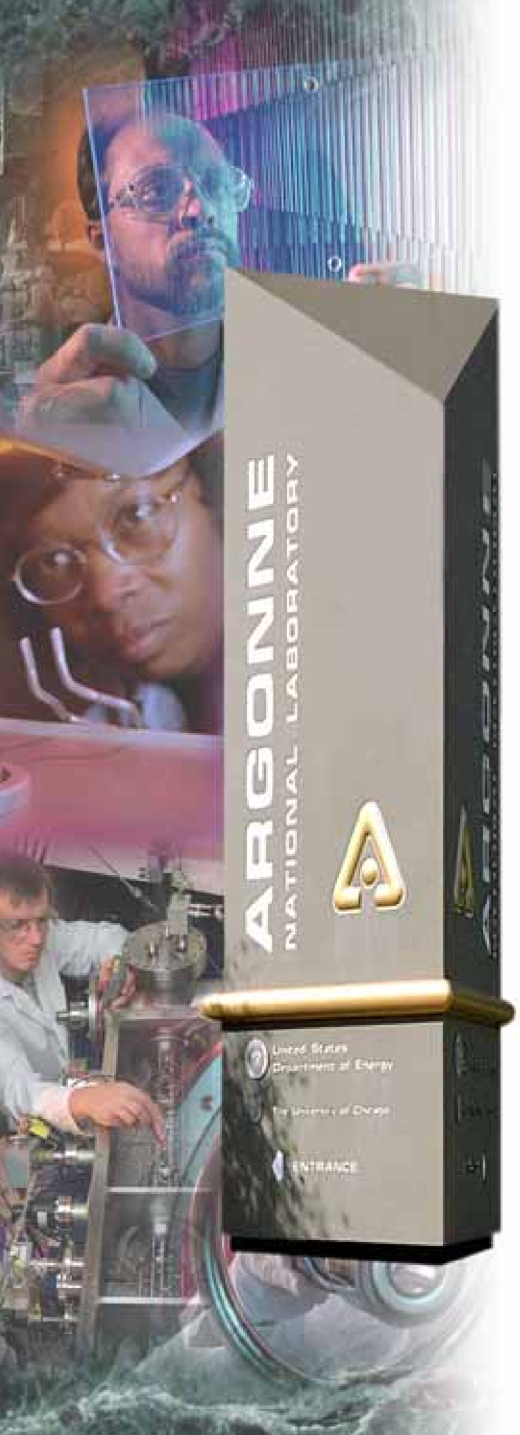

Synchrotron-based Studies of Geomicrobiology

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Molecular Environmental Science Group,
Environmental Research Division

APS/Users Monthly Operations Meeting

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Argonne National Laboratory

Office of Science
U.S. Department of Energy

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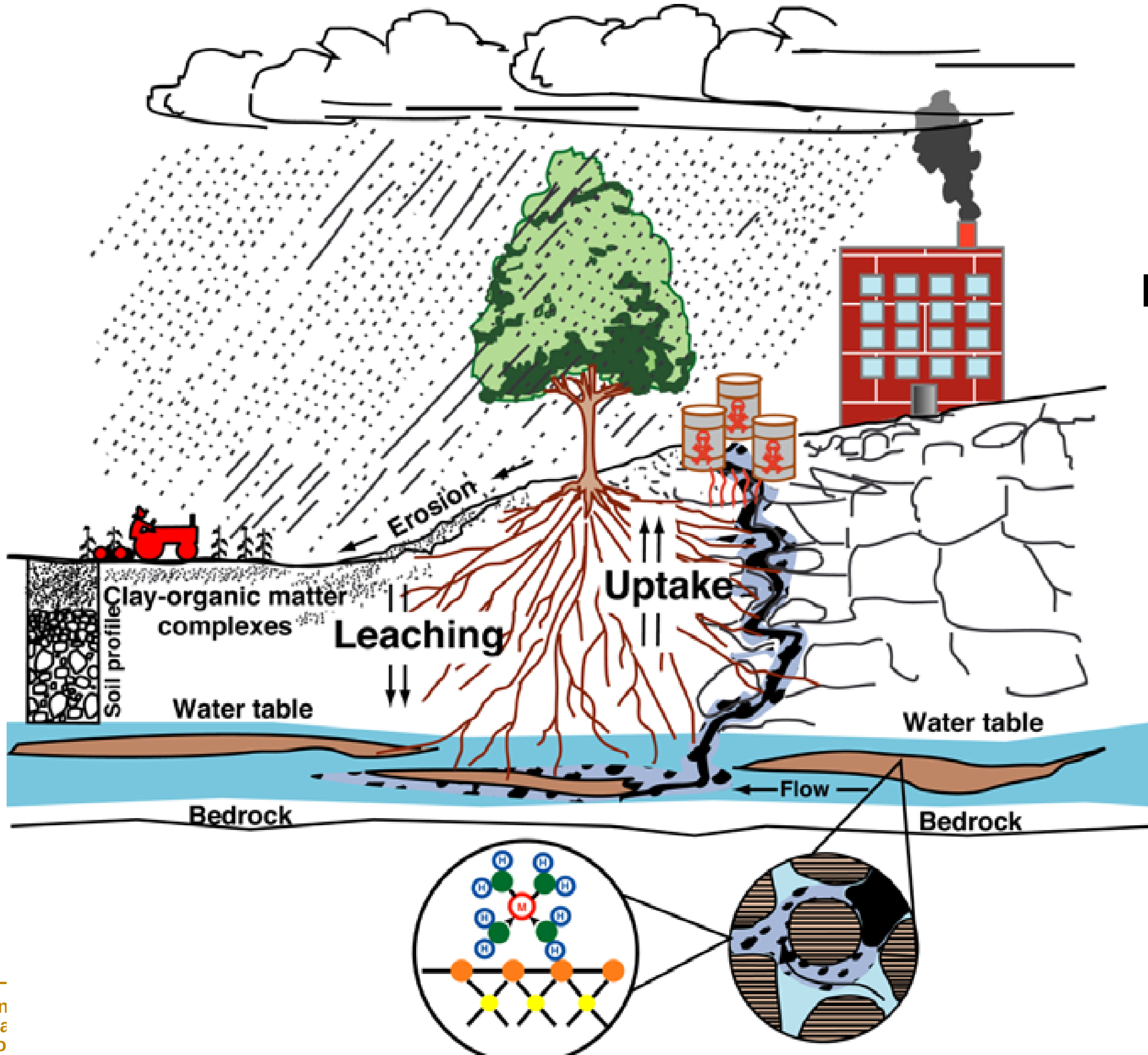
Outline

- What is Environmental Science?
- Introduction to Geomicrobiology and its role in Environmental Science
- The use of hard synchrotron x-ray (spectro)microscopy to investigate Geomicrobiology systems
- Final thoughts

What is Environmental Science?



