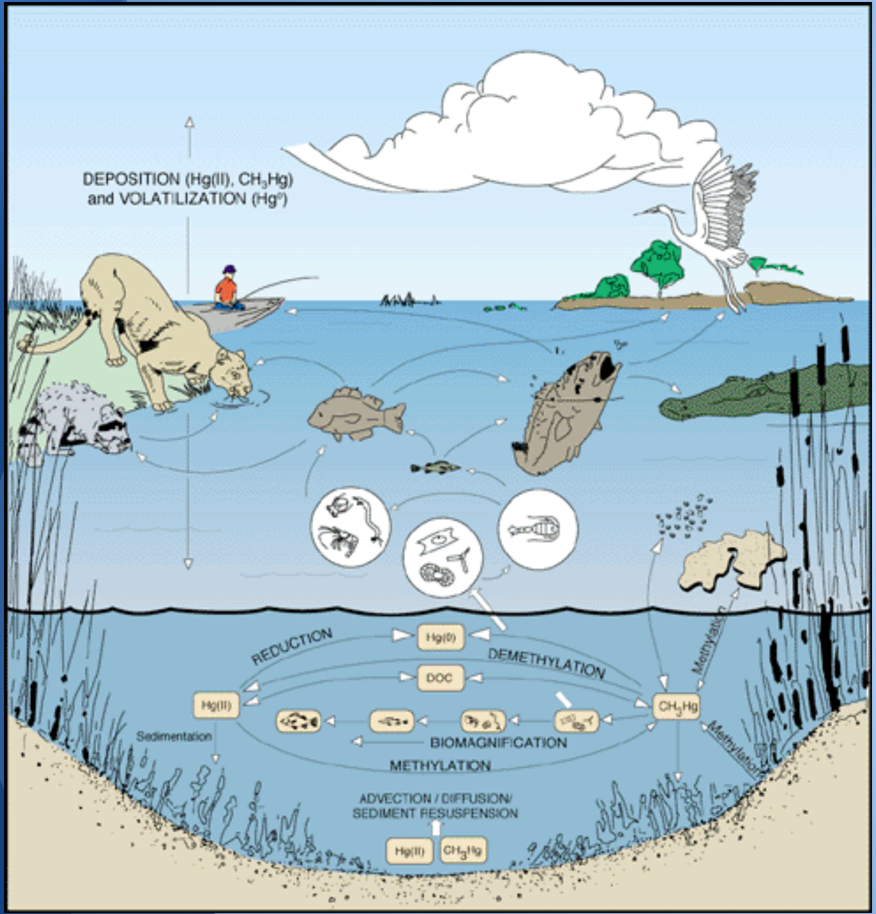
*http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2004/ucm108267.htm

Total Mercury Content from FDA Sampling

SPECIES	MERCURY CONCENTRATION MEAN (PPM)	MERCURY CONCENTRATION MEDIAN (PPM)	MERCURY CONCENTRATION STDEV (PPM)	MERCURY CONCENTRATION MIN (PPM)	MERCURY CONCENTRATION MAX (PPM)	NO. OF SAMPLES	SOURCE OF DATA
TILEFISH (Gulf of Mexico)	1.45	N/A	N/A	0.65	3.73	60	NMFS REPORT 1978
SWORDFISH	0.995	0.87	0.539	ND	3.22	636	FDA 1990-2010
SHARK	0.979	0.811	0.626	ND	4.54	356	FDA 1990-2007
MACKEREL KING	0.73	N/A	N/A	0.23	1.67	213	GULF OF MEXICO REPORT 2000
TUNA (FRESH/FROZEN, BIGEYE)	0.689	0.56	0.341	0.128	1.816	21	FDA 1991 - 2005
ORANGE ROUGHY	0.571	0.562	0.183	0.265	1.12	81	FDA 1991-2009

http://www.fda.gov/food/foodborneillnesscontaminants/metals/ucm115644.htm
http://www.fda.gov/Food/FoodborneIllnessContaminants/Metals/ucm191007.htm

Bioconcentration of Organic Mercury in Oceans



Methylation of Hg by bacteria

→ consumption by zooplankton

→ long biological half-life of Me-Hg and high uptake rates by zooplankton*

→ increased concentrations in tissue at higher levels in food-chain

More on the details later...

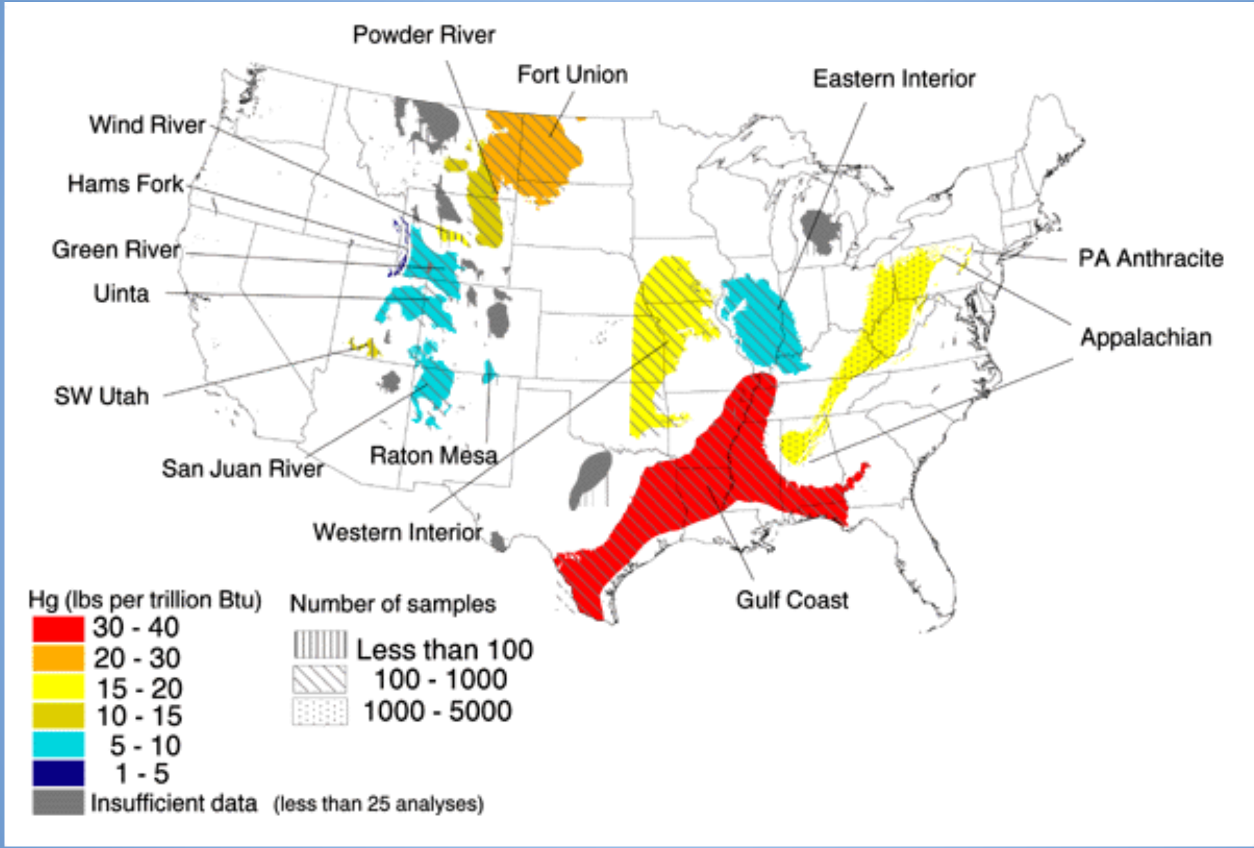
Image Credit: USDA. http://www.usgs.gov/themes/factsheet/146-00/. Mercury in the Environment: Fact Sheet. (October 2000)

