Investigation of New Habitats for Mercury Methylation
- case studies at rice paddy and landfill leachate environments

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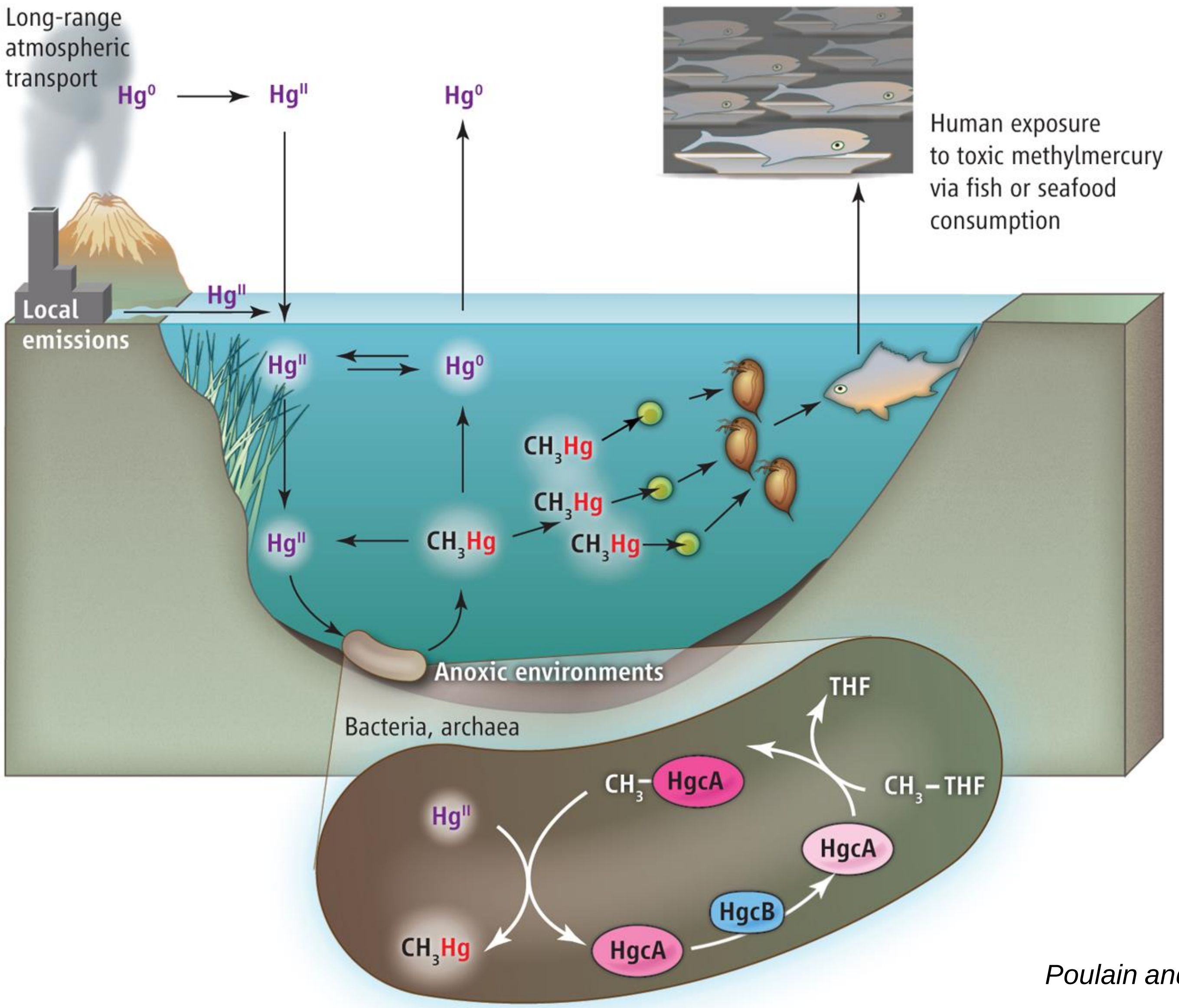
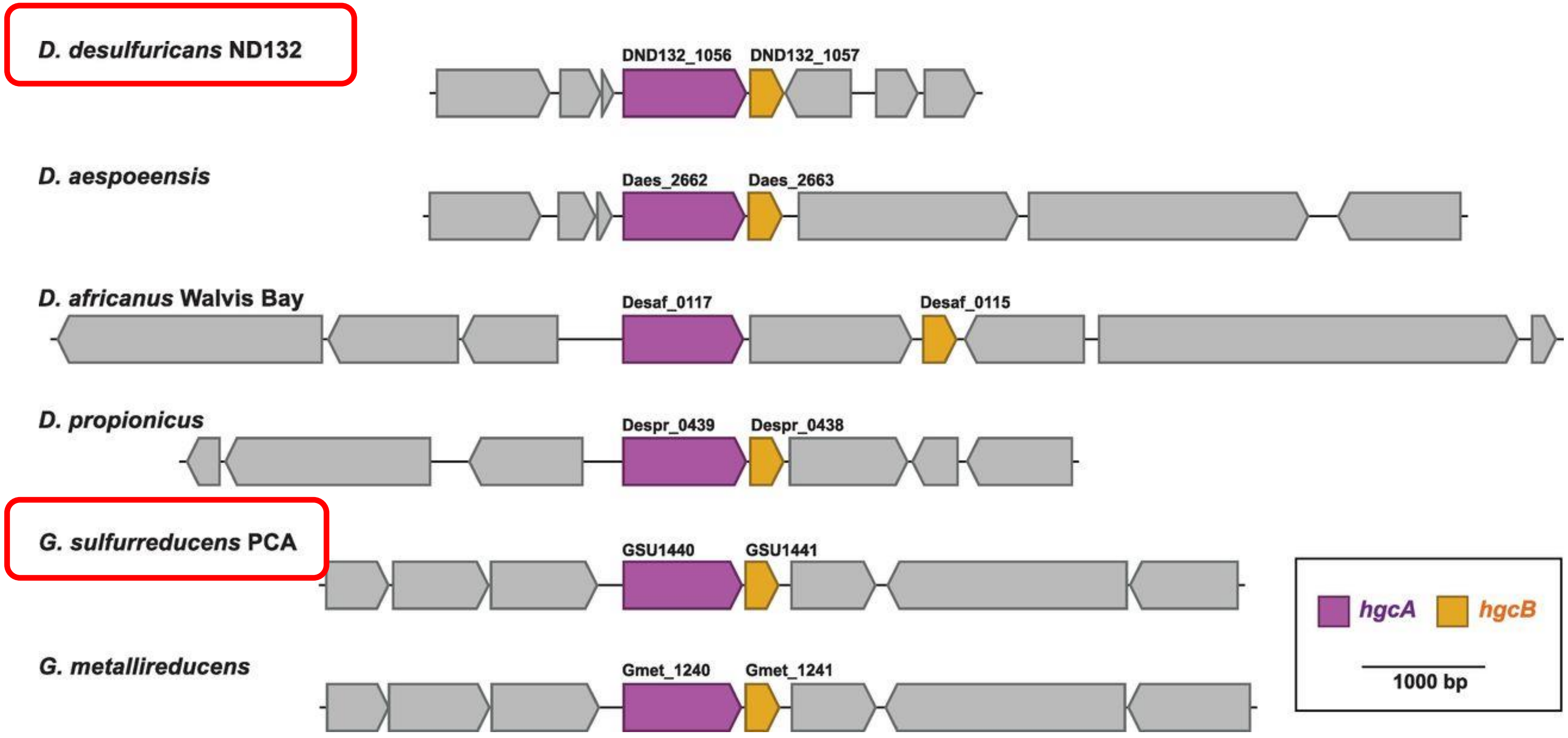
To date, much of what we learn about Hg biogeochemistry and bioaccumulation pathways have come from the studies mostly with the lake ecosystem.



Poulain and Barkay (2013) Science

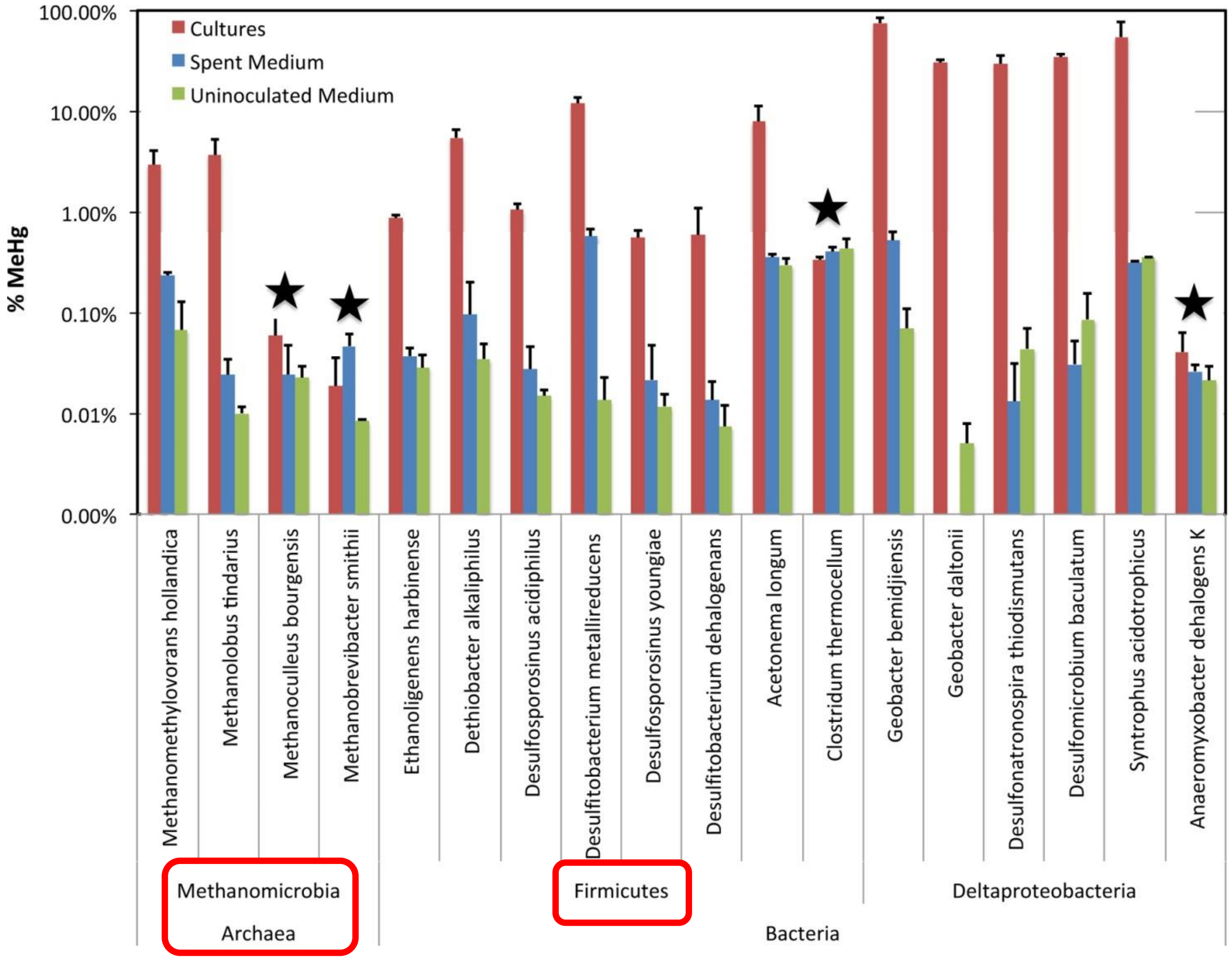
Putative mercury methylation gene cluster and genomic context

