Investigation of Indigenous Metal-reducing Bacteria and the Chemistry of Native Metal Oxides at the NABIR FRC

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Summary
In November 2001 we were provided with sediment samples from both the Background Area and Area 3 of the NABIR FRC, Oak Ridge, TN. Three samples from the Background Area (FWB303-02-22, FWB303-05-14, and FWB303-07-18) were provided representing three depth intervals: surface (24-44 in.), vadose (96-113 in.), and saturated (168-192 in.) zones. Two samples from Area 3 (FWB100-02, FWB100-06-13) were provided from two depth intervals: 381-382 in. and 548-550 in. Our goals are to determine the diversity of indigenous metal-reducing bacteria and to understand the nature of the metal oxides available for reduction by the metal-reducing populations. Chemical analyses include sequential extractions of Fe and Mn, and simultaneously extracted trace metals, secondary-ion mass spectrometry (SIMS) to identify surface adsorbed species, and X-ray absorption spectroscopy (XAS) to identify the species intimately associated with the metal oxides. Microbiological analyses include enrichments for Fe-, Mn-, and sulfate-reducing bacteria, plate counts of aerobic heterotrophs, and construction of Bacterial and Archaeal 16S rDNA libraries. The results to date are summarized below.

Fe phase partitioning, from Table 1

- All samples were predominantly oxidized, as little Fe(II) could be detected in any extraction.
- Total Fe varied between 2.25% and 5.15% (by wt), with the greatest amount in the saturated zone of the Background Area and the least in the deep sample from Area 3.
- Small amounts of weak acid-extractable Fe(III) (0.5 M HCl) were detected in all samples, with the highest concentration found in the saturated zone at the Background Area.
- Greater concentrations of strong acid-extractable Fe(III) (5 M HCl) were found in all samples, with the highest concentration in the deep sediments at Area 3.
- The lowest concentrations of both weak and strong acid-extractable Fe(III) were found in the surface sediments at the Background Area, even though this sediment contains the second most total Fe.
- Ppb levels of Mg-exchangeable Fe were measured in all samples, with the greatest amount in the shallow Area 3 sediments and the least amount in the deep Area 3 sediments. This is not surprising considering the oxidizing nature of the sediments.
- While ammonium oxalate (pH 3) extracted more Fe(II) than either HCl extraction, and more than the $MgCl_2$ extraction, far less Fe(III) was extracted by this method than by the HCl extractions. The greatest amount of oxalate-extractable Fe(III) was found in the deep Area 3 sediments and the least was found in the surficial Background Area sediments.
- Of the 5 extractions used, citrate-bicarbonate-dithionite extracted the most Fe, with the highest concentration in the vadose zone of the Background Area and the least in the shallow Area 3 sediments.
- The extractions used here accounted for between 4.5% and 37% of the total Fe in each sample, **indicating that large quantities of the Fe in the systems are tied up in recalcitrant mineralogical forms.**

Mn phase partitioning, from Table 2

- Total Mn ranged from 640 mg kg^{-1} to 2000 mg kg^{-1}, with the highest concentration in the Background Area saturated zone (same as Fe), and the lowest in the shallow sample from Area 3.
- Unlike total Fe, the deep Area 3 sample contained high concentration of total Mn.

- The two sequential HCl extractions accounted for >100% of the total Mn in the saturated zone at the Background Area (the discrepancy in mass balance is almost certainly due to local heterogeneity within the sample we were provided, since total Mn was determined on a different subsample than the HCl extractions).
- Likewise, the HCl extractions accounted for nearly 100% (94.5%) of the total Mn in the deep Area 3 sediments.
- Mg exchanged far more Mn than it did Fe, with the greatest amount exchanged from the deep Area 3 sediment.
- Unlike Fe, very little oxalate-extractable Mn could be found in the deep Area 3 sediments.
- CBD extracted far less Mn than it did Fe, with the greatest in the deep Area 3 sediments.
- **The Mn data suggest that a large fraction of the Mn at both Areas is in a potentially bioavailable form.**