Anthropogenic Sources of Hg

- Human activity has greatly contributed to Hg additions to the environment.*
- Coal-fired power plant emissions are a major contributor **
- As Dr. Jared Cohon noted, however, controls for coal-fired power plants are getting better (at least in the US) → **What about sources of Hg pollution from coal mining sites?** - more direct route of pollution of water supply systems

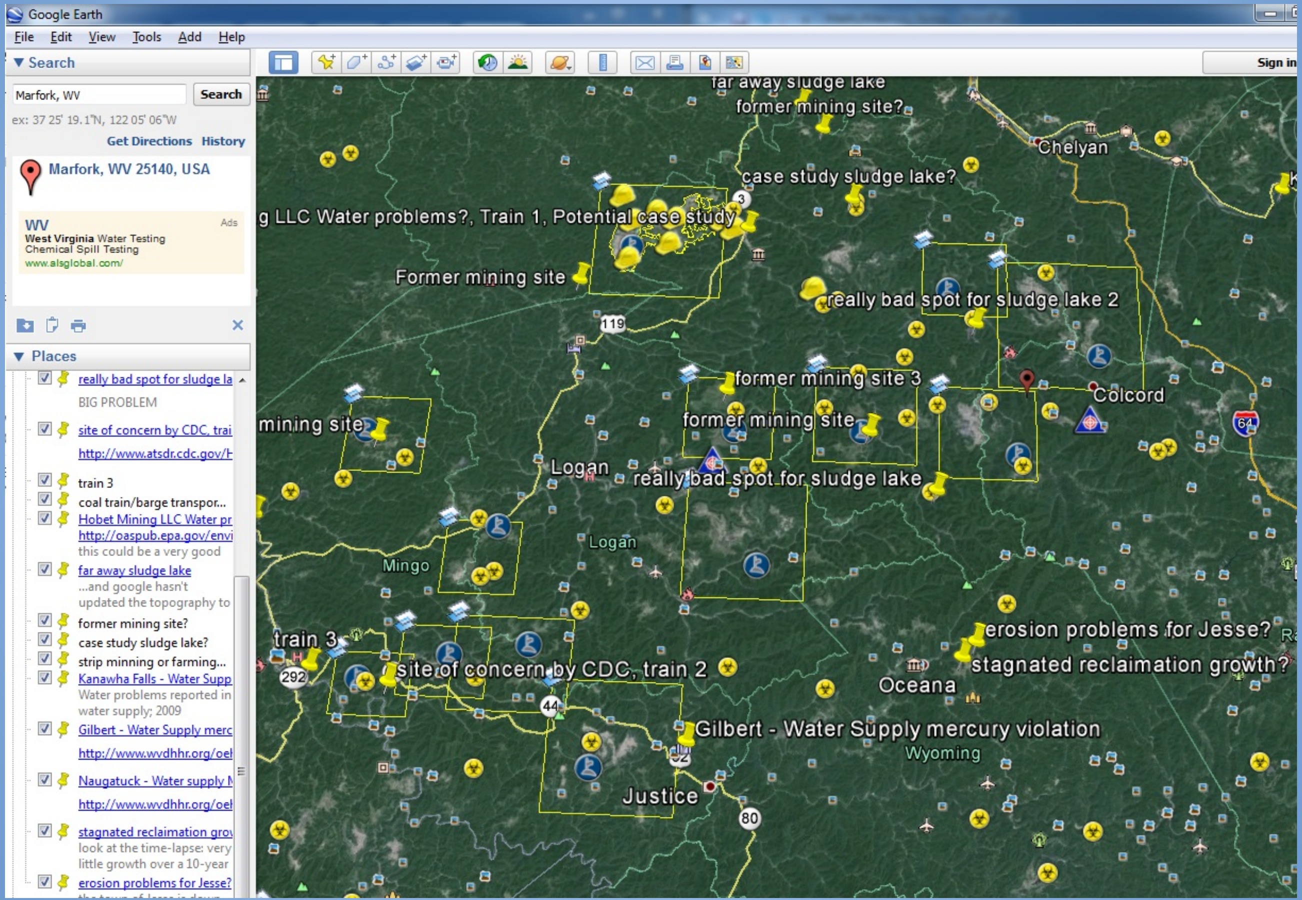
* United Nateions Environment Programme. Global Mercury Assessment 2013
** Northeast Statees for Coordinated Air Use Management. Mercury Emissions From Coal-Fired Power Plants

Hg Concentrations in Coal

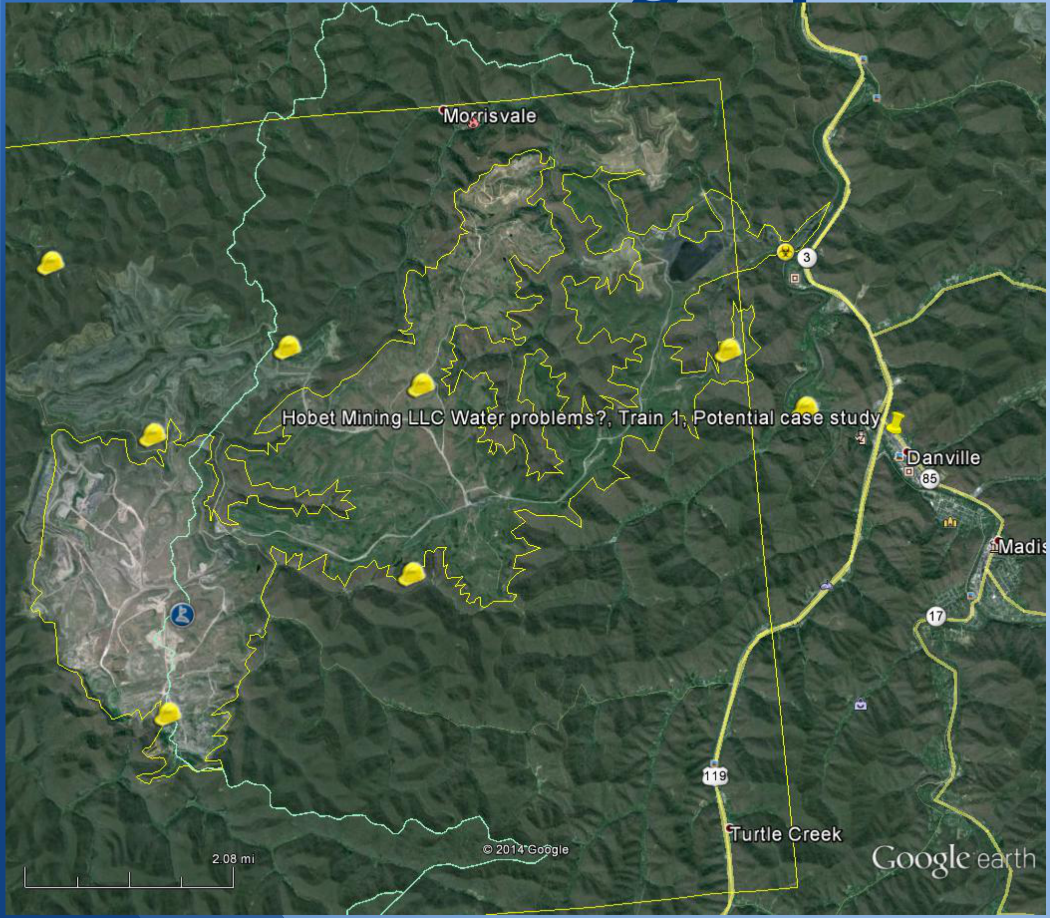


USGS, http://pubs.usgs.gov/of/1998/of98-772/map.htm

Google Earth GIS Shameless Plug



Coal Mining Operations in WV



- “Strip mining” or “mountain-top removal” strategy: destroy mountains with nitrogen-based explosive charges → filter and wash debris

see advocacy group: ilovemountains.org

Coal Mining Operations in WV

- Impoundments for coal slurry/sludge - effluent from coal washing process
- Most Impoundments are ponds formed by mountains and artificial dams of refuse material, but there are ground injection sites as well
- Impoundments generally have on the order of 10^9 gallons maximum capacity
- Treatment and controlled release management