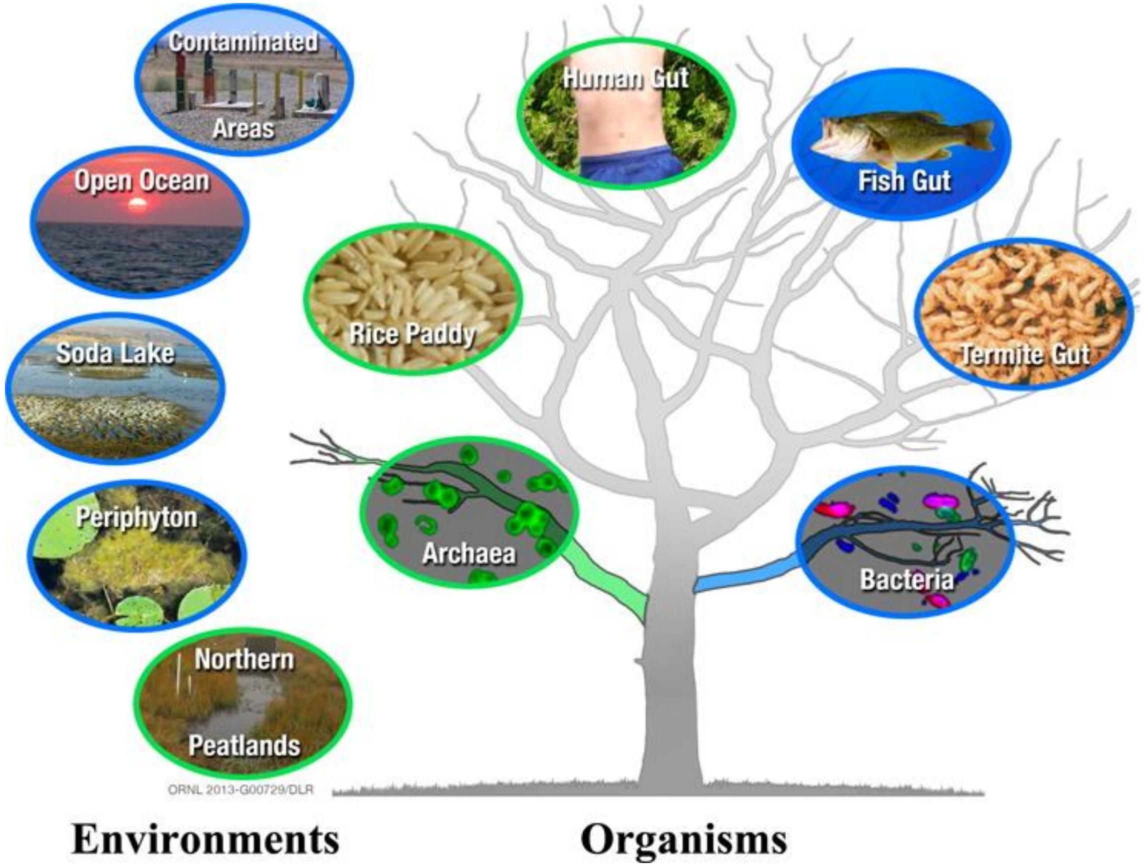
HgcA: a putative methyltransferase corrinoid protein; HgcB: a putative [4Fe-4S] ferredoxin



Parks et al. (2013) Science

The Hg-methylating gene cluster, *hgcAB*, is a reliable bio-marker that would be helpful in the development of monitoring and management stratigies.



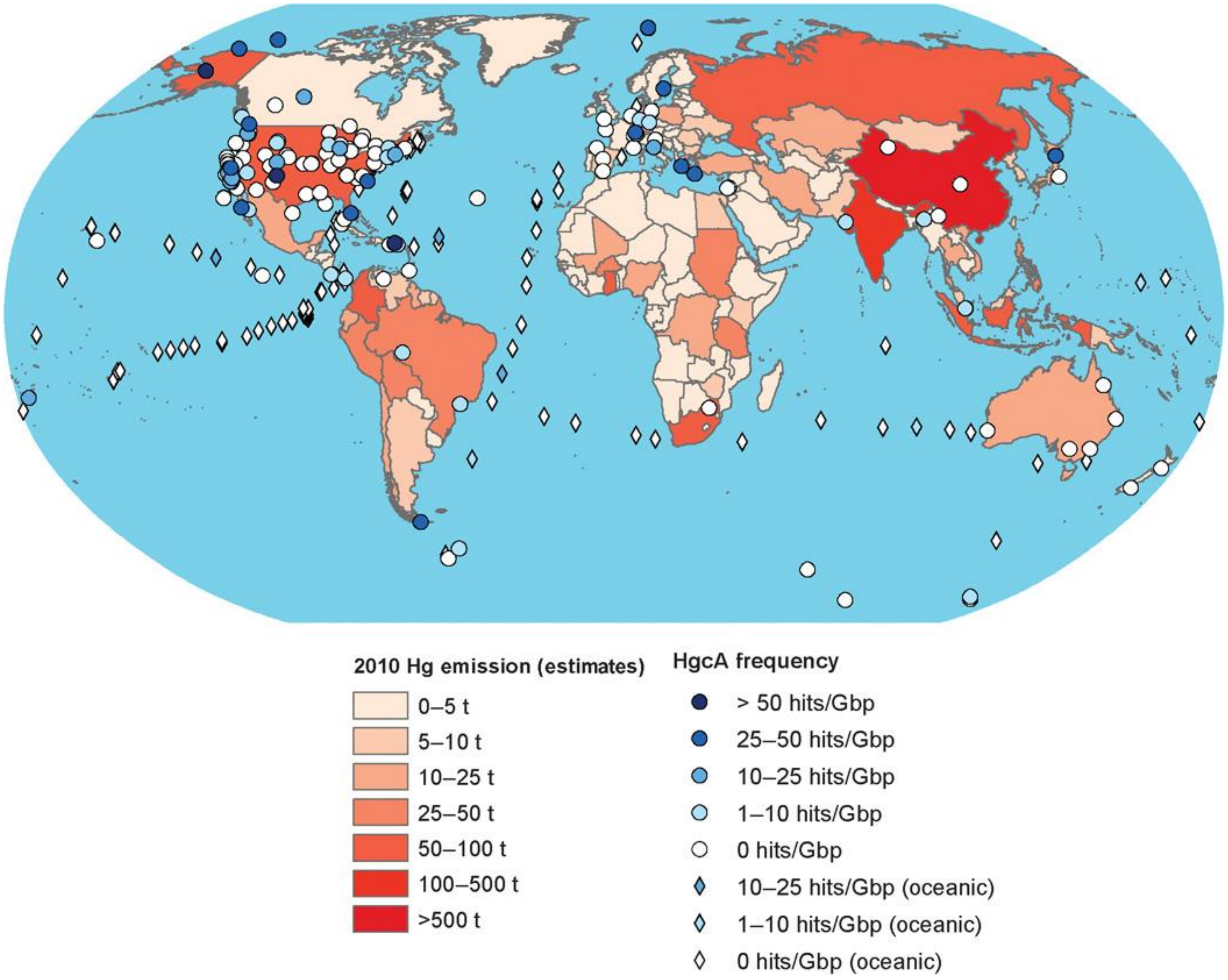


Gilmour et al.(2013) ES&T

The UN Environment Programme recently identified two pressing global issues with regard to mercury pollution (2013):

(1) establishing the link among deposition, methylation, and uptake by living organisms;

(2) characterizing methylation and demethylation and how these reactions are affected by climate change.

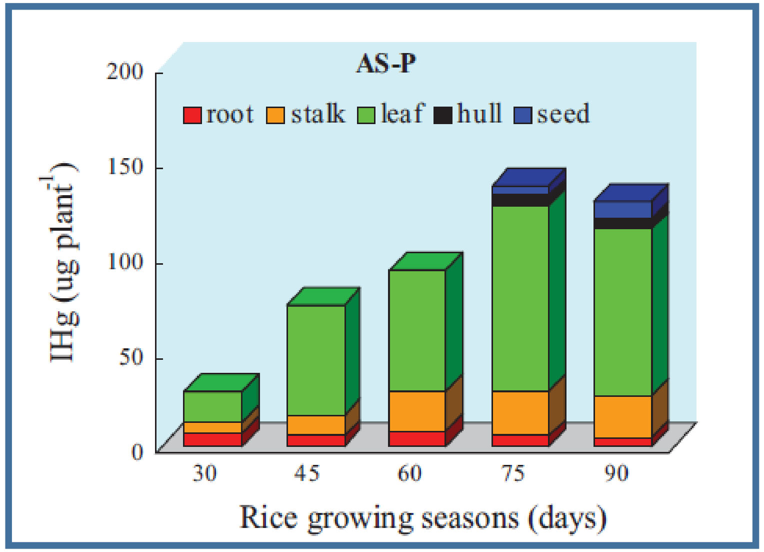


Podar et al.(2015) Sci.Adv.

Hg methylation potential in the rice paddy system

Hg bioaccumulation in the terrestrial food web has been considered negligible. However, in inland mining areas of China:

- Hair Hg levels significantly correlate with rice MeHg intake
- Other crops have 10-100 fold lower MeHg in the edible portion
- Rice seeds have the highest capacity to accumulate MeHg
- Accumulation pathways of IHg and MeHg in rice are different

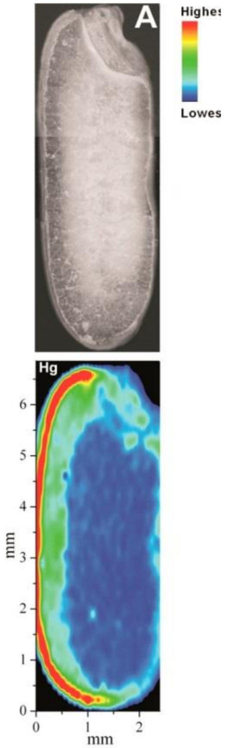


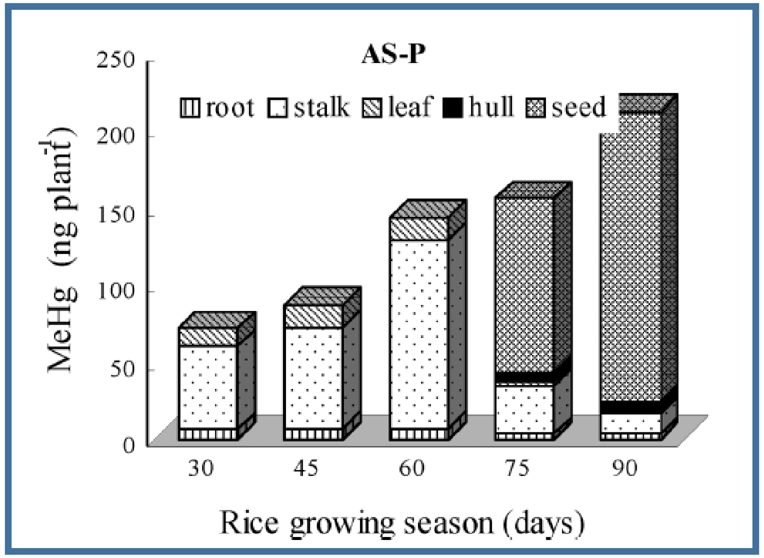
Meng et al. (2012) Environ. Toxicol. Chem.