Oceans?
Microbes?

Acknowledgements

- **ANL-Environmental Research Division (Molecular Environmental Science Group)**
  - E. O'Loughlin (Environmental Chemist)
  - S. Kelly, M. Boyanov (X-ray Physicists)
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  - S. Glasauer, T. Beveridge (Microbiologists, Geomicrobiologists)

Why are microbes/bacteria important in Environmental Science?

- **~3600 identified minerals, yet vastly more microbial species have already been identified (less than 0.1% culturable)**
- **Size (~1 micron), large surface to volume ratio**
- **Microbes can use a wide variety of electron donors and exploit many different alternative oxidants (catalyze numerous reactions).**
- **10^6 - 10^9 cells/gram of soil or subsurface material**
- **Microbes can transform poisons (heavy metals) into harmless compounds, or repackage them so they are physiologically unavailable (bioremediation).**
- **Microbes degrade organic pollutants, restore key nutrients to depleted soil, or act as a sink for greenhouse gases (CO2), from the atmosphere.**
- **Profound effects on major societal issues such as groundwater quality, environmental contamination, the loss of productive agricultural lands, and global warming.**

Why use hard x-rays for investigating environmental systems?

- **Hard x-rays (i.e. greater than ~2 keV) interact "weakly" with matter (relative to charge particle probes) and enable the investigation of hydrated and/or buried samples.**
- **Hard x-rays enable highly sensitive elemental analysis on extremely small objects.**
- **High sensitivity of x-rays enables x-ray absorption spectroscopy (i.e. interrogation of chemistry)**

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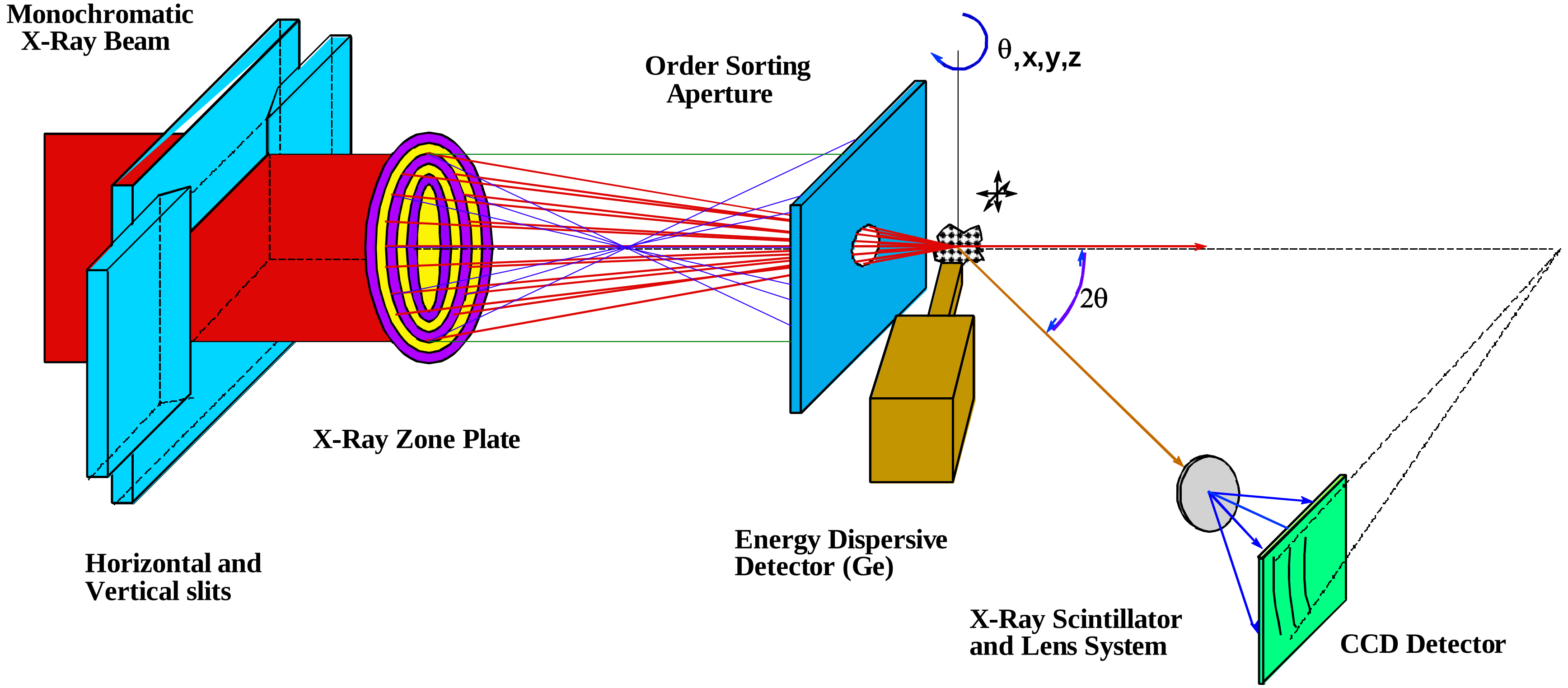
Differences between planktonic and surface-adhered bacteria to heavy metal exposure

- **Attachment of cells to surfaces during biofilm formation leads to major changes in metabolism, resistance, and survivability.**
- **Although microbes appear to be able to catalyze almost any reaction from which energy can be obtained, it is difficult to determine the mechanisms whereby catalysis occurs at the microbe-substrate interface.**
- **It is difficult to quantify the concentrations of metals, their cellular locations, and their redox states.**