Ohio Valley Environmental Coalition, Sludge Safety Project:
http://www.sludgesafety.org/photos?&page=2

"On February 26, 1972, a coal waste impoundment failed at Buffalo Creek, West Virginia resulting in the deaths of 125 people and leaving over 4,000 homeless. The area downstream of the impoundment was affected for a distance of over 15 miles. The failure occurred because of deficiencies in the design, construction, and inspection of the impounding structure."*

"On October 11, 2000, a coal waste impoundment broke into an underground coal mine in Martin County, Kentucky, releasing over 300 million gallons of slurry. Slurry poured into the mine and discharged from two mine portals, contaminating miles of creeks and rivers. Fortunately, no miners were in the mine at the time of the failure, and no one was physically injured downstream. However, aquatic life was killed, environmental damage occurred, and the water supplies for several communities were disrupted. The failure occurred because the barrier between the mine workings and the impoundment was inadequate."*

* Mine Safety and Health Administration. MSHA COAL MINE IMPOUNDMENT INSPECTION AND PLAN REVIEW HANDBOOK. October 2007

Personal Interest

US and global concern over both Hg has prompted a large amount of research regarding its use and physical distribution, thus making it an ideal case study to develop more robust environmental contamination models.

Questions to Answer:

Initial Questions:

1. **What are the permissible (MCL) of Mercury in water supplies? What are the toxicological effects?**
2. **What compounds and minerals are present in coal slurry/sludge and in what concentrations?**
3. **Is there currently any evidence of groundwater infiltration into water supplies?**

Quintessential Model-Specific Questions:

4. How water soluble is methyl mercury? Hg-II? Hg(0)?
5. What bacteria can take Mg 2+ --> methyl mercury in soils? (Methylation? Demethylation?)
6. How readily does methylmercury bind to soil minerals? Hg 2+? Hg-0?
7. How does pH effect solubility and formation potential of Me-Hg?
8. How can we create more accurate flood-routing models?

Remediation Questions:

9. What current remediation practices are available?
10. What organisms can demthylize Hg?

What are the permissible (MCL) of Mercury in water supplies? What are the toxicological effects?

EPA limits on drinking water supplies (http://water.epa.gov/drink/contaminants/index.cfm#one):

Contaminant	MCLG1(MG/L)2	MCL or TT1(MG/L)2	Potential Health Effects from Long-Term Exposure Above the MCL (unless specified as short-term)	Sources of Contaminant in Drinking Water
Mercury (inorganic)	0.002	0.002	Kidney damage	Erosion of natural deposits; discharge from refineries and factories; runoff from landfills and croplands

EPA does not track organic mercury compounds like Me-Hg in water supplies**

What compounds and minerals are present in coal slurry/sludge and in what concentrations?

Aken, Benoit. et al. Environmental Contaminants in Coal Slurry Intended for Underground Injection in the State of West Virginia. Journal of Environmental Engineering. August. 2014

Analysis of data from:

An Evaluation of the underground Injection of Coal Slurry in West Virginia. West virginia Department of Environmental Protection. Senate concurrent resolution - 15

Table 1. Analysis of Liquid Phases of Coal and Coal Slurry in Samples from Three Injection and Coal Preparation Sites in West Virginia: Southern Minerals, Loadout, and Panther

	Southern minerals			Loadout			Panther		
	SM slurry (liquid)		SM coal leachate	LL slurry (liquid)		LL coal leachate	PL slurry (liquid)		PL coal leachate
Contaminant	Dissolved	Total		Dissolved	Total	Dissolved	Dissolved	Total	Dissolved
Metals (mg/L)									
Aluminum[a]	**0.1950**	**0.651**	NA	**0.1500**	**2.37**	**0.0540**	**0.029**	**0.0460**	**0.398**
Antimony[a]	**0.0220**	**0.0215**	NA	0.0057	0.0059	0.0019	**0.0146**	**0.016**	0.0012
Arsenic[a]	0.0039	0.0043	NA	0.0042	0.0047	0.0041	**0.0104**	**0.0113**	**0.012**
Barium	0.0809	0.114	NA	0.0974	0.133	0.0055	0.243	0.269	0.0129
Beryllium	0.0002	0.0004	NA	ND	ND	ND	ND	ND	ND
Cadmium	ND	ND	NA	ND	ND	ND	ND	0.0011	ND
Calcium	51.4	51.7	NA	62.10	63.7	2.42	2.83	3.51	0.464
Chromium	0.0013	0.0016	NA	ND	ND	0.0013	0.0272	0.0342	ND
Cobalt	0.0021	0.0024	NA	ND	0.0016	ND	0.0142	0.0161	ND
Copper	0.0012	0.0018	NA	0.0016	0.0034	ND	0.0248	0.0278	ND
Iron[a]	ND	**0.91**	NA	ND	**0.828**	ND	0.068	0.089	ND
Lead[a]	ND	0.0008	NA	ND	0.0016	ND	**0.0762**	**0.0775**	ND
Magnesium	20.8	21	NA	19.8	20.6	0.705	0.591	0.771	ND
Manganese[a]	0.0141	0.0177	NA	**0.0860**	**0.097**	ND	0.021	0.028	ND
Mercury	ND	ND	NA	ND	ND	ND	ND	ND	ND
Molybdenum	0.0176	0.0178	NA	0.0447	0.0466	0.0090	0.198	0.217	ND
Nickel	0.0043	0.0052	NA	0.0067	0.0073	ND	0.0386	0.0432	ND
Potassium	6.90	7.07	NA	13.9	14.3	5.02	5.38	7.05	1.23
Selenium	0.0082	0.0082	NA	0.0268	0.0278	0.0195	0.0224	0.0255	0.0087
Silicon	3.3	3.76	NA	2.3	8.54	11.1	0.346	0.358	0.384
Silver	ND	ND	NA	ND	ND	0.0005	ND	ND	ND
Sodium	58.8	55.5	NA	265	267	4.88	266	341	10.1
Strontium	1.16	1.17	NA	1.44	1.47	0.0159	0.571	0.632	0.0222
Thallium	ND	0.0002	NA	0.0003	0.0004	ND	ND	ND	ND
Vanadium	0.0018	0.0021	NA	0.0013	0.0025	0.0044	0.0103	0.0131	0.007
Zinc	0.016	0.027	NA	ND	0.008	0.008	0.019	0.014	ND
General chemistry (mg/L)									
Nitrogen, nitrate	0.45	—	NA	1.85	—	0.07	0.59	—	0.03
Nitrogen, nitrite[a]	**2.32**	—	NA	0.35	—	0.17	ND	—	ND
Chloride[a]	0.18	—	NA	84.80	—	1.45	**423.00**	—	7.12
Fluoride[a]	**8.39**	—	NA	ND	—	0.55	1.53	—	0.51
Sulfate[a]	157.00	—	NA	**849.00**	—	7.40	**261.00**	—	2.60
Nitrogen, ammonia	0.18	—	NA	1.27	—	0.34	1.96	—	0.44
Specific conductance[b]	702.00	—	NA	1,840	—	57.20	5,000.00	—	170.00
Total dissolved solids[a]	423.00	—	NA	**933.00**	—	21.00	**2,540.00**	—	87.00
Total suspended solids	5,440.00	—	NA	191.00	—	1.00	74.00	—	6.00
Acidity, total	6.80	—	NA	6.90	—	ND	ND	—	ND
Alkalinity, bicarbonate	180.00	—	NA	102.00	—	25.50	412.00	—	42.00
Alkalinity, carbonate	1.40	—	NA	ND	—	6.00	7.10	—	14.30
Alkalinity, total	181.00	—	NA	103.00	—	32.70	420.00	—	58.20
pH[a, b]	7.93	—	NA	7.88	—	**9.40**	8.26	—	**9.56**

