Strategic Environmental Research and Development Program (SERDP)

Final Technical Report

Immobilization Of Energetics On Live Fire Ranges (CU-1229)

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Revision 1.0

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List of Acronyms

2-ADNT / 4-ADNT	2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene
2,4-DANT / 2,6-DANT	2,4- and 2,6-diaminonitrotoluene
2,4-DNT / 2,6-DNT	2,4- and 2,6-dinitrotoluene
DNX	hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine
DoD	Department of Defense
EPA	Environmental Protection Agency
EOD	explosive ordnance disposal
HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
MMR	Massachusetts Military Reservation
MNX	hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine
OB/OD	open burn/open detonation
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
2,4,6-TAT	2,4,6-triaminotoluene
TNT	2,4,6-trinitrotoluene
TNX	hexahydro-1,3,5-trinitroso-1,3,5-triazine
UXO	unexploded ordnance

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1. Executive Summary

This project resulted in development and proof-of-concept laboratory testing of cost-effective technology to immobilize and biodegrade energetic compounds (TNT, RDX, HMX, and breakdown products) released as residues at firing ranges to prevent their migration to groundwater. The technology is comprised of a sorbent material to immobilize newly generated explosives residues at the soil surface, and a biostimulant to enhance the biotransformation and biodegradation of the explosive compounds before they can migrate into the soil and down to the groundwater. Using a tiered approach, multiple potential sorbents and biostimulants were screened. The most effective combination of sorbent and biostimulant was determined to be *Sphagnum* peat moss plus crude soybean oil, mixed at a ratio of approximately 0.5 g crude soybean oil per gram of peat moss. A 0.5-inch layer of this material reduced the aqueous pore-water concentrations of TNT, RDX, and HMX at 10 cm below the soil surface of a repacked soil column by 100%, 60%, and 40%, respectively, compared to the control without the treatment layer. A model developed during this project based on experimental results indicated that a 1-inch layer of this material would reduce the relative mass loading of TNT, RDX, and HMX to soil by 90%, 80%, and 70%, respectively over a one period, at an estimated materials cost of $4200 per acre.

2. Objective

The objective of this project was to develop a cost-effective technology to immobilize and biodegrade energetic compounds (TNT, RDX, HMX, and breakdown products) released as residues at firing ranges to prevent their migration to groundwater. The goal was to develop an inexpensive soil treatment that can be readily applied over wide and remote areas prior to firing range activities.

3. Background

The Department of Defense's impact ranges, including that at the Massachusetts Military Reservation (MMR) on Cape Cod, Massachusetts, are used for testing new ordinance and training personnel in the handling and firing of mortars, rockets, and other munitions. Munitions contain a number of different explosive compounds. For example, a 60-mm mortar round contains 2,4,6-trinitrotoluene (TNT) in the primer, 2,4- and 2,6-dinitrotoluene (DNT) in the propellant charge, TNT and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the filler, and RDX and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) in the fuse (24). After detonation of a high explosive, residues of these materials remain in the impact area. TNT and its various nitroaromatic breakdown products (i.e., 4-amino-2,6-dinitrotoluene (4-ADNT), 2-amino-4,6-dinitrotoluene (2-ADNT), 2, 6-diamino-4-nitrotoluene (2,6-DANT) , 2,4-diamino-6-nitrotoluene (2,4-DANT), and 2,4,6-triaminotoluene (2,4,6-TAT)) have been shown to sorb strongly to clay and organic matter, and are usually retained close to the soil surface (10, 20, 23, 27, 35). In contrast, the nitramine explosive RDX and its nitroso-containing breakdown products – hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) – appear to sorb to a significantly lesser degree, allowing them to percolate through the subsurface and end up in groundwater (1, 20, 38, 39). HMX, although sparingly soluble in water, is also expected to be mobile in subsurface environments due to sorption characteristics similar to RDX (20). In sandy soils with little organic matter or clay content, such as those present on Cape Cod, transport of

residual TNT, RDX, and HMX to the vadose zone and ultimately to groundwater is possible. Recent reports of groundwater contamination at MMR with low levels of RDX confirm this assumption (33).

The U.S. Environmental Protection Agency (USEPA) has listed RDX and 2,4- and 2,6-dinitrotoluene (2,4-DNT, 2,6-DNT) – two breakdown products of TNT – on both the Draft Drinking Water Candidate Contaminant List and the Unregulated Contaminant Monitoring Regulation List (2). Minimal reporting levels for 2,4- and 2,6-DNT have been set at 2 µg/L, and a reporting level for RDX is currently being developed. Drinking water criterion and minimal reporting levels for other explosive compounds, while not specifically addressed at this point, will likely be similar.

The low concentrations at which explosive compounds in drinking water are likely to be regulated reflect the understanding of the threat that these compounds pose to human and ecological health. Chronic occupational exposure of humans to TNT, and controlled exposures of laboratory animals to TNT or RDX have resulted in similar adverse effects: liver damage, blood damage (caused by methemoglobinemia and associated cyanosis), anemia, cataracts, allergenic dermatitis, discoloration of hair and skin, and nausea (15, 19, 37, 44). Most of the explosive compounds examined have been shown to be toxic and/or mutagenic at concentrations considerably below their respective solubility limits (26, 41, 42). These effects lend urgency to research efforts focused on removing these compounds from the environment.

Incineration is the current preferred method for treating explosive compounds in contaminated soils, but it is both costly and environmentally unfriendly. Based on extensive research that has been done on the biodegradation and biotransformation of explosive compounds by bacteria (for review see refs (14, 18, 21)), fungi (5, 6, 12, 32, 36, 40, 46) and plants (8, 16, 22, 30, 45), other approaches for remediating explosive compounds-contaminated soils have been developed. These include the *ex situ* processes of windrow composting (7, 34), biotreatment in aerobic/anoxic (9, 17, 28) or anaerobic (13, 25) soil slurry reactors, and by "in place" land

farming (43). Abiotic reactor approaches also have been proposed (3, 4, 11, 29, 31, 43, 47). However, the presence of unexploded ordinance on impact ranges, as well as their broad expanse (MMR is 140,000 acres) makes all these technologies impractical and prohibitively expensive for soil treatment. In addition, although these technologies deal with the current sources of explosives in the soil, they are not applicable for the prevention of groundwater contamination by explosives residues generated during ongoing training activities.

Therefore, this project seeks to develop an appropriate technology to immobilize and biodegrade explosives residues (TNT, RDX, HMX and their respective breakdown products) at or near the soil surface, thereby preventing the downward migration of the compounds into the underlying groundwater. The technology must be inexpensive, both in terms of materials and deployment, due to the large areas that need to be treated.

4. Material and Methods

This project has been conducted in a tiered fashion, as illustrated in Figure 1. The general approach was to screen multiple materials for their ability to sorb explosive compounds or promote the biotransformation/biodegradation of explosive compounds. The materials that yielded the best results were advanced to further testing in soil microcosms. Finally, a determination was made that peat moss was the best sorbent material and crude soybean oil was the most effective bio-stimulant. These two materials were tested in soil columns operated under unsaturated flow conditions. Additional experiments using "mini-columns" operated under continuous flow conditions were performed to determine kinetic parameters needed for development of a predictive model of treatment effectiveness under a variety of conditions.

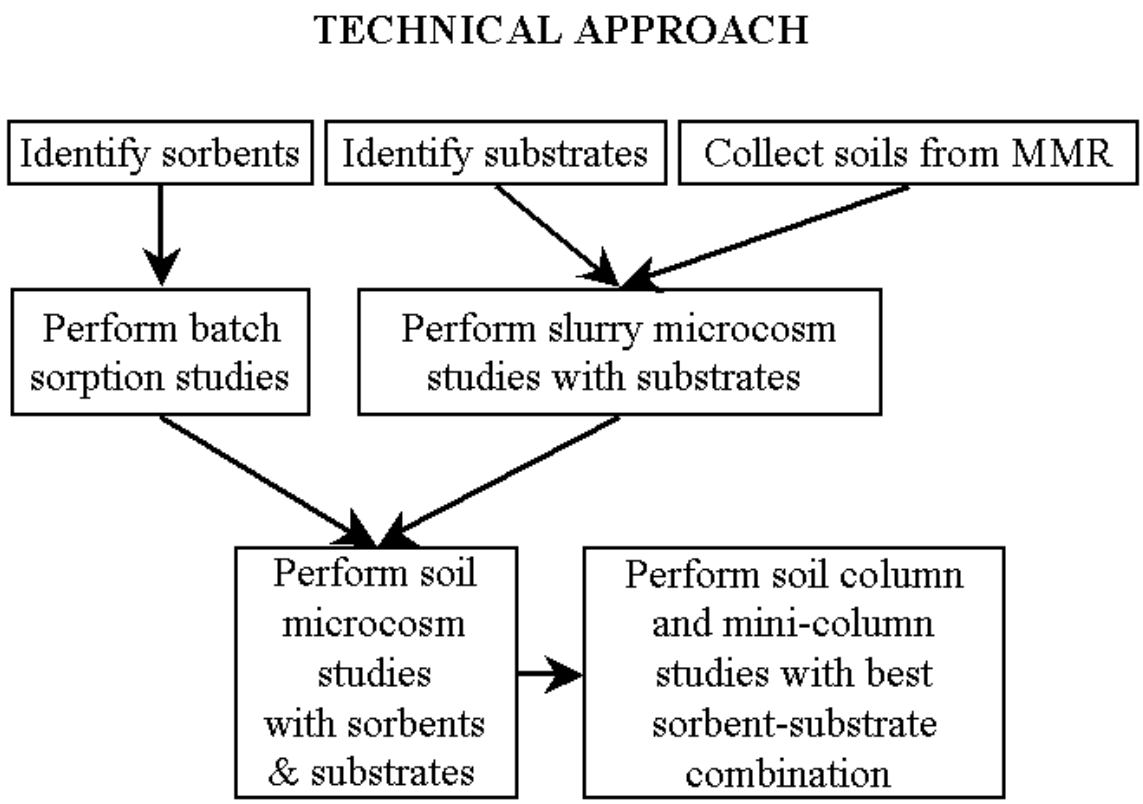

Figure 4-1. Flowchart illustrating the general approach used during this project to develop and evaluate the technology.

Reprints and/or drafts of the manuscripts that have come from this research are included in Appendix B, which constitutes the main presentation of the methods and materials of this project. Detailed methods and results for each aspect of the project are provided in the form of the Quarterly Progress Reports included in Appendix A.

5. Results and Accomplishments

Two manuscripts presenting the major results evaluating the potential sorbents and cosubstrates have been published in the journal *Environmental Toxicology and Chemistry* in January 2004. Two additional manuscripts have been prepared and submitted for publication in *Environmental Engineering Science* and *Soil & Sediment Contamination*. Reprints and/or drafts of these manuscripts are included in Appendix B.

A summary of the most important findings are as follows:

5.1. SORBENT SCREENING

A variety of materials were screened as potential sorbents, as detailed in Table 5.1. The list of potential sorbents to be shortened by performing an initial 24 h sorption screening. These results are presented in Table 5.2 and Figure 5.1 (for TNT only). The clays sorbed the compounds to much lower degree than the organic materials.

Sorption-desorption isotherms were performed using the materials deemed to be the most effective sorbents. The isotherms are presented in Figures 5.2, 5.3, and 5.4 for TNT, RDX, and HMX, respectively. The results in terms of Freundlich sorption and desorption coefficients are summarized in Table 5.3. In general, the clays sorbed TNT to a much higher degree than RDX or HMX. For comparison, the sorption-desorption coefficients for TNT and RDX to soil samples from the Massachusetts Military Reservation are presented in Table 5.4. TNT sorbed minimally to the soils, and RDX did not sorb at all.

Sorption-desorption isotherm results indicated that *Sphagnum* peat moss, and to a lesser degree sawdust, were highly effective sorbent materials for the three explosives TNT, RDX, and HMX. [Please see Hatzinger *et al.*, 2004 reprint in Appendix B for more details.]

Table 5.1. Information on sorbent materials used for this research.

Material	Source	Processing
Montmorillonite clay, KSF[a]	Aldrich Chemical Company	None
Sphagnum peat moss	Nirom Peat Moss, Inc.	Sieved[b]
Ground rubber tires #30	Oklahoma rubber recycling plant	None
Sawdust (conifer/deciduous mix)	Cape Cod sawmill	Sieveda
Rice hulls	Arkansas rice processor	None
Ground rubber tires #30-40	Oklahoma rubber recycling plant	None
Wheat straw	New Jersey	Cut/sieved
Montmorillonite clay, K10[c]	Aldrich Chemical Company	None
Seaweed	Cape Cod beach	Cut/sieved
Lobster shells	Cape Cod restaurant	Washed/crushed/sieved
Vermiculite	Standard packing material	Sieved
Kaolin clay	Aldrich Chemical Company	None
Clam shells	Cape Cod clam processing plant	Washed/crushed/sieved

[a]Bulk density = 300-370 g/L; surface area = 220-270 m^2/g.

[b]Sieving performed to produce material bewteen 18 mesh (1 mm) to 5 mesh (4 mm) in size.

[c]Bulk density = 800-850 g/L; surface area = 20-40 m^2/g.

Table 5.2. Summary of initial adsorption and desorption results.

	TNT			RDX			HMX		
Sorbent	Initial (mg/L)	Ads. (%, 24 h)	Des.[a] (%, 48 h)	Initial (mg/L)	Ads. (%, 24 h)	Des. (%, 48 h)	Initial (mg/L)	Ads. (%, 24 h)	Des. (%, 48 h)
Montmorillonite clay, KSF	75	93	15	37	23	-	3	62	72
Sphagnum peat moss	80	82	22	37	65	58	3	69	50
Ground rubber tires #30	80	77	29	37	19	-	3	17	-
Sawdust	80	76	25	37	47	64	3	53	54
Rice hulls	86	75	32	37	47	56	3	60	53
Ground rubber tires #30-40	75	75	29	-	-	-	3	10	-
Wheat straw	75	74	28	37	50	45	3	62	55
Montmorillonite clay, K10	75	54	58	-	-	-	3	44	94
Seaweed	80	50	42	-	-	-	3	36	-
Lobster shells	80	18	-[b]	-	-	-	3	21	-
Vermiculite	80	7	-	-	-	-	3	13	-
Kaolin clay	80	5	-	-	-	-	3	10	-
Clam shells	75	6	-	-	-	-	3	3	-

[a]Cummulative compound desorbed from solid phase after two replacements of aqueous $CaCl_2$ phase over 48 h.

[b]Not determined.

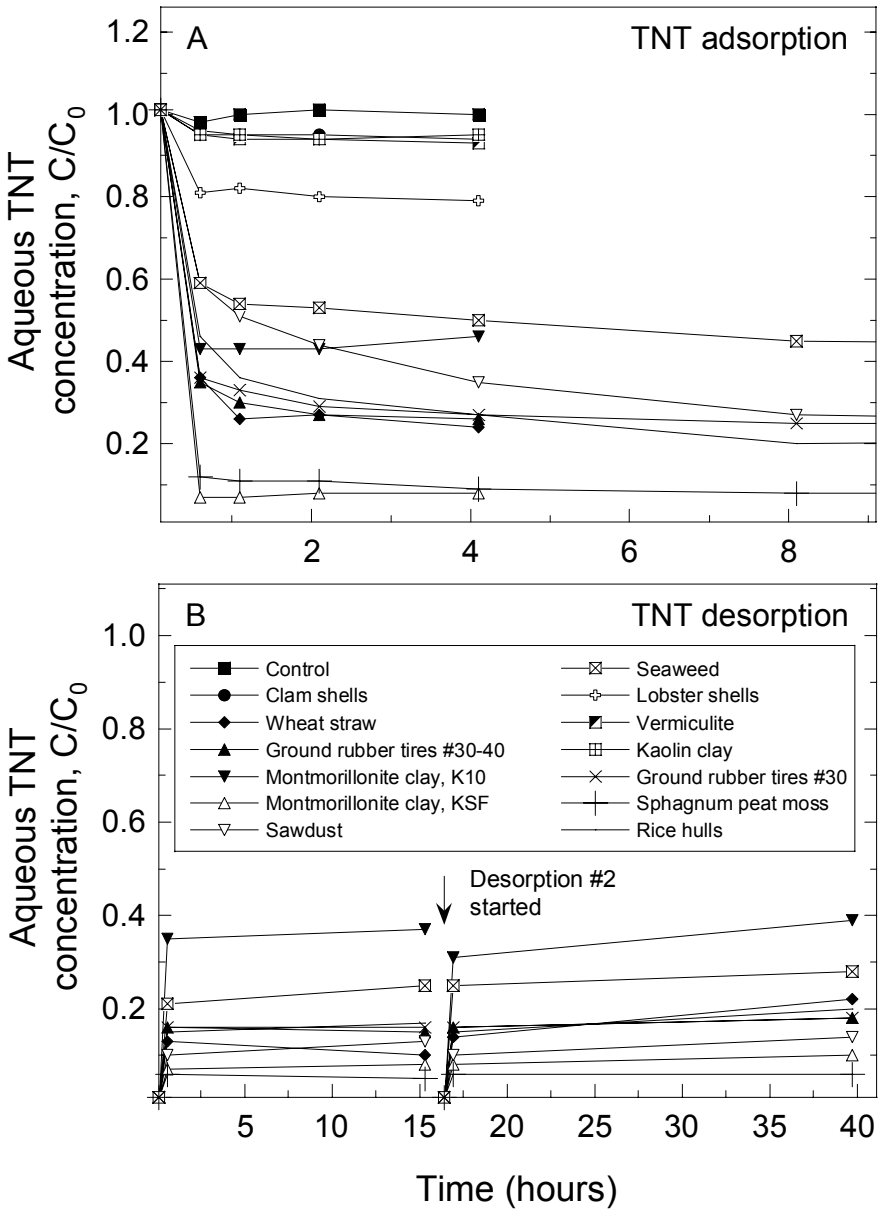

Figure 5.1. Kinetics of TNT sorption to (A) and desorption from (B) test sorbents.

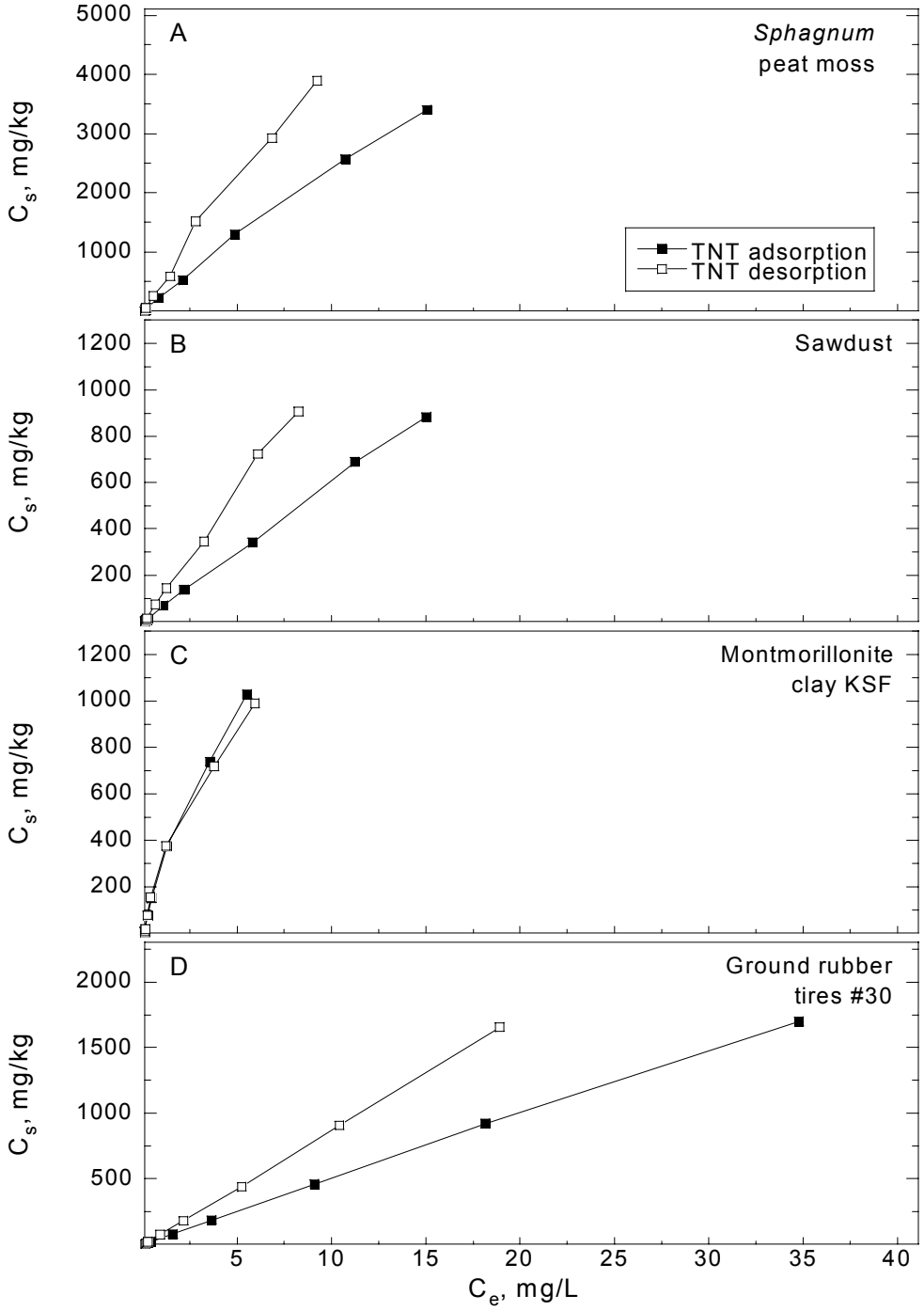

Figure 5.2. TNT sorption and desorption isotherms for peat moss (A), sawdust (B), montmorillonite clay KSF (C), and ground rubber tires #30 (D).