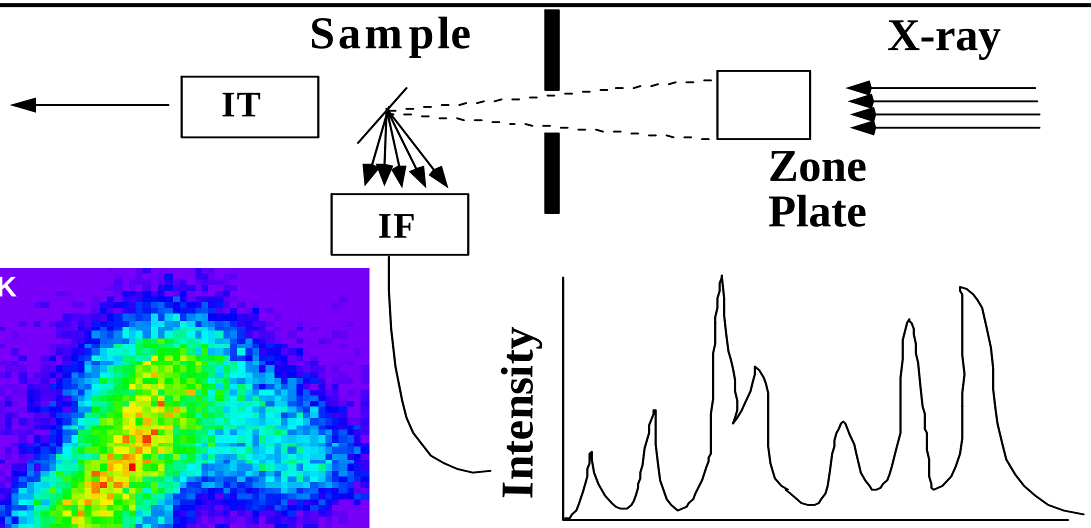
- ***Can XRF microscopy identify differences between planktonic and surface-adhered bacteria upon exposure to heavy metals and changes to heavy metal speciation upon exposure to bacteria?***

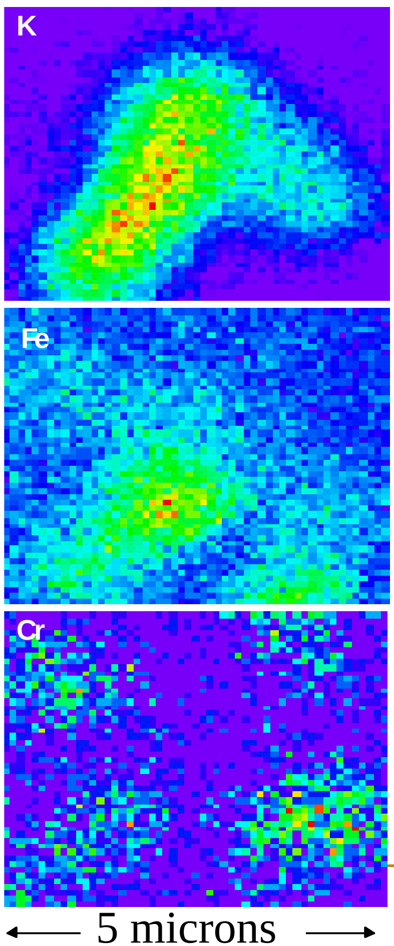
XRM with Fresnel zone plates:

X-RAY MICROPROBE BEAMLINE AT APS (2-ID-D/E)



2-D X-ray Fluorescence Imaging of Individual hydrated Bacterium with Zone Plates at the APS





What can it do for me?

Spatially resolve (150 nm):