Trace metal phase partitioning, from Tables 3-6

- Total arsenic (As) ranged from 1 to 2.5 mg kg^{-1}, but was not detected in any subsequent extraction, suggesting that it is tied up in a highly recalcitrant mineral phase.
- Mercury (Hg) was consistently below detection (0.15 mg kg^{-1}).
- Interference with Fe caused the detection limits of Pb and U to be extremely high (100 mg kg^{-1}), and Cu to be 5 mg kg^{-1}. Thus, Cu, U, and Pb were undetectable in all samples.
- Total technetium (Tc) was measured at approx. 9 pCi g^{-1} in the FWB100-06-13 (deep Area 3) sediments, and below detection (i.e., < 3 pCi g^{-1}) in all others.
- The detection limits for Ni, Co, Zn, and Cr varied with Fe content of the extract.
- Total Ni, Co, and Zn concentrations are lower at Area 3 than in the Background sediments.
- Total Cr is comparable at both Areas.
- As much as 22% of the Ni could be accounted for in the 0.5 M and 5 N HCl extractions.
- Likewise, greater than 10% of the total Ni in the deep Area 3 sediments was exchanged by $MgCl_2$.
- Oxalate and CBD did not extract any more Ni than did the acid extractions.
- In the background Area, 5 N HCl extract of Ni varies linearly as a function of 5 N HCl Fe with a slope of +0.0076 and a correlation coefficient of 0.9906 (**Figure 1**).
- $MgCl_2$-exchangeable Ni varies linearly with 0.5 N HCl-extractable Fe with a slope of +0.0011 and a correlation coefficient of 0.9997 (**Figure 2**).
- CBD-extracted Ni varies linearly with CBD-extracted Mn with a slope of +0.017 and a correlation coefficient of 0.9997 (**Figure 3**).
- **These correlations suggest that Ni is bound in some potentially bioavailable forms with both Fe and Mn.**
- The greatest percentage of total Cr was extracted from the vadose zone of the Background Area with CBD (23%).
- No Cr was detected by Mg exchange.
- As much as 75% of the total Co was extracted in the HCl extractions from the Background Area saturated zone.
- In most samples, >10% of the total Co was Mg-exchangeable.
- CBD extracted more than 100% of the total Co from the Background Area vadose, and more than half from the saturated zone.
- **Co may also be in a highly bioavailable form.**

- A large fraction (>30%) of the total Zn was accounted for in the HCl extractions.
- >100% of the total Zn was accounted for in the HCl extractions from the deep Area 3 sediments.
- Small amounts of Mg-exchangeable Zn were detected in the Background Area vadose sediments and the deep Area 3 sediments.
- Oxalate extracted very little Zn, while CBD extracted about the same amount as the 0.5 M HCL extractions.

Plate counts of viable aerobic heterotrophs

- Aerobic heterotrophs were counted using the dilution plate count method with both R2A and LBA media (circumneutral).
- The lowest dilution plated, 10^{-4}, produced no colonies from Area 3 sediments.
- In the Background Area, plate counts increased with depth from $2.55 \times 10^5 \pm 1.57 \times 10^5$ cells per gram wet sediment to $3.15 \times 10^6 \pm 1.00 \times 10^6$ cells per gram (on LBA). Counts on R2A were only slightly lower (**Figure 4**).
- Both sets of plate counts (LBA and R2A) were dominated by 1 or 2 colony types. Eight putatively unique colony types have been isolated and are currently being identified.

Enrichments

- Circumneutral enrichments for Fe-, Mn-, SO_4-, and NO_3-reducing bacteria were initiated with H_2, acetate, or lactate as electron donor. When H_2 was the electron donor the cultures were provided 10 mM formate as an organic carbon source.
- From the Background Area, multiple enrichments are reducing Fe, Mn, and sulfate from all depths.
- No nitrate-reducing enrichments show signs of activity from either Area.
- From Area 3, the only positive enrichment is one with sulfate and H_2.
- These enrichments will continue to be cultivated and an attempt to isolate individual organisms is forthcoming.

Clone libraries

- DNA was extracted from all 5 sediment samples and a Bacterial 16S rDNA (full length) clone library has been constructed for the saturated zone in the Background Area.
- Of 50 clones screened by RFLP, only 8 unique ribotypes were identified.
- Two ribotypes predominated the library, comprising 46% and 42% of the clones screened.
- Each of the other 6 ribotypes appeared only once in the library.
- The 8 unique clones are currently being sequenced.
- Libraries will be constructed for the remaining samples.