Table 1. Concentrations of IHg and MeHg in Fractions of Rice Grain from Different Sampling Sites (Mean ± S.D, $\mu g\ kg^{-1}$)

sampling sites	fractions	Hg concentration: IHg	Hg concentration: MeHg	MeHg/THg(%)
Control Site (n = 10)	hull	6.2 ± 0.51	0.26 ± 0.05	4.1 ± 0.73
	bran	24 ± 1.4	3.0 ± 0.48	11 ± 1.8
	white rice	2.7 ± 0.25	2.4 ± 0.16	48 ± 3.3

Meng et al.(2014) ES&T 2014

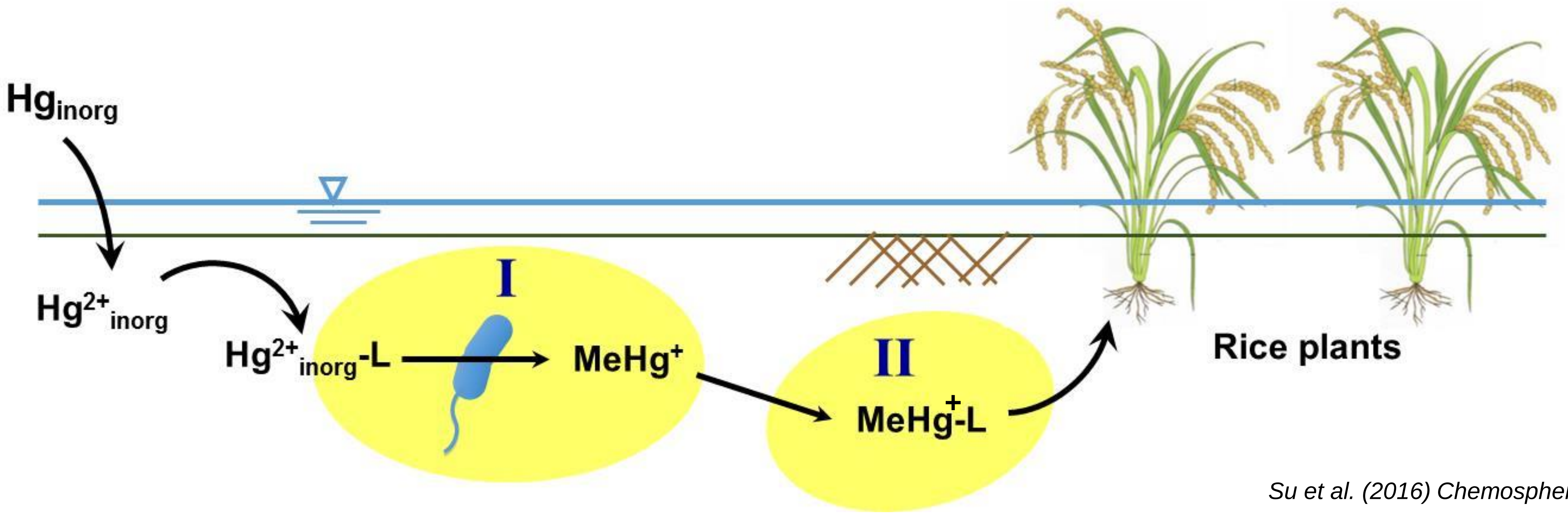




Meng et al.(2011) ES&T 2011

Given that we do not yet have a complete picture of the mechanisms that underlie the formation, uptake and accumulation of MeHg in the paddy ecosystem, in this study we sought to examine and explore:

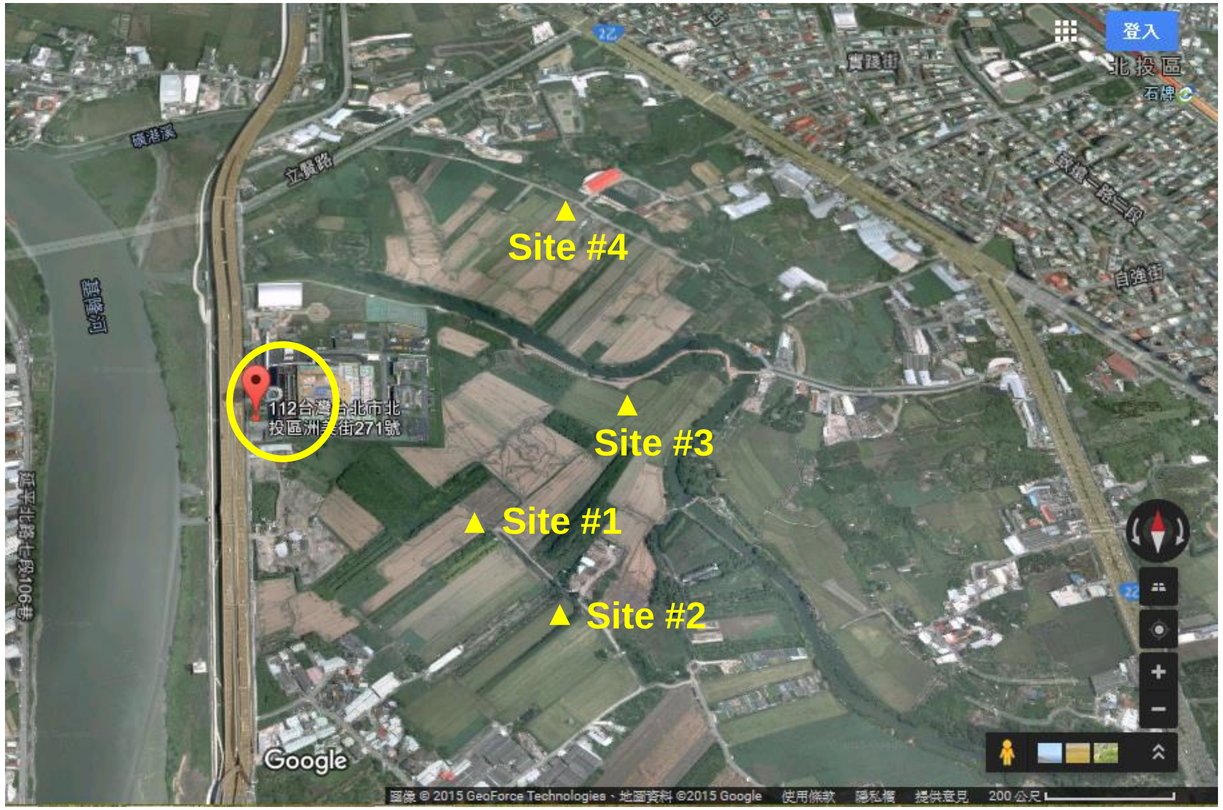
- why rice paddies are conductive for Hg methylation?
- what are the major biogeochemical factors involved in this process?
- who are the primary *in situ* Hg-methylators in the paddy rhizosphere?
- what is the role of porewater coordination chemistry in MeHg uptake by rice roots?



Su et al. (2016) Chemosphere

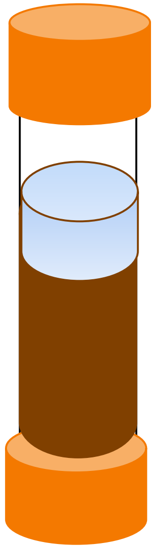
Approaches: to answer these questions, we conducted field campaigns over a rice growing season at

The Beitou Municipal Solid Waste Incinerator (2013)



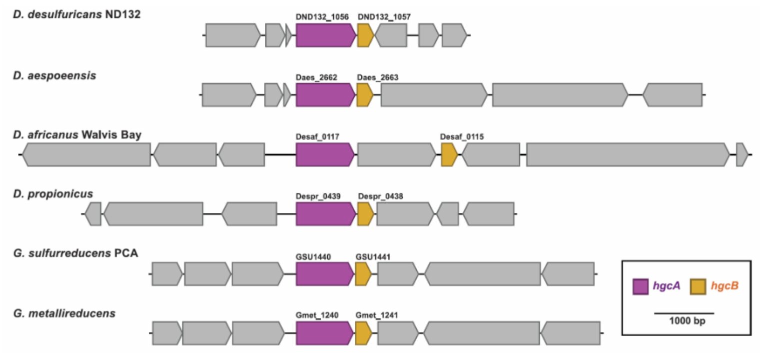
The Taichung Coal-Fired Power Station (2014)

Approaches: we carefully processed the field samples using strict anaerobic techniques in the lab



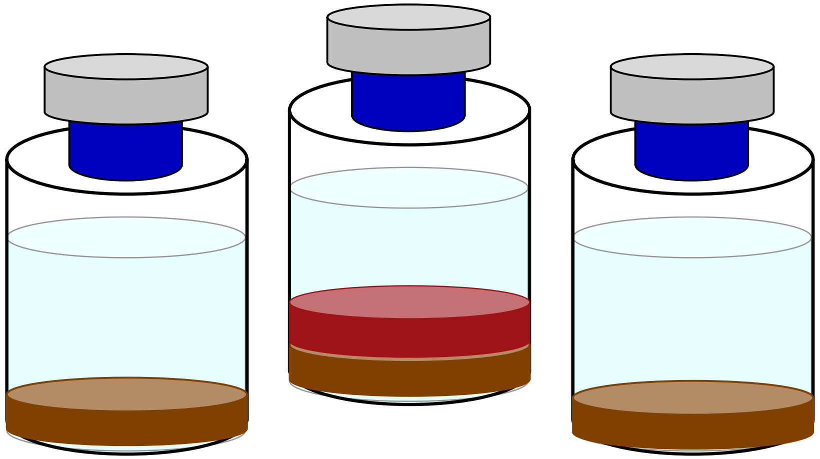
Took sediment cores while maintaining the redox status

Extracted **porewater** and quantified the concentrations of total Hg, MeHg, and ancillary geochemical parameters (iron, sulfur, organic carbon, pH…)



Parks et al. (2013) Science

Other **soil samples**: in addition to the exactly the same geochemical parameters, we also designed the primers targeting the *hgcA* gene



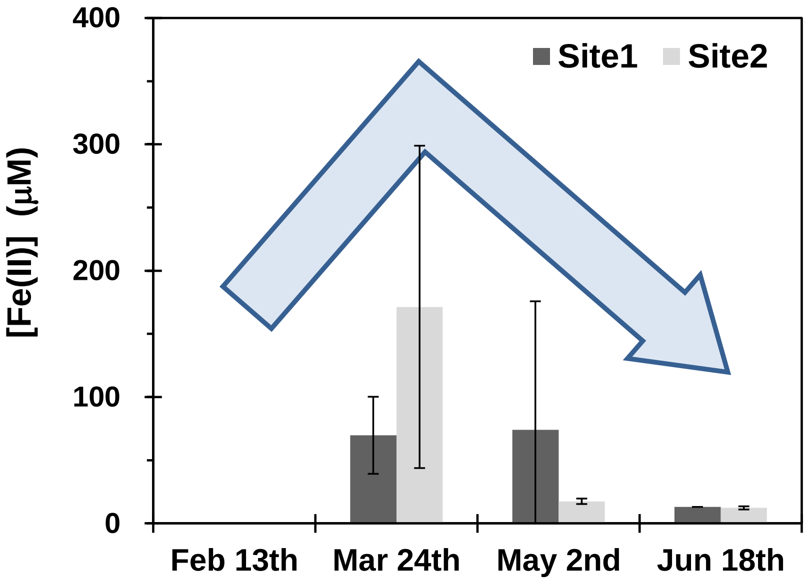
Set up incubation microcosm tests to

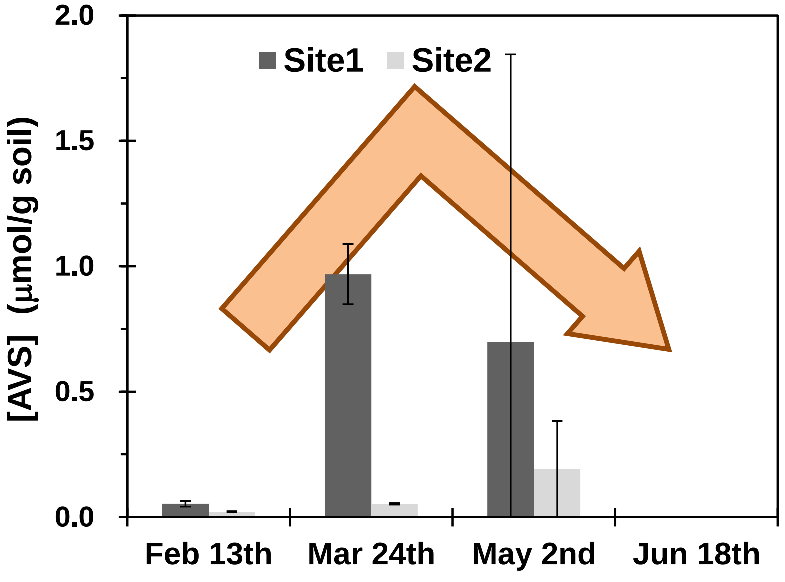
- Stimulate/inhibit SRB: SO_4^{2-}/molybdate
- Stimulate FeRB: ferrihydrite
- Stimulate/inhibit MPA: H_2 + CO_2/BESA