- ***Distribution***
- ***Valence state***
- ***Chemical speciation***

of elements

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Biological Abundance

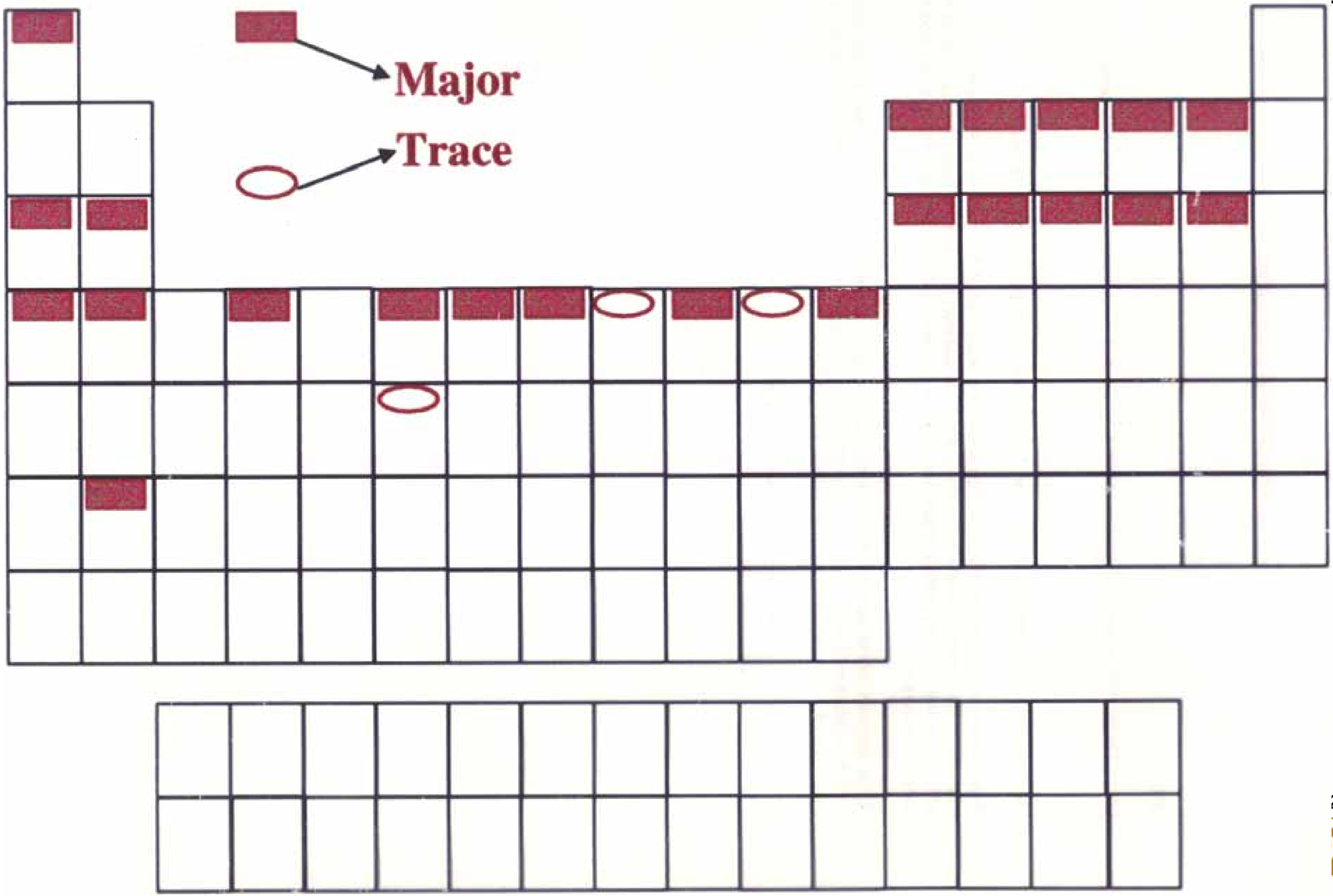
Major

Trace

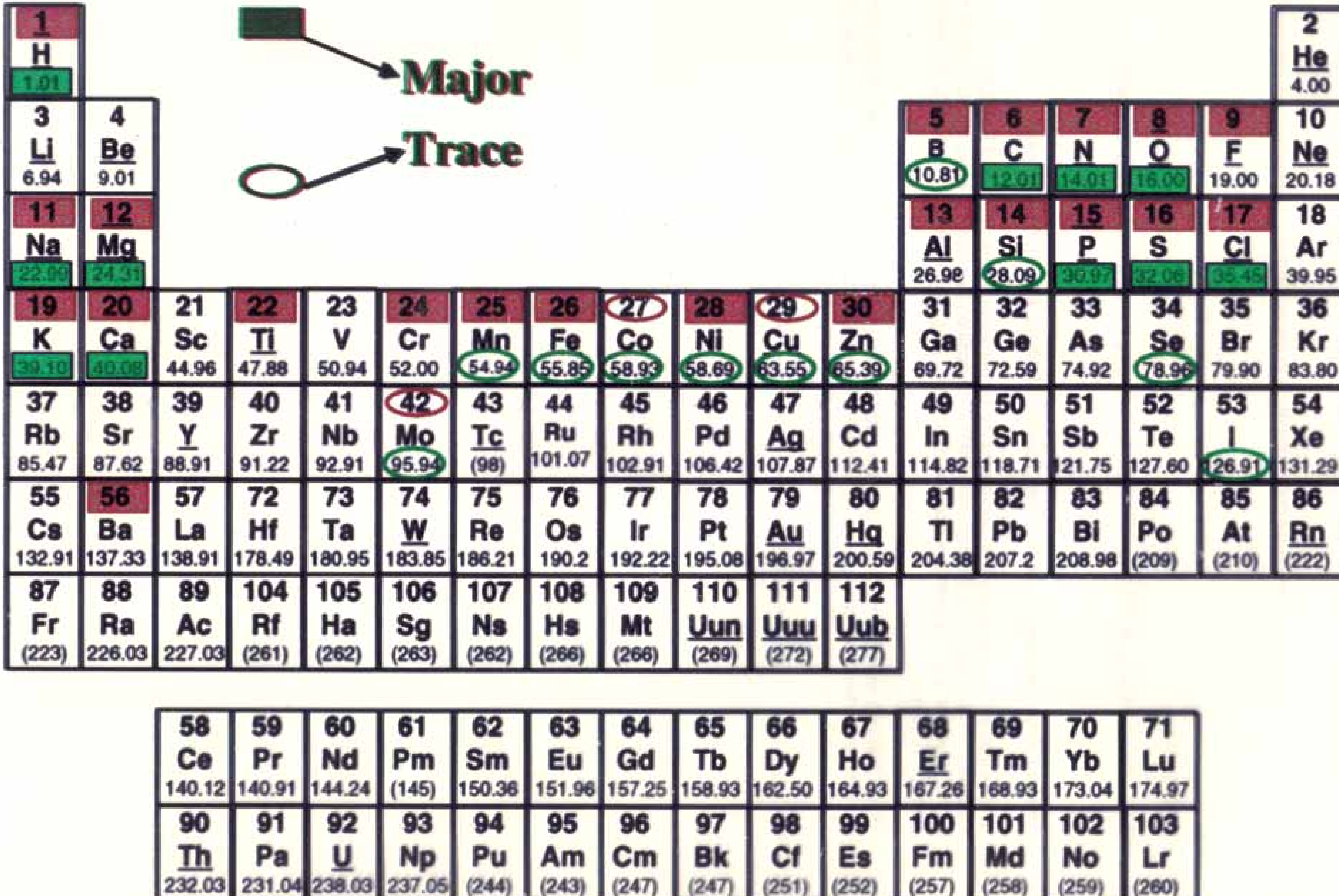
1 H 1.01																	2 He 4.00
3 Li 6.94	4 Be 9.01											5 B 10.81	6 C 12.01	7 N 14.01	8 O 16.00	9 F 19.00	10 Ne 20.18
11 Na 22.99	12 Mg 24.31											13 Al 26.98	14 Si 28.09	15 P 30.97	16 S 32.06	17 Cl 35.45	18 Ar 39.95
19 K 39.10	20 Ca 40.08	21 Sc 44.96	22 Ti 47.88	23 V 50.94	24 Cr 52.00	25 Mn 54.94	26 Fe 55.85	27 Co 58.93	28 Ni 58.69	29 Cu 63.55	30 Zn 65.39	31 Ga 69.72	32 Ge 72.59	33 As 74.92	34 Se 78.96	35 Br 79.90	36 Kr 83.80
37 Rb 85.47	38 Sr 87.62	39 Y 88.91	40 Zr 91.22	41 Nb 92.91	42 Mo 95.94	43 Tc (98)	44 Ru 101.07	45 Rh 102.91	46 Pd 106.42	47 Ag 107.87	48 Cd 112.41	49 In 114.82	50 Sn 118.71	51 Sb 121.75	52 Te 127.60	53 I 126.91	54 Xe 131.29
55 Cs 132.91	56 Ba 137.33	57 La 138.91	72 Hf 178.49	73 Ta 180.95	74 W 183.85	75 Re 186.21	76 Os 190.2	77 Ir 192.22	78 Pt 195.08	79 Au 196.97	80 Hg 200.59	81 Tl 204.38	82 Pb 207.2	83 Bi 208.98	84 Po (209)	85 At (210)	86 Rn (222)
87 Fr (223)	88 Ra 226.03	89 Ac 227.03	104 Rf (261)	105 Ha (262)	106 Sg (263)	107 Ns (262)	108 Hs (266)	109 Mt (266)	110 Uun (269)	111 Uuu (272)	112 Uub (277)						

