THE EFFECT OF APATITE II™ ON THE BIODEGRADATION OF TNT AND PERCHLORATE IN CONTAMINATED SOIL SAMPLES

Marissa N. Martinez[1], Shaun M. Hightower[1], Geoffrey B. Smith[1], Wolfgang Mueller[1],
James L. Conca[1] and
Judith Wright[2]

[1]New Mexico State University, [2]PIMS NW, Inc.

DOD Contaminated Sites

- Contain soil contaminated with 2,4,6-Trinitroluene (TNT), unexploded ordnance, and perchlorate
- Research shows that TNT remains a hazardous carcinogen and toxic substance long after an explosion has occurred
- 40% of hazardous waste sites are co-contaminated with metal pollutants.

Biodegradation of Organic Pollutants

- Some microorganisms such as bacteria or fungi have the ability to biodegrade TNT
  - White rot fungus *Phanerochaete chrysosporium* (Bumpus et al)
  - Fungal strain identified as *Penicillium* (This Work)
- Apatite II, a natural phosphate material developed from fish bones, promotes biodegradation
  - acts as a nutrient for microorganisms
  - produces a Phosphate-Induced Metal Stabilization (PIMS) effect on metals such as lead (Pb), cadmium (Cd), zinc (Zn), copper (Cu), uranium (U) and plutonium (Pu), which can be found in contaminated sites

What is Apatite II?

- **Manufactured from processed fish bones.**

- **The nominal composition of Apatite II is:**

  $Ca_{10-x}Na_x(PO_4)_{6-x}(CO_3)_x(OH)_2$

  where x < 1.

- **(U.S. Patent #6,217,775)**

- **Nominal Cost $30-$50/ treated ton**

SEM micrograph

PURPOSE

- Determine influence of Apatite II nutrient on the biodegradation of 2,4,6-Trinitroluene (TNT) and Perchlorate.

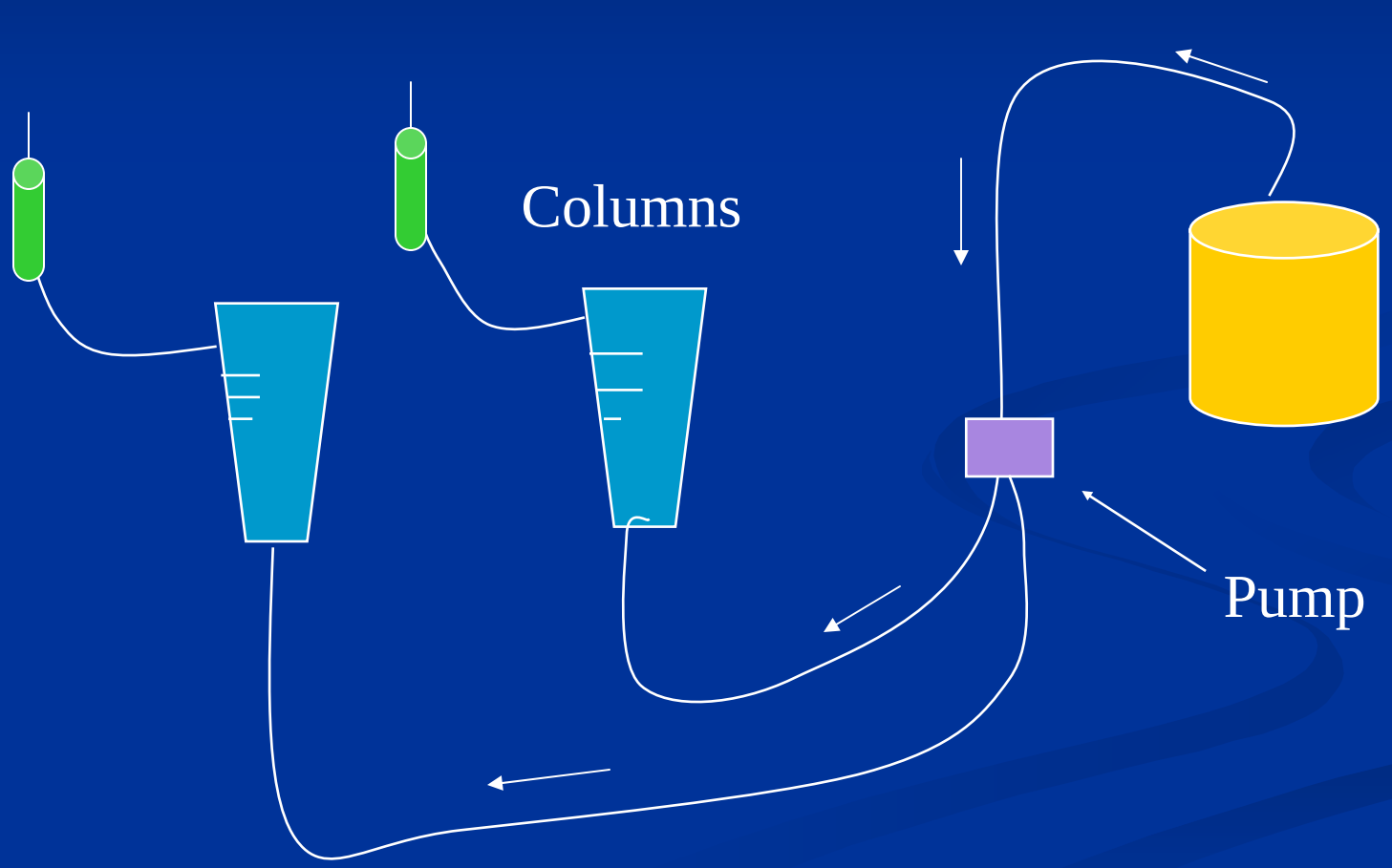
- Significance- promote simultaneous bioremediation of inorganic and organic contaminants, such as, perchlorate, and TNT in soil contaminated gunnery ranges or ammunition repositories