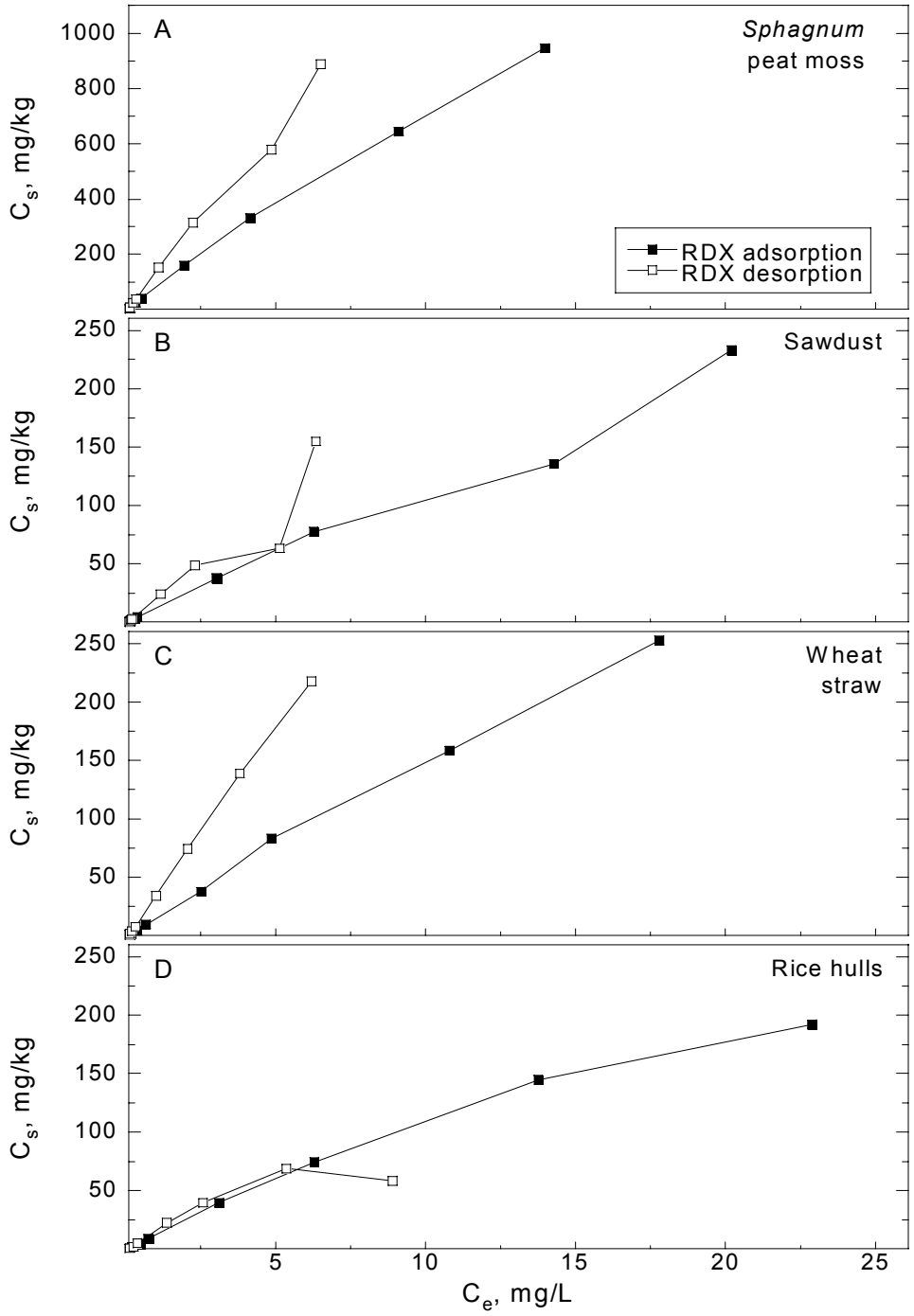

Figure 5.3. RDX sorption and desorption isotherms for peat moss (A), sawdust (B), wheat straw (C), and rice hulls (D).

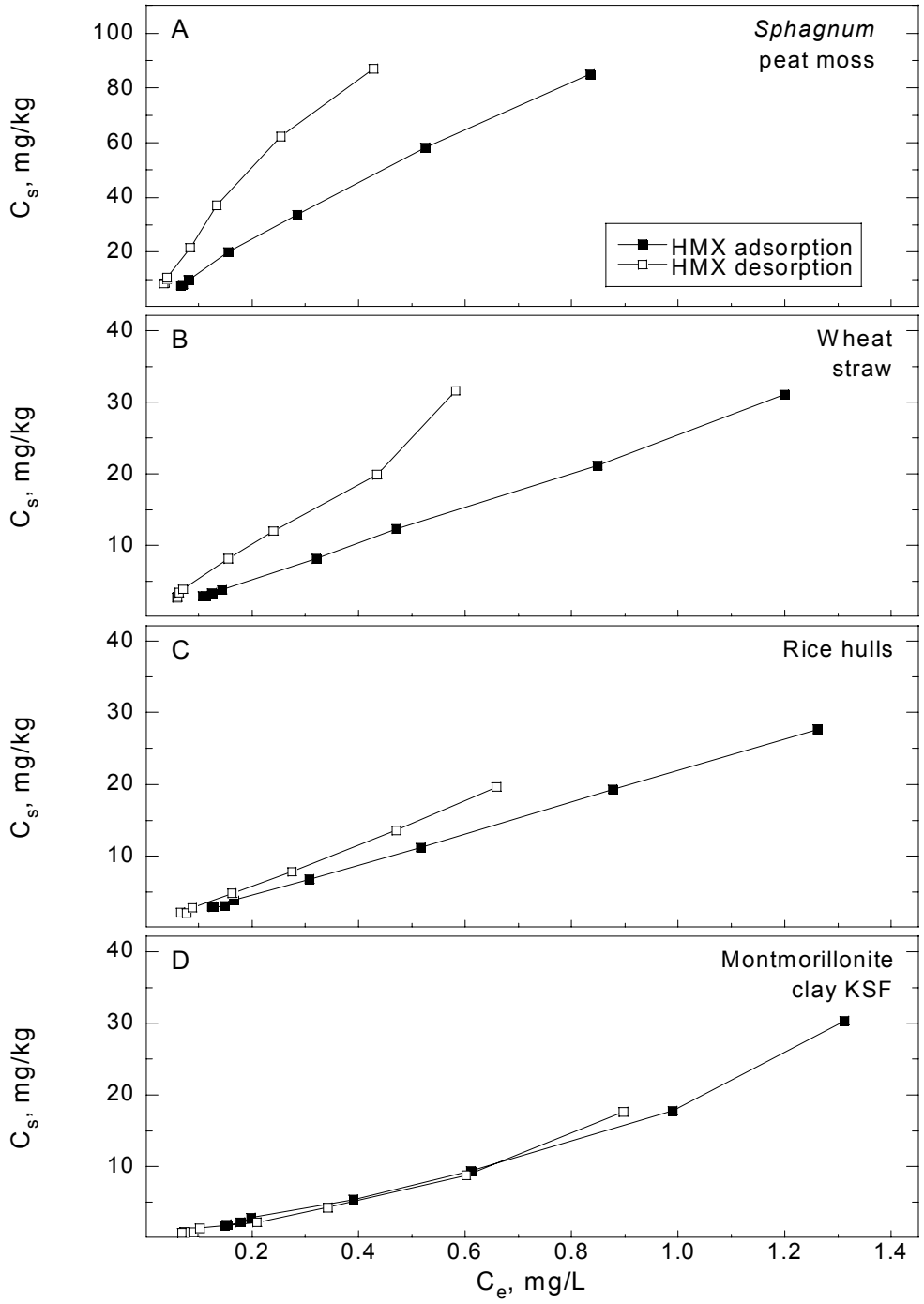

Figure 5.4. HMX sorption and desorption isotherms for peat moss (A), wheat straw (B), rice hulls (C), and montmorillonite clay KSF (D).

Table 5.4. Freundlich model fits for explosive-soil sorption-desorption isotherms.

A. Adsorption

Sorbent	TNT			RDX[a]		
	log K^s_d, L/kg	n	r^2	log K^s_d, L/kg	n	r^2
Grassy soil (uncontaminated)	0.27	0.93	1.00	-0.28	0.96	0.97
Colored patch soil (contaminated)	0.50	0.82	1.00	-0.65	0.78	0.83

B. Desorption

Sorbent	TNT			RDX[b]		
	log K^d_d, L/kg	n	r^2	log K^d_d, L/kg	n	r^2
Grassy soil (uncontaminated)	1.51	0.95	0.99	1.26	0.78	0.96
Colored patch soil (contaminated)	1.62	1.20	0.99	0.40	0.67	0.61

[a]Values of parameters were obtained from the regression line, using 7 points of data.

[b]Values of parameters were obtained from the regression line, using 5 points of data.

5.2. COSUBSTRATE SCREENING

A variety of materials were screened as potential carbon sources, as detailed in Table 5.5. Soil slurry microcosm experiments (Figure 5.5) indicated that crude soybean oil, and to a lesser extent molasses, were the best carbon sources for stimulating the biotransformation and biodegradation of the explosive compounds TNT, RDX, and HMX. Results are presented in Figure 5.6, and summarized in Table 5.6. TNT mineralization was minimal in all cases, in agreement with previous research, but > 95% of TNT was biotransformed to breakdown products in most experiments. RDX was mineralized to the greatest extent (~35-40%), while HMX mineralization was somewhat lower (~8-10%). [see Fuller *et al.*, 2004 reprint in Appendix B for more details]

5.3. COMBINED SORBENT / COSUBSTRATE SCREENING

Unsaturated soil microcosms (Figure 5.7), which compared combinations of the sorbent materials peat moss and sawdust with the carbon sources crude soybean oil and molasses, indicated that maximum degradation occurred with crude soybean oil. The extent of degradation was only slightly reduced (35% vs. 42%) when peat moss was present, most likely due to some irreversible binding of the explosives to the peat moss. [see Fuller *et al.*, 2004 reprint in Appendix B for more details]

5.4. UNSATURATED SOIL COLUMN EXPERIMENT

Experiments were conducted with unsaturated repacked soil columns (see Figure 5.8 for illustration). These experiments indicated that both peat moss and peat moss plus crude soybean oil reduced the downward mobility of explosive residues applied to the soil surface. Peat moss plus soybean oil reduced the dissolved concentrations of TNT, RDX and HMX detected at 10 cm depth by 100%, 60%, and 40%, respectively, compared to the no-treatment control column (Figure 5.9). The RDX metabolites MNX, DNX, and TNX were detected periodically, but were always lower in the peat moss plus soybean oil column than in the peat moss only treatment and the no treatment control (Figure 5.10). Peat moss only initially reduced the concentrations of

explosives observed at 10 cm depth relative to the control, but resulted in slightly higher concentrations than the control by the end of the experiment. [see Fuller *et al.*, draft manuscript in Appendix B for more details]

Table 5.5. Information on cosubstrates used for this research.

Material	Source	Cost per kg[d], $	Concentration
LIQUID COSUBSTRATES			%, vol:vol[a]
Molasses	Zook Molasses Company	0.03	0.5[b]
Corn steep liquor	Grain Processing Corporation	0.03	0.5[b]
Soybean oil-crude	Cargill	0.07	0.5
SoyClear 1500	AG Environmental Products, LLC	0.39	0.5
SoyGold 1000	AG Environmental Products, LLC	0.34	0.5
Safflower oil	California Oils Corporation	0.23	0.5
DRY COSUBSTRATES			%, wt:wt[c]
Potato starch	Lance Products, Inc.	0.12	2.0
Solulac	Grain Processing Corporation	0.02	2.0
Corn starch	Grain Processing Corporation	0.04	2.0
Unrefined chitin (practical grade)	Sigma Chemical Company	6.00	2.0

[a]Volume of cosubstrate:liquid volume of microcosm slurry.
[b]Molasses and corn steep liquor added as 25% soln to facilitate pipetting.
[c]Weight of cosubstrate:weight of soil in microcosm slurry.
[d]Cost does not reflect bulk discount or transport from source to location of use.

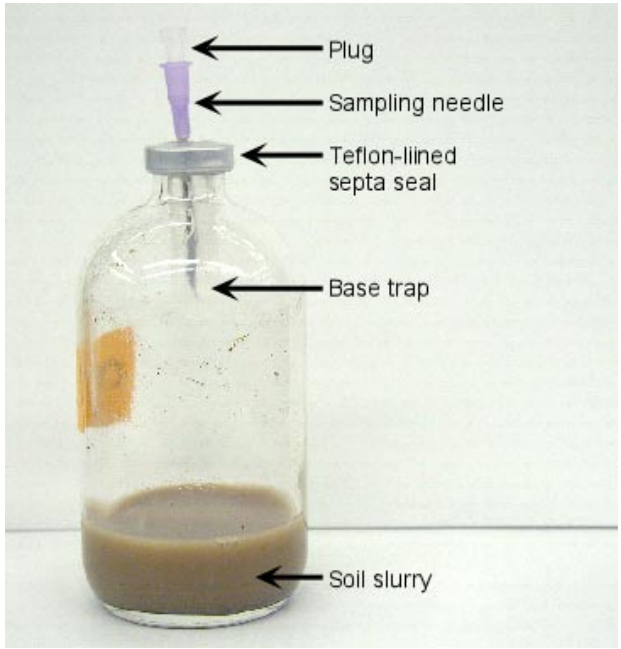

Figure 5.5. Photograph of the soil slurry microcosms.

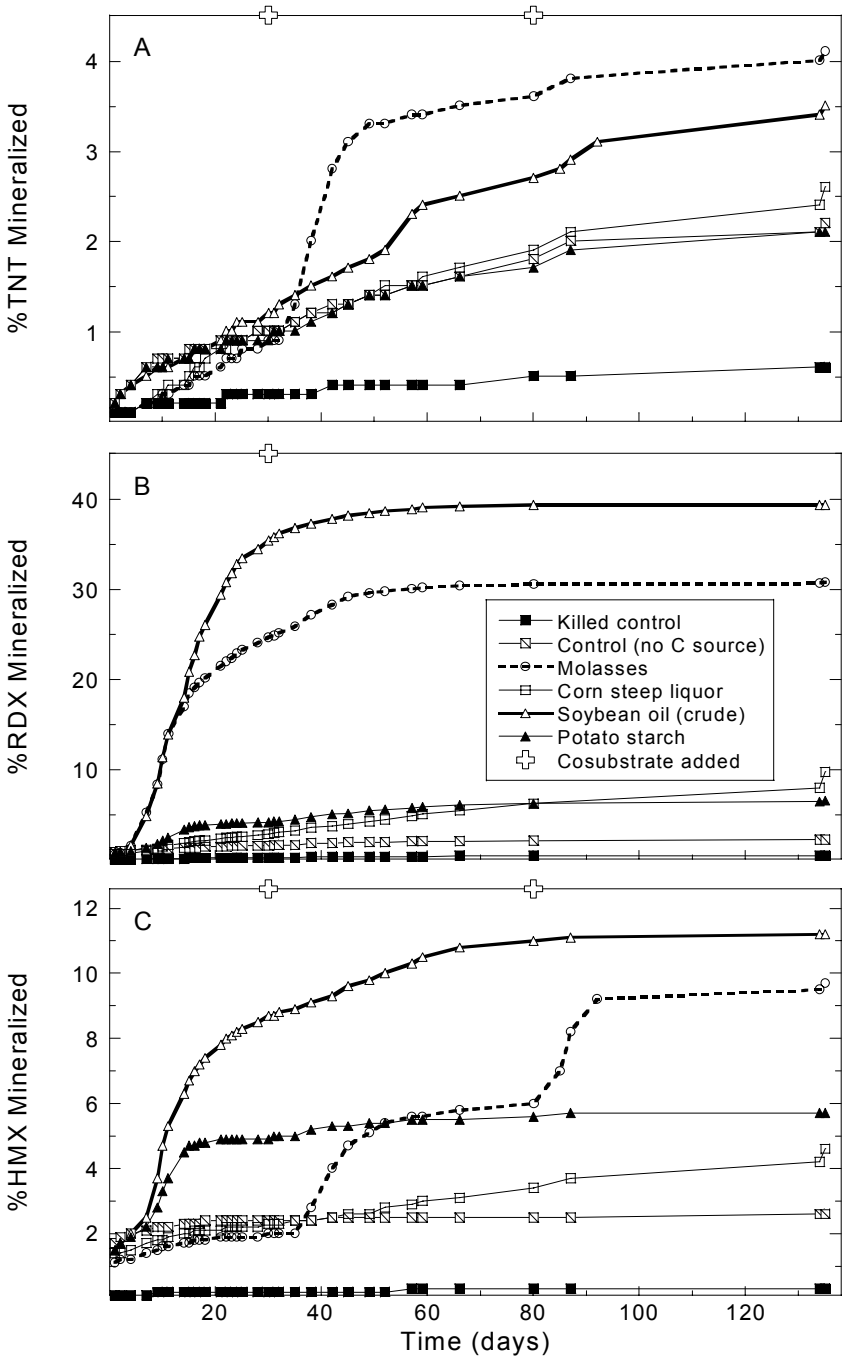

Figure 5.6. Mineralization of TNT (A), RDX (B), and HMX (C) in MMR soil slurries. The "+" indicates when the slurries received additional cosubstrate.

Table 5.6. Percentage of [^{14}C] in different fractions for slurries prepared using uncontaminated soil.

EXPLOSIVE & TREATMENT	Percent of initial dpm[a]					
	[^{14}C]CO_2	WATER SOLUBLE	ACETONITRILE EXTRACTABLE	DICHLOROMETHANE EXTRACTABLE	HEXANE EXTRACTABLE	MASS BALANCE[b]
TNT						
Killed Control (HgCl2, Na Azide)	1 (0)	53 (1)	67 (5)	33 (1)	1 (1)	68 (5)
Control (no C source)	2 (0)	17 (0)	20 (4)	10 (0)	1 (0)	23 (4)
Molasses	4 (0)	6 (1)	9 (1)	4 (0)	1 (0)	13 (1)
Corn steep liquor	3 (0)	14 (2)	10 (2)	4 (1)	1 (1)	12 (2)
Soybean oil-crude	3 (0)	4 (1)	6 (0)	24 (8)	1 (0)	9 (1)
Potato starch	2 (0)	10 (2)	23 (3)	8 (3)	1 (0)	25 (3)
RDX						
Killed Control (HgCl2, Na Azide)	0 (0)	89 (0)	93 (6)	61 (6)	2 (0)	93 (6)
Control (no C source)	2 (0)	88 (1)	85 (3)	60 (2)	1 (0)	87 (3)
Molasses	31 (5)	1 (0)	1 (0)	0 (0)	0 (0)	32 (4)
Corn steep liquor	10 (5)	66 (7)	58 (11)	45 (12)	1 (0)	67 (7)
Soybean oil-crude	39 (3)	1 (0)	3 (1)	9 (5)	1 (1)	42 (4)
Potato starch	7 (0)	72 (1)	57 (3)	21 (1)	1 (1)	63 (3)
HMX						
Killed Control (HgCl2, Na Azide)	0 (0)	66 (2)	76 (2)	6 (0)	2 (-)	76 (2)
Control (no C source)	3 (0)	62 (1)	76 (3)	7 (2)	0 (0)	78 (3)
Molasses	10 (4)	30 (8)	40 (10)	1 (1)	1 (0)	50 (6)
Corn steep liquor	4 (1)	53 (0)	62 (3)	2 (0)	1 (0)	67 (4)
Soybean oil-crude	11 (3)	28 (1)	51 (1)	28 (8)	0 (0)	62 (4)
Potato starch	6 (0)	54 (0)	69 (6)	3 (0)	1 (0)	75 (5)

[a]Results presented as average percent (± difference between duplicate bottles / 2).
[b]Mass balance is the sum of [^{14}C]CO_2 and acetonitrile extractable values.

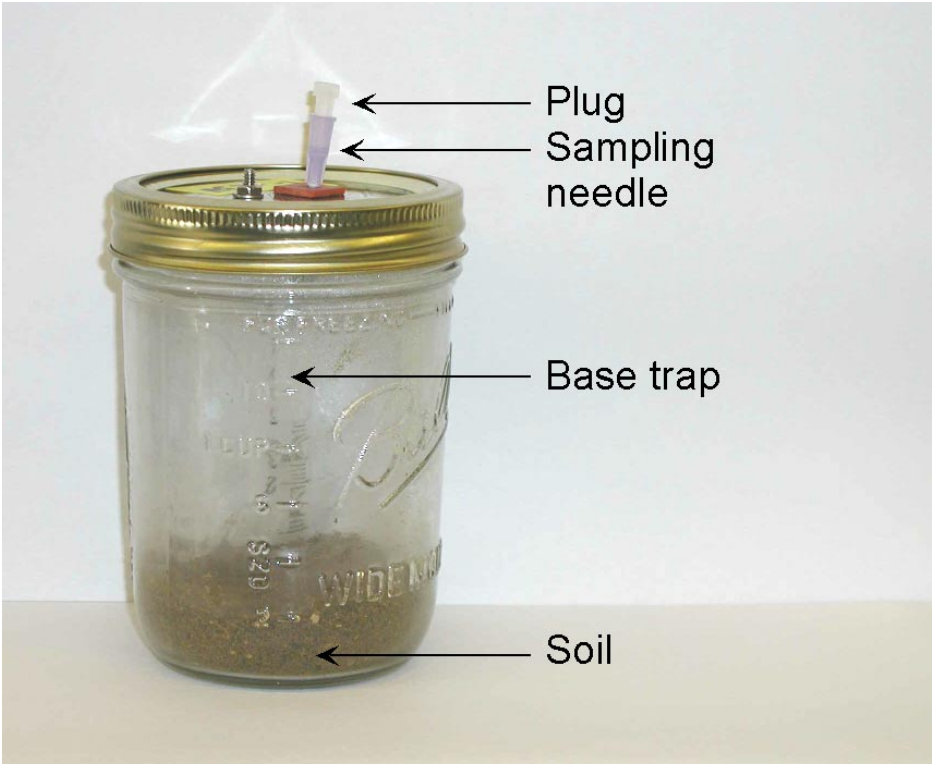

Figure 5.7. Unsaturated soil microcosms.