58 Ce 140.12	59 Pr 140.91	60 Nd 144.24	61 Pm (145)	62 Sm 150.36	63 Eu 151.96	64 Gd 157.25	65 Tb 158.93	66 Dy 162.50	67 Ho 164.93	68 Er 167.26	69 Tm 168.93	70 Yb 173.04	71 Lu 174.97
90 Th 232.03	91 Pa 231.04	92 U 238.03	93 Np 237.05	94 Pu (244)	95 Am (243)	96 Cm (247)	97 Bk (247)	98 Cf (251)	99 Es (252)	100 Fm (257)	101 Md (258)	102 No (259)	103 Lr (260)

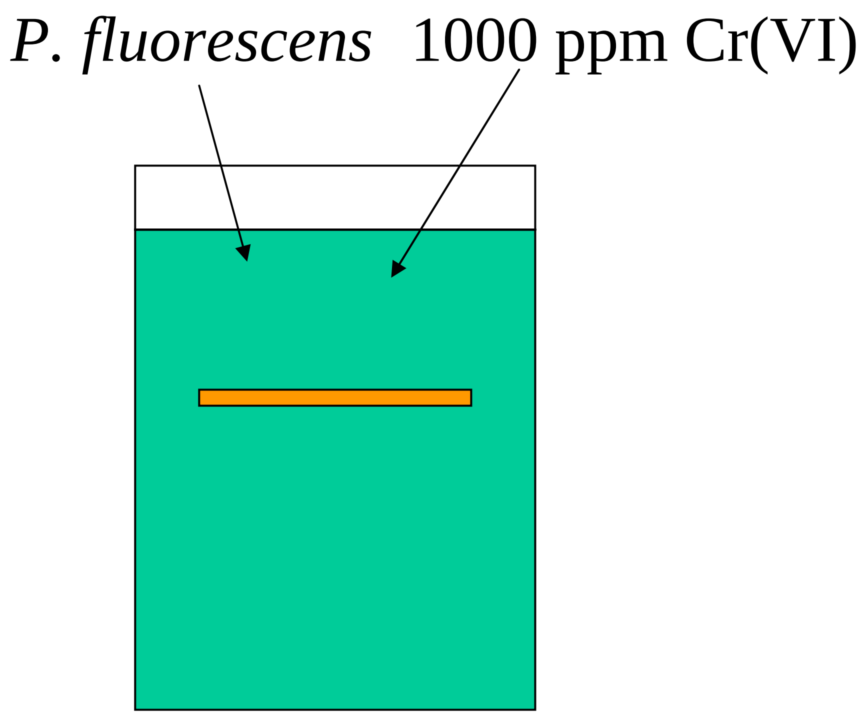
Crustal Abundance



Biological Abundance



What is the role of the physiological state of a microbe (planktonic versus surface-adhered) on its tolerance to heavy metals?



Elements required for life: H, C, N, O, P, Ca, S, Fe, Ni, Cu….

These elements should be in cells.

Elemental distribution in planktonic *P. fluorescens* w/ and w/out addition of Cr(VI)