MATERIALS & METHODS

Biodegradation Column Set-up

- Following content was added to four up-flow columns (5 through 8) :
  1. column 5 contains 25 cc of soil, and 25 cc of Apatite II
  2. column 6 contains 25 cc of soil, 25 cc of Apatite II and fungus.
  3. column 7 contains 50 cc of soil
  4. column 8 contains 50 cc of soil, inoculated with fungus
- Columns were supplied with 10 ppm TNT in sterile water at a rate of 0.75ml/min (50 min retention time).
- Once a week the influent and effluent was sampled and analyzed:
  1. Columns ran for 2 hours without stopping
  2. First sample was taken after the first ten minutes
  3. A second sample was taken at the end of a 2-hr period (represents degradation during 50 minute column retention time)

Laboratory Setup



Soil columns with and without Apatite II (Ap) infused with TNT-contaminated groundwater

MATERIALS & METHODS

Analysis of Apatite II and PCR Amplification of TNT degrading fungus

- Analysis of Apatite II was performed by using Soxlet Extraction Method over a 24-hr period

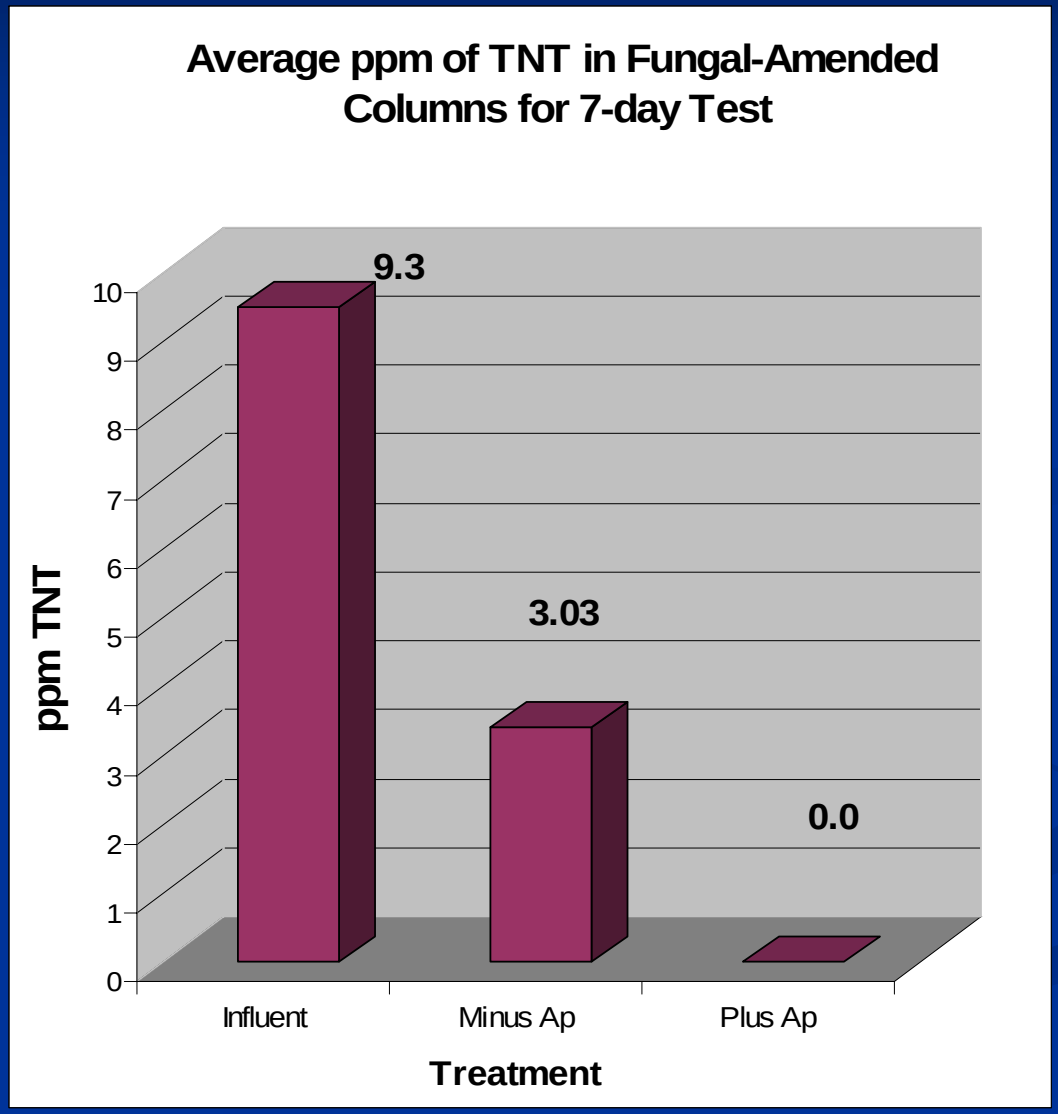
- TNT degrading fungus was identified through the PCR Amplification process.
  - This fungus was isolated from Sphagnum peat moss
  - Fungus was identified through analysis of
    - ribosomal DNA intergenic spacer (ITS) region
    - β-tubulin gene
  - Fungus has conidiospores and rRNA sequence consistent with that of ***Penicillium***

RESULTS

7-day Test Results, Initial 10-minute Run— TNT Remaining ppm Amounts

7 Day test		TNT ppm				
			Column 5	Column 6	Column 7	Column 8
Date	Sample Set	Influent (ppm)	25 cc of soil & 25 cc of Apatite II	25 cc of soil & 25 cc of Apatite II & fungi	50 cc of soil	50 cc of soil & fungi
8/26/04	1	6.8	0.3	0.0	5.7	3.3
9/13/04	2	10.2	0.8	0.0	3.3	2.9
9/23/04	3	11.4	1.4	0.0	2.2	1.9
9/30/04	4	10.6	1.5	0.0	2.4	3.1
10/7/04	5	9.2	1.6	0.0	2.9	4.1
10/14/04	6	8.6	2.6	0.0	3.4	5.2
10/28/04	7	10.3	1.5	0.0	4.6	3.9
11/8/04	8	8.1	0.0	0.0	3.5	1.8
11/30/04	9	9.5	0.0	0.0	3.0	2.3
12/14/04	10	10.31	0.0	0.0	3.62	2.43
1/27/05	11	7.9	0.0	0.0	2.96	2.37
	AVERAGE	9.3	.88	0.0	3.42	3.03