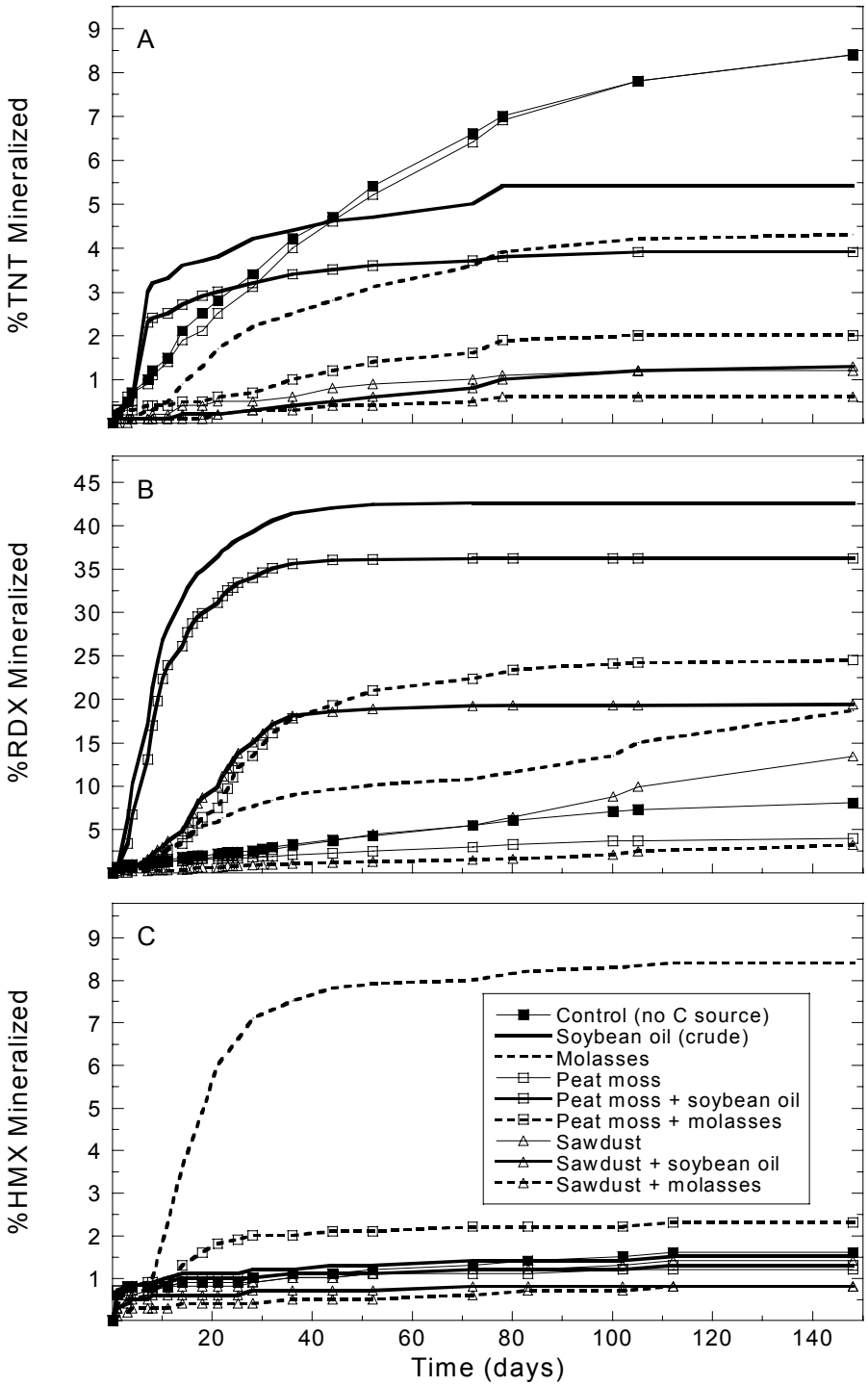

Figure 5.8. Mineralization of TNT (A), RDX (B), and HMX (C) in isolation in unsaturated MMR Soil microcosms amended with sorbents and cosubstrates.

5.5. MINI-COLUMN EXPERIMENTS

Mini-column experiments were performed using large diameter syringes packed with peat moss or a mixture of peat moss plus soybean oil (Figure 5.11). Experiments were performed under water-saturated conditions. The results indicated that uptake into the peat moss significantly reduced the migration of dissolved TNT, RDX, and HMX, and that uptake into the peat moss was mass transfer limited. Addition of the crude soybean oil had a negligible effect on the transport of RDX and HMX, but had a significant impact on the transport of TNT through the mini-columns (Figure 5.12). The relatively large impact of crude soybean oil on TNT transport was due, in part, to enhanced biotransformation of TNT. The TNT biotransformation rate constant in the presence of crude soybean oil was approximately 10-times greater than in the presence of peat alone, and at least 10-times greater than the biotransformation rate constant of RDX or HMX. The presence of the crude soybean oil increased the rate of TNT mass transfer to kinetically-controlled sorption sites, resulting in an overall decrease in TNT flux from the peat plus crude soybean oil mini-columns.

A diffusion model was developed to describe the impact of crude soybean oil addition on the transport of energetic compounds. Model results indicated that mass transfer limitations in peat moss are the result of liquid phase diffusion, as viscosity-dependent liquid diffusion coefficients were shown to correlate with mass transfer rates of the energetic compounds. Based on this model, predictions of the reductions in mass loading of explosive residues to soil over a 1 year timeframe due the presence of a peat moss/soybean oil treatment layer placed on top of soil ranged from >70% for HMX to >90% for TNT (Figure 5.13). This simulation assumed a rainfall rate of 120 cm/year (corresponding to the average annual rainfall total at MMR), and initial TNT, RDX, and HMX concentrations of approximately 50, 2, and 1 mg/L generated from dissolving explosive debris deposited on the surface. [see Schaefer *et al.*, draft manuscript in Appendix B for more details]

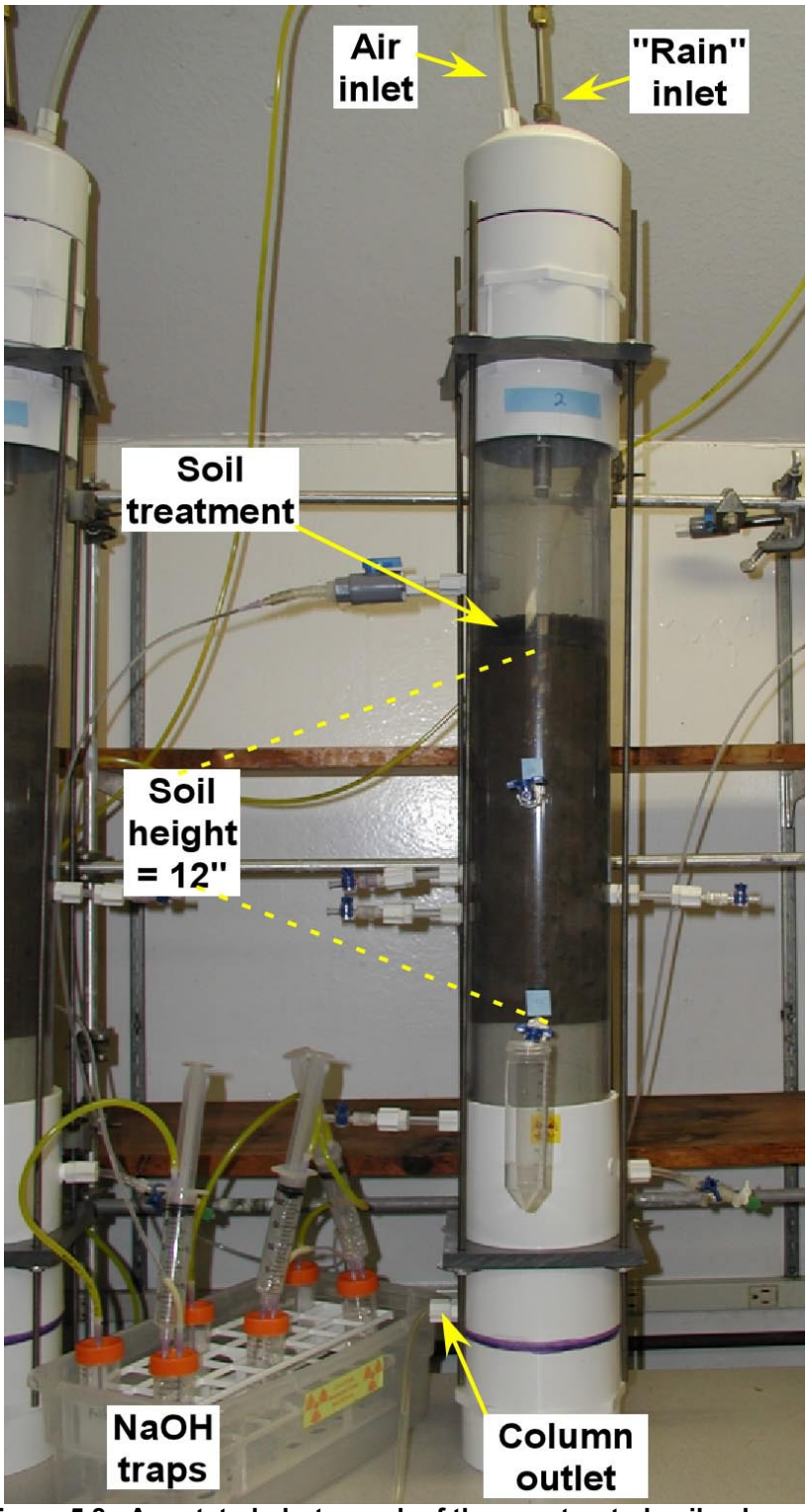

Figure 5.8. Annotated photograph of the unsaturated soil columns.

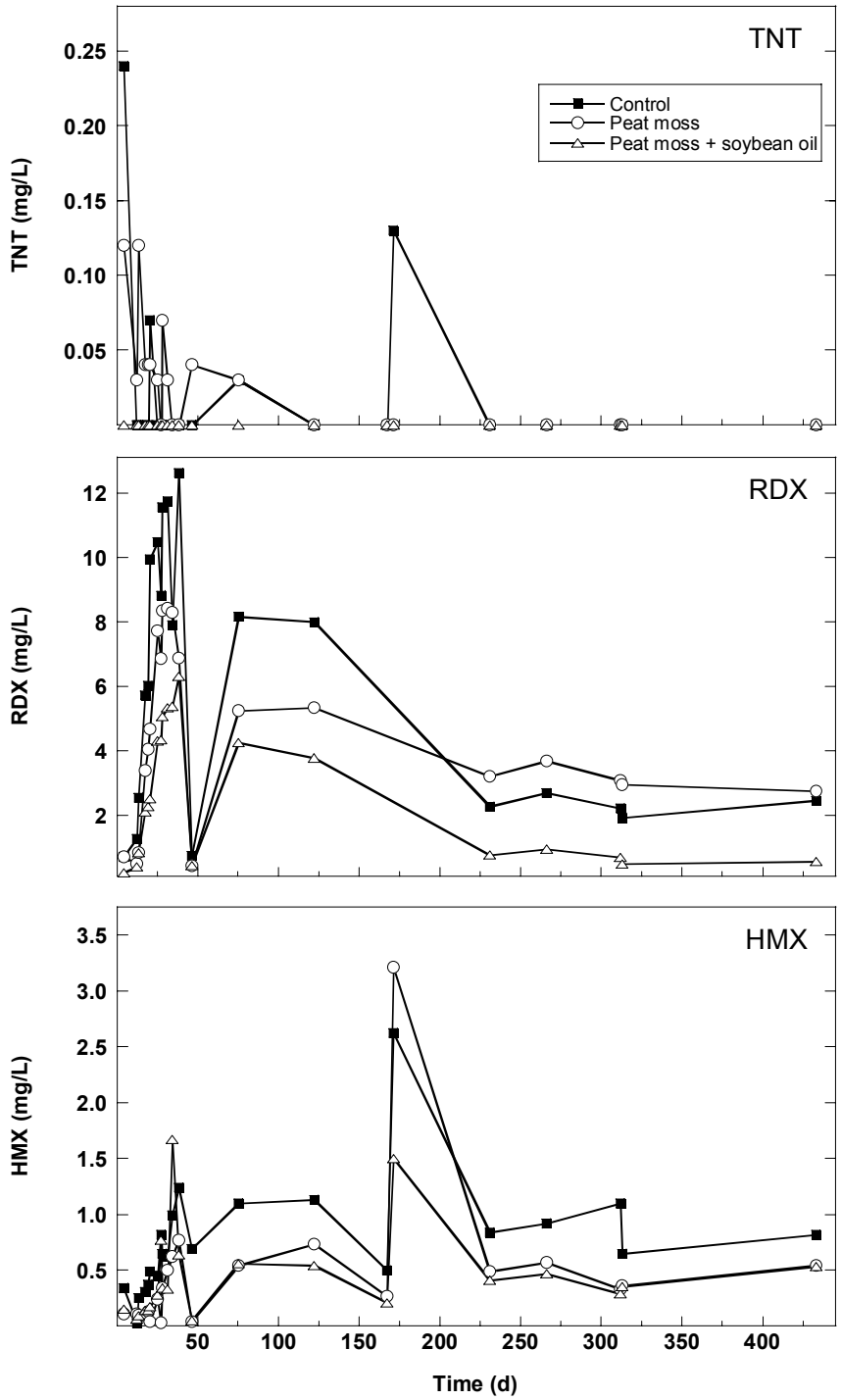

Figure 5.9. Concentrations of explosive compounds in pore water from a depth of 10 cm below the surface in the three columns over time.

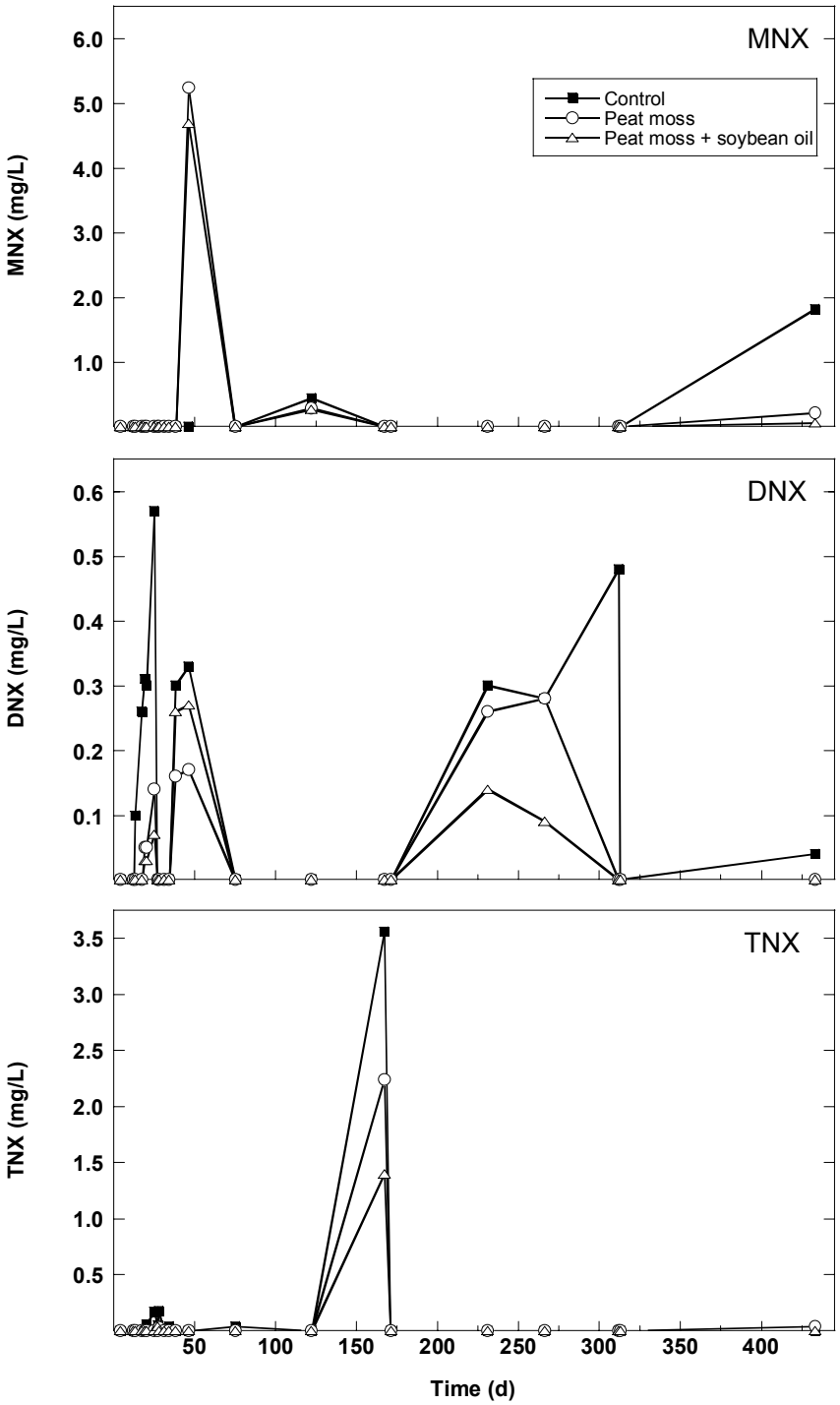

Figure 5.10. Concentrations of RDX metabolites in pore water from a depth of 10 cm below the surface in the three columns over time.

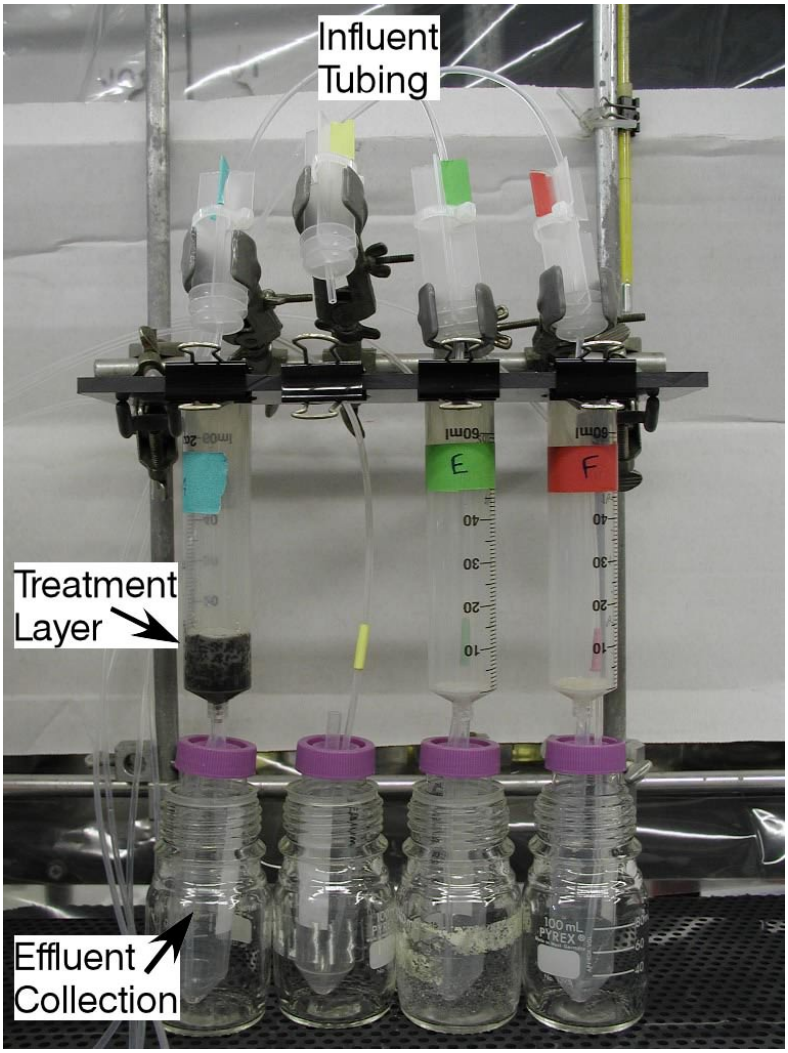

Figure 5.11. Photograph of the mini-column experimental set-up.

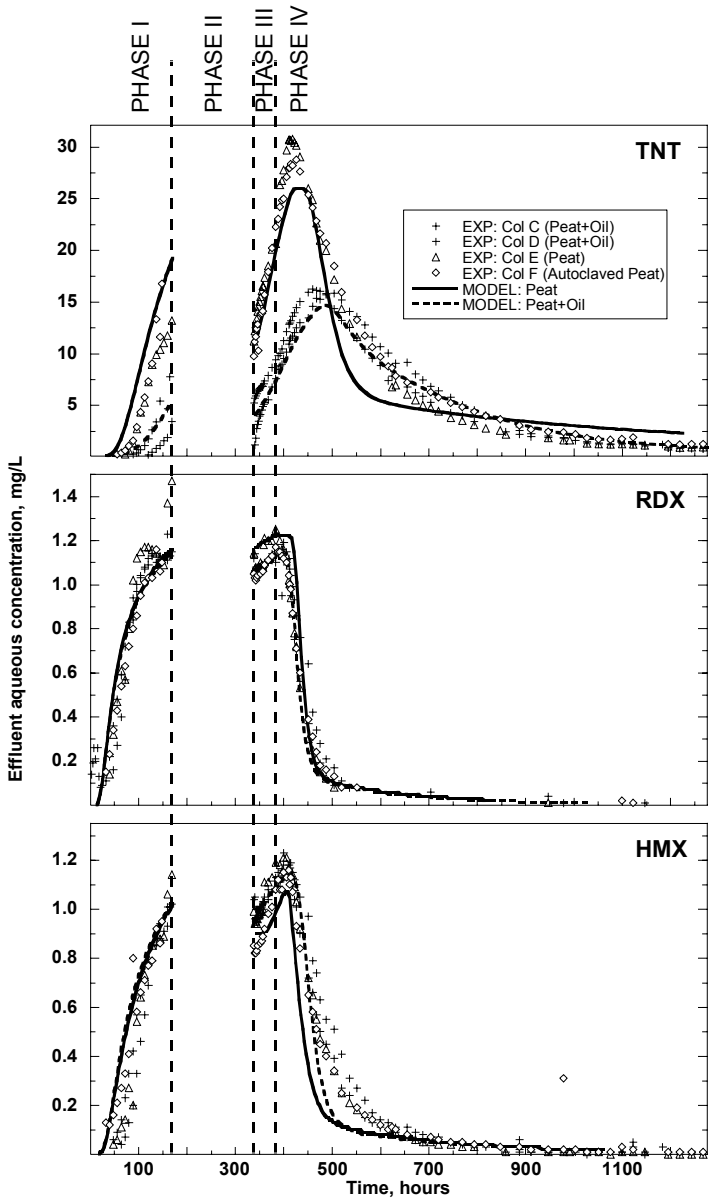

Figure 5.12. Predicted and actual concentration profiles for TNT, RDX, and HMX passing through the mini-columns. Phases: I, loading; II, no-flow; III, loading; IV, desorption.