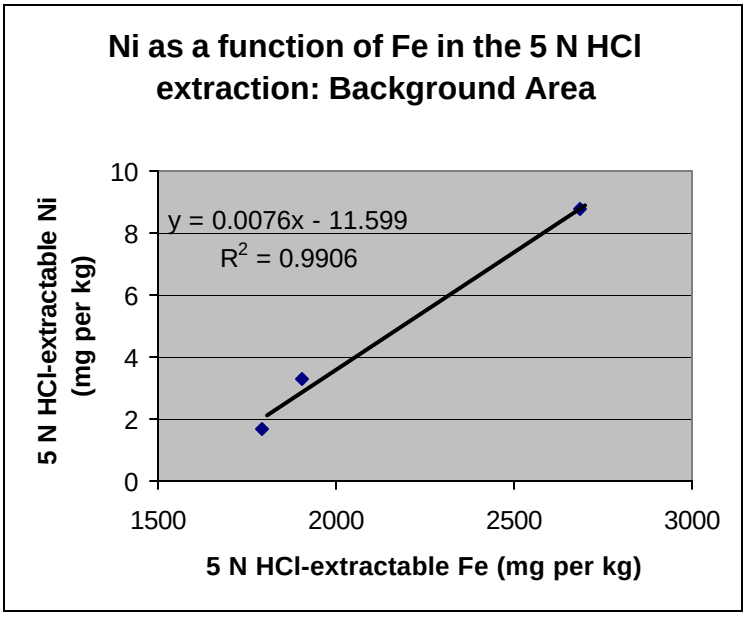

Figure 1. Correlation between Ni and Fe in FRC Background Area sediments digested in 5 N HCl.

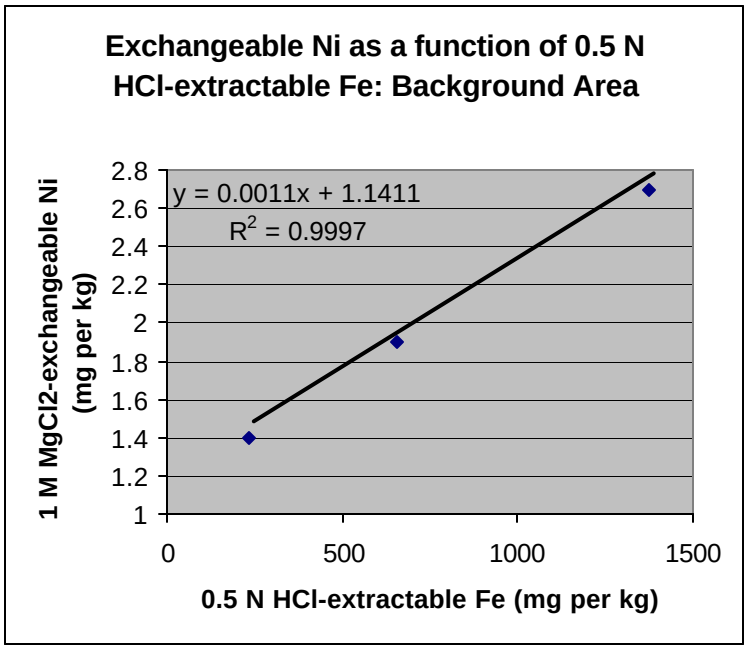

Figure 2. Correlation between $MgCl_2$-exchangeable Ni and 0.5 N HCl-extractable Fe in FRC Background Area sediments.