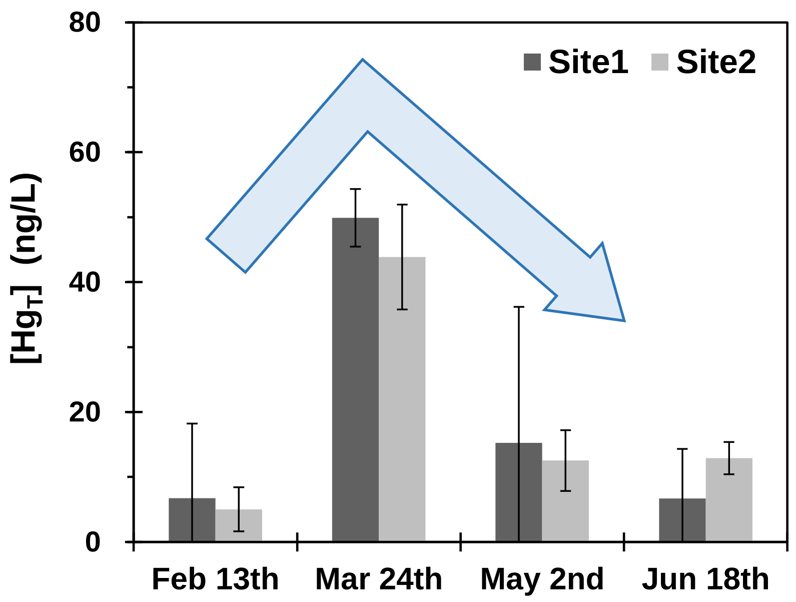
We also conducted hydroponic experiments by cultivating rice in a defined nutrient solution amended with fixed MeHg and varying levels of MeHg-binding ligands

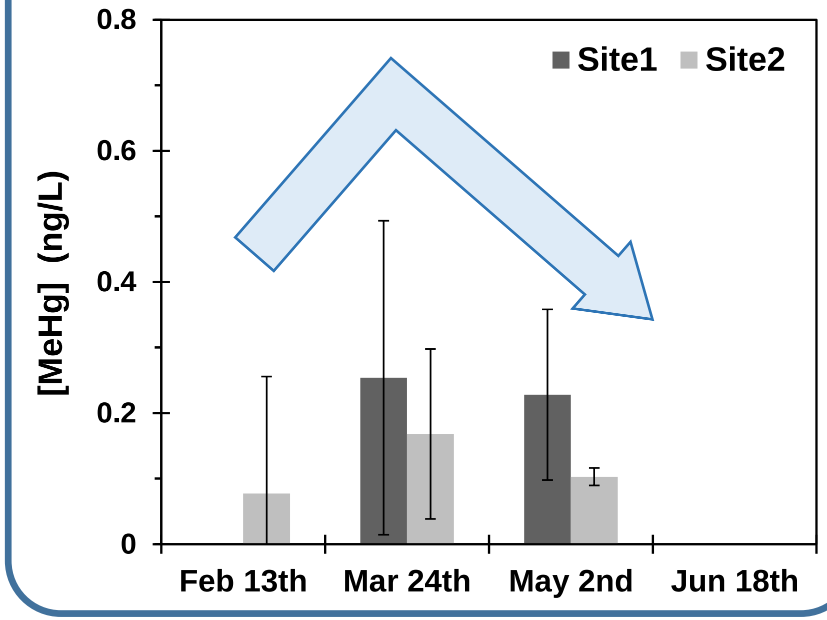
We observed **Hg cycling associated with rhizosphere biogeochemical dynamics in the paddy**:

- Total Hg and MeHg levels in paddy soil and rice grains did not exceed the control standards for farmland soil and edible rice in Taiwan.
- *In situ* bioavailability of inorganic Hg and activity of Hg-methylating microbes in the rhizosphere increased from the early-season and peaked at the mid-season, presumably due to the anoxia created under flooded conditions and root exudation of organic compounds.





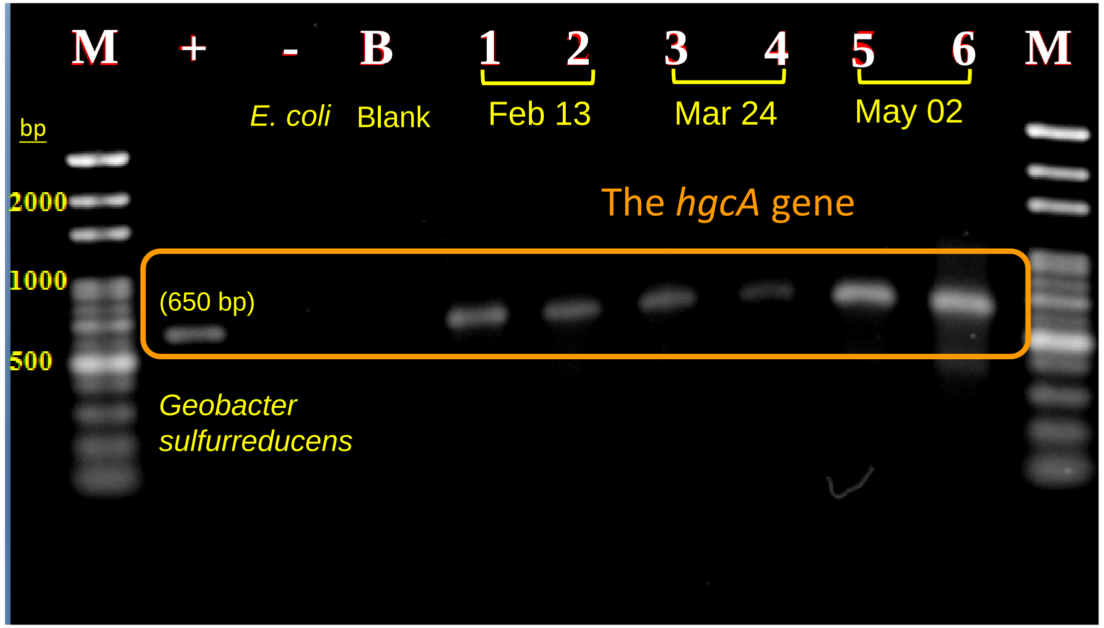




Su et al.(2016) Chemosphere

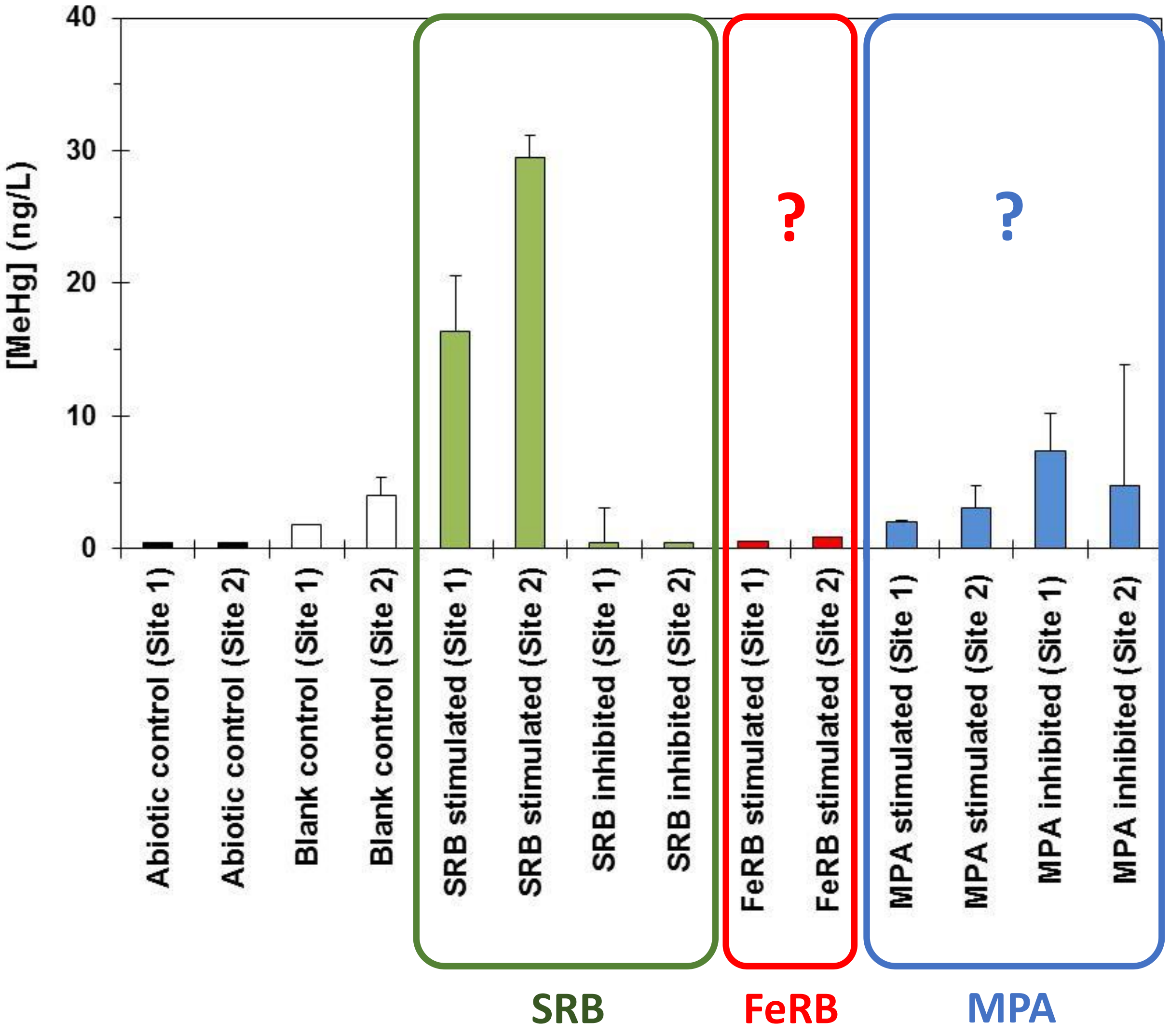
In addition, we identified **the potential primary *in situ* Hg-methylators in the paddy rhizosphere:**

- The presence of Hg-methylators was also confirmed by the detection of the bacterial Hg-methylating gene, *hgcA*, in all root soils.
- Microcosm incubation tests revealed that sulfate reducers might have been the primary Hg-methylating guild at our study sites.