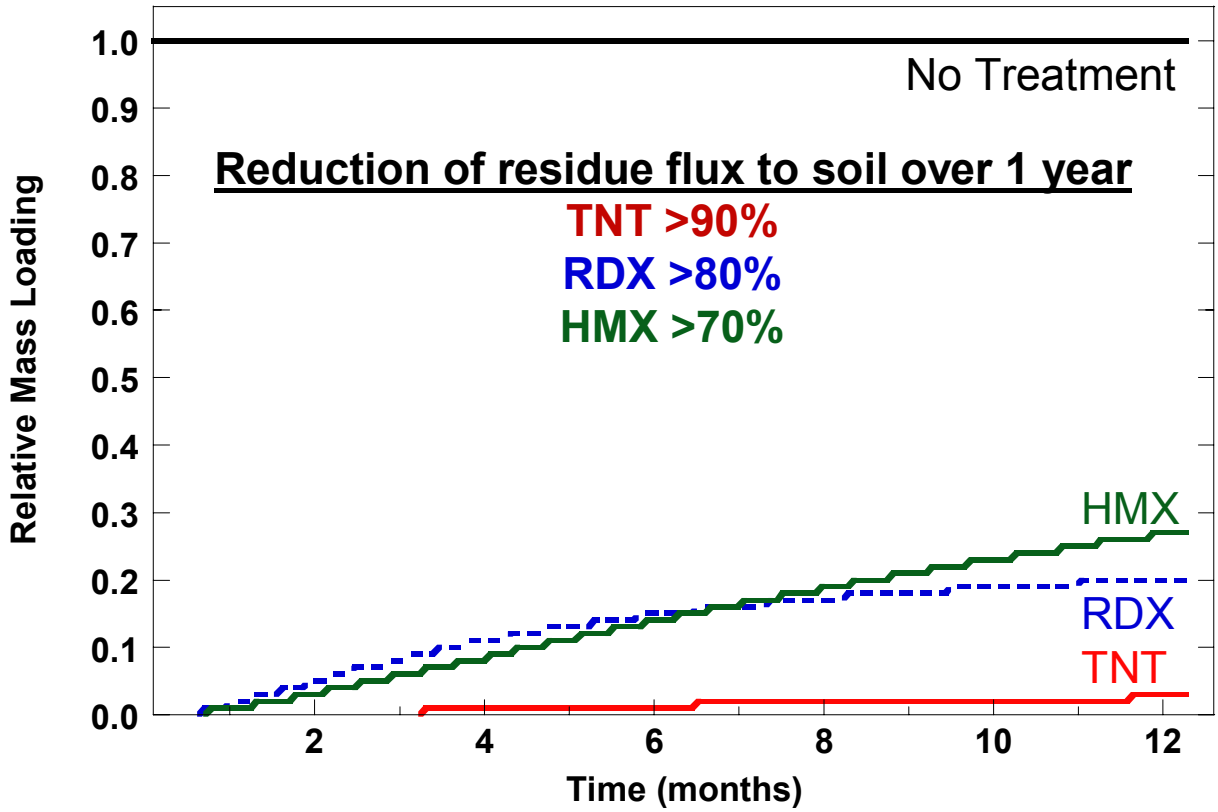

Figure 5.13. Modeling results illustrating the predicted reduction in vadose zone pore water concentrations of TNT and RDX at a depth of 10 feet below ground surface with no in place treatment versus a one inch layer of peat moss plus soybean oil. A rainfall rate of 120 cm/year was used in the simulation (corresponding to the average annual rainfall total at MMR).

6. Conclusions

This project has developed and demonstrated proof-of-concept of a new, surface-applied technology that will greatly reduce the potential for additional groundwater contamination with explosive compounds as a result live fire training activities. The combination of two materials, peat moss and crude soybean oil, both of which are inexpensive, readily available, and environmentally benign, proved to be effective for immobilizing explosive residues at the soil surface and reducing their downward movement and transport.

This technology was specifically designed to be used on live fire ranges to capture and enhance the degradation of explosive residues produced from partial and low-order detonations. However, we believe it has the potential to enhance the degradation of new and existing residues in hand-grenade ranges, mortar firing points, and OB/OD and EOD areas, especially if it can be mixed into the surface layer of soil. This combination of material may also be effective when added to the backfill during UXO excavation and removal activities to stimulate the degradation

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Appendix A

Supporting Data

Quarterly Progress Report

SERDP Project 1229 - Immobilization of Energetics on Live Fire Ranges
Year 2001 – Third Quarter
October 15, 2001

The SERDP contract for FY 2001 was awarded to Envirogen on August 1, 2001. This report covers technical progress for SERDP Project 1229 from August 1 – September 30, 2001.

The objective of this project is to develop a cost-effective technology to immobilize energetic compounds (TNT, RDX, HMX, and breakdown products) released as residues at firing ranges to prevent their migration to groundwater. The goal is to develop an inexpensive soil treatment that can be readily applied over wide and remote areas prior to or immediately following firing range activities.

During the current quarter, activities have focused on gathering the needed materials and supplies, and developing the methods to complete the proposed research. Initial sorption experiments have also been performed, which have yielded very promising results. The results described below focus on TNT sorption; procurement of unlabeled and radiolabeled RDX and HMX occurred during the first weeks of October and will be reported in the next Quarterly Report.

I. EVALUATE ADSORPTION OF TNT, RDX, AND HMX TO SOLID PHASE SORBENTS.

1. SORBENT SELECTION. The treatment of live fire ranges is complicated by their large size, remoteness, and vegetated nature. Therefore selected sorbents must be inexpensive, available locally in large quantities, and resistant to deterioration. We have selected several sorbents for testing based on these criteria.

The first potential sorbent that has been selected is ground rubber from used tires. Envirogen has used ground tires to adsorb environmental contaminants, and has received two U.S. patents on this application. Adsorption of the contaminants reduces their aqueous phase concentration and allows biodegradation of the sorbed contaminants either while on the rubber surface or as they slowly diffuse back into the aqueous phase. Ground tires are also used extensively in landscaping, as padding around playgrounds, and as surfaces in horse training facilities. They are resistant to deterioration and have proven to be environmentally safe. Whole used tires represent a significant waste problem in the United States, and they are noted for their attraction of mosquitoes and other vermin. Thus, if successful, this technology represents a significant application for recycling used tires to help reduce the tire disposal problem.

Another potential sorbent being examined is saw dust. Saw dust is a waste product of the lumber industry and available in bulk throughout New England and many other areas of the country. It is resistant to deterioration, but completely biodegradable. The presence of humic acids and other phenolic compounds in sawdust allows it to adsorb both hydrophobic and polar contaminants (such as the reduced transformation products of TNT). Sawdust has also been shown to adsorb heavy metals that are common soil and groundwater contaminants at live fire ranges.

Chitin from the lobster shells has been obtained for evaluation; chitin is also available in bulk from the crab and shrimp processing industry. Chitin is a positively charged organic polymer (polyanacetylglucosamine) that is resistant to deterioration but completely biodegradable through the action of soil bacteria and fungi. Lobster shells contain 50% mineral matter (primarily calcium carbonate), 25% protein, and 25% chitin. The protein in these materials may support the growth of energetics degrading microorganisms, while its charged nature may aid its adsorption of parent energetics or their reduced degradation products. Chitin also is capable of chelating heavy metals such as those found in soils of live fire ranges.

Two agricultural byproducts, wheat straw and rice hulls, have been obtained. These materials are readily available in agricultural areas of the United States. Again, they are biodegradable, but they are less resistant to deterioration than sawdust, chitin, and rubber. The addition of these

materials are expected to limit the mobilization of energetics and some heavy metals present at ranges by sorption, while at the same time stimulating the growth and activity of microorganisms that can biodegrade the energetics.

Peat and seaweed, two additional plant-based materials, have been included in this research. Seaweed is a waste problem near beaches and it is also harvested on an industrial scale directly from the sea. Its resistance to deterioration, stimulation of microbial population, and adsorption of energetics is expected to be similar to straw and rice hulls. Peat is a mining product that is used extensively as a soil additive. Its cost ultimately may be prohibitive for large scale application in some areas, but its humic composition should aid adsorption of energetics and/or their daughter products. Both products have water-holding capacities that could prolong microbial degradation of energetics in surface soils.

Kaolinite and two types of montmorillonite clays have been purchased for testing. These materials are mining products that can be purchased inexpensively in bulk quantities. Large clay producing facilities are located near some large live fire ranges (e.g., Georgia, South Carolina, and Utah). Clays are natural products, they are resistant to deterioration, and they have large charged surface areas. Clays also can adsorb water to minimize its migration to the subsurface and to support and prolong microbial activities in surface soils.

The sources of the potential sorbents mentioned above, and any processing steps that have been performed prior to their use in the laboratory are listed in Table 1. Additional processing activities included washing, drying, crushing, cutting or chopping, and sieving. A photo of the sorbents is presented in Figure 1. Sufficient quantities for the experiments to be performed during the initial phase of this SERDP project have been prepared.

2. SORPTION KINETICS. An initial screening of the sorbents was performed using TNT, with [^{14}C]TNT as a tracer. The objective of these screening experiments was to determine the time needed for TNT to reach equilibrium between the sorbed and dissolved phase with each of sorbent.

2.1 METHODS:

Experiments were performed in 30 ml Teflon Oak Ridge centrifuge tubes. Air dry sorbents (~1 g) were weighed into duplicate tubes and autoclaved (1 h, 121°C, 20 psi) to inhibit biological activity (i.e., TNT transformation) during the experiment. Tubes containing no sorbent were prepared to control for abiotic losses and sorption onto the inside of the tubes. Fifteen milliliters of $CaCl_2$ (3 mM) solution containing 70 to 80 mg/L TNT and a trace amount of [^{14}C]TNT (~100,000 dpm/ml) was added to each tube, and the tubes were sealed. The actual concentration of TNT in each experiment was determined by HPLC. Tubes were shaken horizontally (250 rpm) at room temperature. Samples were removed periodically for determination of aqueous phase radioactivity. One hundred microliters of particulate-free sample was added to 5 ml of Liquiscint scintillation cocktail (National Diagnostics, Atlanta, GA) and analyzed using a Pharmacia LKB Model 1209 Rackbeta scintillation counter (Pharmacia LKB Nuclear, Gaithersburg, MD). Samples were initially filtered with using 0.2 µm nylon filters to remove particulates of each sorbent prior to scintillation counting, but this procedure was shown to result in a significant loss (~60%) of the TNT to the filter membrane. Therefore, samples were centrifuged in all subsequent experiments (14,000 x g at 4°C for 10 minutes for small subsamples; 10,000 x g at 4°C for 20 minutes for entire 30 ml tubes).

Once the sorption of TNT had reached equilibrium (i.e., constant aqueous concentration of [^{14}C]TNT), and sorption measurements were complete, the desorption of TNT from the sorbents was quantified. Only sorbents with >20% sorption of TNT were examined for desorption. To perform desorption measurements, the aqueous phase was removed from each tube, and fresh $CaCl_2$ solution was added to each sorbent. The tubes were sealed and shaken horizontally, and aqueous samples were removed periodically and analyzed for [^{14}C]TNT as described above. During some experiments, a second desorption step was performed by removing the aqueous phase and adding another aliquot of fresh $CaCl_2$.

2.2 RESULTS:

Sorption at each timepoint was calculated by dividing the dpm/100 µL at a given time by the dpm/100 µL at the beginning of the sorption phase of the experiment (i.e., C/C_0). Desorption was calculated by dividing the total aqueous dpm at a given time by the total dpm remaining sorbed to the solid sorbent at the start of the desorption phase of the experiment. Results were converted to percentages to allow comparison among all of the different sorbents, and between experiments with differing starting concentrations of unlabelled and [^{14}C]-labeled TNT. Graphical and tabular sorption results are presented in Table 2 and Figure 2, respectively. Desorption results are presented in Table 2 and Figure 3.

Sorption of TNT reached equilibrium within 30 minutes (the earliest timepoint examined) for both KSF and K10 montmorillonite clays, kaolin clay, and vermiculite. In contrast, TNT sorption onto all the other sorbents exhibited varying degrees of biphasic behavior characterized by a rapid initial phase followed by very slow second phase (Figure 2). Sorption onto all sorbents appeared to level off after 1 day except for seaweed and sawdust; these latter two materials required 2 days to level off.

The extent of TNT sorption varied extensively among the variety of sorbents (Table 2). TNT exhibited the greatest sorption (>75%) to montmorillonite KSF clay, followed by *Sphagnum* peat moss, ground rubber tires #30, sawdust, rice hulls, ground rubber tires #30-40, and wheat straw. TNT sorbed the least, and to a similar degree (<10%) to vermiculite, kaolin clay, and clam shells. Moderate sorption (~20 to 50%) was observed with lobster shells, seaweed, and montmorillonite K10 clay.

As with adsorption, TNT desorption was rapid (Figure 3). The total percentage of sorbed TNT that was observed to desorbed varied with respect to the sorbent (Table 2). For example, only 15% of sorbed TNT desorbed from montmorillonite KSF clay, compared to 58% from montmorillonite K10 clay. TNT also exhibited moderate desorption from the other sorbents examined, ranging from 20% to 40%.

The rapid initial sorption and desorption of TNT observed with most of the materials examined likely indicates that TNT initially sorbed to the surface of the sorbent (via hydrophobic or

electrostatic interactions). This surface associated TNT is able to rapidly equilibrate with the aqueous phase. The second slower phase of TNT sorption and desorption suggests that some of the TNT slowly diffused into the sorbent matrix through interparticle pores. This internal TNT requires longer periods of time to equilibrate with the aqueous phase. The desorption data collected to date also indicate that irreversible binding of TNT to the sorbents occurs only to a small degree, if any.

2.3 CONCLUSIONS:

The results from these kinetic experiments have allowed procedures for TNT sorption experiments to be developed. Full sorption/desorption TNT isotherms for the most promising sorbents will be reported in the next quarter.

II. TABLES AND FIGURES.

The tables and figures supporting this document have been provided to the SERDP Office as a separate attachment.

TABLES & FIGURES for SERDP PROJECT 1229 – THIRD QUARTER REPORT, 2001

Table 1. General information on sorbents being used during this research.

Sorbent	**Source**	**Processing**
Ground rubber tires #30	Oklahoma rubber recycling plant	None
Ground rubber tires #30-40	Oklahoma rubber recycling plant	None
Sawdust (conifer/deciduous mix)	Cape Cod sawmill	Sieved[a]
Rice hulls	Arkansas rice processor	None
Wheat straw	New Jersey	Cut/sieved
Vermiculite	Standard packing material	Sieved
Sphagnum peat moss	Nirom Peat Moss, Inc.	Sieved
Lobster shells	Cape Cod restaurant	Washed/crushed/sieved
Actica clam shells	Cape Cod clam processing plant	Washed/crushed/sieved
Seaweed	Cape Cod beach	Cut/sieved
Kaolin	Aldrich Chemical Company	None
Montmorillonite K10[b]	Aldrich Chemical Company	None
Montmorillonite KSF[c]	Aldrich Chemical Company	None

[a]Sieving performed to produce material bewteen 18 mesh (1 mm) to 5 mesh (4 mm) in size.
[b]Bulk density = 800-850 g/L; surface area = 20-40 m^2/g.
[c]Bulk density = 300-370 g/L; surface area = 220-270 m^2/g.

Table 2. Summary of TNT adsorption and desorption results.

Sorbent	**Sorbent mass (g)**	**Initial TNT (mg/L)**	**Adsorption (%, 24 h)**	**Desorption[a] (%, 24 h)**
Montmorillonite clay, KSF	0.96	75	93	15
Sphagnum peat moss	0.38	80	82	22
Ground rubber tires #30	0.99	80	77	29
Sawdust	0.91	80	76	25
Rice hulls	0.94	86	75	32
Ground rubber tires #30-40	0.98	75	75	29
Wheat straw	0.91	75	74	28
Montmorillonite clay, K10	0.97	75	54	58
Seaweed	0.84	80	50	42
Lobster shells	0.94	80	18	ND[b]
Vermiculite	0.99	80	7	ND
Kaolin clay	0.99	80	5	ND
Clam shells	0.99	75	6	ND

[a]Cummulative TNT desorbed after two replacements of aqueous $CaCl_2$ phase over 48 h.
[b]ND, not determined.

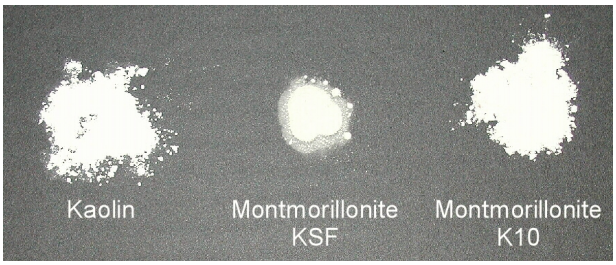
Figure 1. Photograph of the different materials being tested as sorbents.

Figure 1 (cont.)

Seaweed

Wheat straw

Vermiculite



Clays

Figure 2. Comparison of TNT sorption kinetics for different sorbents. Each point represents average of two replicates. A, Four hour incubation. B, Twenty four hour incubation.

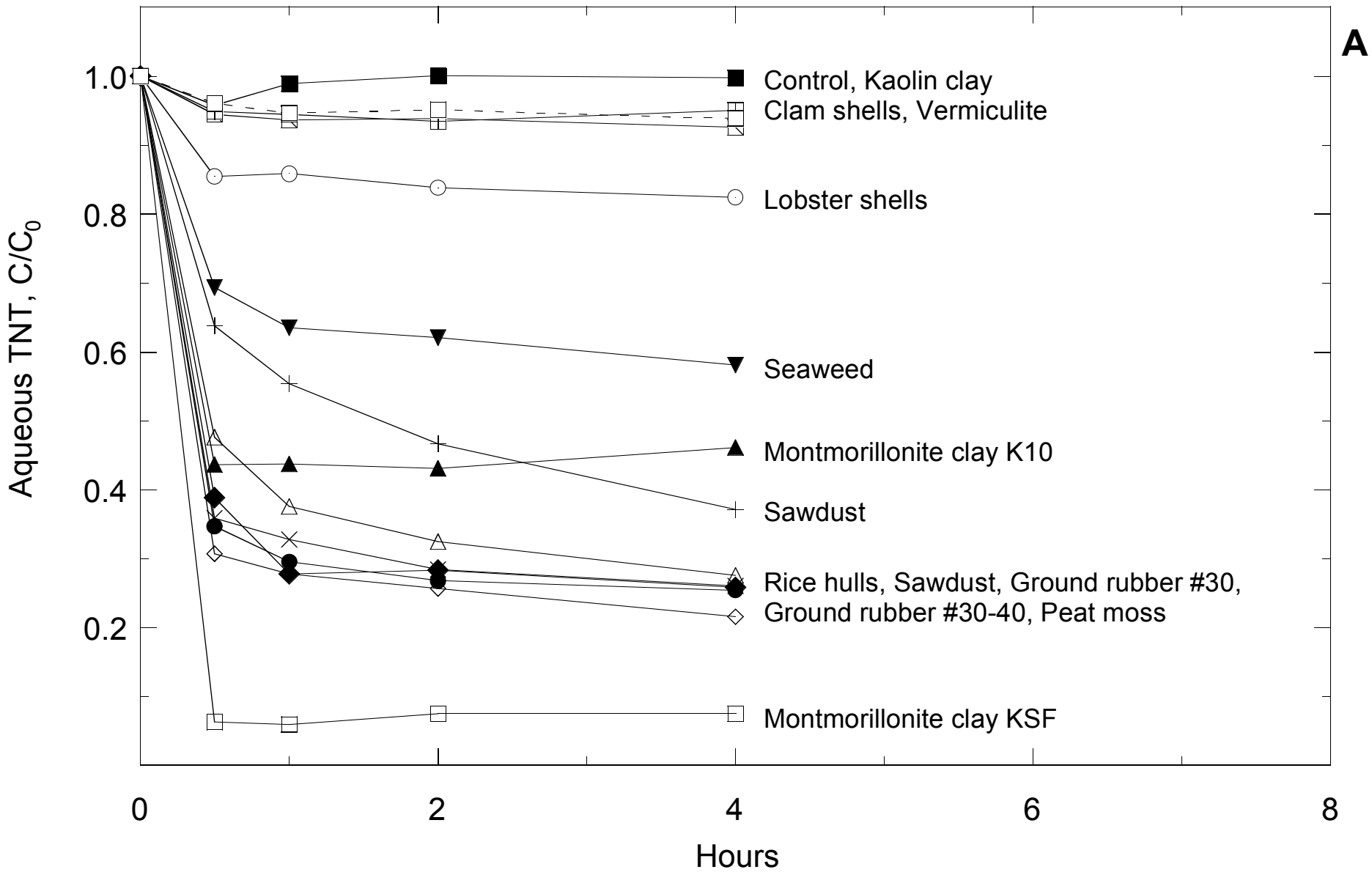


Figure 2 (cont.)