Volatile organic compounds (μg/L)									
2-Butanone	ND	—	NA	ND	—	ND	68.4	—	ND
Acetone	ND	—	NA	ND	—	ND	16.7	—	9.9
Acrolein	ND	—	NA	7	—	14.8	ND	—	ND
Benzene	ND	—	NA	ND	—	ND	1.8	—	1.6
m,p-Xylene	ND	—	NA	ND	—	ND	0.8	—	0.4
Methylene chloride	ND	—	NA	1.4	—	1.0	ND	—	ND
o-Xylene	ND	—	NA	ND	—	ND	0.6	—	0.3
Toluene	ND	—	NA	0.6	—	0.7	2.8	—	2.1
Semivolatile organic compounds (mg/L)									
Bis(2-ethylhexyl) phthalate	ND	—	NA	ND	—	ND	ND	—	ND
Naphthalene	ND	—	NA	0.0143	—	ND	ND	—	ND
Phenanthrene	ND	—	NA	0.061	—	ND	ND	—	ND
Miscellaneous (mg/L)									
TPH (diesel range)	ND	—	NA	16.60	—	ND	0.51	—	ND
TPH (oil range)	ND	—	NA	19.40	—	ND	ND	—	ND
Sulfate-reducing bacteria[b]	NA	—	NA	NA	—	ND	ND	—	ND

Note: ND = not detected; NA = not analyzed.
[a]Analytes detected at least once at or above one environmental guideline comparison value are marked in bold.
[b]The specific conductance is given in mS/cm, the pH is given in SU, and the bacterial numbers are given in CFU/mL.

Metals: Metals in the samples were determined by inductively coupled plasma–mass spectrometry (ICP-MS) according to U.S. EPA (NERL) Method 200.8 (revision 5.4, 1994) and inductively coupled plasma–atomic emission spectrometry (ICP-AES) according to U.S. EPA (NERL) Method 200.7 (revision 4.4, 1994). The methods are applicable to the determination of a wide variety of dissolved elements in groundwater, surface waters, drinking water, wastewaters, sludges, and soils samples. For the analysis of trace metals in the solid fractions of the coal slurry and raw coal, the samples were subjected to acid digestion according to U.S. EPA method 3050 B.

Is there currently any evidence of groundwater infiltration into water supplies?

- Sites chosen because of differences in duration of time ground injection sites had been in use and variation in mining activity so as to provide comparisons
- Data only collected over 1 year period
- lack of MSDS on materials used in coal preparation plants made sampling difficult
- **Virtually no Hg detected**
- Note the low pH at all sampled sites
- Unable to establish causal effect of ground injection on groundwater supplies

Table 2. Analysis of Liquid Phases of Coal and Coal Slurry in Samples from Three Injection and Coal Preparation Sites in West Virginia: Power Mountain, Coresco, and Marfork

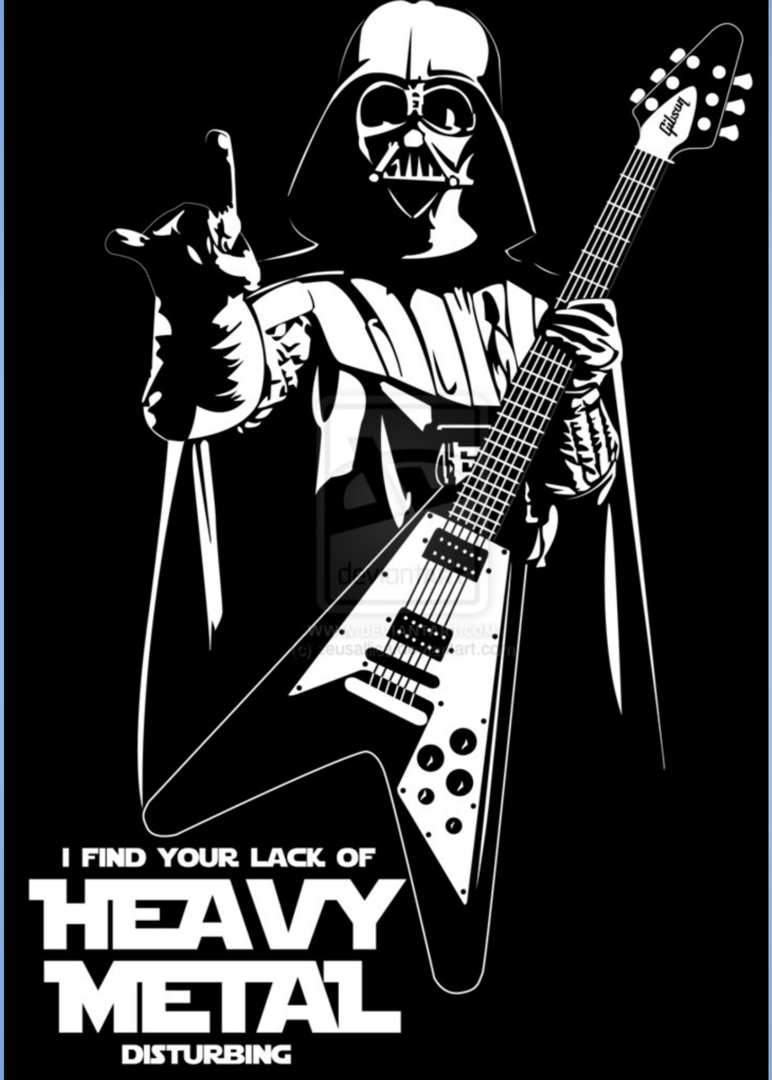
	Power mountain			Coresco			Marfork		
	PM slurry (liquid)		PM coal leachate	CL Slurry (liquid)		CL coal leachate	MF slurry (liquid)	MF coal leachate	
Contaminant	Dissolved	Total	Dissolved	Dissolved	Total	Dissolved		Dissolved	Total
Metals (mg/L)									
Aluminum[a]	**0.509**	**0.564**	**0.214**	**0.532**	**0.644**	**0.356**	NA	**0.146**	**1.190**
Antimony[a]	0.0004	0.0005	0.0018	**0.0069**	**0.0071**	0.0005	NA	0.0015	0.0011
Arsenic[a]	ND	ND	**0.0141**	ND	ND	0.0019	NA	0.0198	**0.246**
Barium	0.0523	0.0634	0.0079	0.0677	0.0713	0.0047	NA	0.0227	0.695
Beryllium	ND	ND	ND	ND	ND	ND	NA	ND	0.002
Cadmium	ND	ND	ND	ND	ND	ND	NA	ND	ND
Calcium	124.00	123.00	0.552	111	115	4.820	NA	0.2840	1.260
Chromium	ND	ND	ND	ND	ND	ND	NA	ND	0.0054
Cobalt	0.0037	0.0039	ND	0.0027	0.0029	ND	NA	ND	0.0067
Copper	0.0015	0.0016	ND	0.0021	0.0021	ND	NA	ND	0.0248
Iron[a]	0.030	0.195	0.038	ND	0.174	0.022	NA	0.050	**13.200**
Lead[a]	ND	0.0004	0.0004	ND	ND	ND	NA	0.0003	**0.2170**
Magnesium	81.40	82.20	ND	38.90	40.00	0.29	NA	ND	2.21
Manganese[a]	**0.921**	**0.921**	ND	**0.133**	**0.138**	ND	NA	0.001	**0.142**
Mercury	ND	ND	ND	ND	ND	ND	NA	ND	ND
Molybdenum	0.0023	0.0024	0.0035	0.0290	0.0297	0.0020	NA	0.0029	0.0021
Nickel	0.0092	0.0096	ND	0.0073	0.0074	ND	NA	ND	0.011
Potassium	15.50	15.50	0.380	5.01	5.16	1.080	NA	0.321	0.925
Selenium	0.0057	0.0059	0.0082	0.0024	0.0024	0.0019	NA	0.0043	0.0040
Silicon	3.27	5.31	7.59	1.14	3.91	0.43	NA	13.20	71.00
Silver	0.0006	0.0006	ND	ND	ND	ND	NA	ND	ND
Sodium	236.0	237.0	75.5	272.0	279.0	12.6	NA	48.1	6.7
Strontium	1.63	1.74	0.0043	31.9	3.27	0.16	NA	0.115	0.135
Thallium	0.0002	0.0003	ND	ND	0.0002	ND	NA	0.0002	0.0004
Vanadium	ND	ND	0.0052	ND	ND	0.0015	NA	0.0031	ND
Zinc	0.032	0.041	ND	ND	ND	0.003	NA	ND	0.038
General chemistry (mg/L)									
Nitrogen, nitrate	3.45	—	ND	0.83	—	ND	NA	ND	—
Nitrogen, nitrite[a]	ND	—	0.14	0.16	—	ND	NA	0.10	—
Chloride[a]	77.10	—	1.71	32.80	—	0.60	NA	1.43	—
Fluoride[a]	0.56	—	0.42	ND	—	ND	NA	0.31	—
Sulfate[a]	**853.00**	—	3.44	**1,110.00**	—	14.00	NA	4.55	—
Nitrogen, ammonia	1.16	—	0.35	0.72	—	0.14	NA	0.10	—
Specific conductance[b]	2,110	—	100	ND	—	ND	NA	86.7	—
Total dissolved solids[a]	**1,470**	—	21	**1,340**	—	51	NA	15.0	—
Total suspended solids	9	—	1	22	—	1	NA	1	—
Acidity, total	8.7	—	ND	5.4	—	ND	NA	ND	—
Alkalinity, bicarbonate	146.0	—	34.3	143.0	—	32.1	NA	23.2	—
Alkalinity, carbonate	ND	—	10.0	ND	—	6.8	NA	6.0	—
Alkalinity, total	147.0	—	45.8	144.0	—	40.0	NA	30.6	—
pH[ab]	7.75	—	**9.49**	7.71	—	**9.35**	NA	**9.44**	—