Results After 7-day Incubation

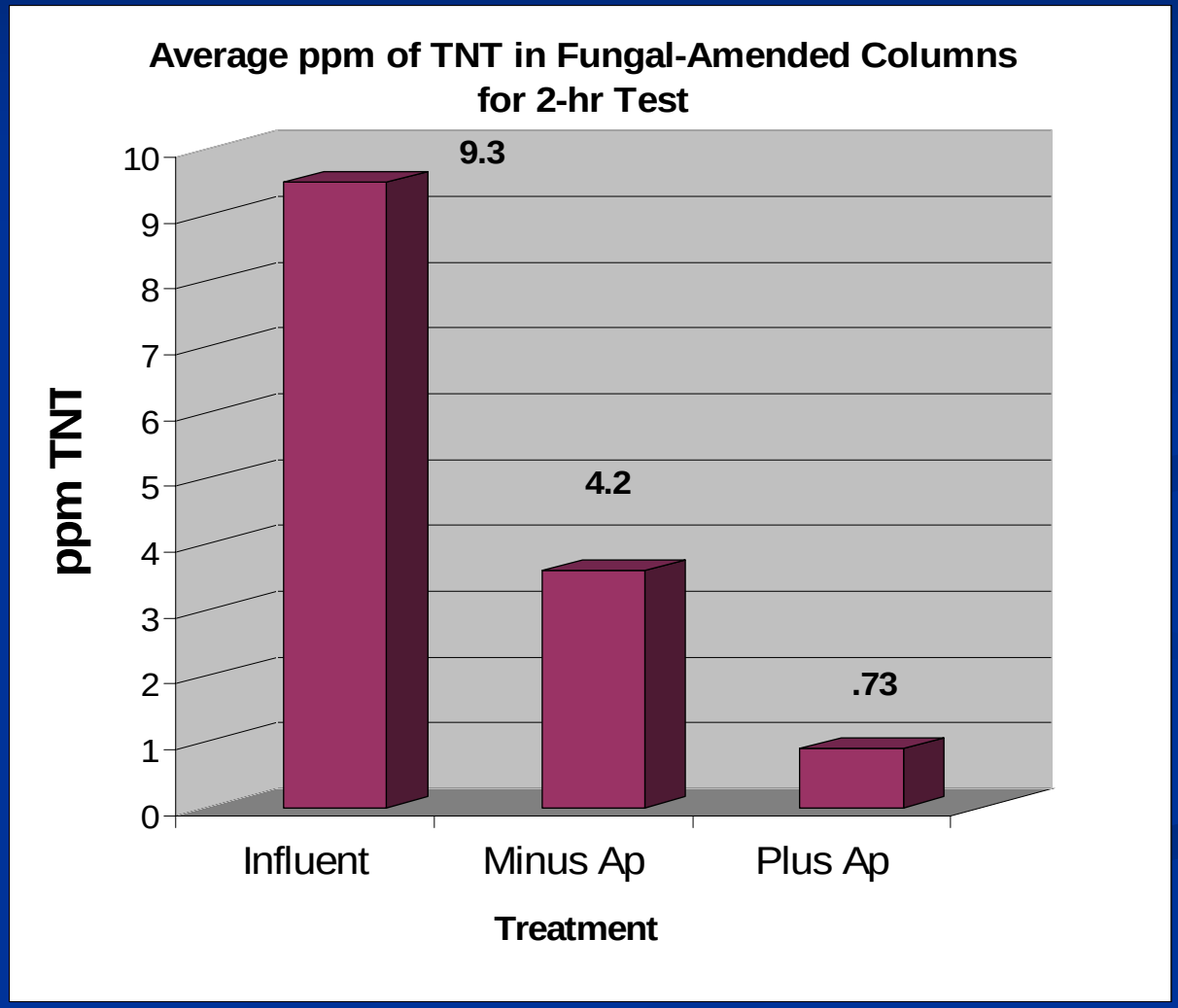


RESULTS

2-Hr Test Result, Recycled Run
TNT Remaining ppm Amounts

2 hr. Test		Ppm				
			Column 5	Column 6	Column 7	Column 8
Date	Sample Set	Influent (ppm)	25 cc of soil & 25 cc of Apatite II	25 cc of soil & 25 cc of Apatite II & fungi	50 cc of soil	50 cc of soil & fungi
8/26/2004	1	6.8	4.9	1.6	6.2	4.7
9/13/2004	2	10.2	1.5	0	5.1	2
9/23/2004	3	11.4	2	1.3	5.3	3.2
9/30/2004	4	10.6	2.4	0.8	5.4	4.2
10/7/2004	5	9.2	1.8	0.6	4.6	2.7
10/14/2004	6	8.6	3.6	0.9	5.3	4.8
10/28/2004	7	10.3	3.8	1.4	6.6	6.2
11/8/2004	8	8.1	1.5	1	4.6	4.4
11/30/2004	9	9.5	2.94	0.49	5.06	5.15
12/14/2004	10	10.31	1.55	0	5.42	4.98
1/27/2005	11	7.9	2.07	0	4.3	4
	AVERAGE	9.3	2.55	0.73	5.26	4.21