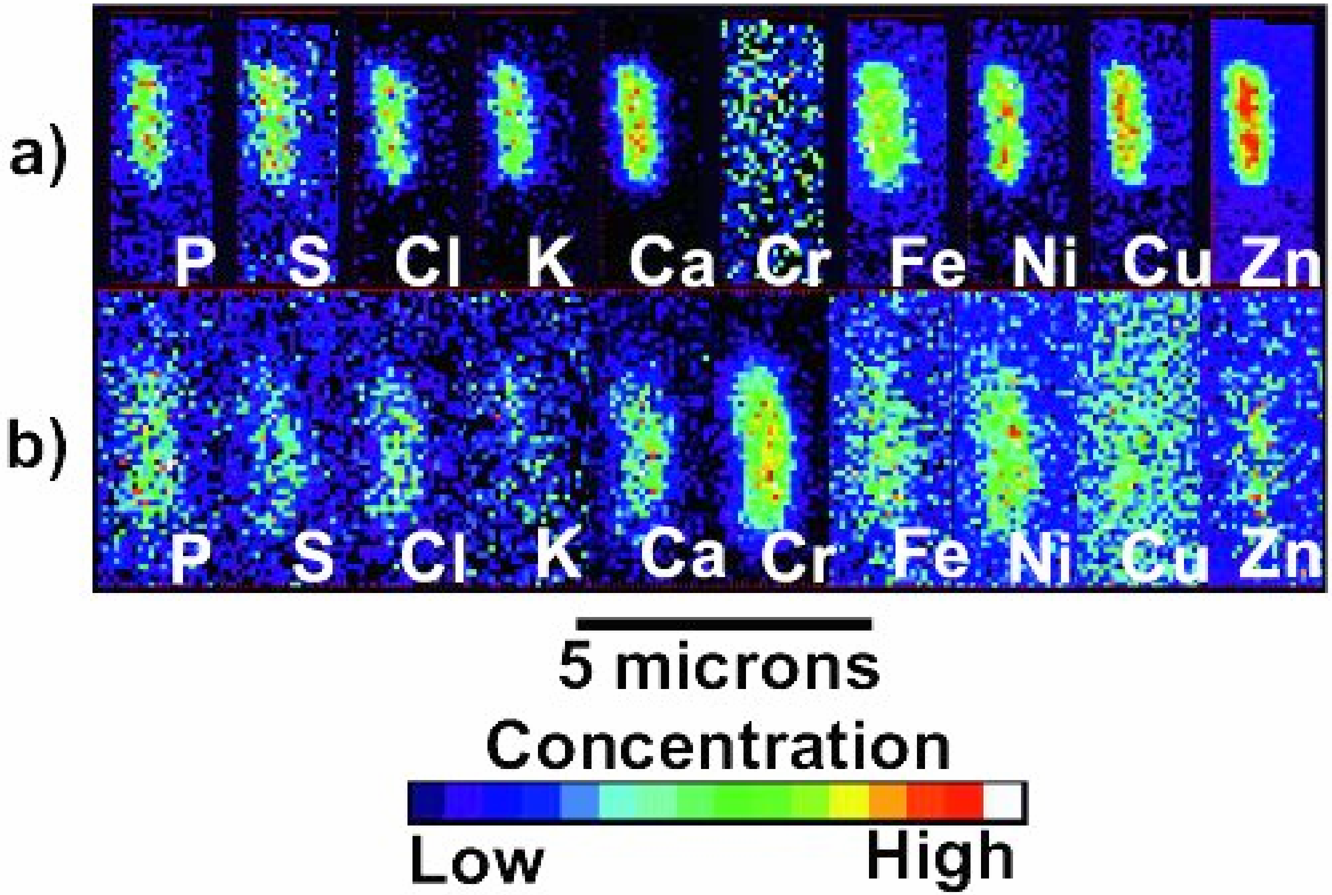


Fig. 1

K. M. Kemner, S. D. Kelly, B. Lai, J. Maser, E. J. O'Loughlin, D. Sholto-Douglas, Z. Cai, M. A. Schneegurt, C. F. Kulpa, Jr., K. H. Nealson, "*Elemental and Redox Analysis of Single Bacterial Cells by X-ray Microbeam Analysis*," *Science* **306** 686-687, 2004.

Elemental distribution in surface-adhered *P. fluorescens* w/ and w/out addition of Cr(VI)

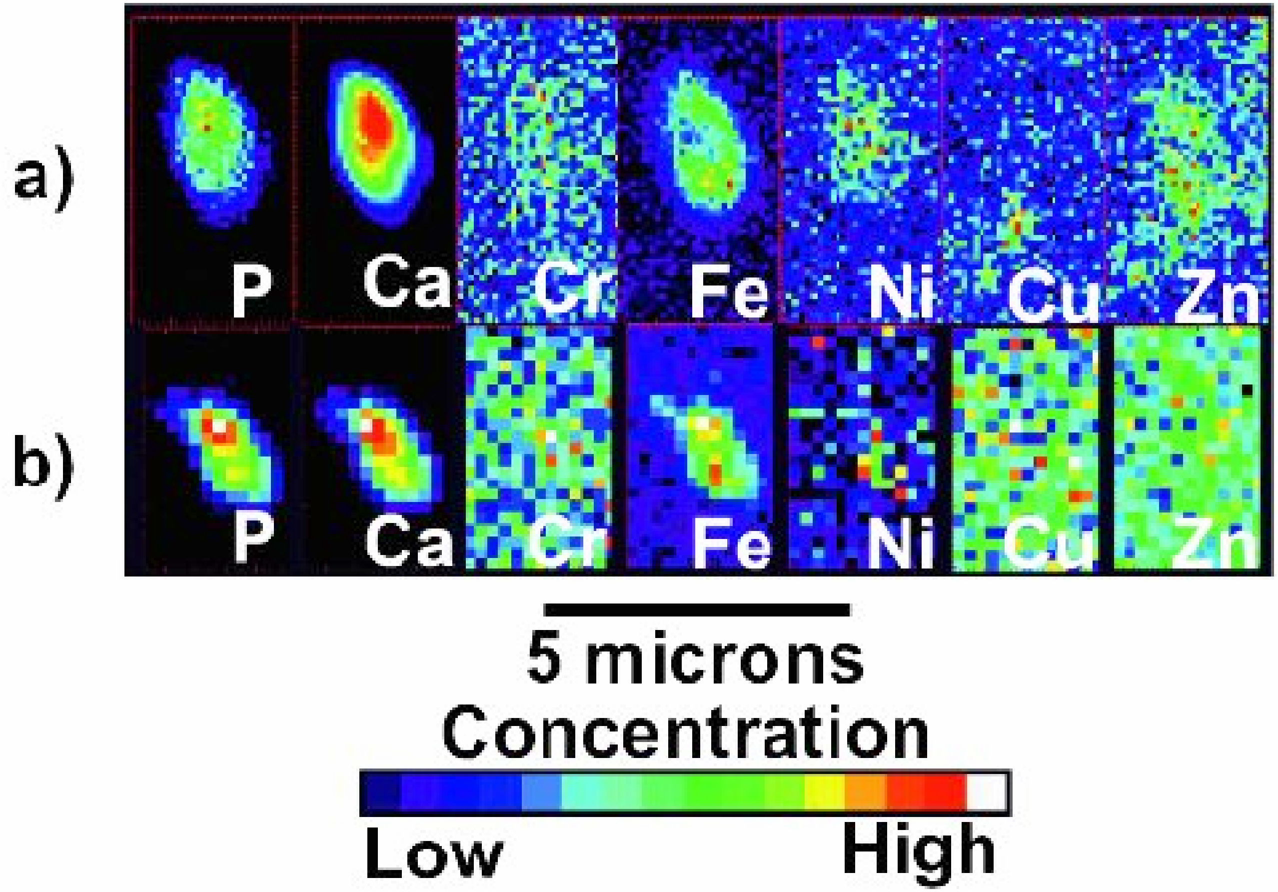
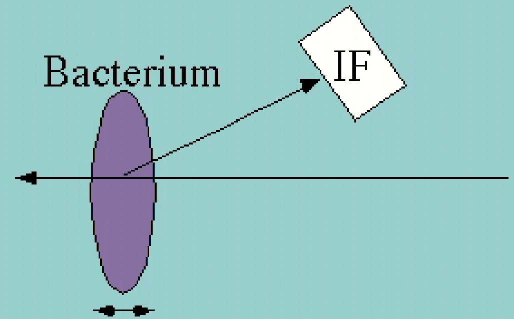