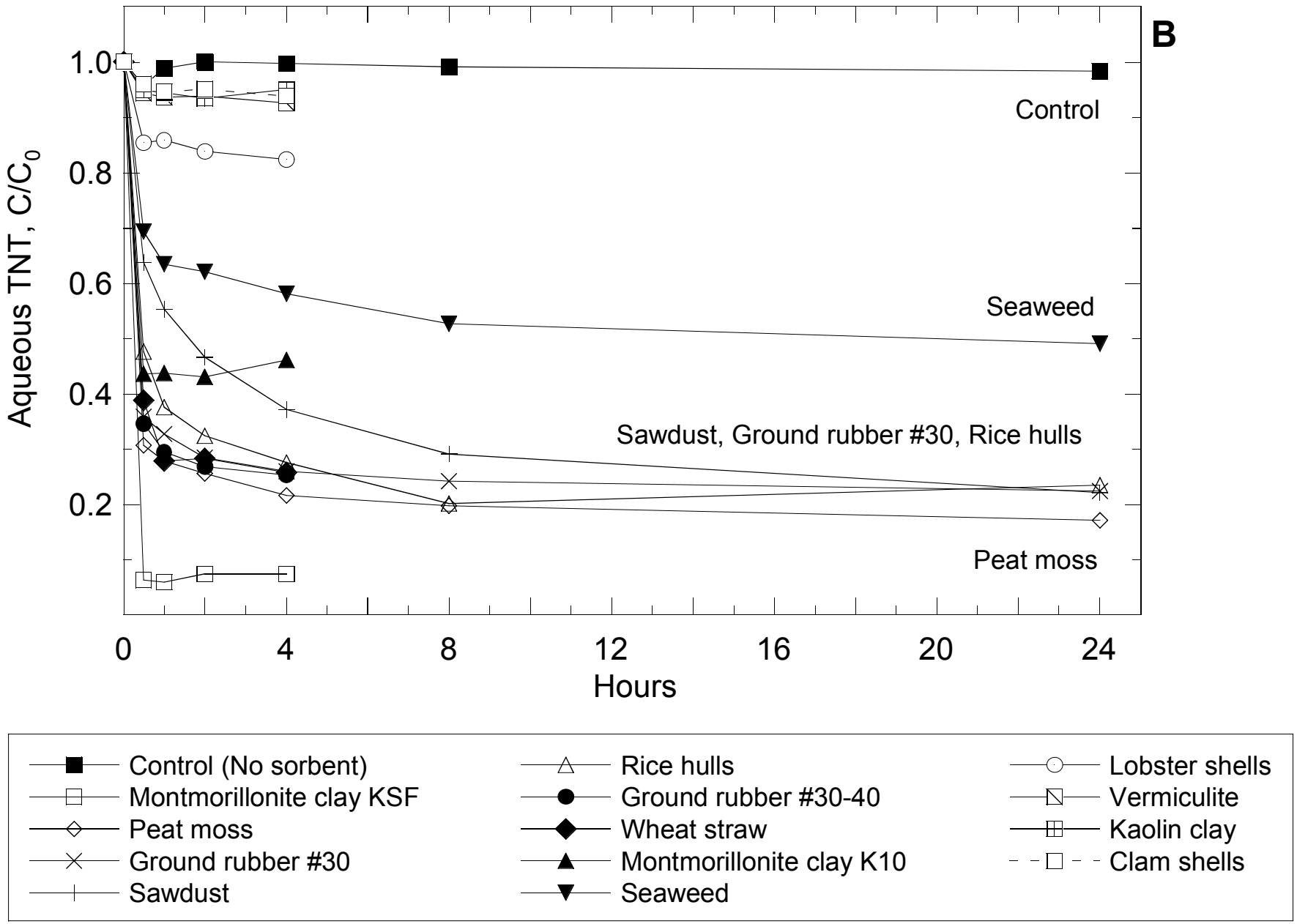


Figure 3. Comparison of TNT desorption kinetics for different sorbents. Each point represents average of two replicates. Percent desoprtion observed with second addition of $CaCl_2$ (----) is relative to amount of TNT remaining sorbed after first desorption was complete.

A, Single desorption with all sorbents. B & C, Double desorption during separate experiments with sorbent subsets.

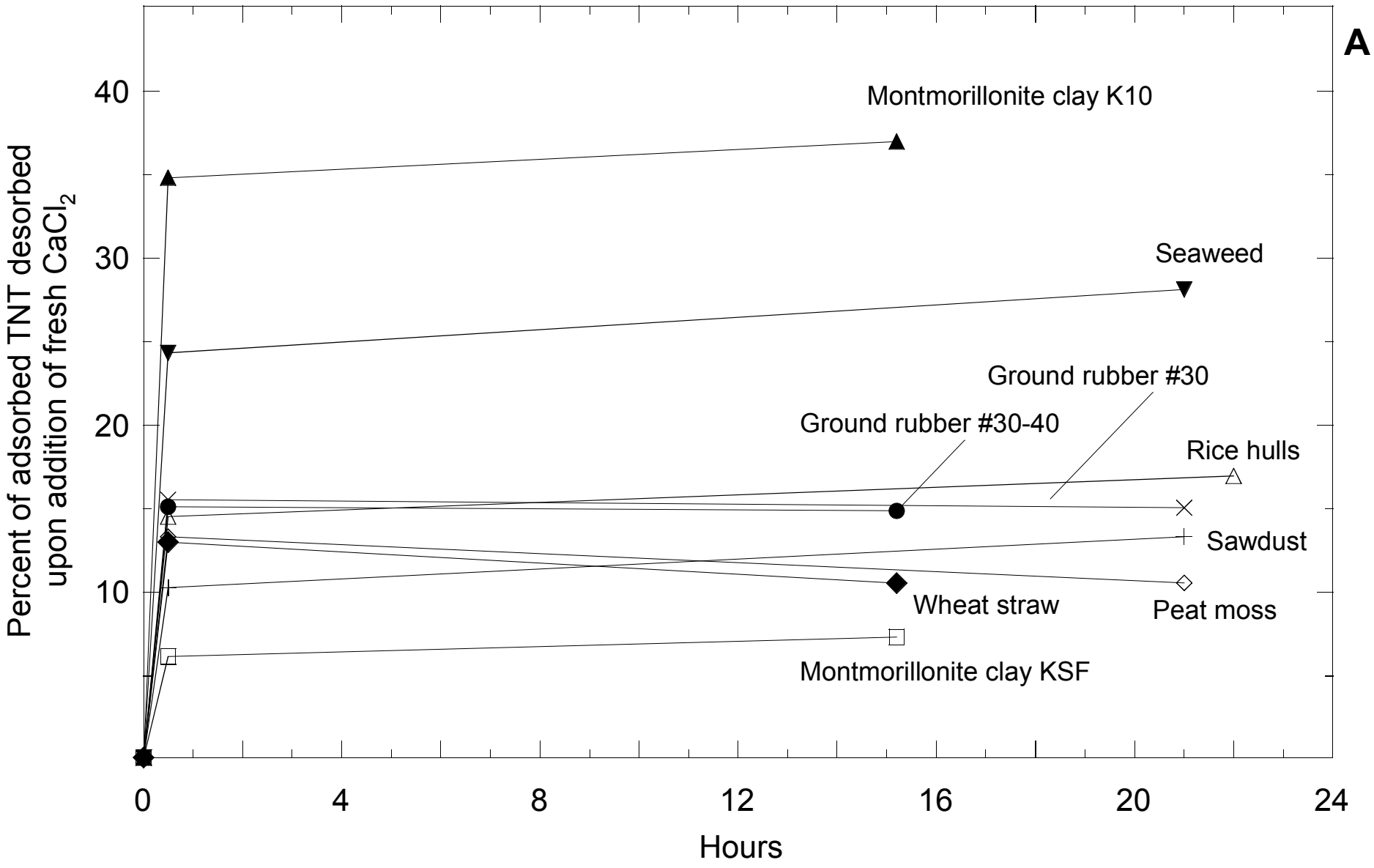


Figure 3 (cont.)

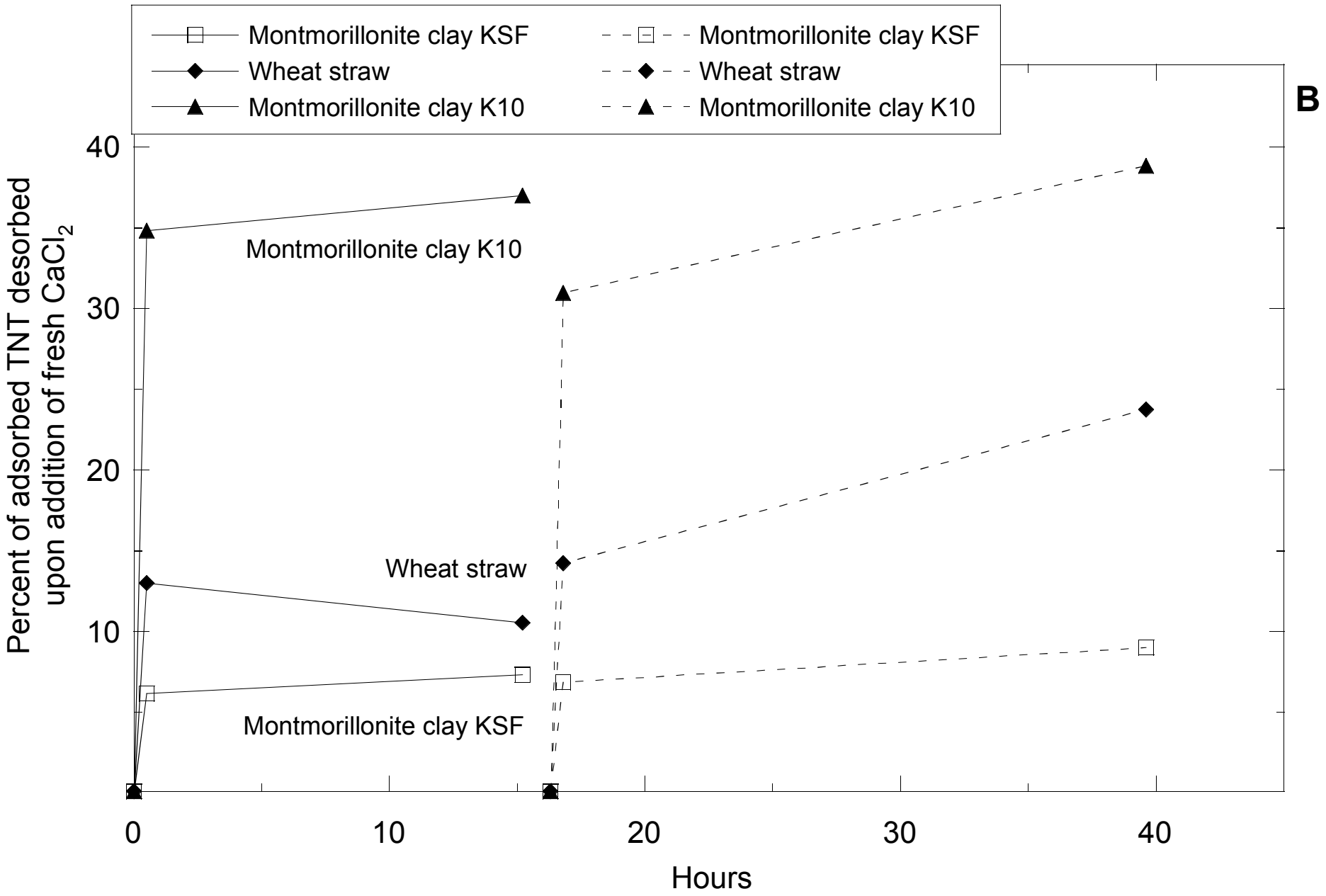
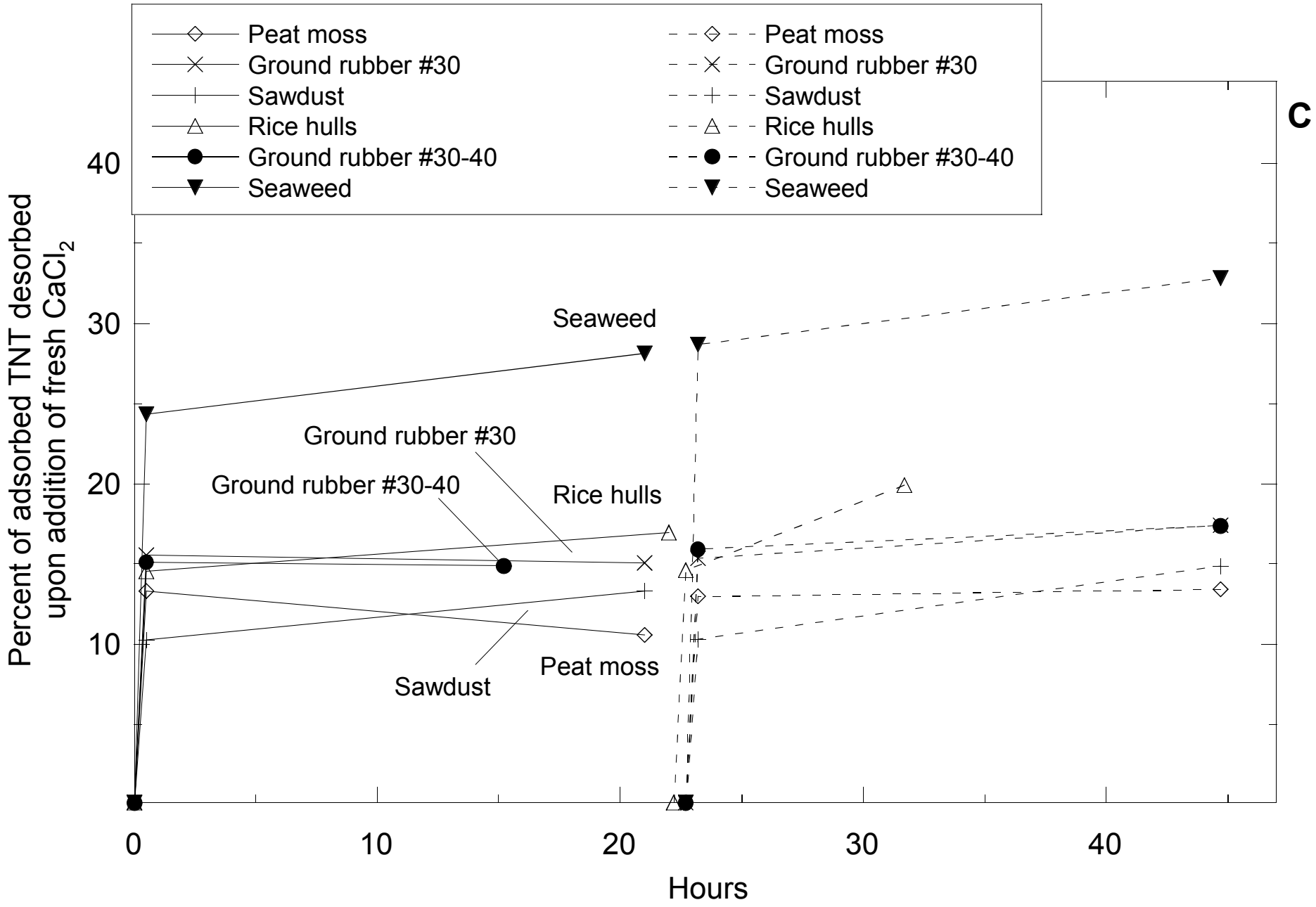


Figure 3 (cont.)



Quarterly Progress Report

SERDP Project 1229 - Immobilization of Energetics on Live Fire Ranges
Year 2001 – Fourth Quarter
January 15, 2002

The SERDP contract for FY 2001 was awarded to Envirogen on August 1, 2001. This report covers technical progress for SERDP Project 1229 from October 1, 2001 – January 15, 2001.

The objective of this project is to develop a cost-effective technology to immobilize energetic compounds (TNT, RDX, HMX, and breakdown products) released as residues at firing ranges to prevent their migration to groundwater. The goal is to develop an inexpensive soil treatment that can be readily applied over wide and remote areas prior to or immediately following firing range activities.

During the current quarter, activities have focused on completing screening of potential sorbents for the explosives, conducting full sorption-desorption isotherms, and screening the potential cosubstrates for their ability to stimulate biodegradation of TNT, RDX and HMX in microcosms.

I. EVALUATE ADSORPTION OF TNT, RDX, AND HMX TO SOLID PHASE SORBENTS.

1. POTENTIAL SORBENT SCREENING.

Screening of potential sorbents has been completed for TNT, RDX, and HMX using [^{14}C]-labelled compounds as tracers. Table 1, which presents the type, source and processing of the materials examined, is included from the previous quarterly report for reference.

1.1 METHODS:

Methods used to screen the materials for their ability to sorb the target explosives were presented in detail in Section 2.2 of the Third Quarter Report. Briefly, 1 g of the potential sorbent and 15 of a $CaCl_2$ solution containing unlabelled and a trace amount of [^{14}C]-labelled explosive was added to a teflon tube. The tube was then shaken for 24 hours, at which time, the amount of radioactivity remaining in solution was determined. This allowed the extent of sorption to be determined. All the remaining fluid was then removed from the tube, and fresh CaCl2 containing no explosives was added. The tube was shaken for 24 hours, at which time the amount of radioactivity coming into solution was determined. This allowed the extent of desorption to be determined.

1.3 RESULTS & DISCUSSION:

A complete summary of the screening results is presented in Table 2. For RDX and HMX, *Sphagnum* peat moss proved to be the best sorbent (65% and 69% adsorption, respectively), whereas montmorillonite KSF clay was a slightly better sorbent than peat moss for TNT (93% vs. 82% adsorption, respectively). Overall, RDX and HMX sorbed to most of the materials to a lesser degree than TNT, ranging from 1.5- to 7-fold less. Exceptions were observed, with 20%,

70%, and 100% more HMX than TNT sorption to lobster shells, vermiculite, and kaolin clay, respectively.

Desorption of RDX and HMX occurred to a larger extent than TNT from all the materials examined, by a factor of 1.5- to almost 5-fold. For example, 50+% of the adsorbed RDX and HMX desorbed from peat moss, compared to only about 20% of the adsorbed TNT. The biggest differential was observed with montmorillonite clay, from which 72% of the adsorbed HMX desorbed, compared to only 15% of the adsorbed TNT.

The desorption results are not wholly unexpected based on the current literature, and the growing number of reports of RDX, as opposed to TNT, in the groundwater underlying contaminated soils.

2. SORPTION-DESORPTION ISOTHERMS.

Based on the results of the initial screening of the potential sorbents, detailed sorption-desorption isotherms were performed for the best four sorbents for each explosive.

2.1 METHODS:

Sorption-desorption isotherms were performed in a similar manner as the initial screening. Air dry sorbents (~1 g) were weighed into duplicate 30 ml Teflon Oak Ridge centrifuge tubes and autoclaved (1 h, 121°C, 20 psi) to inhibit biological activity (i.e., TNT transformation) during the experiment. Tubes containing no sorbent were prepared to control for abiotic losses and sorption onto the inside of the tubes. Eight concentrations of each explosive in a $CaCl_2$ solution (3 mM) were used for each isotherm. These were prepared by adding a known volume of a concentrated explosive stock (in acetonitrile) to sterile glass bottles, evaporating the solvent under a stream of nitrogen, adding a known volume of $CaCl_2$, and sonicating/mixing until all the explosive had dissolved. Nominal concentrations of TNT, RDX, and HMX are given in Table 3; actual concentrations of each solution were determined by HPLC. [^{14}C]-labelled explosive was then added to the solution to achieve approximately ~100,000 dpm/ml. The actual concentration of TNT in each experiment was determined by HPLC, and the concentration was adjusted to account for the amount of [^{14}C]-labelled explosive compound added (calculated based on the specific activities of the radiolabelled compounds). Tubes were sealed and shaken horizontally (250 rpm) at room temperature for 24 h. Tubes were centrifuged (10,000 x g, 4°C, 20 min), and 100 µl of the cleared supernatant was removed for determination of aqueous phase radioactivity as described above.

The desorption isotherm was then initiated. The remainder of the aqueous phase was removed and 15 ml of fresh $CaCl_2$ solution was added to each tube. The tubes were sealed and shaken horizontally (250 rpm) at room temperature. After 24 h, the tubes were centrifuged, and the radioactivity in the aqueous phase was measured as described above.

To assess whether the autoclaving procedure used to sterilize the sorbent materials prior to use significantly affected the sorption-desorption isotherms – by changing the physical or chemical properties of the material though the action of high heat and pressure – isotherms were repeated using sterile and nonsterile *Sphagnum* peat moss and sawdust with three concentrations of TNT. These two sorbents, and this explosive, were assumed to represent the worst-case scenario. Only non-radiolabelled TNT was used for these experiments so that aqueous samples could be analyzed by HPLC for the presence of TNT breakdown products.

The bulk of the RDX used for this research contained ~7% HMX as an impurity formed during the manufacturing process. Therefore, the effects of trace HMX impurities on RDX sorption and desorption isotherms was also determined. Isotherms were determined for *Sphagnum* peat moss and wheat straw using RDX with a purity of 93%, as well as with 99% pure RDX recrystallized from an analytical standard solution.

2.2 RESULTS & DISCUSSION:

Freundlich and Langmuir models were fitted to the sorption and desorption data using Microsoft Excel. Results are presented in Figures 1, 2, and 3, and in Tables 4 and 5. The presence of the 7% HMX impurity in the RDX used for these experiments had no significant influence on in the resulting sorption-desorption isotherms (data not shown). Autoclaving the peat moss and sawdust also resulted in no significant differences in the isotherm results compared to unautoclaved materials (data not shown), and this was assumed to be applicable to the other materials as well.