Degradation During 50-minute Retention Time

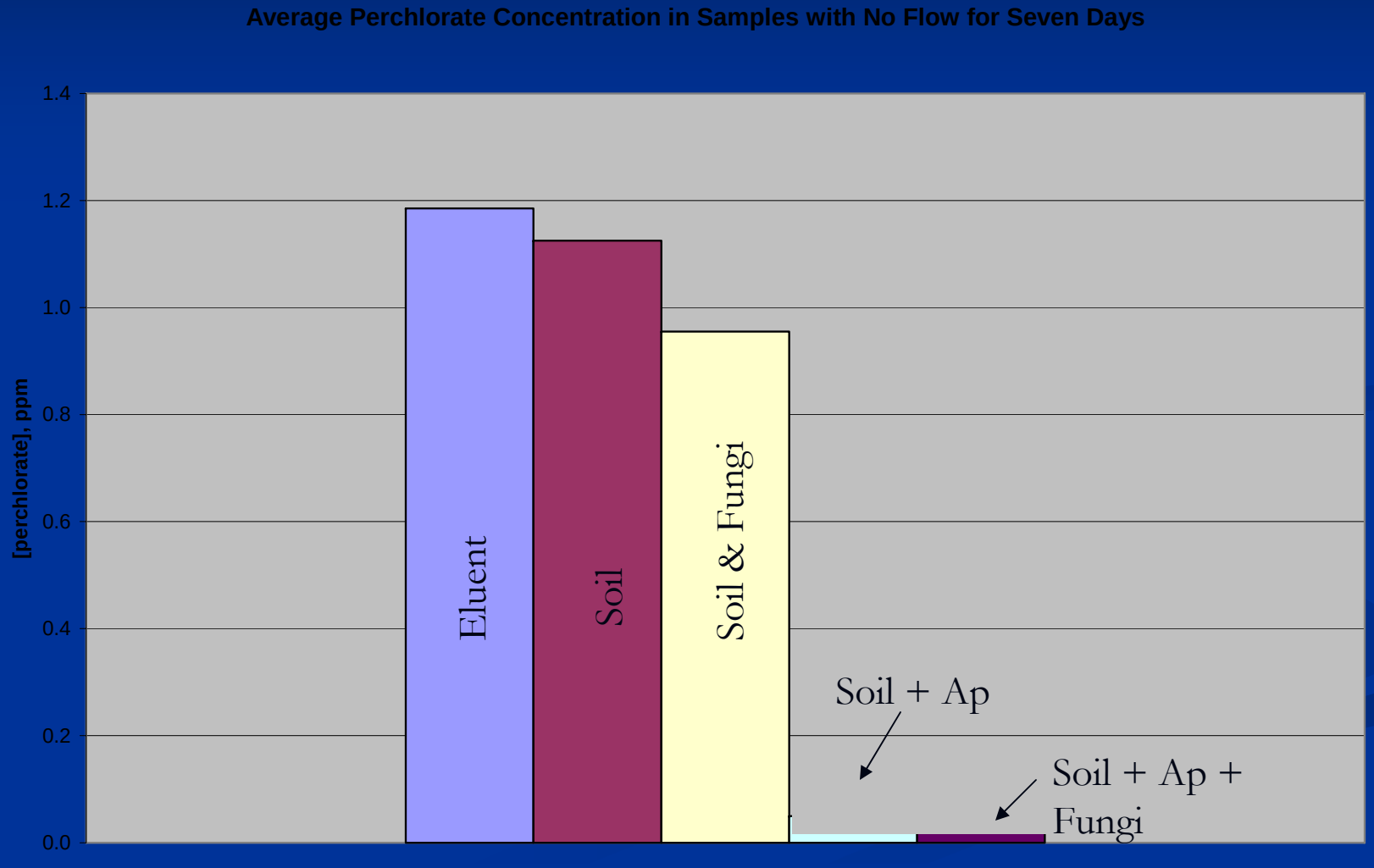


RESULTS

Levels of TNT and Perchlorate in Columns (after 7-day incubation)

7 Day test		ppm of TNT or Perchlorate Remaining			
Date		Column 5	Column 6	Column 7	Column 8
	Influent (ppm)	25 cc of soil & 25 cc of Apatite II	25 cc of soil & 25 cc of Apatite II & fungi	50 cc of soil	50 cc of soil & fungi
7/26/05	11 ppm TNT	0.0	0.0	4.0	2.2
7/26/05	1.1 ppm Perchlorate	0.0	0.0	1.1	0.9
8/2/05	11.1 ppm TNT	0.0	0.0	3.9	2.0
8/2/05	0.9 ppm Perchlorate	0.0	0.0	1.2	1.1

Results for Perchlorate After 7-day Incubation



RESULTS

Levels of TNT in Columns Co-contaminated with Lead

	Sample	Column 5 mg/L	Column 6 mg/L	Column 7 mg/L	Column 8 mg/L	Influent
7-Day Test	2/22/2006	1.77	3.18	6.8	4.49	9.44
	3/8/2006	0.07	0.76	5.53	6.67	9.06
2-Hour Test	2/22/2006	2.66	3.13	7.38	8.86	9.44
	3/8/2006	4.18	8.8	7.5	6.53	9.06

RESULTS

Levels of Perchlorate in Columns Co-contaminated with Lead

	Sample	Column 5 mg/L	Column 6 mg/L	Column 7 mg/L	Column 8 mg/L	Influent
10 Min. Sampling	1/27/2006	0.06	0.1	0.95	0.95	0.87
7 Day Test	2/3/2006	0.94	0.1	0.96	0.98	0.98
	2/15/2006	0.29	0.33	1.01	1.03	1.08
	2/22/2006	0.7	0.58	1.08	1.08	0.96
	3/8/2006	0.21	0	0.84	0.8	0.92
	Average	0.44	0.22	0.97	0.97	0.96
2 Hour Test	1/27/2006	0.71	0.75	0.98	0.99	0.87
	2/3/2006	0.61	0.64	1.02	1.01	0.98