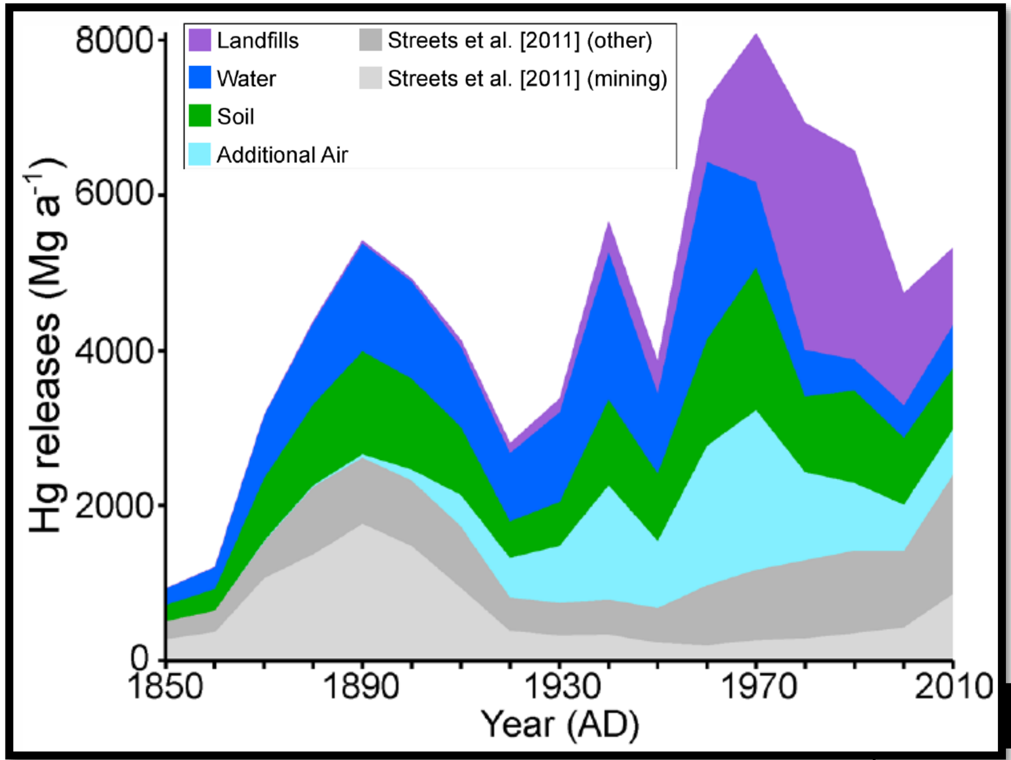
Su et al.(2016) Chemosphere

- **Should there be a more significant role for FeRB and MPA to play in MeHg production in paddies?**

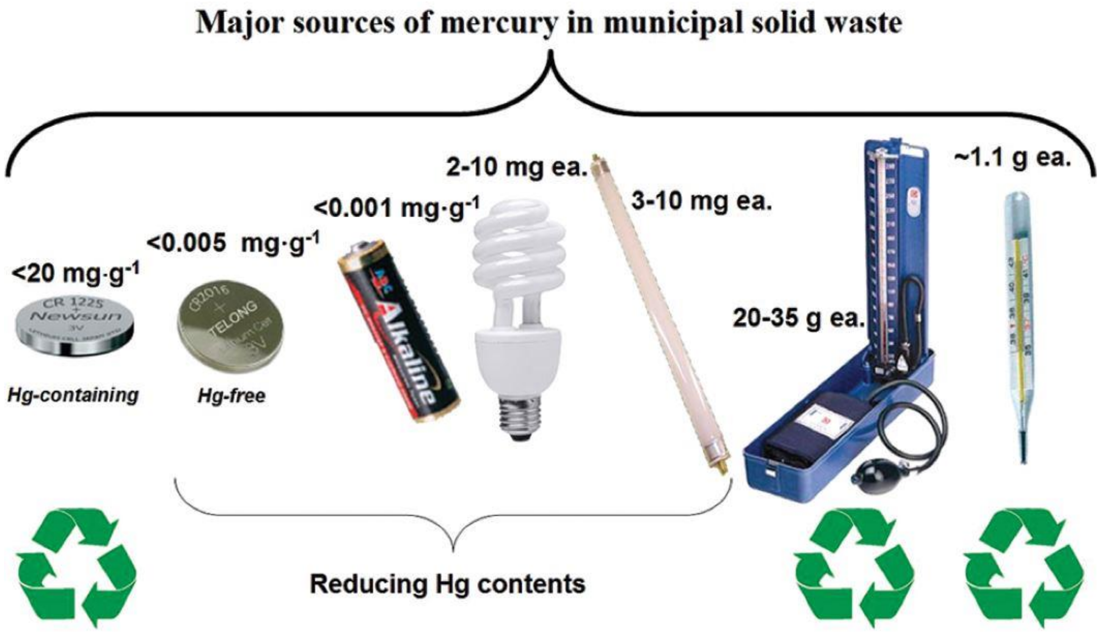


Hg methylation potential in the landfill system

Hg-containing products may be disposed of at landfills



Horowitz et al. (2014) ES&T



Cheng et al. (2012) ES&T

reservoirs	Hg (Gg)
Mining (air)	215
air, water, soil	310
Landfill	230
total	755

Levels of mercury detected in landfill leachates

Environmental level ~ 1-500 ng/L

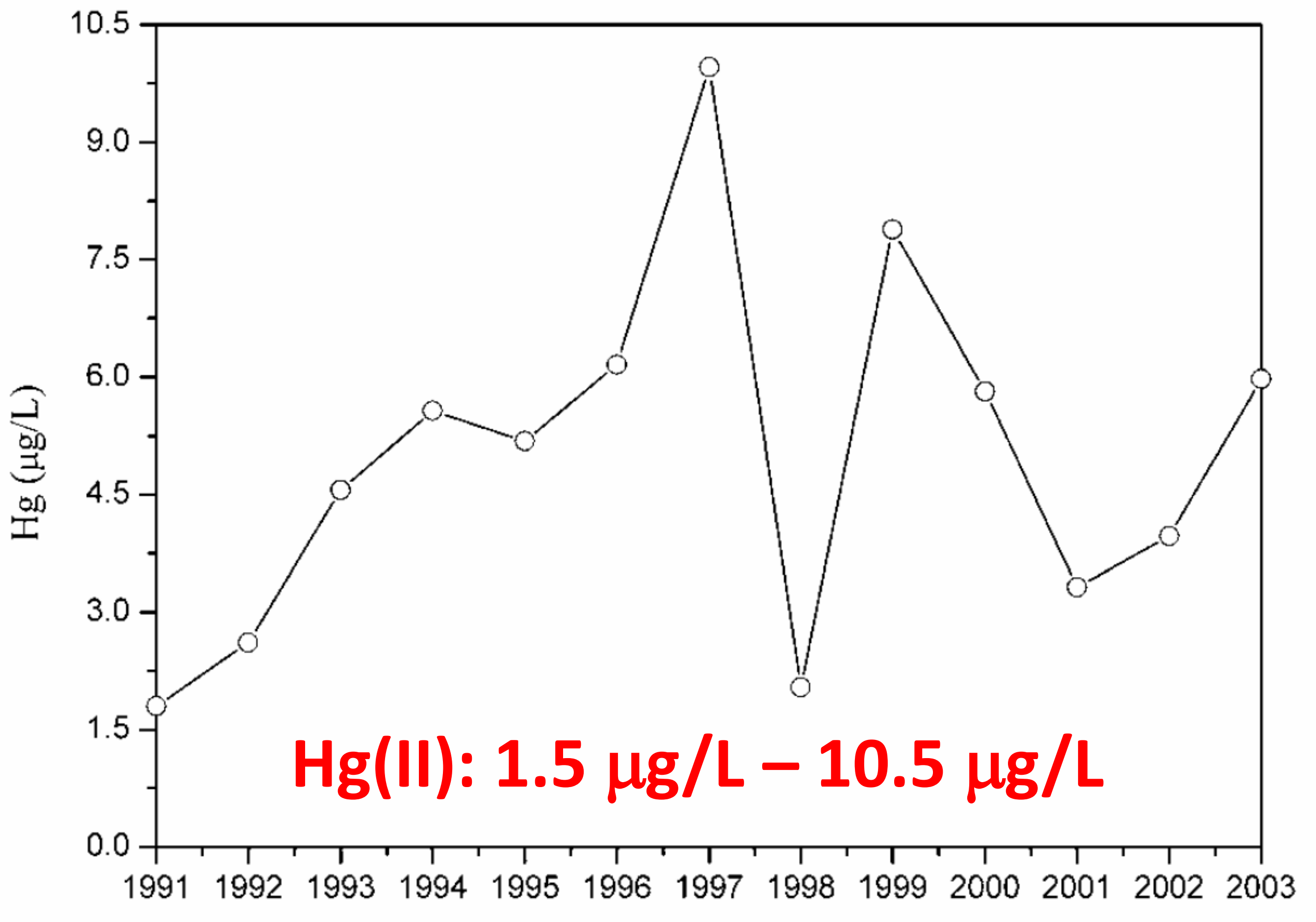


Fig. 1 The mercury concentration of landfill leachate (the discharge standard of Hg in landfill leachate is 0.001 mg l^{-1}).

Xiaoli et al. (2011) J. Environ. Monit.

Composition of landfill leachate[a] *(Christensen et al., Appl. Geochem. 2001)*

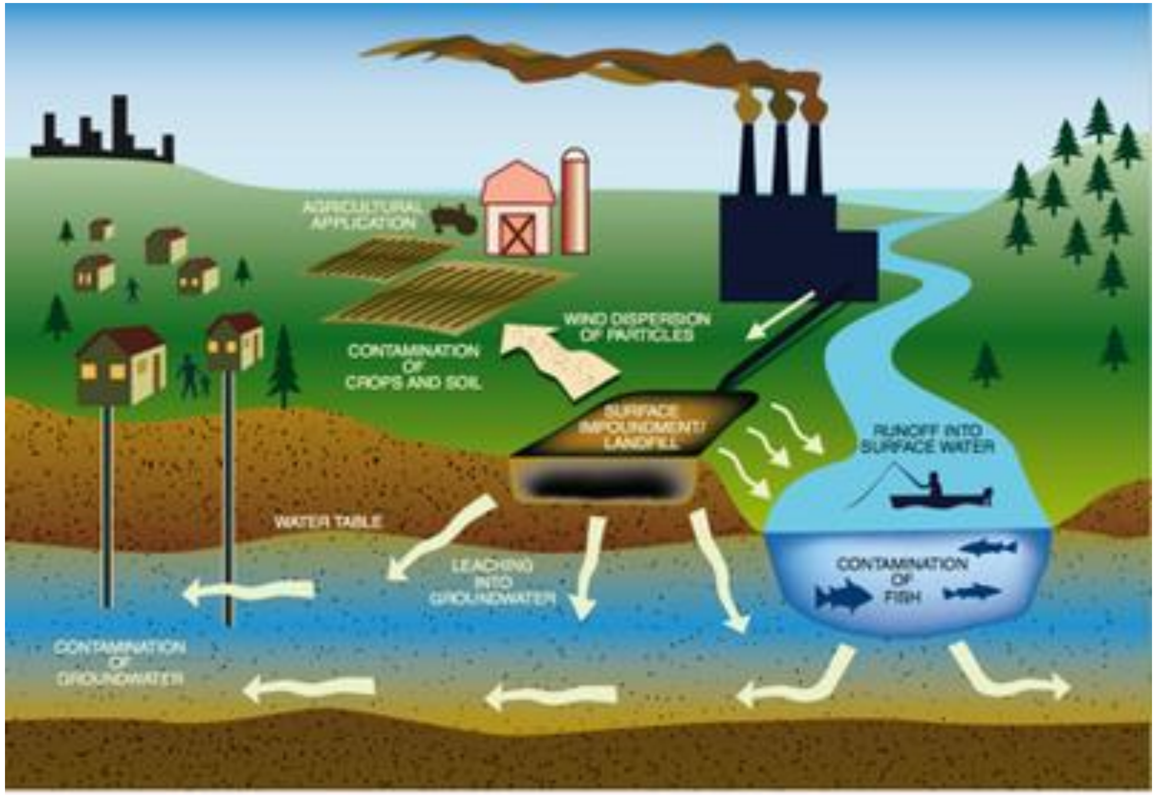
Parameter	Range
pH	4.5–9
Specific conductivity ($\mu S\ cm^{-1}$)	2500–35 000
Total solids	2000–60 000
Organic matter	
Total organic carbon (TOC)	30–29 000
Biological oxygen demand (BOD_5)	20–57 000
Chemical oxygen demand (COD)	140–152 000
BOD_5/COD (ratio)	0.02–0.80
Organic nitrogen	14–2500
Inorganic macrocomponents	
Total phosphorous	0.1–23
Chloride	150–4500
Sulphate	8–7750
Hydrogencarbonate	610–7320
Sodium	70–7700
Potassium	50–3700
Ammonium-N	50–2200
Calcium	10–7200
Magnesium	30–15 000
Inorganic trace elements	
Arsenic	0.01–1
Cadmium	0.0001–0.4
Chromium	0.02–1.5
Cobolt	0.005–1.5
Copper	0.005–10
Lead	0.001–5
Mercury	0.00005–0.16
Nickel	0.015–13
Zinc	0.03–1000