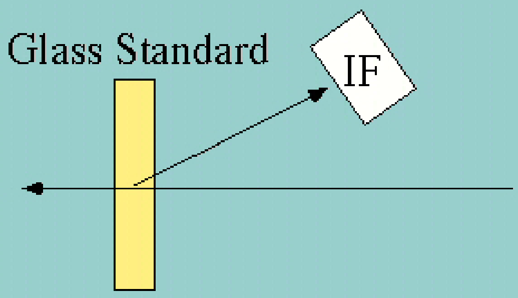
Fig. 2

XRF Elemental Microanalysis of a Bacterium



thickness$_{microbe}$ ~ 1 micron

$\rho_{microbe}$ ~ 1 g/cm^3



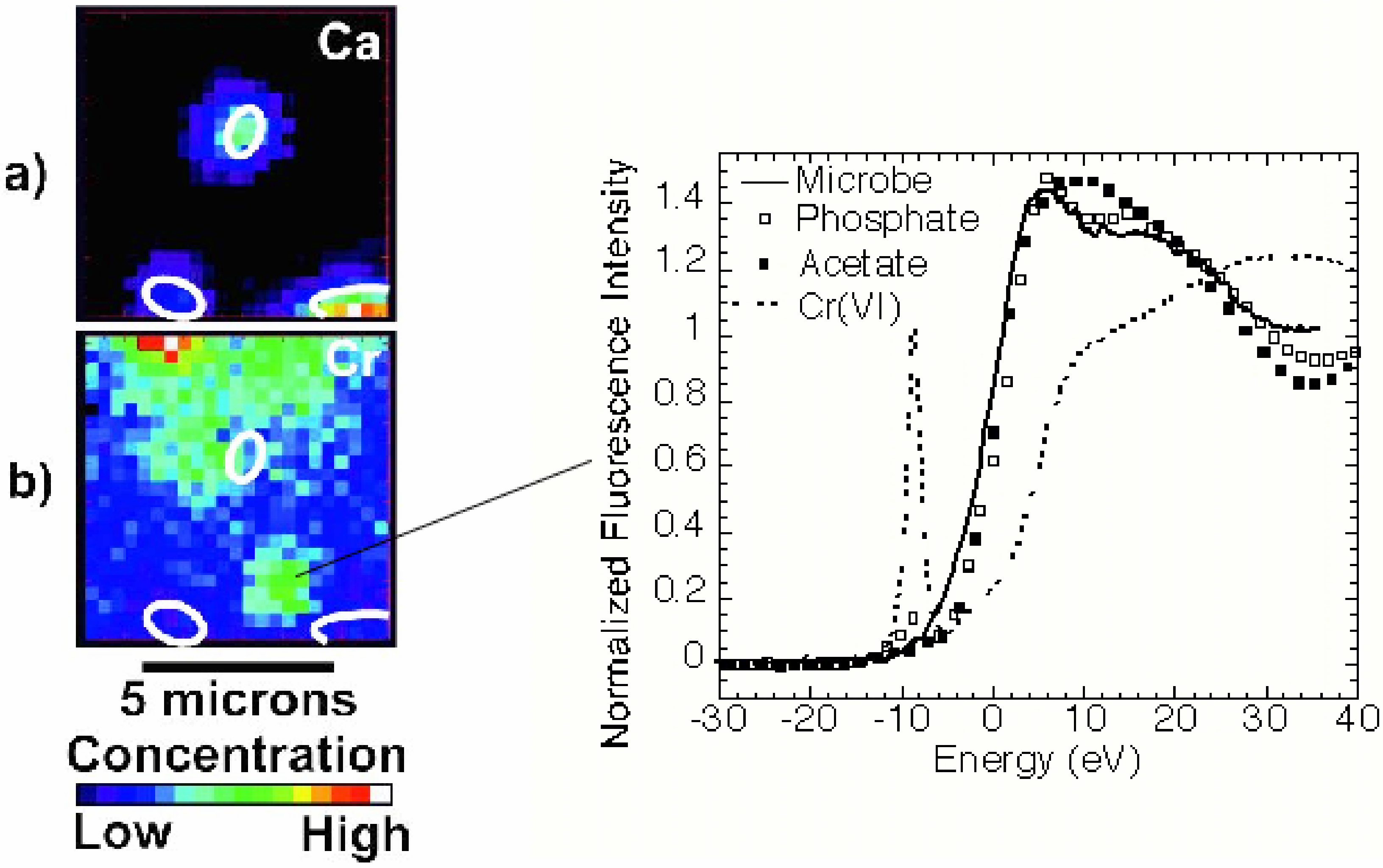
Known elemental area density = σ^2 μg/cm^2

$$[\ \ ] = \frac{(IF_{microbe})}{(\rho_{microbe} * thickness_{microbe})} \frac{(\sigma^2_{glass})}{(IF_{glass})}$$

Results of quantitative XRF elemental analysis of single cells

	[P]	[S]	[Cl]	[K]	[Ca]	[Cr]	[Mn]	[Fe]	[Co]	[Ni]	[Cu]	[Zn]
Planktonic (5)	16,048 (2,446)	6,625 (1,117)	8,421 (2,628)	3,604 (1,173)	3,815 (392)	9 (2)	22 (4)	156 (23)	190 (37)	120 (33)	201 (46)	1,175 (176)
Planktonic + Cr(VI) (6)	6,156 (1,034)	3,719 (1,516)	3,908 (1,814)	2,201 (1668)	673 (230)	949 (323)	22 (4)	58 (29)	13 (12)	26 (18)	105 (76)	94 (30)
Surface-Adhered (8)	661,032 (139,416)	*	*	*	570,855 (92,831)	32 (10)	40 (7)	360 (216)	14 (7)	26 (10)	0 (14)	25 (13)
Surface-Adhered +Cr(VI) (10)	419,034 (362,728)	*	*	*	427,987 (147,983)	24 (15)	23 (8)	326 (177)	12 (7)	18 (9)	2 (5)	15 (7)

Spatial distribution and valence state of Cr relative to Surface-adhered cells



Summary

- The integration of new techniques/tools such as the Advanced Photon Source with multiple scientific disciplines provides new and exciting opportunities for addressing a variety of highly relevant Environmental Science/Geomicrobiology issues.
- Hard x-ray (micro)(spectro)scopy offers many exciting possibilities for future investigations.
- The integration of the strengths of both x-ray and electron microscopies to investigate geomicrobiological systems is especially promising.