Volatile organic compounds ($\mu g/L$)									
2-Butanone	ND	—	ND	ND	—	ND	NA	ND	—
Acetone	ND	—	ND	ND	—	ND	NA	ND	—
Acrolein	ND	—	ND	ND	—	ND	NA	ND	—
Benzene	ND		ND	ND	—	ND	NA	ND	—
m,p-Xylene	ND	—	0.4	ND	—	ND	NA	ND	—
Methylene chloride	ND	—	ND	ND	—	ND	NA	ND	—
o-Xylene	ND	—	0.3	ND	—	ND	NA	ND	—
Toluene	ND	—	1.9	ND	—	ND	NA	0.2	—
Semivolatile organic compounds (mg/L)									
Bis(2-ethylhexyl)phthalate	ND	—	0.0091	ND	—	ND	NA	0.0108	—
Naphthalene	ND	—	ND	ND	—	ND	NA	ND	—
Phenanthrene	ND	—	ND	ND	—	ND	NA	ND	—
Miscellaneous (mg/L)									
TPH (diesel range)	0.26	—	ND	ND	—	ND	NA	ND	—
TPH (oil range)	ND	—	ND	ND	—	ND	NA	ND	—
Sulfate-reducing bacteria[b]	NA	—	NA	NA	—	5,500	NA	NA	—

Note: ND = not detected; NA = not analyzed.

[a]Analytes detected at least once at or above one environmental guideline comparison value are marked in bold.

[b]The specific conductance is given in mS/cm, the pH is given in SU, and the bacterial numbers are given in CFU/mL.


I FIND YOUR LACK OF
HEAVY
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MADISON, WV 25130

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Safe Drinking Water Information System (SDWIS)

- Operated by the EPA
- Violations are frequently "Monitoring, Regular" (MR)
- Very few instances, if any, of MLC violations

"**NOTICE:** EPA is aware of inaccuracies and underreporting of some data in the Safe Drinking Water Information System. We are working with the states to improve the quality of the data."

Type of Violation	Compliance Period Begin Date	Compliance Period End Date	Drinking Water Rule or Contaminant	Violation ID
PN Violation for NPDWR Violation	JAN-20-2005	JUL-26-2012	Public Notice	193105

Follow-up Action	Date of Response
St Compliance achieved	JUL-26-2012
St AO (w/o penalty) issued	OCT-07-2008
St Formal NOV issued	FEB-05-2005

Type of Violation	Compliance Period Begin Date	Compliance Period End Date	Drinking Water Rule or Contaminant	Violation ID
Monitoring, Regular	JAN-01-2005	DEC-31-2007	Arsenic	194509

Follow-up Action	Date of Response
St AO (w/o penalty) issued	OCT-07-2008
St Compliance achieved	AUG-20-2008
St Public Notif received	FEB-20-2008
St Formal NOV issued	FEB-02-2008
St Public Notif requested	FEB-02-2008

Type of Violation	Compliance Period Begin Date	Compliance Period End Date	Drinking Water Rule or Contaminant	Violation ID
Monitoring, Regular	JAN-01-2005	DEC-31-2007	1,2,4-Trichlorobenzene	194510

Let’s consider the what could be happening in theory….

Formation Potential - Microbial Processes

- Anaerobic microbes have the greatest potential for the methylation of Hg, especially those with metabolic pathways for sulfates.*
- decreased pH increases formation potential of Me-Hg*
- increased DOC increases formation potential of Me-Hg*

"...insoluble mercuric sulfide (HgS) will be methylated in aerobic sediments at rates 100 to 1,000 times slower than for the less strongly bound $HgCl_2$ (Olson and Cooper 1976)."*

- A sulfate concentration of 200-500 µM in the water column is optimal for mercury methylation by SRB in sediment (Gilmour and Henry 1991).
- Humic and Fulvic acid interactions → Sulphur group of Humic substances bound to Hg, but released at low pH

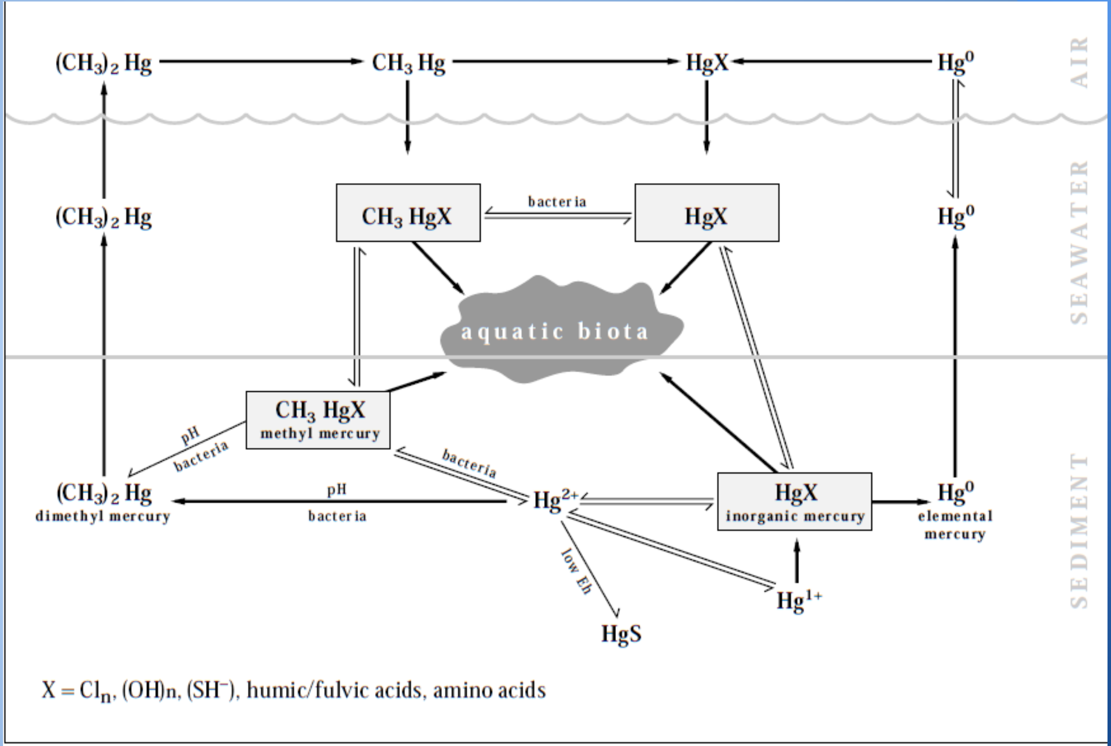
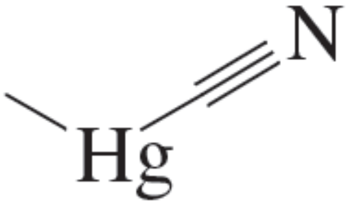