Hg(II): 50 ng/L - 160 µg/L

Mercury Transformations in Landfill Sites

- **Recent available data prompt a need to re-examine the landfill environment as a potential hot-spot for MeHg production, given that the mechanisms underlying Hg transformations in this system have not been studied.**
- To approach and address this issue, the following factors/processes have to be determined:
    - existence of the *hgcAB* gene pair
    - existence of a Hg(II)$_{(aq)}$ pool
    - bioavailability of Hg(II)$_{(aq)}$
    - net MeHg production
- <u>Our hypothesis</u>: the extent of MeHg formation in this system is still a function of both the activity of Hg-methylating bacteria and Hg(II)-bioavailability.

$$\mathrm{Hg(0)} \underset{\text{reduction}}{\overset{\text{oxidation}}{\rightleftharpoons}} \mathrm{Hg(II)} \underset{\text{demethylation}}{\overset{\text{methylation}}{\rightleftharpoons}} \mathrm{MeHg}$$



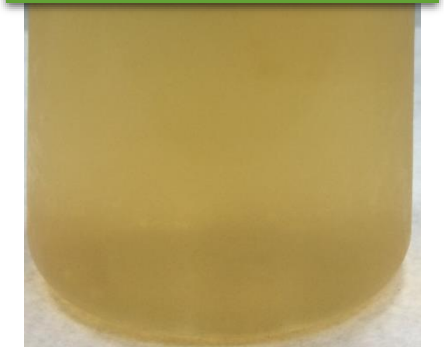
Source ：catawbariverkeeper.org

Site C

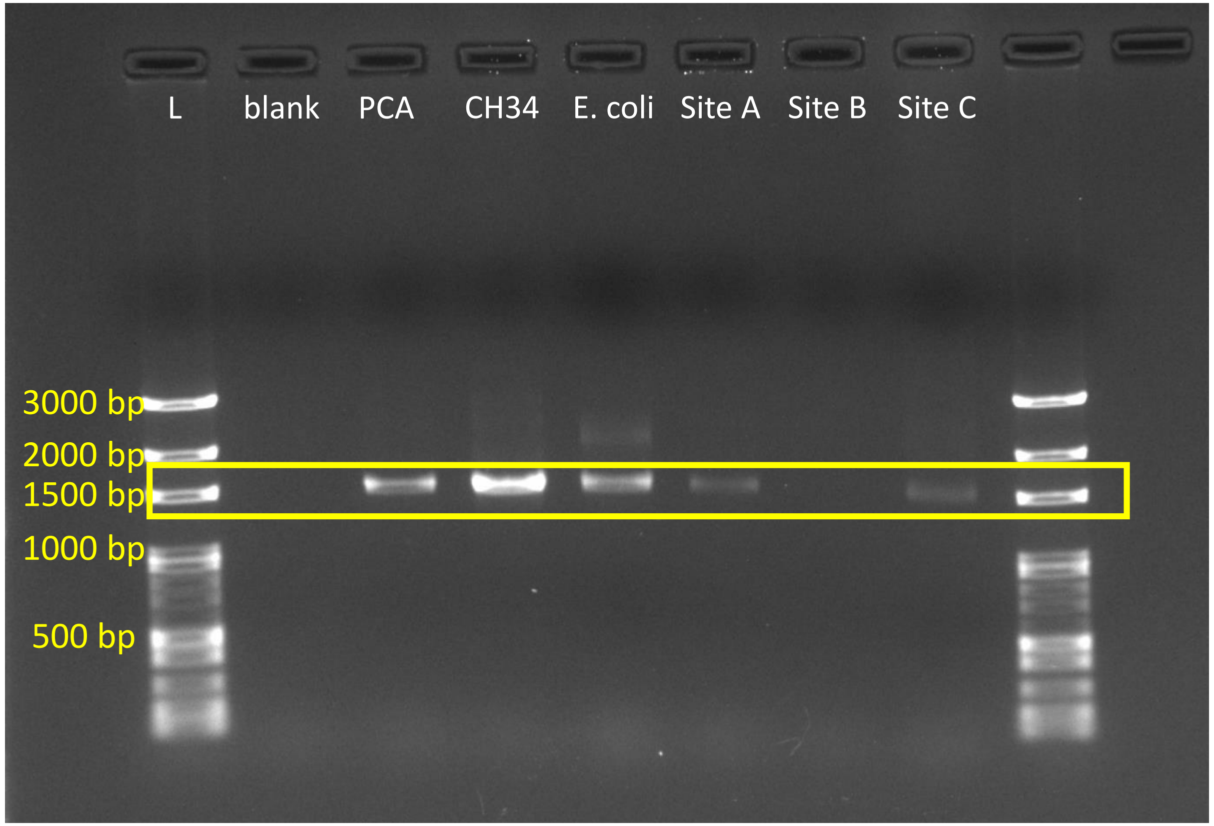
Geochemical conditions

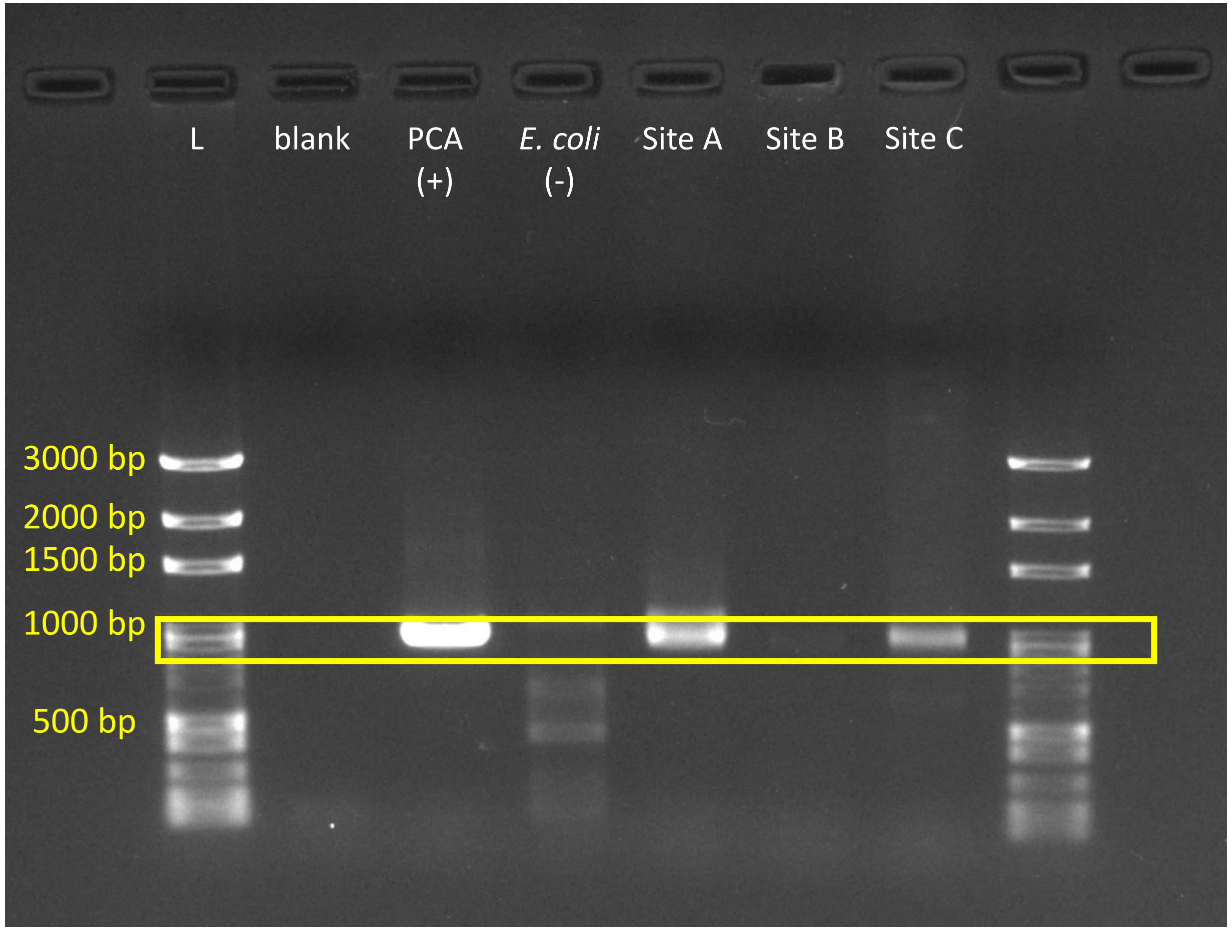
		Site A	Site B	Site C
Temp	$^{\circ}$C	25.6	24.7	20.8 - 27.3
pH	---	7.32	7.62	6.96 - 7.06
EC	μS/cm	5652 - 7612	3054 - 4132	8634 - 14789
DO	mg/L	0.07 - 5.18	0.10 - 0.23	0.16 - 0.73
ORP	mV	205 - 220	207 - 219	23.4 - 221
COD	mg/L as COD	357	147 - 247	453 - 988
TOC	mg/L as C	81-116	35 - 154	109 - 153
Sulfate	mg/L as SO_4^{2-}	2.08	< 2.00	< 2.00
Sulfide	μg/L as S^{2-}	36	20 - 141	54 - 65
Nitrate	mg/L as NO_3^--N	0.39	7.07	6.80
Nitrite	mg/L as NO_2^--N	0.10	1.74	5.35
TFe	mM	< 10	< 10	< 10