The Freundlich equation fit the sorption and desorption data very well, yielding coefficients of determination (r^2) of >0.98 or greater for all the explosive-sorbent combinations tested. The distribution coefficients (K_d) for both adsorption (K^s_d) and desorption (K^d_d) were always in the order TNT > HMX > RDX for a given sorbent. The K^d_d's were higher (and the slopes of the desorption isotherms were steeper) than the corresponding adsorption K^s_d's, indicating some degree of hysteresis. The K^s_d value for TNT, however, is only 57% of that previously reported, likely reflecting differences in the cation substitution of the two clays.

In contrast, the Langmuir equation did not fit the data very well, except in the case of TNT and HMX with montmorillonite clay KSF as the sorbent (r^2 >0.90). Even with the weaker fits, the Langmuir model results indicated that maximum total amount (parameter b) of explosive that could be adsorbed was in the order TNT > RDX > HMX. The fact that the Langmuir equation yielded good results only with clay probably reflects the fact that this equation was originally developed to explain simple surface processes. However, the interaction of the explosives and the other sorbents likely involves both adsorption to surfaces, as well as absorption of explosive molecules within the structure of the sorbents.

The adsorption values for TNT and RDX for all the sorbents tested are greater than those reported in the literature for various types of soils, giving a general indication that they will indeed serve to retard the migration of all three explosives down through the soil and into the subsurface. This will likely hold true for HMX as well due to its structural similarity to RDX, although literature values are not readily available for comparison. The desoprtion results also

indicate that all the explosives are liekly to desorb from the sorbents when they come in contact with a clean liquid phase (i.e., during and after a rain event), with less desorption of TNT compared to RDX and HMX. This desorbed explosive would then be available for biodegrdation.

II. CONDUCT MICROCOSM STUDIES.

As well as examining the ability of inexpensive, readily available materials to sorb explosives compounds to prevent their migration into the subsurface, another aspect of this project is to evaluate several materials for their ability to promote the biotransformation of explosives.

The selection of potential cosubstrates is based on literature reports and cost/availability information. To be an effective cosubstrate, the material must be biodegradable, inexpensive, and readily available. The ideal cosubstrate will provide all the nutrients (C,N,S,P, etc.) required by indigenous soil bacteria be very metabolically active. The cosubstrate also needs to be added at a concentration such that suboxic and anoxic microsites within the soil will be generated as the cosubstrate is consumed, or due to the physical properties of the cosubstrate (i.e., oil forming a thin layer around soil particles). Finally, the material needs to be acceptable in terms of its intentional use as a soil amendment.

Molasses, a byproduct of the sugar processing industry, has been shown in many studies to support explosives biodegradation in both laboratory and field bioslurry reactors. It has also proven effective under simulated land-farming conditions in the laboratory. Molasses is rich in sugars/carbohydrates, proteins, amino acids, vitamins and minerals, and it is widely used as an animal feed supplement.

Corn steep liquor is a waste product of the corn processing industry, and is rich in protein, lactic acid, amino acids, vitamins, and minerals. It has been widely used as a fermentation media in the pharmaceutical industry, and is also being examined as a medium for the anaerobic production of fuel grade ethanol, as well as other compounds such as butanol, acetate, etc. Corn steep liquor is expected to stimulate extensive explosives biodegradation.

Crude/unrefined soybean and safflower oils are inexpensive and readily available cosubstrates that not only provide carbon and other nutrients to the soil microbes to promote explosives biodegradation, but also may serve to coat soil particles, thereby enhancing the formation of anoxic microsites ideal for biological transformation of explosives. SoyGold 1000 and SoyClear 1500 are soybean oil derivatives that have been developed as environmentally friendly replacements for organic solvents for many industrial applications. These products are nontoxic and highly biodegradable, although the fact that they have been refined to some degree means they will likely provide less trace nutrients to the soil microbes than the unrefined oils.

Solulac® is a registered product comprised of distillers dried grains with solubles, high in protein, lactic acid, amino acids, and vitamins, and the major minerals. It is used as a feed supplement for animals. This complex material is a dry solid, so release of the nutrients to the

soil microbes is expected to be slower than for the liquid cosubstrates and dependent on the moisture level.

Potato starch and unmodified corn starch are inexpensive and widely available agricultural products. They are used in the food, paper and pharmaceutical industries for a variety of purposes. These materials are partially refined dry solids, so release of nutrients is expected to be slower than for the liquid cosubstrates, and other nutrients are expected to be minimal.

Finally, chitin is a solid byproduct of the seafood processing industry, derived from crab, lobster, and shrimp shells. The practical grade used for this research is unrefined crab shells. Chitin has been shown to be an effective electron donor and growth substrate for the promotion of anaerobic dechlorination of PCE, TCE, etc. It therefore seems likely it will be a good cosubstrate for stimulating explosives biodegradation.

1. COSUBSTRATE SCREENING.

The initial part of this aspect of the research was to evaluate the ability of multiple substances to serve as cosubstrates for TNT, RDX and HMX biodegradation under optimal conditions in soil slurries.

1.1. METHODS:

A list of the cosubstrates examined, their sources, cost per unit, and the concentrations used for the screening experiments is given in Table 6. A photograph of the different materials is presented in Figure 4.

The soils used for this research were obtained from the Massachusetts Military Reservation (MMR). The soils we collected from an assumed uncontaminated grassy area (soil G) and a discolored soil area which was assumed to be contaminated (soil C). Initial concentrations of explosives in the soils were obtained using a modified EPA Method 8330 with HPLC analysis. Pertinent soil parameters are presented in Table 7. Only a subset of the cosubstrates tested with the contaminated soil (C) were tested using the clean soil (G).

Cosubstrate screening was performed in microcosms, as illustrated in Figure 5. The microcosms for the screening consisted of 10 g (wet wt.) soil, made into a slurry with 25 ml of NP solution, which consisted of deionized water containing 40 g/L $(NH_4)_2HPO_4$ and 20 g/L $(NH_4)H_2PO_4$. This resulted in final concentrations of nitrogen-N and phosphorus-P of approximately 80 and 100 mg/kg, respectively. Three aliquots of the NP solution were amended with unlabelled and [^{14}C]-labelled TNT, RDX, and HMX, resulting in final explosive concentrations of 50.6, 21.0 and 3.2 mg/L, respectively. The “killed control” treatment received the same NP solution amended with $HgCl_2$ and sodium azide to achieve final concentrations of 2500 mg/kg (or 1000 mg/L) in each microcosm. TNT, RDX, and HMX added to the killed control NP solution resulted in concentrations of 41.6, 21.0, and 5.0 mg/L, respectively. The total radioactivity added to each of the microcosm was approximately 100000 dpm; triplicate subsamples of each [^{14}C]explosives-amended NP solution were analyzed to determine the actual initial dpm added to each treatment.

Solid cosubstrates were added to the microcosms before the soils were slurried, whereas liquid cosubstrates were added after the NP solution had been added to the soil in each bottle. The control and killed control treatments received no cosubstrate additions. Two duplicate bottles of each treatment were prepared. After sealing the bottles, 0.5 ml of base (0.5 N KOH) was added to each base trap through the sampling needle, and the sampling needle was closed with a plastic luer plug. Bottles were placed in closed boxes and incubated at room temperature with gentle shaking (150 rpm).

During sampling, the liquid in each base trap was removed using a 1 ml tuberculin syringe and expelled into a 20 ml vial containing 4.5 ml of OptiPhase HiSafe 3 liquid scintillation cocktail (National Diagnostics, Atlanta, GA). A separate syringe was dedicated for removing the base from each pair of bottles. After sampling, a fresh aliquot of KOH was placed in each base trap, and the incubation was continued. All the sample vials were sealed, vortexed well, and analyzed using scintillation counting.

The scintillation data was used to calculate the cumulative mineralization of the explosive compounds in each microcosm, expressed as the dpm collected as $^{14}CO_2$ in the base trap divided by the total dpm added to the bottle. Mineralization for each cosubstrate was calculated as the average of duplicate bottles.

1.2. RESULTS & DISCUSSION:

The cumulative percent mineralization of each explosive after incubation for approximately 14 days with each cosubstrate in the two different soils is presented in Table 8. The biocides used in the killed control were effective at suppressing microbial activity, as revealed by the very low percentages of $^{14}CO_2$ recovered from these microcosms.

Mineralization was always greater in the uncontaminated soil (G) compared to the contaminated soil (C). There are several possible reasons for this. Firstly, the concentration of culturable bacteria in soil G were approximately 6-fold higher than in soil C. These bacteria likely represent those bacteria most likely to respond to the cosubstrate addition, and hence faster and more pronounced mineralization could be expected to occur in the uncontaminated soil. Secondly, since the contaminated soil also contained significant concentrations of the explosives which were added in the NP solution. These pools of endogenous contaminants may have acted to dilute the added [^{14}C]-labelled explosives used as tracers, and hence less $^{14}CO_2$ was liberated just due to competition between of [^{14}C]-labelled explosives and unlabelled explosives and the degradative enzymes in the bacteria. In fact, if the total initial mass of TNT, RDX, and HMX in each microcosm is multiplied by the percent mineralization, the total mass of each explosive mineralized is approximately double in the contaminated soil compared to the uncontaminated soil (data not shown). Thirdly, the contaminated soil resulted in a higher pH solution when slurried compared to the uncontaminated soil slurry (7.9 vs. 6.7). This may be resulting in a slow release (and capture) of $^{14}CO_2$ from the contaminated soil microcosms. Fourthly, the amount of mineralization in the uncontaminated soil slurry which received no cosubstrate was greater for all the explosives than in the contaminated soil. This indicates that there may be a pool of nutrients in the uncontaminated soil that can support explosives biodegradation. However, this pool of nutrients is not sufficient to account for some of the dramatic differences observed between the

uncontaminated and contaminated soils (i.e., RDX mineralization with crude soybean oil as the cosubstrate). Finally, the soil characteristics of the two soils may be different, leading to less TNT, RDX, and HMX being bioavailable in the microcosms prepared with contaminated soil compared those prepared with uncontaminated soil. This phenomena will be more fully explored as time and resources permit, but they are very promising in that they show that little or no pre-exposure to the target compounds, and no acclimation time, is required to stimulate significant explosives biodegradation.

Looking at the data on a compound-specific basis, the extent of mineralization after approximately 14 days was RDX > HMX >> TNT. The minimal mineralization of TNT (<1%) in both soils is in agreement with most of the current literature. The transformation of TNT to amino-containing metabolites with subsequent polymerization and/or binding of the metabolites to other organic material seems to be more common than TNT mineralization. However, there may be a slow mineralization of the transformation products over time. The amount of RDX mineralization in the uncontaminated soils microcosms seems to be more than observed with pure bacterial cultures, but is comparable to what other investigators have seen in nutrient-amended soils and sludges. However, the preliminary rates of RDX mineralization by the soil slurries may be faster than previously observed. The moderate amount of HMX biodegradation at this point also appears to be in line with the limited literature reports.

The stimulation of explosives mineralization varied greatly with cosubstrate type. For the uncontaminated soil, crude soybean oil ("soybean oil" henceforth) was by far the best cosubstrate for RDX and HMX biodegradation. Soybean oil stimulation of TNT biodegradation was not definitive after about 2 weeks given that the control microcosms which received no cosubstrate released the same percentage of $^{14}CO_2$. Molasses, which has been used many times as a cosubstrate for explosives degradation, supported slightly less mineralization of RDX than soybean oil (20% vs. 25%), followed by potato starch (4% mineralization). HMX mineralization stimulation with potato starch was second behind soybean oil (5% vs. 7%), with all other cosubstrates resulting in no significant increases in $^{14}CO_2$ production relative to the control. However, For the contaminated soil, all the cosubstrates (including soybean oil) have proven to be equally effective at stimulating mineralization of TNT, RDX, and HMX. However, the relatively low total percent of mineralization makes comparisons difficult.

These microcosm experiments will be continued until the rates of mineralization become negligible, at which time they will be processed and analyzed for explosive metabolites and different pools of residual ^{14}C in order to close the mass balance. Samples will also be taken for culturable bacterial counts and isolation of potential explosive-degrading strains.

Overall, while the mineralization data is promising, complete destruction of the explosives is not as crucial to the success of this project as is finding the optimal conditions that promote the greatest protection of groundwater underlying firing ranges. It may be that a combination of explosives transformation and irreversible sequestration (adsorption), or slow release and degradation, of the explosives will provide the necessary level of risk reduction.

III. TABLES AND FIGURES.

The tables and figures supporting this document have been provided to the SERDP Office as a separate attachment.

Table 1. General information on sorbent materials used for this research.

Material	Source	Processing
Montmorillonite clay, KSF[a]	Aldrich Chemical Company	None
Sphagnum peat moss	Nirom Peat Moss, Inc.	Sieved[b]
Ground rubber tires #30	Oklahoma rubber recycling plant	None
Sawdust (conifer/deciduous mix)	Cape Cod sawmill	Sieveda
Rice hulls	Arkansas rice processor	None
Ground rubber tires #30-40	Oklahoma rubber recycling plant	None
Wheat straw	New Jersey	Cut/sieved
Montmorillonite clay, K10[c]	Aldrich Chemical Company	None
Seaweed	Cape Cod beach	Cut/sieved
Lobster shells	Cape Cod restaurant	Washed/crushed/sieved
Vermiculite	Standard packing material	Sieved
Kaolin clay	Aldrich Chemical Company	None
Clam shells	Cape Cod clam processing plant	Washed/crushed/sieved

[a]Bulk density = 300-370 g/L; surface area = 220-270 m^2/g.

[b]Sieving performed to produce material bewteen 18 mesh (1 mm) to 5 mesh (4 mm) in size.

[c]Bulk density = 800-850 g/L; surface area = 20-40 m^2/g.

Table 2. Summary of adsorption and desorption results

Sorbent	TNT Initial (mg/L)	TNT Adsorption (%, 24 h)	TNT Desorption[a] (%, 48 h)	RDX Initial (mg/L)	RDX Adsorption (%, 24 h)	RDX Desorption[a] (%, 48 h)	HMX Initial (mg/L)	HMX Adsorption (%, 24 h)	HMX Desorption[a] (%, 48 h)
Montmorillonite clay, KSF	75	93	15	37	23	-	3	62	72
Sphagnum peat moss	80	82	22	37	65	58	3	69	50
Ground rubber tires #30	80	77	29	37	19	-	3	17	-
Sawdust	80	76	25	37	47	64	3	53	54
Rice hulls	86	75	32	37	47	56	3	60	53
Ground rubber tires #30-40	75	75	29	-	-	-	3	10	-
Wheat straw	75	74	28	37	50	45	3	62	55
Montmorillonite clay, K10	75	54	58	-	-	-	3	44	94
Seaweed	80	50	42	-	-	-	3	36	-
Lobster shells	80	18	-[b]	-	-	-	3	21	-
Vermiculite	80	7	-	-	-	-	3	13	-
Kaolin clay	80	5	-	-	-	-	3	10	-
Clam shells	75	6	-	-	-	-	3	3	-

[a]Cummulative compound desorbed from solid phase after two replacements of aqueous $CaCl_2$ phase over 48 h.

[b]Not determined.

Best material based on sorption screening results

TNT	% Adsorption
Montmorillonite clay, KSF	93
Sphagnum peat moss	82
Ground rubber tires #30	77
Sawdust	76

RDX	% Adsorption
Sphagnum peat moss	65
Wheat straw	50
Sawdust	47
Rice hulls	47

HMX	% Adsorption
Sphagnum peat moss	69
Montmorillonite clay, KSF	62
Wheat straw	62
Rice hulls	60

Table 3. Explosives concentrations[a] employed for isotherms.

TNT		RDX		HMX	
Nominal	Actual Range	Nominal	Actual Range	Nominal	Actual Range
0.10	0.09 - 0.12	0.05	0.06 - 0.11	0.005	0.268 - 0.299
0.50	0.46 - 0.69	0.10	0.07 - 0.21	0.010	0.252 - 0.299
1.00	0.92 - 1.31	0.50	0.38 - 0.85	0.050	0.310 - 0.327
5.00	4.93 - 6.53	1.00	0.54 - 1.38	0.100	0.318 - 0.396
10.00	9.92 - 15.54	5.00	3.70 - 5.79	0.500	0.650 - 0.809
25.00	25.03 - 39.35	10.00	7.63 - 12.23	1.000	1.113 - 1.209
50.00	50.65 - 78.59	20.00	15.22 - 24.90	2.000	1.960 - 2.121
70.00	68.48 - 146.74	30.00	22.66 - 37.25	3.000	2.930 - 3.258

[a]All concentrations as mg/L.

Table 4. Model fits for explosive-sorbent isotherms - Freundlich Equation

Freundlich Equation $C_s = K(C_e)^n$

C_s = amount of compound sorbed

C_e = amount of compound in water in equilibrium with C_s

K (or K_d) = the adsorption or distribution coefficient

n = constant

A. Adsorption

	TNT			RDX			HMX		
Sorbent	**log K^s_d, L/kg**	**n**	**r^2**	**log K^s_d, L/kg**	**n**	**r^2**	**log K^s_d, L/kg**	**n**	**r^2**
Peat moss	2.48	0.89	1.00	1.94	0.94	1.00	2.03	0.95	1.00
Montmorillonite clay KSF	2.46	0.85	1.00	-	-	-	1.28	1.26	0.99
Sawdust	1.82	0.97	1.00	1.10	0.96	1.00	-	-	-
Ground rubber #30	1.72	0.98	1.00	-	-	-	-	-	-
Wheat	-[a]	-	-	1.20	0.98	1.00	1.41	0.99	1.00
Rice Hulls	-	-	-	1.08	0.94	1.00	1.34	1.00	1.00

B. Desorption

	TNT			RDX			HMX		
Sorbent	**log K^d_d, L/kg**	**n**	**r^2**	**log K^d_d, L/kg**	**n**	**r^2**	**log K^d_d, L/kg**	**n**	**r^2**
Peat moss	2.73	0.90	1.00	2.17	0.94	1.00	2.34	0.94	0.99
Montmorillonite clay KSF	2.47	0.80	0.99	-	-	-	1.23	1.23	0.99
Sawdust[b]	2.08	0.97	1.00	1.31	0.95	0.99	-	-	-
Ground rubber #30	1.96	0.98	1.00	-	-	-	-	-	-
Wheat	-	-	-	1.56	0.99	1.00	1.71	1.01	0.99
Rice Hulls[c]	-	-	-	1.12	0.91	0.98	1.46	0.99	0.99

[a]Not determined

[b]Equation parameters for RDX desorption excluding the outlier 7th point were: log K_d, 1.36; n, 0.99; R^2, 1.00.

[c]Equation parameters for RDX desorption excluding the outlier 8th point were: log K_d, 1.17; n, 0.96; R^2, 0.99.

Table 5. Model fits for explosive-sorbent isotherms - Langmuir Equation

Langmuir Equation $C_s = (kbC_e) / (1 + kC_e)$

C_s = amount of compound sorbed

C_e = amount of compound in water in equilibrium with C_s

k, b = constants

A. Adsorption

	TNT			RDX			HMX		
Sorbent	**b**	**k**	**r^2**	**b**	**k**	**r^2**	**b**	**k**	**r^2**
Peat moss	10000	0.04	0.64	2500	0.04	0.90	500	0.25	0.80
Montmorillonite clay KSF	1667	0.29	0.97	-	-	-	-30	-0.38	0.90
Sawdust	5000	0.01	0.77	909	0.01	0.61	-	-	-
Ground rubber #30	16667	0.00	0.62	-	-	-	-	-	-
Wheat	-[a]	-	-	2000	0.01	0.43	1250	0.02	0.14
Rice Hulls	-	-	-	588	0.02	0.82	-2500	-0.01	0.01

B. Desorption

	TNT			RDX			HMX		
Sorbent	**b**	**k**	**r^2**	**b**	**k**	**r^2**	**b**	**k**	**r^2**
Peat moss	10000	0.07	0.57	3333	0.05	0.63	435	0.62	0.64
Montmorillonite clay KSF	1250	0.47	0.96	-	-	-	-16	-0.59	0.74
Sawdust	5000	0.03	0.50	400	0.06	0.22	-	-	-
Ground rubber #30	25000	0.00	0.24	-	-	-	-	-	-
Wheat	-	-	-	-25000	0.00	0.00	-1667	-0.03	0.01
Rice Hulls	-	-	-	122	0.14	0.59	333	0.09	0.00

[a]Not determined

Table 6. General information on cosubstrates used for this research.

Material	Source	Cost per kg[d], $	Concentration
LIQUID COSUBSTRATES			%, vol:vol[a]
Molasses	Zook Molasses Company	0.01	0.5[b]
Corn steep liquor	Grain Processing Corporation	unavailable	0.5[b]
Soybean oil-crude	Cargill	0.07	0.5
SoyClear 1500	AG Environmental Products, LLC	0.39	0.5
SoyGold 1000	AG Environmental Products, LLC	0.34	0.5
Safflower oil	California Oils Corporation	0.23	0.5
DRY COSUBSTRATES			%, wt:wt[c]
Potato starch	Lance Products, Inc.	0.26	2.0
Solulac	Grain Processing Corporation	unavailable	2.0
Corn starch	Grain Processing Corporation	unavailable	2.0
Unrefined chitin (practical grade)	Sigma Chemical Company	unavailable	2.0

[a]Volume of cosubstrate:liquid volume of microcosm slurry

[b]Molasses and corn steep liquor added as 25% soln to facilitate pipetting

[c]Weight of cosubstrate:weight of soil in microcosm slurry

[d]Cost does not reflect bulk discount or transport from source to location of use. Cost based on various sources.

Table 7. General information about MMR soils used during this research.

Soil ID	H2O[a] %	WHC[b] %	CFU[c] x10^5	Slurry pH[e]	mg/kg TNT	RDX	HMX
Grassy soil	4.7	26.7	3.8	6.7	ND[d]	ND	ND
Colored patch soil	6.0	26.3	0.6	7.9	580±185	1690±315	135±18

[a]Water content, g water per g soil (wet wt).

[b]Water holding capacity, g water per g soil (wet wt).

[c]Colony forming units of culturable heterotrophs on R2A agar plates per g soil (wet wt).

[d]None detected

[e]pH of 10 g (wet wt) soil mixed with 25 ml of NP solution (no explosives, biocides or cosusbstrates added).