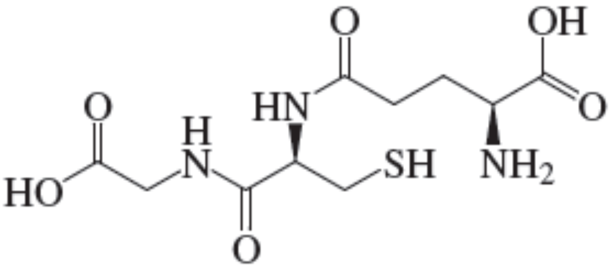
Figure 1. Important pathways of mercury speciation in the aquatic environment.

*Contaminants in Aquatic Habitats at Hazardous Waste Sites: Mercury. NOAA Technical Memorandum ORCA 100. December. 1996

Example Methylmercury Compounds



Cyanomethylmercury



Glutathione

A potential methylmercury compound

Very important transporter for plants; sulfur group particularly important for Hg binding affinity

- David R. Lide, ed., CRC Handbook of Chemistry and Physics, Internet Version 2005, <http://www.hbcpnetbase.com>, CRC Press, Boca Raton, FL, 2005.

More Common Examples in Aquatic Systems:

$$CH_3HgOH \longleftrightarrow CH_3HgCl$$

Salinity: Dependence on Cl^- concentration

Nonetheless, the most important factor in methylation of Hg is presence and reactivity of microbes

M. Ranchou-Peyruse et al Overview of Mercury Methylation Capacities among Anaerobic Bacteria Including Representatives of the Sulphate-Reducers: Implications for Environmental Studies. Geomicrobiology Journal, 26: 1-8, 2009. Taylor and Francis Group

Abstract:

- Methylation only by delta-Proteobacteria
- Taxonomy/phylogeny does not predict methylation potential (16s rRNA analysis)

Introduction:

- Acetyl-CoA pathway is a primary method of methylation
- Complete mechanistic descriptions of methylation still not understood
- Prior studies have varying initial concentrations of Hg^{2+} → this study sought to observe methylation at low initial concentration: 10 µg * L^{-1}

TABLE 1
List of the strains tested, protein production and number of divisions during the mercury methylation experiments.

Strain	Name	Protein production (mg. L^{-1})	n
DSM 2032	*Desulfobulbus propionicus* strain 1 pr3	40.7	5.0
DSM 2603	*Desulfovibrio africanus*	15.8	4.1
ADR 13	*Desulfovibrio africanus*	21.9	2.5
DSM 6949	*Desulfovibrio desulfuricans* subsp. *desulfuricans*	18.4	2.1
DSM 644	*Desulfovibrio vulgaris* strain Hildenborough	14.1	3.1
BEROc 1	*Desulfovibrio caledoniensis*	15.1	1.6
DSM 10707	*Desulfomicrobium escambiense*	18.3	2.6
ADR 19	*Desulfomicrobium* sp.	12.3	1.9
ADR 21	*Desulfomicrobium salsuginis*	15.4	2.3
ADR 28	*Desulfomicrobium salsuginis*	18.2	4.5
DSM 3379	*Desulfobacter curvatus*	30.3	3.1
DSM 771	*Desulfotomaculum acetoxidans*	2.3	1.3
DSM 574	*Desulfotomaculum nigriFicans*	1.3	0.8
ADR 30	*Desulfosporosinus* sp.	9.8	2.3
DSM 525	*Clostridium pasteurianum*	7.2	1.6
ADR 31	*Clostridium* sp.	6.3	2.3
DSM 10017	*Syntrophobacter fumaroxidans*	4.0	1.6

TABLE 2

Comparison of the mercury methylation capacities determined for two reference strains *Desulfobulbus propionicus* (DSMZ 2032) and *Desulfovibrio africanus* (DSMZ 2603).

Strain	Hg^{2+} added ($\mu g \cdot L^{-1}$)	Methylation rate ($ng \cdot L^{-1} \cdot h^{-1}$)	Methylation yield ($\mu g. mg^{-1}$ of protein)	Reference
Desulfobulbus propionicus (1 pr3 / DSM 2032)	1	10.6 ± 1.5		Ekstrom *et al.*, 2003
	10	86.04 ± 4.72	0.104 ± 0.001	This study
	100	1.05 ± 30.4		King *et al.*, 2000
Desulfovibrio africanus (DSM 2603)	1	12.9 ± 5.6		Ekstrom *et al.*, 2003
	10	22.81 ± 2.50	0.072 ± 0.007	This study

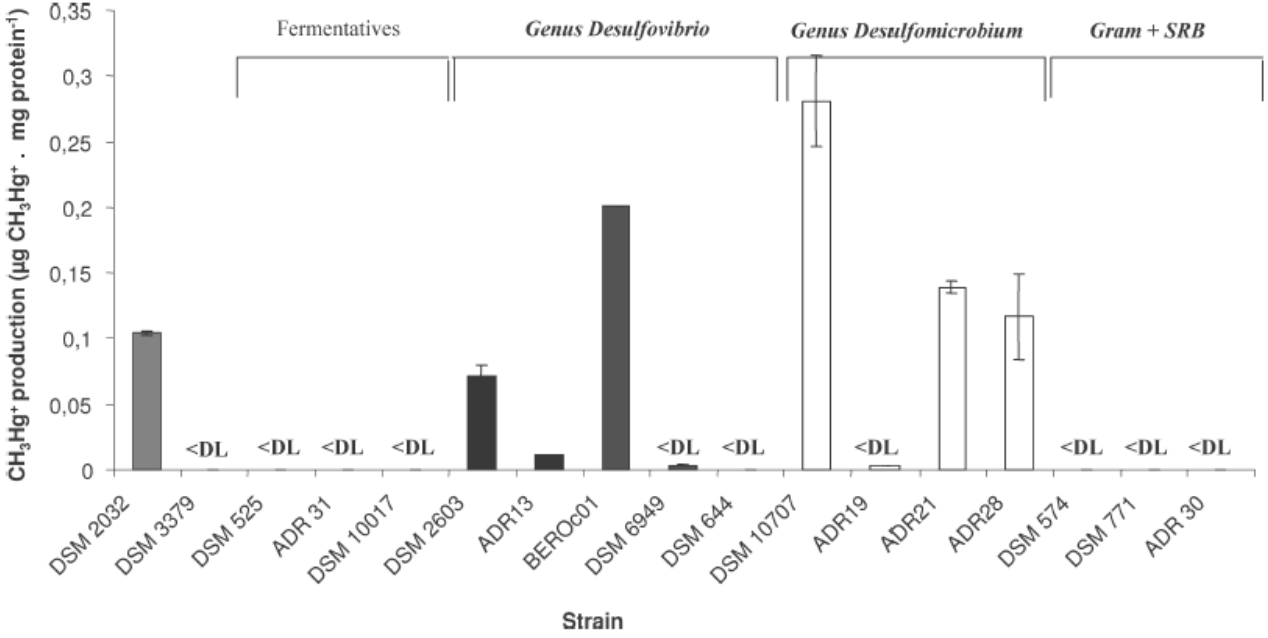


FIG. 1. Mercury methylation specific activities determined for the strains tested. The strains are grouped according to their metabolic or taxonomic affiliation. Strains DSMZ 2032 (*Desulfobulbus propionicus*) and DSMZ 3379 (*Desulfobacter curvatus*) were the positive and negative controls, respectively.