THg & MeHg

		Site A	Site B	Site C
				
total Hg	pM	68.7 (9.70 - 249)	82.7 (8.72 - 259)	245.8 (39.1 - 483)
dissolved Hg	pM	12.5 (6.00 - 20.1)	14.4 (5.29 - 54.4)	92.4 (88.3 – 96.7)
(% in THg)		18.2%	17.5%	23.31%
non-purgeable Hg	pM	18.0 (12.0 - 26.2)	80.0 (75.3 – 86.4)	266.6 (168 - 336)
(% in THg)		96.6%	69.0%	70.4%
MeHg	pM	1.07 $\pm$ 0.102	1.07 $\pm$ 0.11	0.53 $\pm$ 0.11
(% in THg)		1.56%	1.30%	0.22%

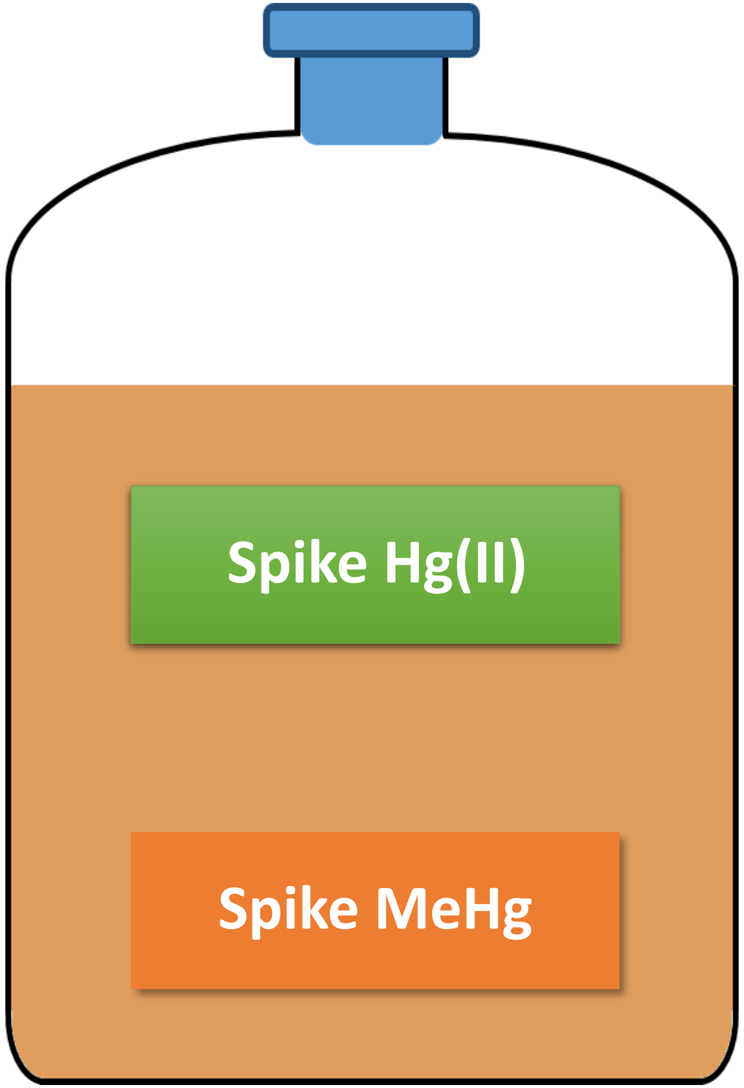
Detection of the *hgcAB* gene cluster



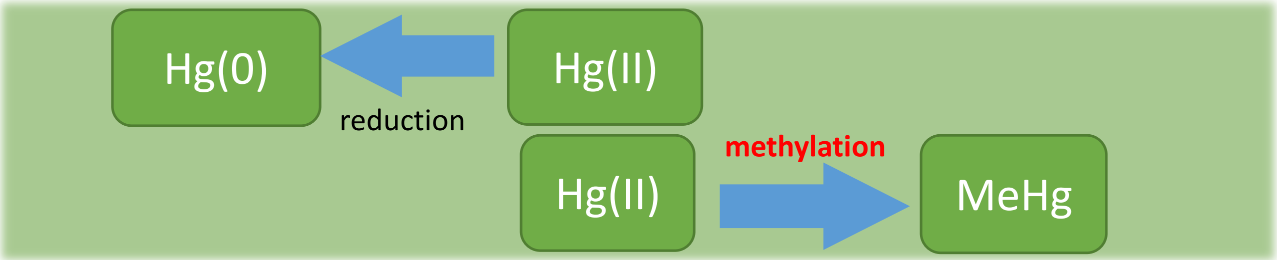


Blank: DDW as sample
PCA: *Geobacter sulfurreducens* PCA
CH34: *Cupriavidus metallidurans* CH34
E. coli: *Escherichia coli*

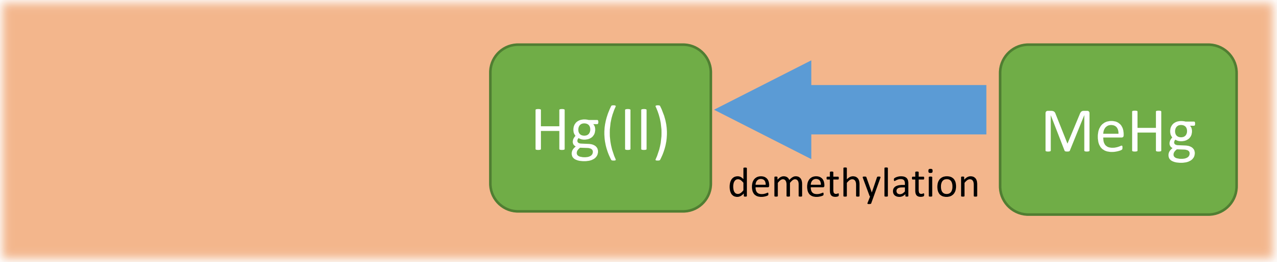
Microcosm tests



in the dark under anaerobic conditions
at static, room temperature

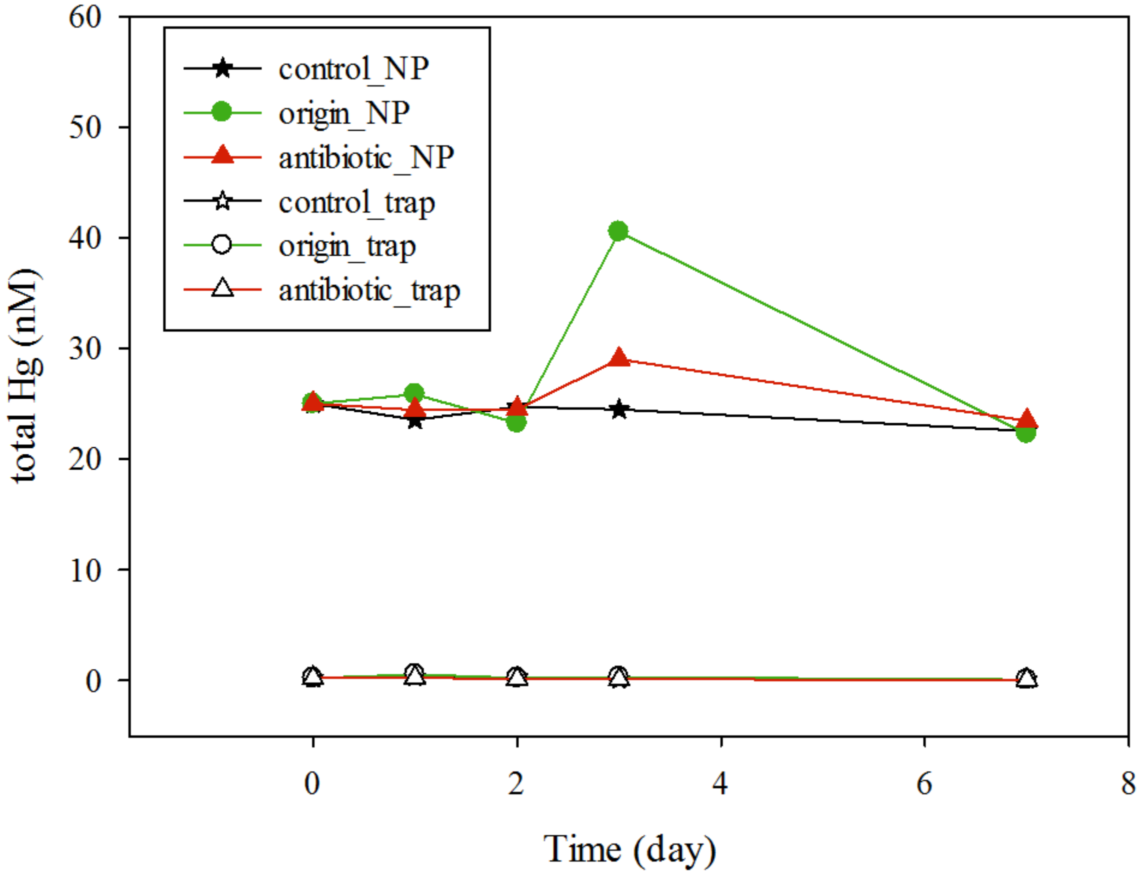


- Hg(II) reduction
- Hg(II) methylation (Potential)
- Hg(II) methylation (Energy)
- Hg(II) methylation (Bioavailability): PCA