	2/15/2006	0.79	0.77	1.07	1.12	1.08
	2/22/2006	0.9	0.76	1.01	1.04	0.96
	3/8/2006	0.62	0.59	0.91	0.92	0.92
	Average	0.73	0.7	1	1.02	0.96

CONCLUSIONS

1. Results show that the Apatite II nutrient can be used to promote the biodegradation of TNT and perchlorate, separately and as co-contaminants.

2. Simultaneous TNT and perchlorate biodegradation was stimulated by the presence of Apatite II, since in its absence perchlorate was not degraded at all and TNT was only partially degraded.

3. The effect of apatite amendment was long-lived since the TNT had been continuously degraded over the past 20 months with no build-up of intermediates and no feedback inhibitions.

4. Apatite II showed promise as a natural means of enhancing TNT and perchlorate bioremediation processes. Apatite II does not pose any adverse environmental impacts and can be easily mixed with contaminated soil.

5. Results show that upon the addition of lead, TNT and Perchlorate degradation continued to occur in the columns that contain Apatite II.

Future Plans

- Continue TNT and Perchlorate biodegradation analysis over the next year, determining the effect of the different variables, i.e. fungus.
- Perform *in situ* testing and evaluation like at Ft.Bliss or White Sands
- Perform more biodegradation experiments using different conditions such as sterile environment.

QUESTIONS?

THANK YOU!