Table 8. Results of cosubstrate screening in MMR soils.

Explosive	Soil	%Explosive mineralization ($^{14}CO_2$ dpm / total dpm x 100)											
		Killed Control	Control	Molasses	Corn steep liquor	Soybean oil crude	SoyClear 1500	SoyGold 1000	Safflower oil	Potato starch	Solulac	Corn starch	Unrefined chitin
TNT	Contaminated (C)	0.1	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.1	0.3	0.3
	Clean (G)	0.2	0.8	0.5	0.6	0.8	-[a]	-	-	0.8	-	-	-
RDX	Contaminated (C)	0.1	0.7	0.7	0.3	0.5	0.4	0.3	0.5	0.7	0.3	0.7	0.6
	Clean (G)	0.2	1.3	19.6	2.1	24.7	-	-	-	3.8	-	-	-
HMX	Contaminated (C)	0.1	1.5	0.9	0.7	1.2	0.9	0.9	1.1	1.5	0.6	1.4	1.2
	Clean (G)	0.1	2.2	1.7	2.0	7.1	-	-	-	4.7	-	-	-

[a]Not determined

Figure 1. TNT-sorbent isotherms.

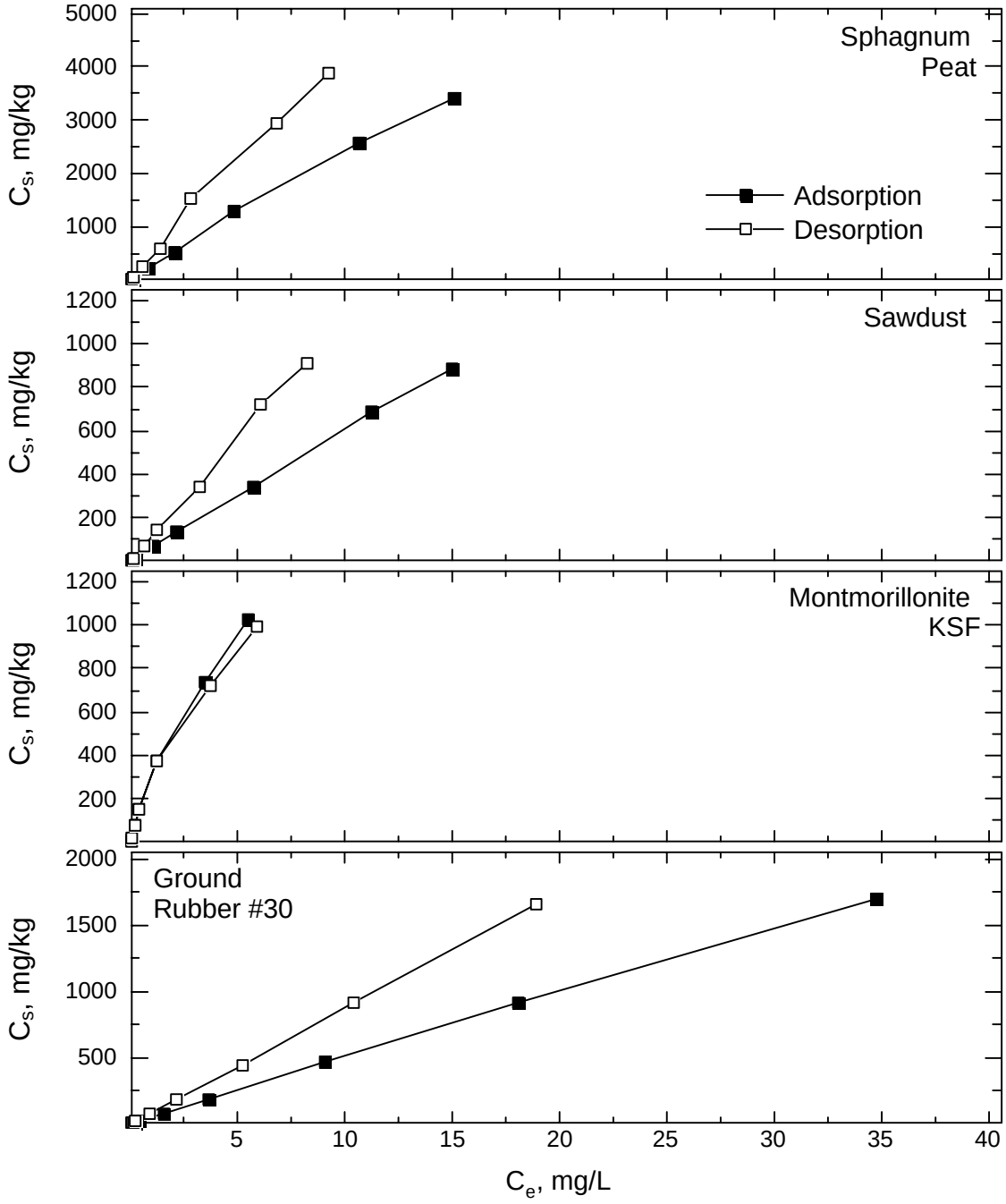


Figure 2. RDX-sorbent isotherms.

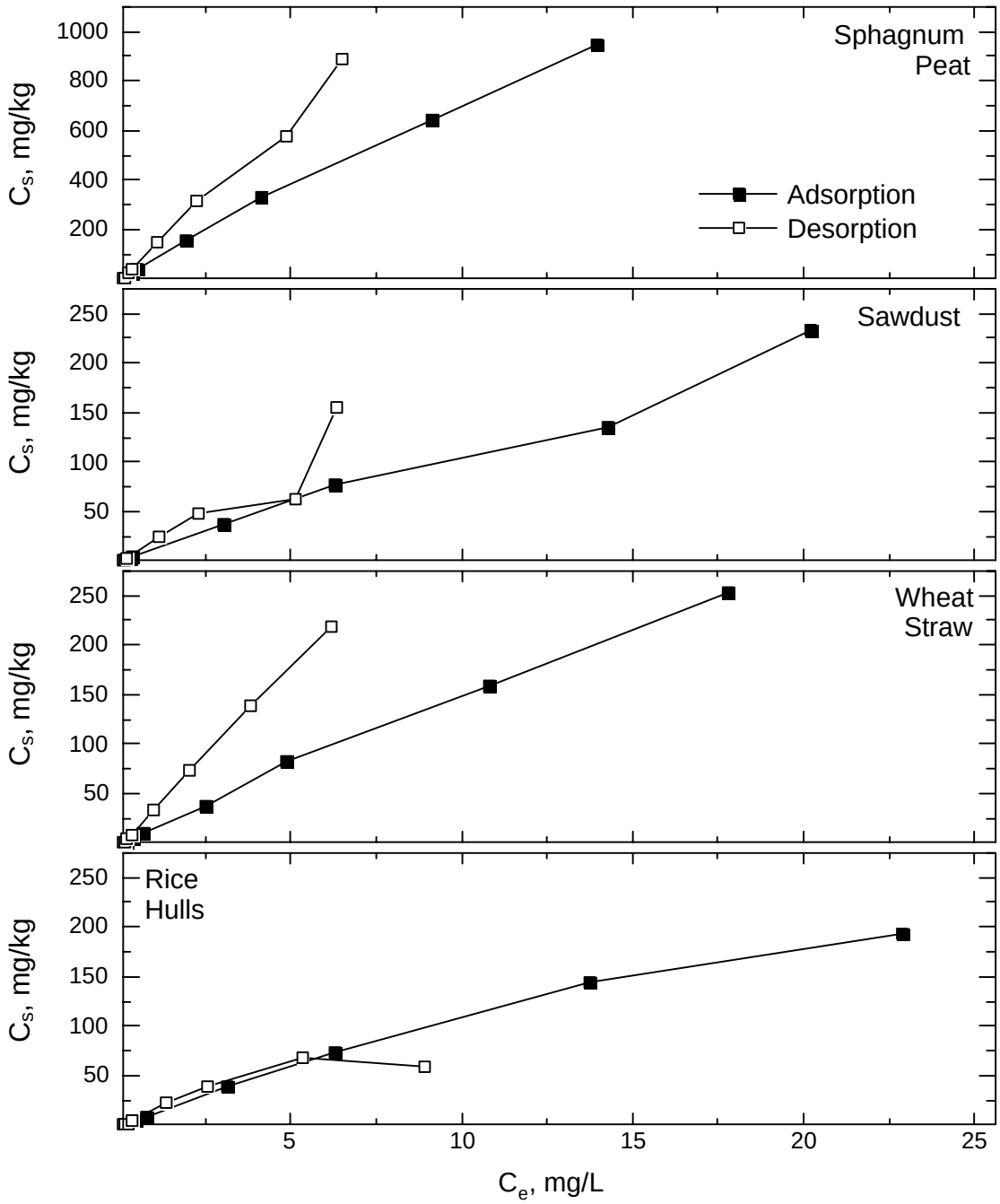


Figure 3. HMX-sorbent isotherms.

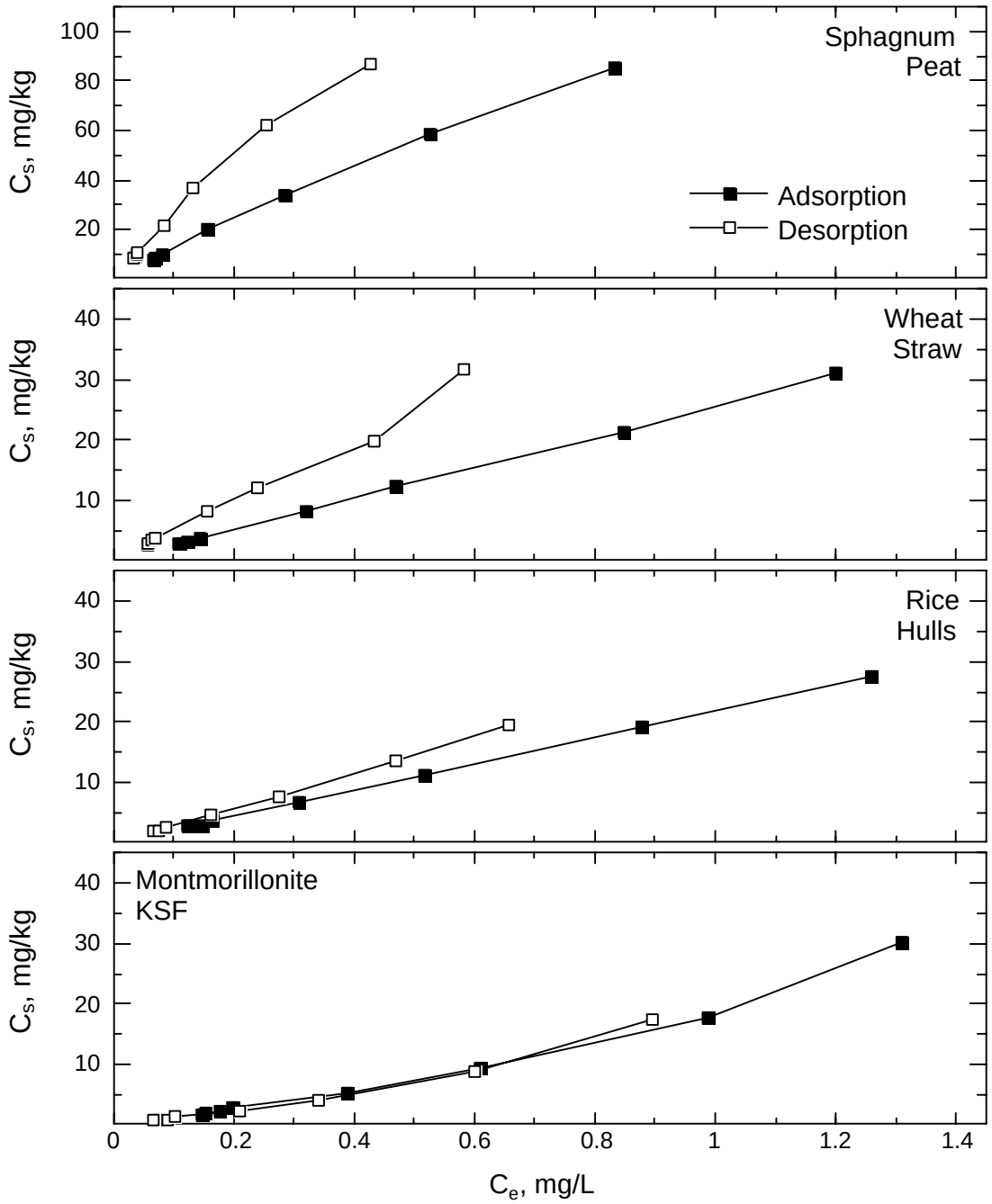


Figure 4. Photograph of the various cosubstrates.

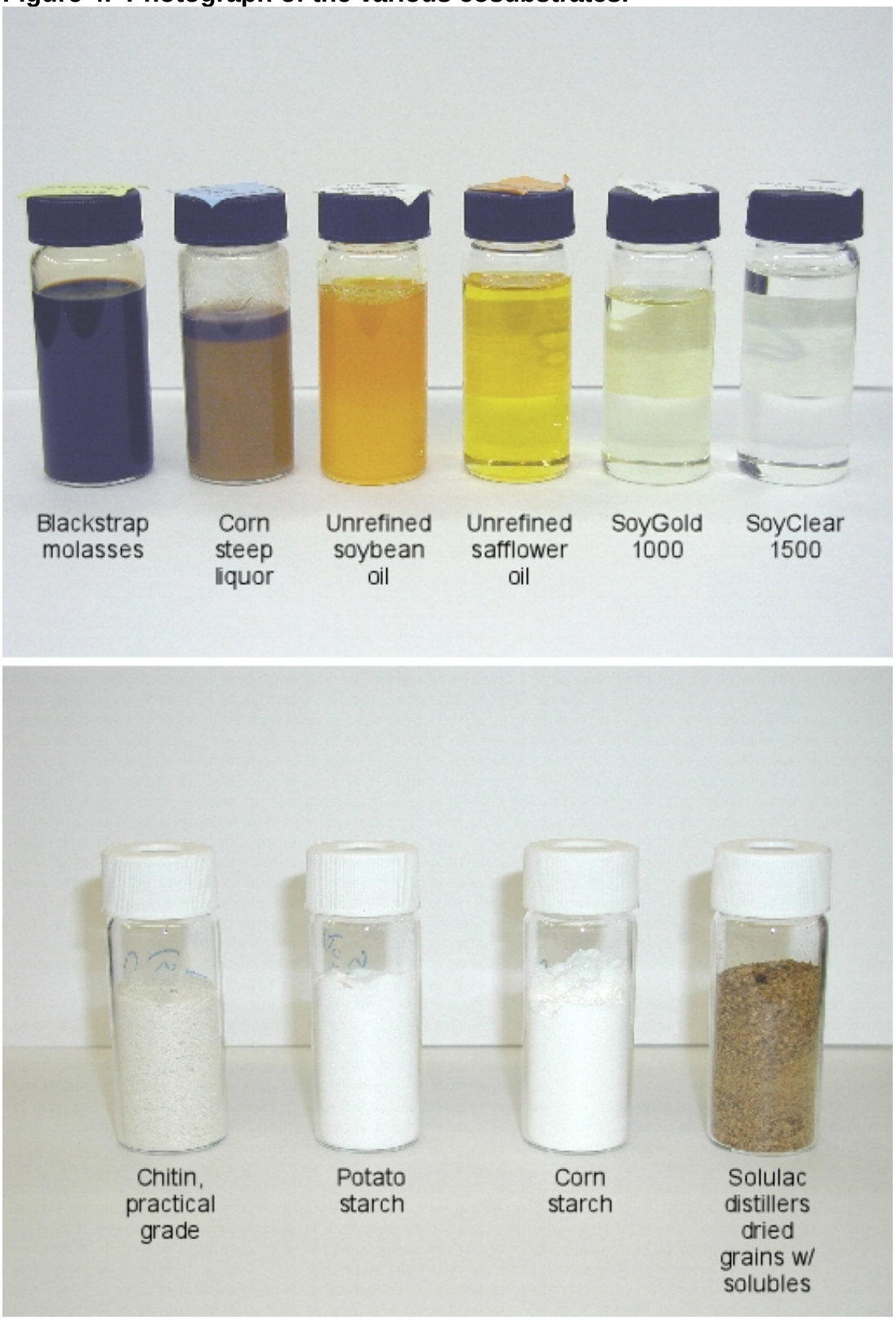
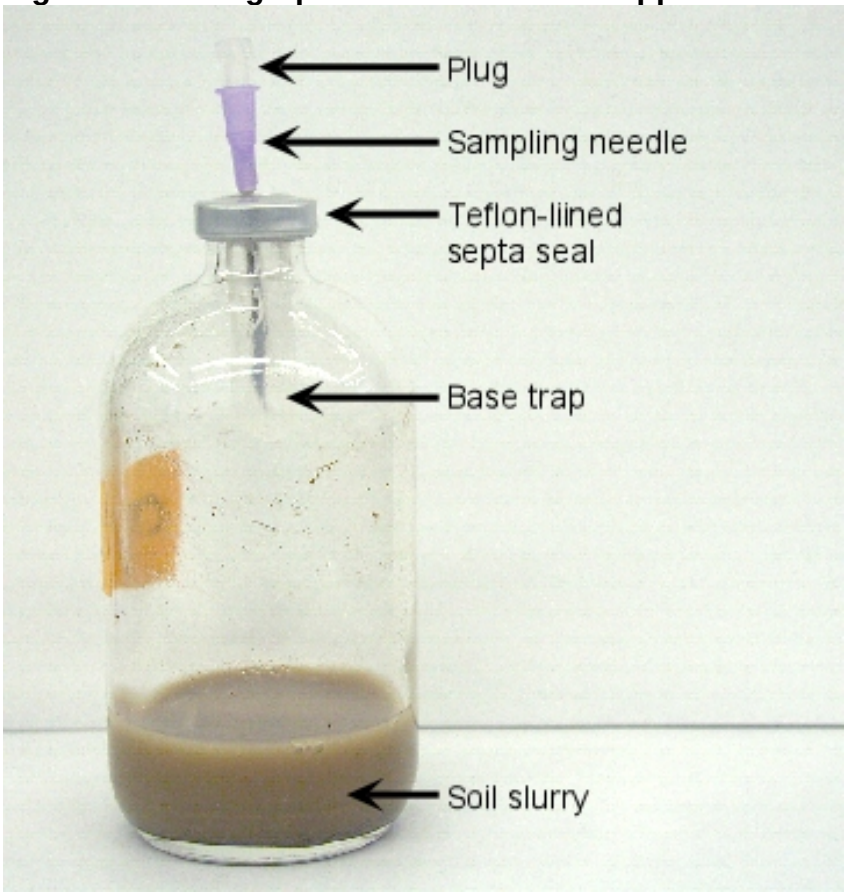


Figure 5. Photograph of the microcosm apparatus.



Quarterly Progress Report

SERDP Project 1229 - Immobilization of Energetics on Live Fire Ranges
Year 2002 – First Quarter
April 15, 2002

The SERDP contract for FY 2001 was awarded to Envirogen on August 1, 2001. This report covers technical progress for SERDP Project 1229 from January 15, 2002 – April 15, 2002.

The objective of this project is to develop a cost-effective technology to immobilize energetic compounds (TNT, RDX, HMX, and breakdown products) released as residues at firing ranges to prevent their migration to groundwater. The goal is to develop an inexpensive soil treatment that can be readily applied over wide and remote areas prior to or immediately following firing range activities.

During the current quarter, activities have focused on completing all sorption isotherm experiments, finishing the screening of potential cosubstrates, examining the interaction of the most effective sorbents and cosubstrates on explosives biodegradation, and designing and constructing the columns to evaluate explosive transport and biodegradation under unsaturated flow conditions

I. EVALUATE ADSORPTION OF TNT, RDX, AND HMX TO SOLID PHASE SORBENTS.

1. SORPTION-DESORPTION ISOTHERMS.

Based on the results of the initial screening of the potential sorbents, detailed sorption-desorption isotherms were developed for the best four sorbents for each explosive. The sorption-desorption isotherms of TNT and RDX to the MMR soils used during this project were also determined.

1.1 METHODS:

Sorption-desorption isotherm studies were performed in a similar manner as the initial screening experiments, as described in Section I.2.1 of the Fourth Quarter Report 2001 for this project. Based on revised specific activity values of the [^{14}C]TNT, [^{14}C]RDX, and [^{14}C]HMX, the explosive concentrations used for the sorption isotherm experiments were determined to not cover the low to mid µg/L (ppb) concentration range. Since these concentrations are environmentally relevant, we decided to perform additional isotherm experiments with very low concentrations of these explosives. Low concentration HMX isotherms were prepared with data from two separate experiments, one with moderately low concentrations, and one with very low concentrations. The revised nominal and actual concentrations used for both the high and low concentration experiments are presented in Table 1A and 1B, respectively.

The methodology used to prepare the sorption-desorption isotherms of TNT and RDX with the MMR soils was the same as that used for the individual sorbents.

1.2 RESULTS & DISCUSSION:

The Freundlich and Langmuir equations were used to model the isotherm data. Freundlich model parameters for the high concentration isotherms (revised data) and the low concentration isotherms (new data) are presented in Tables 2 and 3, respectively. The corresponding Langmuir model isotherm parameters for these isotherm data are presented in Tables 4 and 5, respectively.

The Freundlich model fit the isotherm data better than the Langmuir model. Differences in the log K^s_d values obtained with the high explosive concentrations compared to the low concentrations were usually only between 5%.and 15%, whereas a few differences in the log K^d_d were close to 25%. This indicates that the values obtained with the higher explosive concentrations are generally applicable to the lower concentrations as well.

Freundlich and Langmuir model parameters for the TNT and RDX adsorption-desorption isotherms with the MMR soils are presented in Tables 6 and 7, respectively. The adsorption coefficients of TNT and RDX for the soils were much lower than for sawdust and peat, indicating that the explosives sorb much more readily to the sorbents than to the soils. A comparison of the adsorption isotherms for both soils and for these two sorbents is presented in Figure 1.