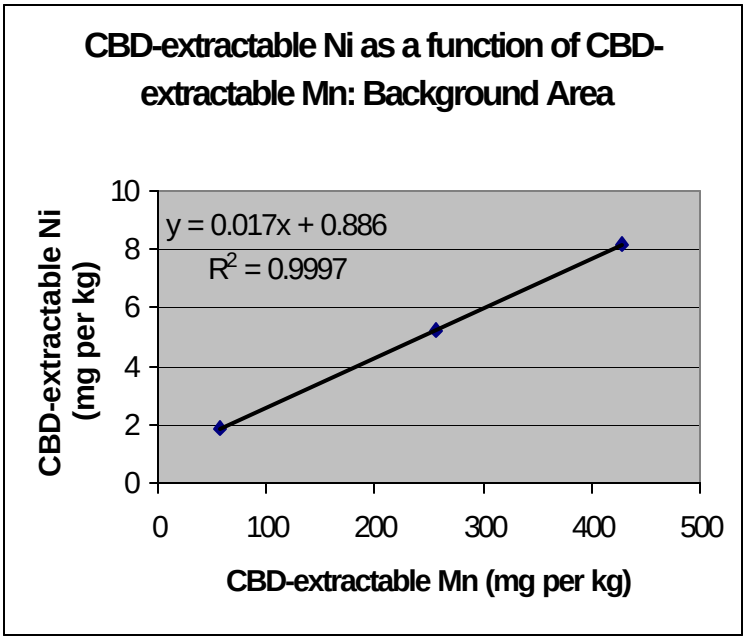

Figure 3. Correlation between Ni and Mn in FRC Background Area sediments digested with citrate-bicarbonate-dithionite (CBD).

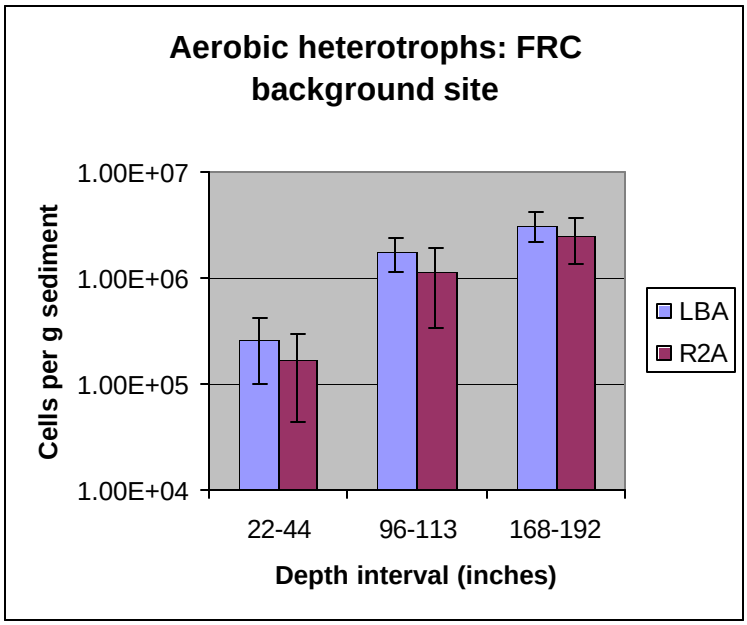

Figure 4. Plate counts of aerobic heterotrophic bacteria in FRC Background Area sediments. Two media were used, a defined medium (R2A) and a complex medium (LBA). Data are means of triplicate dilution series and error bars represent 1 SD from the mean.

Table 1. Partitioning of Fe into operationally-defined phases at the NABIR FRC.

			0.5 M HCl[2]		5 M HCl[2]		1 M $MgCl_2$[3]		Oxalate[4]		CBD[5]	
Area	**Depth (in)**	**Total Fe[1] (%)**	**Fe(II)**	**Total**	**Fe(II)**	**Total**	**Fe(II)**	**Total**	**Fe(II)**	**Total**	**Fe(II)**	**Total**
BKGRD	24-44	4.44	BDL[6]	234	BDL	1790	0.4	0.36	18	227	N/A	3700
BKGRD	96-113	3.73	BDL	656	BDL	1906	0.37	0.52	35	679	N/A	8708
BKGRD	168-192	5.15	BDL	1373	BDL	2685	0.36	0.28	45	831	N/A	7628
3	381-382	3.39	BDL	929	BDL	2363	0.66	0.68	265	480	N/A	2895
3	548-550	2.25	BDL	464	BDL	3241	0.3	0.34	47	1704	N/A	6656

[1] Total Fe was determined by digestion of sediments in boiling HNO3/HF, reported as percent by weight.

[2] 0.5 M HCl and 5 M HCl extractions were performed sequentially on the same sample, mg kg^{-1}.

[3] 1 M $MgCl_2$ extraction was performed on unaltered sediments, followed sequentially by the oxalate and CBD extractions, mg kg^{-1}.