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Results and Discussion

- Rxn rates of methylation varied from prior studies; it should be noted that prior studies used initial ionic Hg concentrations which were 100-10,000 times higher
- Methylation is “strain dependent,” NOT species or genus dependent

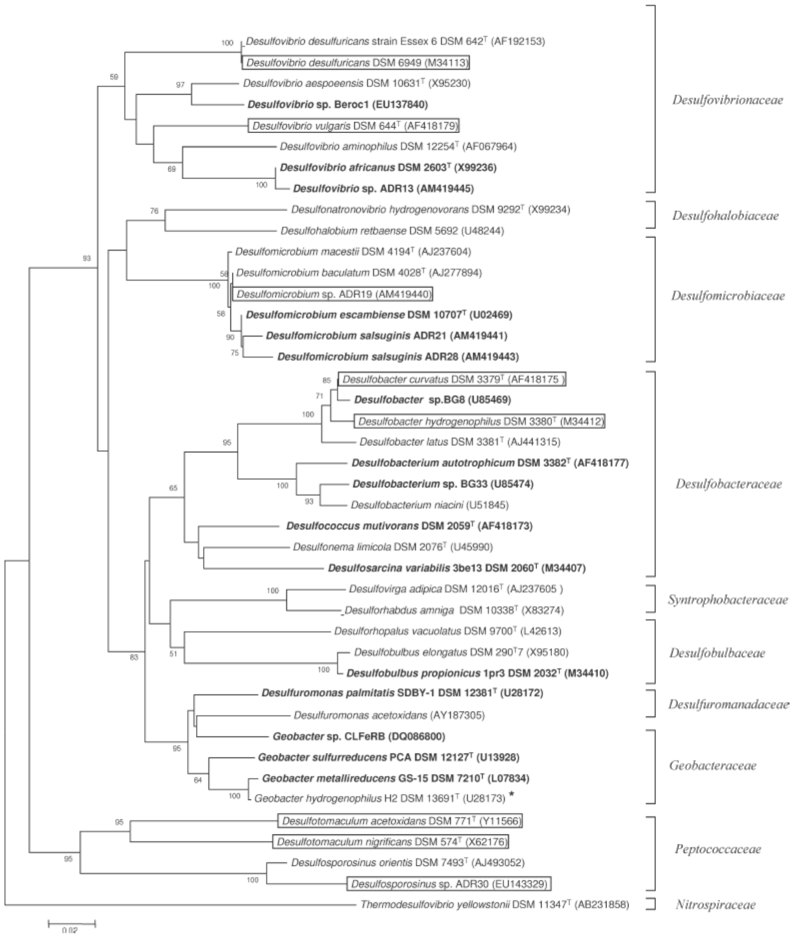
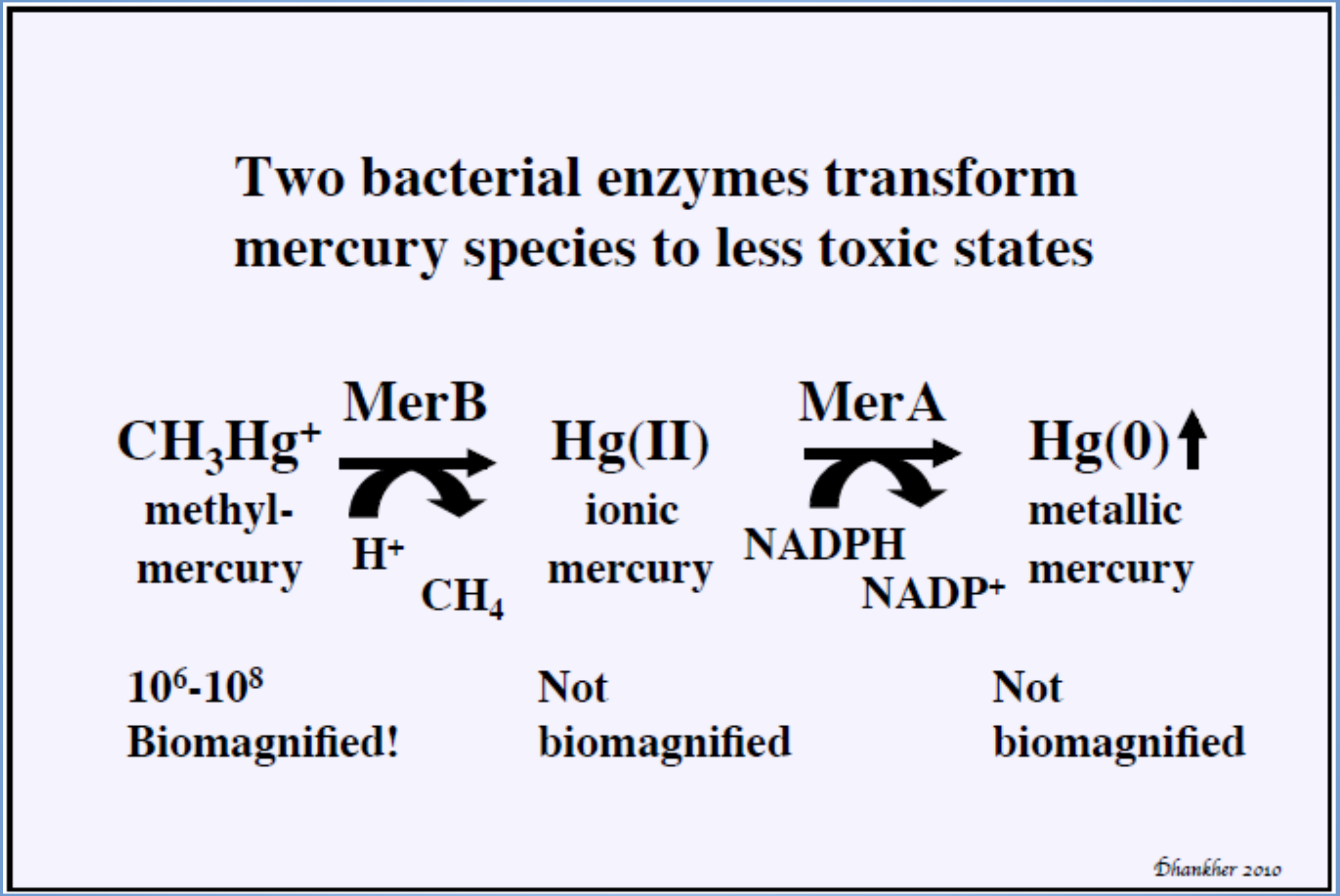