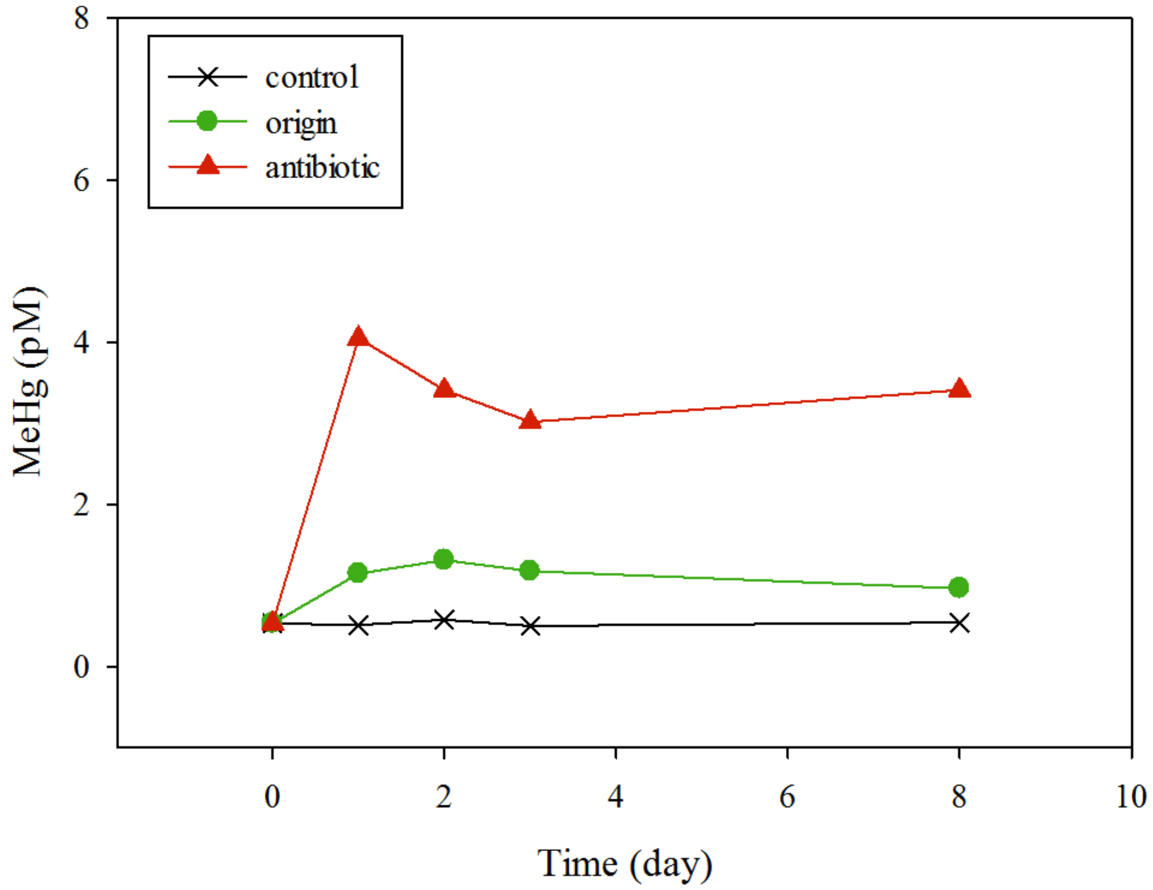


- MeHg demethylation

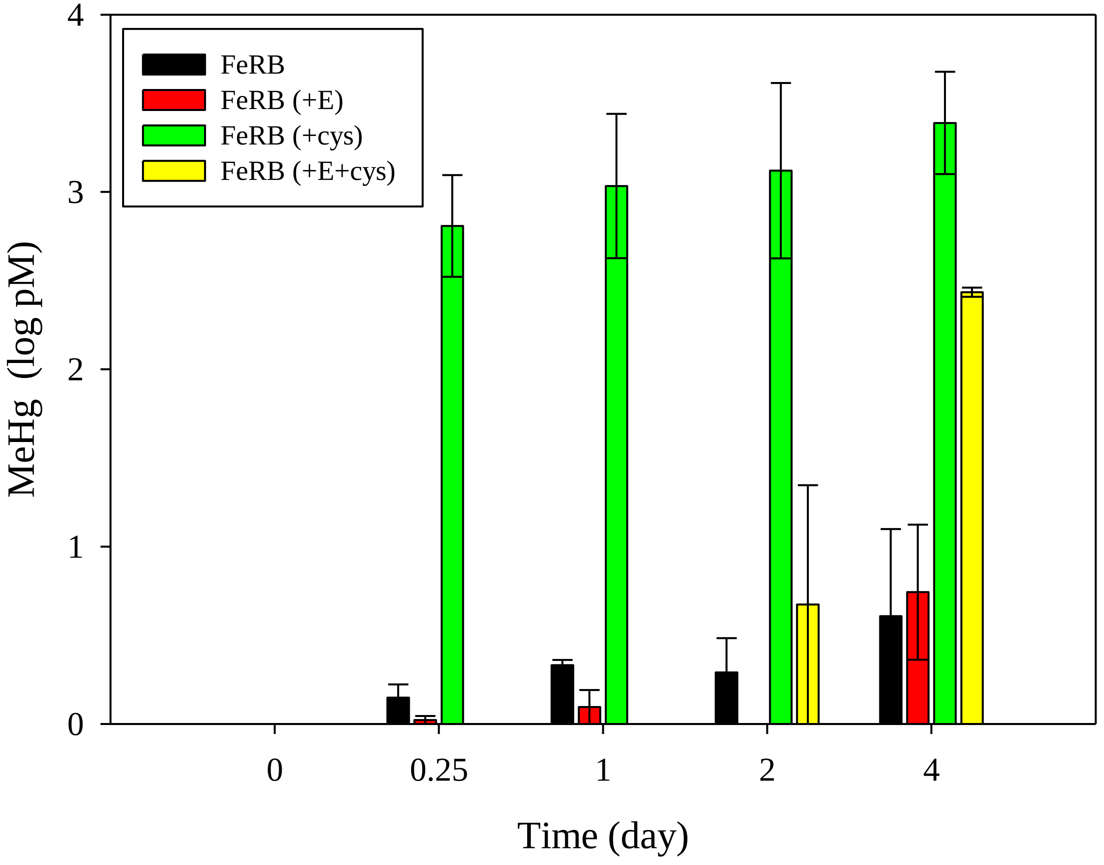
Hg(II) reduction is not significant



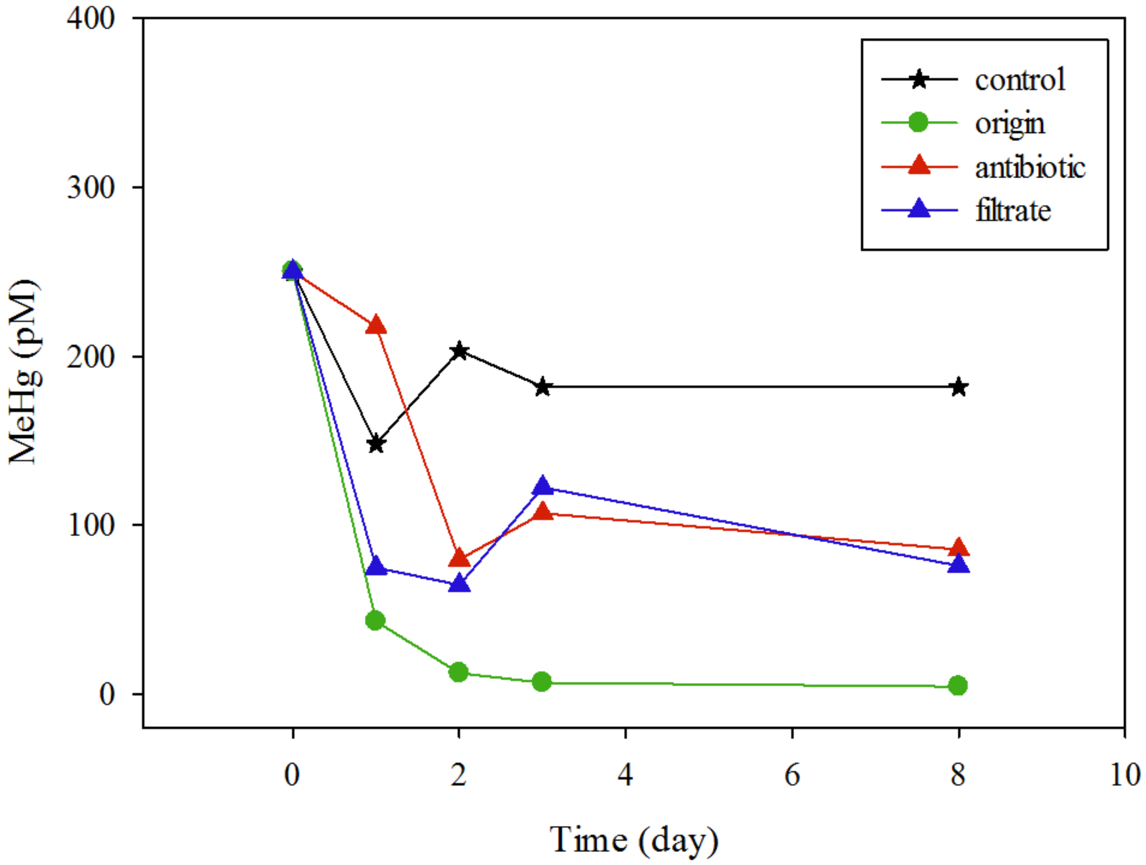
Abitoic Hg methylation is dominant



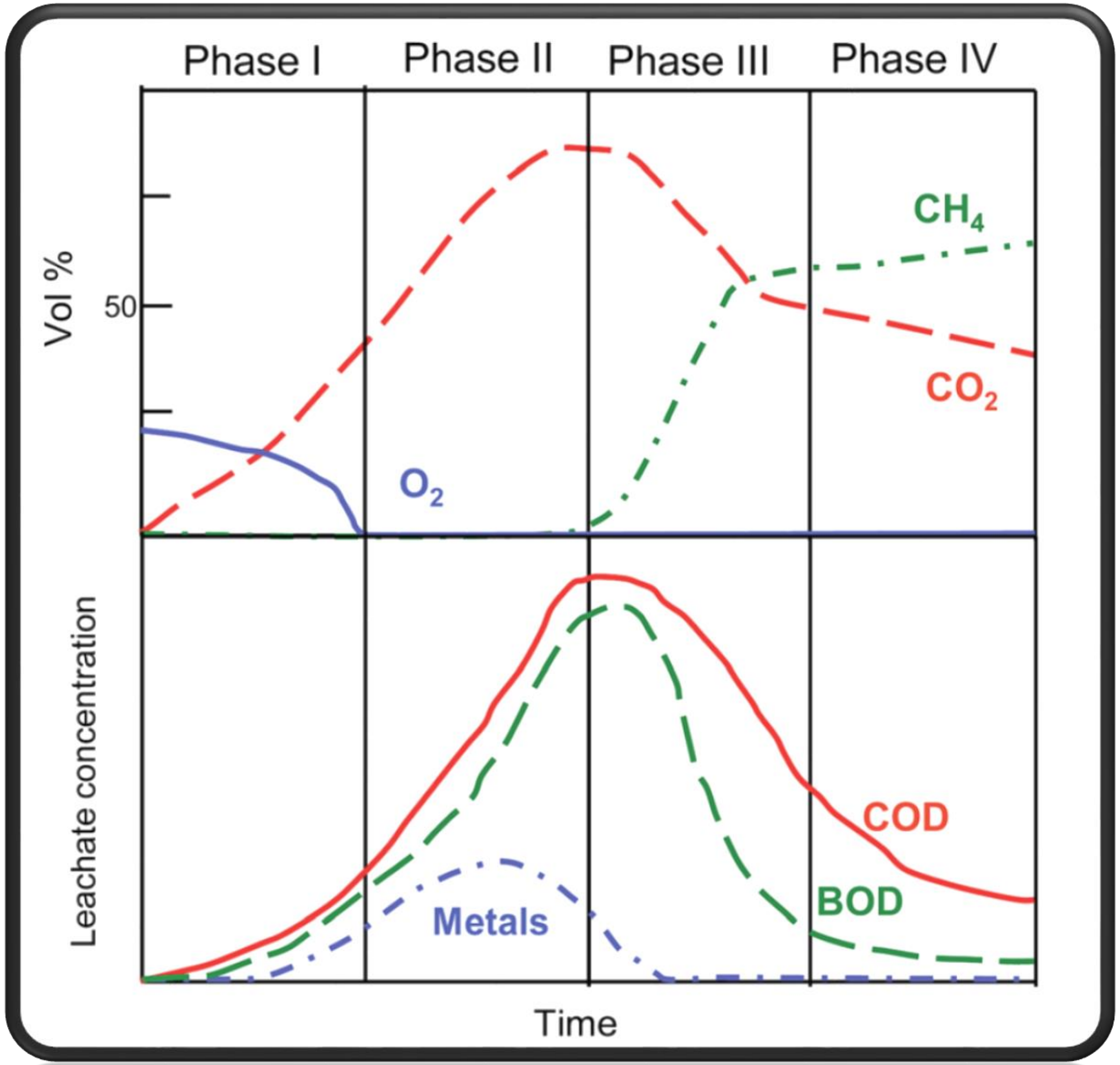
Dissolved Hg(II) is available to microbes



Significantly indigenous MeHg degradation



Landfill maturation process



Summary

- Paddy rhizosphere has higher Hg methylation potential during the rice mid-growing season.
- The role of methanogens in MgHg production in the paddy ecosystem deserves further investigations.
- Hg methylation is not significant in the landfill leachate of the Phase IV and later maturation processes.
- A solid understanding of the mechanisms that underpin the important environmental processes may eventually lead to developments of better ecological assessments and more sound remedial actions.

Thank you for your attention!

Special thanks to

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Yen-Bin Su
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Wei-Chun Chang
(張惟竣)

Chih-Kuen Hsu
(徐志昆)