[4] pH 3 ammonium oxalate, mg kg^{-1}.

[5] Citrate-bicarbonate-dithionite, mg kg^{-1}.

[6] Fe detection limit was 0.5 mg kg^{-1}.

Table 2. Partitioning of Mn into operationally-defined phases at the NABIR FRC.

Area	**Depth (in)**	**Total Mn[1]**	**0.5 M HCl[2]**	**5 M HCl[2]**	**1 M $MgCl_2$[3]**	**Oxalate[4]**	**CBD[5]**
BKGRD	24-44	820	13	245	1.9	254	58
BKGRD	96-113	940	28.5	462	3.0	39.1	257
BKGRD	168-192	2000	166	2270	1.6	905	428
3	381-382	640	39.7	168	25.1	158	51.7
3	548-550	1300	57.2	1172	60.5	4.1	833

[1] Total Mn was determined by digestion of sediments in boiling HNO3/HF, reported as mg kg^{-1}.

[2] 0.5 M HCl and 5 M HCl extractions were performed sequentially on the same sample, mg kg^{-1}.

[3] 1 M $MgCl_2$ extraction was performed on unaltered sediments, followed sequentially by the oxalate and CBD extractions, mg kg^{-1}.

[4] pH 3 ammonium oxalate, mg kg^{-1}.

[5] Citrate-bicarbonate-dithionite, mg kg^{-1}.

Table 3. Partitioning of Ni into operationally-defined phases at the NABIR FRC.

Area	**Depth (in)**	**Total Ni**[1]	**0.5 M HCl**[2]	**5 M HCl**[2]	**1 M $MgCl_2$**[3]	**Oxalate**[4]	**CBD**[5]
BKGRD	24-44	36	5.2	1.7	1.4	BDL[6]	1.9
BKGRD	96-113	36	BDL	3.3	1.9	2.4	5.2
BKGRD	168-192	40	BDL	8.8	2.7	5.5	8.2
3	381-382	34	1.2	4.2	0.78	1.0	2.6
3	548-550	21	BDL	2.7	3.1	BDL	2.0

[1] Total Ni was determined by digestion of sediments in boiling HNO3/HF, reported as mg kg^{-1}.

[2] 0.5 M HCl and 5 M HCl extractions were performed sequentially on the same sample, mg kg^{-1}.

[3] 1 M $MgCl_2$ extraction was performed on unaltered sediments, followed sequentially by the oxalate and CBD extractions, mg kg^{-1}.

[4] pH 3 ammonium oxalate, mg kg^{-1}.

[5] Citrate-bicarbonate-dithionite, mg kg^{-1}.

[6] The detection limit for Ni varied with the Fe content of the extract, between 0.4 and 1.8 mg kg^{-1}.

Table 4. Partitioning of Cr into operationally-defined phases at the NABIR FRC.

Area	**Depth (in)**	**Total Cr[1]**	**0.5 M HCl[2]**	**5 M HCl[2]**	**1 M $MgCl_2$[3]**	**Oxalate[4]**	**CBD[5]**
BKGRD	24-44	66	2.6	BDL[6]	BDL	BDL	2.8
BKGRD	96-113	49	BDL	2.4	BDL	BDL	11.4
BKGRD	168-192	55	BDL	4.4	BDL	BDL	7.3
3	381-382	62	BDL	3.6	BDL	0.52	3.1
3	548-550	42	BDL	BDL	BDL	2.0	4.3

[1] Total Cr was determined by digestion of sediments in boiling HNO3/HF, reported as mg kg^{-1}.

[2] 0.5 M HCl and 5 M HCl extractions were performed sequentially on the same sample, mg kg^{-1}.

[3] 1 M $MgCl_2$ extraction was performed on unaltered sediments, followed sequentially by the oxalate and CBD extractions, mg kg^{-1}.

[4] pH 3 ammonium oxalate, mg kg^{-1}.

[5] Citrate-bicarbonate-dithionite, mg kg^{-1}.

[6] The detection limit for Cr varied with the Fe content of the extract, between 0.4 and 1.8 mg kg^{-1}.

Table 5. Partitioning of Co into operationally-defined phases at the NABIR FRC.