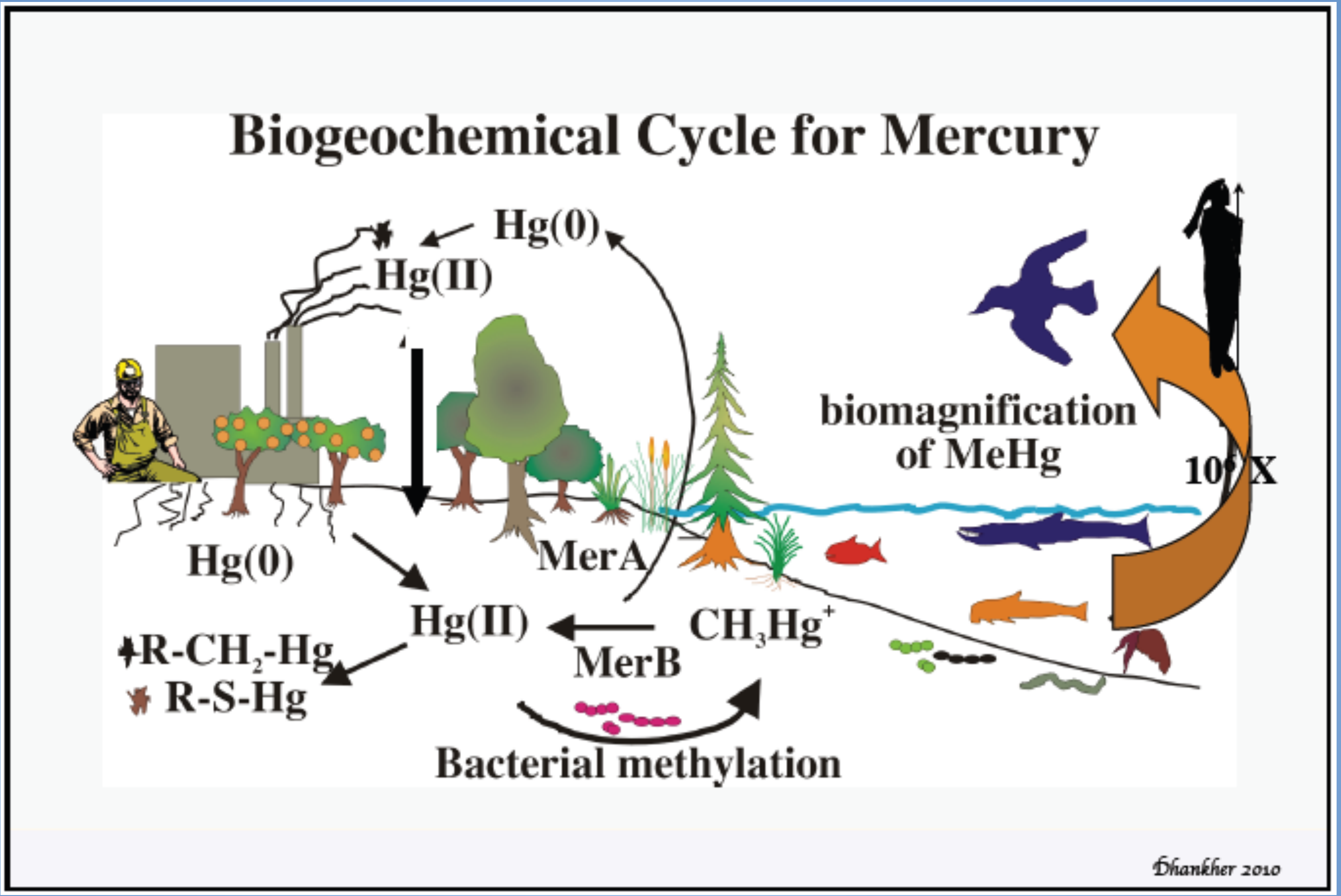
FIG. 2. Phylogenetic tree based on the 16S rRNA gene showing the position of available sequences of strains tested for their mercury methylation capacities within the delta-Proteobacteria and the Clostridia classes. Tree was generated using the neighbour-joining analysis. All accession numbers are indicated. White rectangle: no mercury methylation capacity. Bold typed: mercury methylation capacity. *: These strains were indicated as mercury methylators in Kerin et al. (2006). Nevertheless, methylation levels were extremely low and similar to some of the controls.

Transformation by Organisms - Vice Versa

- Mercurial lyase (encoded by MerB gene): transforms methylmercury (CH_3)-$(Hg)^+$ --> Hg^{+2}; forms by moving e^-'s from Mercury to methyl group to form CH_4
- Mercurial reductase (encoded by MerA gene): reduces $Hg(^{+2})$ --> to Hg^0; forms by oxidizing NADPH --> $NADP^+$
- bacteria with “mer operon”



Credit: Dr. Om Parkash



Credit: Dr. Om Parkash

Transformation by Organisms

Examples:

- Hg^0 --> Hg^{2+} + $2e^-$: non-enzymatic, Bacillus, Pseudomonas
- Hg^{2+} + $2e^-$ --> Hg^0 : detoxification, Pseudomonas, Streptomyces ; Bacillus, Vibro; Alcaligenes, Acinetobacter (Based on Hg resistance)

Eldor A. Paul. Soil Microbiology, Ecology, and Biochemistry. 3rd edition

Organic and Inorganic Forms

Solubility

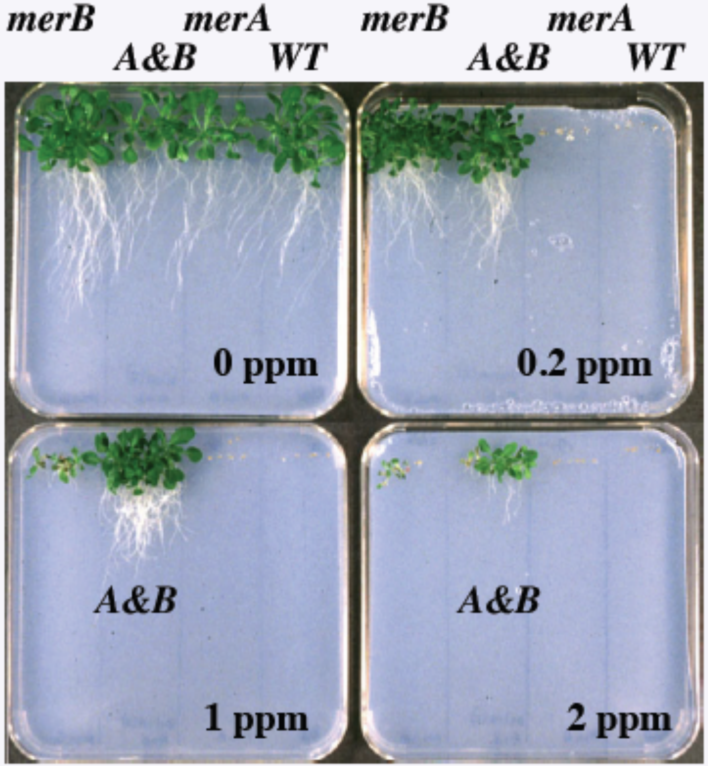
- Hg has a strong binding affinity for Selenium and Sulfur (e.g. glutathione in plants - active sulfur group for transport to cell vacuole)
- FeS_2 (pyrite) commonly found in coal formations
- Note that all sampled ground injection sites had a pH range [7.7, 9.6]*
- low pH necessary to mobilize bound mercury
- soils naturally neutral or acidic (as low as 5.4 pH typical)

*Aken, Benoit. et al. Environmental Contaminants in Coal Slurry Intended for Underground Injection in the State of West Virginia. Journal of Environmental Engineering. August. 2014

Phyto/Bioremediation

- glutathione (GSH) + Hg^{+2} --> GS-Hg (stored in vacuoles); thus if we increase glutathione expression (GSH synthetase, gamma-glutamylcysteine synthetase, and phytochelatin synthase) more mercury will be stored in vacuoles and cause less damage to hyper-accumulating plant.

Phyto/Bioremediation



Co-expression of *merA & merB* produces the highest levels of methylmercury resistance and processing!

Dhankher 2010

Credit: Dr. Om Parkash

Applications to Forming Models and Solutions

- How do we model baseflow/groundwater flow in these regions (West Virginia, Kentucky)? Especially after mountaintop removal alters flood routing and fundamental hydrology of a watershed
- Characterizing the soil and subsoil conditions is critical to predict potential water source contamination
- Understanding metabolic pathways of methylation and demethylation, as well as reaction rates of each, and influencing factors is essential. The primary transformations of Hg are conducted by microbes.

Applications to forming models and solutions - Remediation Strategies

- What plants are currently required and/or being used for mountain-top reclamation?
- How are these plants doing?
- What are soil conditions like when the mining is done and reclamation begins?
- How do these plants modify the soil over time?

- To next lecture