Final Thoughts………..

- **History of this work……**

- ***These results did not occur during a single beam run***

K. M. Kemner, D. B. Hunter, R. Boopathy

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XRF can identify elements in bacterial biomass

W. Yun: "Wait about 3 years"

Note scale bar!

XRF elemental mapping may be able to “image” individual bacterial cells

W. Yun: “Wait about 3-5 years”

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Note scale bar!

XRF elemental mapping can “image” individual bacterial cells

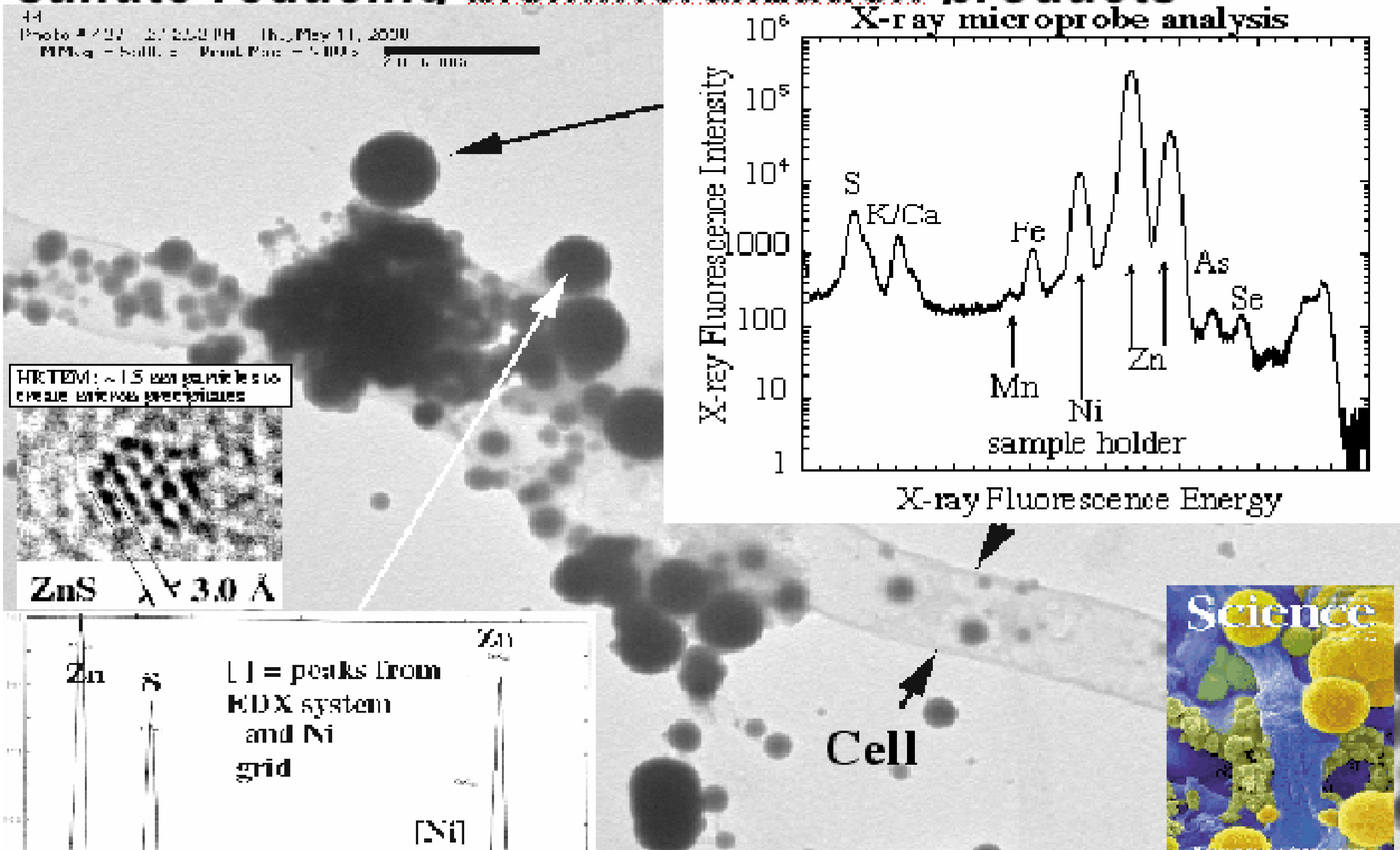
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Note scale bar!

Final Thoughts

- **In 1997, a proposal to "image" individual bacterial cells with high brilliance, hard x-rays was a very strange idea and not well received by many scientists**
  - Most physicists did not care about bacteria
  - Most microbiologists thought the cells would "blow up" when subjected to the x-rays
- **It took over 7 years from the first attempt (~Feb '97) to image bacterial cells until the "big" publication**
- **It took ~4 years from the third attempt (~Nov.'98) until the experiments were routine (lots of kinks in experimental procedure to work out)**
- **A special thanks is owed to W. Yun, B. Lai, and J. Maser (physicists who were willing to try) and M. Schneegurt (a microbiologist who got tired of fighting with me)**
- **Could something like this happen again??????**
  Hopefully, the APS will continue to provide an environment that allows their scientists to explore the very strange ideas

X-ray & electron microscopy investigations of sulfate-reducing biomineralization products



"Formation of Sphalerite (ZnS) Deposits in Natural Biofilms of Sulfate-Reducing Bacteria," M. Labrenz, G. K. Druschel, T. Thomsen-Ebert, B. Gilbert, S. A. Welch, K. M. Kemner, G. A. Logan, R. H. Summons, G. De Stasio, P. L. Bond, B. Lai, S. D. Kelly, J. F. Banfield, *Science* 290, 1744-1747, 2000.