Area	**Depth (in)**	**Total Co**[1]	**0.5 M HCl**[2]	**5 M HCl**[2]	**1 M $MgCl_2$**[3]	**Oxalate**[4]	**CBD**[5]
BKGRD	24-44	14	BDL[6]	3.4	1.4	BDL	2.8
BKGRD	96-113	9	BDL	2.4	1.4	BDL	11.4
BKGRD	168-192	14	2.2	8.3	2.7	BDL	7.3
3	381-382	9	1.2	3.0	0.77	0.52	3.1
3	548-550	11	BDL	2.7	1.6	2.0	4.3

[1] Total Co was determined by digestion of sediments in boiling HNO3/HF, reported as mg kg^{-1}.

[2] 0.5 M HCl and 5 M HCl extractions were performed sequentially on the same sample, mg kg^{-1}.

[3] 1 M $MgCl_2$ extraction was performed on unaltered sediments, followed sequentially by the oxalate and CBD extractions, mg kg^{-1}.

[4] pH 3 ammonium oxalate, mg kg^{-1}.

[5] Citrate-bicarbonate-dithionite, mg kg^{-1}.

[6] The detection limit for Co varied with the Fe content of the extract, between 0.4 and 1.8 mg kg^{-1}.

Table 6. Partitioning of Zn into operationally-defined phases at the NABIR FRC.

Area	**Depth (in)**	**Total Zn[1]**	**0.5 M HCl[2]**	**5 M HCl[2]**	**1 M $MgCl_2$[3]**	**Oxalate[4]**	**CBD[5]**
BKGRD	24-44	58	14.6	14.6	BDL[6]	BDL	7.4
BKGRD	96-113	46	5.2	8.6	0.95	BDL	6.2
BKGRD	168-192	55	5.0	21	BDL	BDL	8.2
3	381-382	44	6.5	11.2	BDL	0.26	3.9
3	548-550	27	13.4	15.2	1.2	0.39	10.6

[1] Total Zn was determined by digestion of sediments in boiling HNO3/HF, reported as mg kg^{-1}.

[2] 0.5 M HCl and 5 M HCl extractions were performed sequentially on the same sample, mg kg^{-1}.

[3] 1 M $MgCl_2$ extraction was performed on unaltered sediments, followed sequentially by the oxalate and CBD extractions, mg kg^{-1}.

[4] pH 3 ammonium oxalate, mg kg^{-1}.

[5] Citrate-bicarbonate-dithionite, mg kg^{-1}.

[6] The detection limit for Zn varied with the Fe content of the extract, between 0.3 and 0.9 mg kg^{-1}.

Table 7. pH and anions in sediments from the NABIR FRC.

Area	**Depth (in)**	**pH[1]**	**Nitrate[2]**	**Sulfate[2]**	**Chloride[2]**
BKGRD	24-44	5.41	BDL[3]	BDL	BDL
BKGRD	96-113	6.69	BDL	BDL	BDL
BKGRD	168-192	7.79	BDL	BDL	BDL
3	381-382	4.11	12000	BDL	2100
3	548-550	4.16	51000	3000	1800

[1] Sediment pH was determined on 5 g of ground sediment dispersed in 25 mL of deionized water.

[2] Anions were determined by boiling ground sediment in dilute KOH, followed by ion chromatography, mg kg^{-1}.

[3] The detection limit for anions was 1000 mg kg^{-1}.

Table 8. Uranium in sediments of the NABIR FRC.

Sample	**Depth (in)**	**Area**	**U (mg kg^{-1})**
FWB303-02-22	24-44	Background	1.2
FWB303-05-14	96-113	Background	1.2
FWB303-07-18	168-192	Background	2.5
FWB100-02	381-382	Area 3	32.5
FWB100-06-13	548-550	Area 3	404

Brief methods

Finely powdered sediment was acid-leached with hot concentrated nitric acid. The resulting slurry was shaken with 25% TBP-in-dodecane and centrifuged. The organic layer was removed and then the uranium was extracted from the aqueous layer in a small volume of an aqueous solution containing 1 M free ammonia and 2 M ammonium carbonate. The goal was to get the uranium away from any concomitants apt to interfere with ICP-AES (e.g., iron) with minimal overall dilution - 1:1 to 1:10.