These results clearly indicate that the sorbents have the potential to greatly enhance the immobilization of explosives residues in the soil.

II. CONDUCT MICROCOSM STUDIES.

During the past quarter, the screening of each of the potential cosubstrates for enhancing the biodegradation of TNT, RDX, and HMX was completed. The degradation of explosives in MMR soil after sorption to peat moss or sawdust and added to unsaturated soil with and without cosubstrate addition was also examined.

1. COSUBSTRATE SCREENING.

The initial objective of this task was to evaluate the ability of several organic materials to serve as cosubstrates for TNT, RDX and HMX biodegradation. The substrates were chosen based on cost, availability, and previous laboratory data. Screening assays were performed under optimal conditions in soil slurries.

1.1. METHODS:

The methodology for screening the cosubstrates was described in Section II.1.1 of the Fourth Quarter Report 2001 for this project. Briefly, soil slurries were prepared from historically contaminated (referred to as “contaminated”) and uncontaminated (referred to as “uncontaminated”) soils from MMR. The soils were spiked with radiolabelled TNT, RDX, or HMX, and the $[^{14}C]CO_2$ that was evolved over time was trapped and measured.

Microcosms were incubated for 30 to 40 days, at which time more cosubstrate was added to each bottle to determine if additional degradation of the explosives could be stimulated. Efforts were undertaken to cause minimal changes to the established headspace conditions. Liquid

cosubstrates were added through the septa using a small syringe. Potato starch and corn starch were also added as thick aqueous suspensions (0.5 g/ml) through the septa using a small syringe. Solulac® and chitin were added as dry solids by opening and resealing the bottles under a nitrogen-flushed headspace. After approximately 80 days of incubation, cosubstrates were added a third time to selected microcosms (see below).

Based on the promising initial results from microcosms amended with the molasses and crude soybean oil, an additional screening was performed to verify the results, and to compare explosives degradation using crude soybean oil versus refined soybean oil (Wesson® cooking oil), and to examine the potential combined effect of crude soybean oil plus molasses. These experiments were performed using the uncontaminated MMR soil only.

1.2. RESULTS & DISCUSSION:

The timecourse of explosives mineralization in contaminated and uncontaminated MMR soils are presented in Figures 2 and 3. The cumulative percent mineralization data for each explosive in the different microcosm treatments are presented in Table 8.

Biodegradation of added [^{14}C]-labelled explosives in the contaminated MMR soil slurries remained low compared to that observed in the uncontaminated soil. Less than 3% of the added radioactivity was recovered as [^{14}C]CO_2 over the course of 80 days. RDX appeared to be mineralized to the greatest extent, followed by TNT and then HMX. The oils stimulated some TNT mineralization, but did not enhance the mineralization of RDX or HMX. The second and third additions of crude soybean oil ("soybean oil" henceforth) may have resulted in increases in TNT degradation. None of the other cosubstrates stimulated more explosive mineralization than was observed in the unamended control. Indeed, mineralization of RDX and HMX was lower in amended microcosms than in the unamended control. The trends indicated that Solulac®, SoyClear, and SoyGold resulted in the least mineralization of RDX and HMX, while Solulac® and molasses caused the smallest release of [^{14}C]TNT as [^{14}C]CO_2.

In contrast, significant amounts of [^{14}C]RDX (up to 40%) and [^{14}C]HMX (up to 10%) were mineralized in the uncontaminated MMR soil slurries. Higher percentages of [^{14}C]TNT (almost 4%) were also converted to [^{14}C]CO_2 in this soil compared to the contaminated soil. Soybean oil proved to be the most effective cosubstrate for stimulating RDX and HMX degradation, followed by molasses, whereas their effectiveness was reversed for TNT. Potato starch was the only other cosubstrate that significantly stimulated greater mineralization of HMX than observed in the unamended control. The second addition of cosubstrates to the slurries after 30 days stimulated increased TNT and HMX mineralization for molasses-amended treatments; a third molasses addition after 80 days resulted in another burst of HMX degradation, but a very limited increase in TNT mineralization.

These microcosms will continue to be monitored for a few weeks. They will then be frozen until they can be destructively sampled in an effort to close the ^{14}C mass balance and determine the extent to which the original TNT, RDX, HMX where converted to known breakdown products (i.e., aminonitrotoluenes, nitroso-containing compounds), and/or microbial biomass. Efforts will

also be directed towards isolation of potential degradative bacterial strains, especially from the contaminated soil slurries.

Based on initial results from the second batch of slurry microcosms, the effectiveness of crude soybean oil has been verified. The total percent RDX mineralization is still low (<1% of initial radioactivity), but almost 10-fold more [^{14}C]CO_2 has been captured from the crude soybean oil amended bottles than from the control and refined Wesson® soybean oil treatments after 7 days of incubation. In addition, crude soybean oil plus molasses resulted in less mineralization than crude soybean oil alone. This may indicate that the type of metabolism stimulated (oil vs. sugar/carbohydrate utilization) directly affects the rate and extent of RDX biodegradation. This pattern of less mineralization in the presence of both crude soybean oil and molasses also appears in the TNT and HMX spiked microcosms, but it is too early to draw any firm conclusions at this point.

2. COMBINED COSUBSTRATE-SORBENT EVALUATION.

One of the goals of this project is to determine if an approach can be designed which leads to both immobilization and biodegradation of explosives residues at or near the soil surface. The results to date indicate that *Sphagnum* peat moss and sawdust are very good sorbents for all three of the target compounds, and that soybean oil and molasses are effective at stimulating the mineralization of RDX and HMX, and to a lesser degree, TNT, in uncontaminated soil under slurry conditions. The questions that remain at this point are as follows:

a) Can explosives sorbed to peat or sawdust be biodegraded by soil microorganisms?
b) Can peat moss or sawdust itself promote mineralization of the explosives?
c) Can soybean oil or molasses enhance explosives degradation in unsaturated soil?
d) How is the biodegradation of one explosives affected by the presence of the other two explosives?

To answer these questions, a treatment matrix consisting of the most effective cosubstrate and sorbent was applied to unsaturated soil microcosms. The results from these experiments should address each of the aforementioned questions.

2.1. METHODS:

The unsaturated soil microcosms were prepared in 1 pint (500 ml) mason jars, as illustrated in Figure 4. The lids were equipped with a base trap assembly for capturing the [^{14}C]CO_2 liberated during radiolabelled explosive mineralization. Solutions of single [^{14}C]-labelled and cold explosives were prepared in distilled water to achieve nominal TNT, RDX, and HMX concentrations of 50, 25, and 3 mg/L and total initial radioactivity of 30,000 to 40,000 dpm/jar. Solutions were also prepared which contained a mixture of three cold explosives, plus one of the radiolabelled explosives (i.e., [^{14}C]TNT + cold TNT, RDX, and HMX). Actual concentrations and radioactivities were determined using HPLC and scintillation counting, respectively, as described previously.

Peat moss and sawdust (2 g/jar, or 0.05 g/g soil) were initially added to the jars along with the explosive solutions (3.2 ml/jar). Controls received no sorbent. The sorbent and solution was

thoroughly mixed, and the jars were sealed and allowed to incubate at room temperature (22-25°C). After 72 hours, the base traps were sampled and counted. Soil (40 g) was added to each jar, soybean oil (2 ml/g soil) or molasses (1 ml/g soil; 2 ml/g soil for some RDX treatments) was added, and the whole mixture was mixed well. Controls received no cosubstrate. Jars were then sealed and base was added to each trap. Jars were incubated at room temperature, and the base was sampled periodically and analyzed using liquid scintillation counting.

2.2. RESULTS & DISCUSSION:

The cosubstrate x sorbent screening experiment has been incubating for 7 days, but some results have already been obtained. The initial data points indicated that no significant [^{14}C]CO_2 was released during the incubation of the explosives with the sorbents prior to the addition of soil and cosubstrates (data not shown). TNT and HMX degradation has been minimal (<1% of initial dpm recovered as [^{14}C]CO_2). However, >10% of the initial [^{14}C]RDX appears to be mineralized in the soybean oil amended treatments in the presence of no sorbent or peat moss, but not in the presence of sawdust.

III. TABLES AND FIGURES.

The tables and figures supporting this document have been provided to the SERDP Office as a separate attachment.

Table 1A. Results of cosubstrate screening in MMR soils.

TNT		RDX		HMX	
Nominal	Actual Range	Nominal	Actual Range	Nominal	Actual Range
0.10	0.47 - 0.48	0.05	0.16 - 0.22	0.005	2.318 - 2.465
0.50	0.83 - 1.05	0.10	0.18 - 0.32	0.010	2.142 - 2.454
1.00	1.28 - 1.67	0.50	0.49 - 0.96	0.050	2.287 - 2.358
5.00	5.30 - 6.89	1.00	0.65 - 1.49	0.100	1.931 - 2.550
10.00	10.29 - 15.90	5.00	3.81 - 5.90	0.500	2.664 - 2.909
25.00	25.40 - 39.71	10.00	7.74 - 12.33	1.000	2.969 - 3.419
50.00	51.00 - 78.97	20.00	15.32 - 25.01	2.000	3.888 - 4.265
70.00	68.84 - 147.11	30.00	22.77 - 37.35	3.000	5.127 - 5.553

[a]All concentrations as mg/L.

Table 1B. Explosives concentrations employed for low concentration isotherms[a].

TNT		RDX		HMX-Moderately Low		HMX-Very Low	
Nominal	Actual Range	Nominal	Actual Range	Nominal	Actual Range	Nominal	Actual Range
0.050	0.039	0.025	0.018	0.050	0.237	0.025	0.020
0.100	0.121	0.050	0.096	0.100	0.310	0.050	0.071
0.500	0.476	0.100	0.183	0.500	0.734	0.100	0.117
1.000	0.956	0.500	0.634	1.000	1.185	0.500	0.535
		1.000	1.216	1.500	1.661		

[a]All concentrations as mg/L.

Table 2. Model fits for high concentration explosive-sorbent isotherms - Freundlich Equation

Table 10. Results of cosut $C_s = K(C_e)^n$

C_s = amount of compound sorbed

C_e = amount of compound in water in equilibrium with C_s

K (or K_d) = the adsorption or distribution coefficient

n = constant

A. Adsorption

	TNT			RDX			HMX		
Sorbent	**log K^s_d, L/kg**	**n**	**r^2**	**log K^s_d, L/kg**	**n**	**r^2**	**log K^s_d, L/kg**	**n**	**r^2**
Peat moss	2.49	0.88	1.00	1.94	0.92	1.00	2.04	0.84	0.99
Montmorillonite clay KSF	2.46	0.83	1.00	-	-	-	0.97	2.05	0.97
Sawdust	1.82	0.96	1.00	1.10	0.95	1.00	-	-	-
Ground rubber #30	1.73	0.98	1.00	-	-	-	-	-	-
Wheat	-[a]	-	-	1.20	0.98	1.00	1.41	0.97	1.00
Rice Hulls	-	-	-	1.09	0.94	1.00	1.34	1.01	0.98

B. Desorption

	TNT			RDX			HMX		
Sorbent	**log K^d_d, L/kg**	**n**	**r^2**	**log K^d_d, L/kg**	**n**	**r^2**	**log K^d_d, L/kg**	**n**	**r^2**
Peat moss	2.73	0.88	1.00	2.17	0.93	1.00	2.31	0.79	0.96
Montmorillonite clay KSF	2.47	0.77	0.99	-	-	-	1.13	1.68	0.95
Sawdust[b]	2.09	0.96	1.00	1.32	0.95	0.99	-	-	-
Ground rubber #30	1.96	0.98	1.00	-	-	-	-	-	-
Wheat	-	-	-	1.56	0.99	1.00	1.70	0.98	0.92
Rice Hulls[c]	-	-	-	1.12	0.90	0.97	1.46	0.98	0.95

[a]Not determined

[b]Equation parameters for RDX desorption excluding the outlier 7th point were: log K_d, 1.36; n, 0.99; R^2, 1.00.

[c]Equation parameters for RDX desorption excluding the outlier 8th point were: log K_d, 1.17; n, 0.96; R^2, 0.99.

Table 3. Model fits for high concentration explosive-sorbent isotherms - Langmuir Equation

Table 10. Results of cosut $C_s = (kbC_e) / (1 + kC_e)$

C_s = amount of compound sorbed

C_e = amount of compound in water in equilibrium with C_s

k, b = constants

A. Adsorption

	TNT			RDX			HMX		
Sorbent	**b**	**k**	**r^2**	**b**	**k**	**r^2**	**b**	**k**	**r^2**
Peat moss	10000	0.29	0.97	2500	0.04	0.90	588	0.23	0.82
Montmorillonite clay KSF	1667	0.01	0.77	-	-	-	-25	-0.30	0.88
Sawdust	5000	0.00	0.62	909	0.01	0.61	-	-	-
Ground rubber #30	16667	0.04	0.64	-	-	-	-	-	-
Wheat	-[a]	-	-	2000	0.01	0.43	1111	0.02	0.16
Rice Hulls	-	-	-	588	0.02	0.82	-2500	-0.01	0.01

B. Desorption

	TNT			RDX			HMX		
Sorbent	**b**	**k**	**r^2**	**b**	**k**	**r^2**	**b**	**k**	**r^2**
Peat moss	10000	0.07	0.96	3333	0.05	0.63	476	0.66	0.75
Montmorillonite clay KSF	1250	0.47	0.50	-	-	-	-2	-4.10	0.72
Sawdust	5000	0.03	0.24	400	0.06	0.22	-	-	-
Ground rubber #30	25000	0.00	0.57	-	-	-	-	-	-
Wheat	-	-	-	-25000	0.00	0.00	3333	0.02	0.00
Rice Hulls	-	-	-	122	0.14	0.59	3333	0.01	0.00

[a]Not determined

Table 4. Model fits for low concentration explosive-sorbent isotherms - Freundlich Equation

Table 10. Results of cosut $C_s = K(C_e)^n$

C_s = amount of compound sorbed

C_e = amount of compound in water in equilibrium with C_s

K (or K_d) = the adsorption or distribution coefficient

n = constant

A. Adsorption

	TNT			RDX			HMX - Moderately Low			HMX - Very Low		
Sorbent	**log K^s_d, L/kg**	**n**	**r^2**	**log K^s_d, L/kg**	**n**	**r^2**	**log K^s_d, L/kg**	**n**	**r^2**	**log K^s_d, L/kg**	**n**	**r^2**
Peat moss	2.72	0.98	1.00	2.03	0.95	1.00	2.06	0.88	1.00	2.07	0.94	1.00
Montmorillonite clay KSF	2.49	0.91	1.00	-	-	-	1.15	1.18	0.99	1.06	1.05	0.99
Sawdust	1.85	0.99	1.00	1.07	0.96	1.00	-	-	-	-	-	-
Wheat	-[a]	-	-	1.13	0.96	1.00	1.49	0.98	1.00	1.54	1.03	1.00

B. Desorption

	TNT			RDX			HMX - Moderately Low			HMX - Very Low		
Sorbent	**log K^d_d, L/kg**	**n**	**r^2**	**log K^d_d, L/kg**	**n**	**r^2**	**log K^d_d, L/kg**	**n**	**r^2**	**log K^d_d, L/kg**	**n**	**r^2**
Peat moss	3.23	0.96	1.00	2.40	0.97	1.00	2.28	0.91	1.00	2.31	0.95	0.99
Montmorillonite clay KSF	2.79	0.95	1.00	-	-	-	1.11	0.89	1.00	1.31	1.10	0.97
Sawdust[b]	2.15	0.98	1.00	1.33	0.96	1.00	-	-	-	-	-	-
Wheat	-	-	-	1.38	0.95	0.99	1.80	1.00	0.99	1.85	1.02	1.00

[a]Not determined

Table 5. Model fits for low concentration explosive-sorbent isotherms - Langmuir Equation

Table 10. Results of cosut $C_s = (kbC_e) / (1 + kC_e)$

C_s = amount of compound sorbed

C_e = amount of compound in water in equilibrium with C_s

k, b = constants

A. Adsorption

	TNT			RDX			HMX - Moderately Low			HMX - Very Low		
Sorbent	**b**	**k**	**r^2**	**b**	**k**	**r^2**	**b**	**k**	**r^2**	**b**	**k**	**r^2**
Peat moss	417	1.41	0.82	213	0.64	0.56	204	0.80	0.95	93	1.67	0.76
Montmorillonite clay KSF	55	9.58	0.98	-	-	-	-24	-0.40	0.89	-39	-0.24	0.06
Sawdust	-1000	-0.07	0.01	56	0.24	0.38	-	-	-	-	-	-
Wheat	-[a]	-	-	58	0.27	0.44	370	0.09	0.67	-77	-0.40	0.48

B. Desorption

	TNT			RDX			HMX - Moderately Low			HMX - Very Low		
Sorbent	**b**	**k**	**r^2**	**b**	**k**	**r^2**	**b**	**k**	**r^2**	**b**	**k**	**r^2**
Peat moss	435	4.60	0.47	294	1.03	0.55	286	0.92	0.88	109	2.42	0.29
Montmorillonite clay KSF	106	8.55	0.74	-	-	-	26	0.71	0.88	-9	-1.42	0.07
Sawdust	833	0.18	0.02	43	0.59	0.17	-	-	-	-	-	-
Wheat	-	-	-	36	0.84	0.20	714	0.09	0.02	-769	-0.09	0.00

[a]Not determined

Table 6. Model fits for explosive-MMR soil isotherms - Freundlich Equation

Table 10. Results of cosubstrate scre $C_s = K(C_e)^n$

C_s = amount of compound sorbed

C_e = amount of compound in water in equilibrium with C_s

K (or K_d) = the adsorption or distribution coefficient

n = constant

A. Adsorption

	TNT			RDX[a]		
Sorbent	**log K^s_d, L/kg**	**n**	**r^2**	**log K^s_d, L/kg**	**n**	**r^2**
Grassy soil (uncontaminated)	0.27	0.93	1.00	-0.28	0.96	0.97
Colored patch soil (contaminated)	0.50	0.82	1.00	-0.65	0.78	0.83

B. Desorption

	TNT			RDX[b]		
Sorbent	**log K^d_d, L/kg**	**n**	**r^2**	**log K^d_d, L/kg**	**n**	**r^2**
Grassy soil (uncontaminated)	1.51	0.95	0.99	1.26	0.78	0.96
Colored patch soil (contaminated)	1.62	1.20	0.99	0.40	0.67	0.61

[a]Values of parameters were obtained from the regression line, using 7 points of data

[b]Values of parameters were obtained from the regression line, using 5 points of data

Table 7. Model fits for explosive-MMR soil isotherms - Langmuir Equation

Table 10. Results of cosubstrate scre $C_s = (kbC_e) / (1 + kC_e)$

C_s = amount of compound sorbed

C_e = amount of compound in water in equilibrium with C_s

k, b = constants

A. Adsorption

	TNT			RDX		
Sorbent	**b**	**k**	**r^2**	**b**	**k**	**r^2**
Grassy soil (uncontaminated)	286	0.01	0.78	-51	-0.01	0.06
Colored patch soil (contaminated)	182	0.02	0.69	3.09	0.08	0.11

B. Desorption

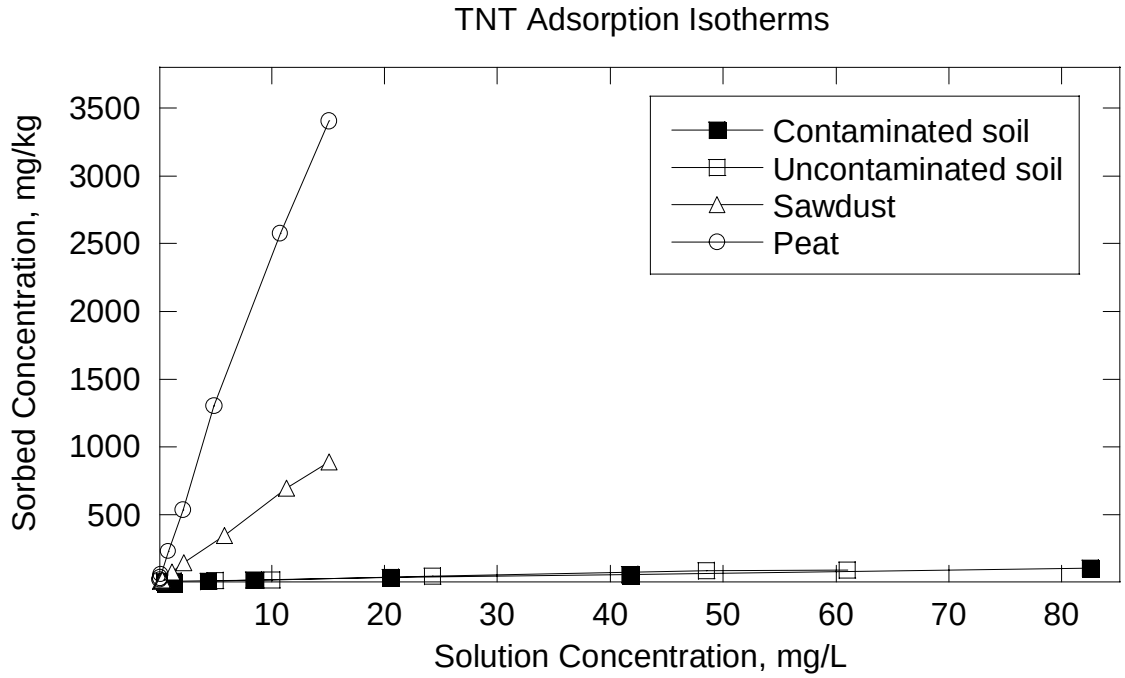
	TNT			RDX		
Sorbent	**b**	**k**	**r^2**	**b**	**k**	**r^2**
Grassy soil (uncontaminated)	169	0.23	0.65	26	1.77	0.21
Colored patch soil (contaminated)	-70	-0.36	0.78	0.43	38.49	0.47

Table 8. Results of cosubstrate screening in MMR soils.

		%Explosive mineralization ($^{14}CO_2$ dpm / total dpm x 100)											
Explosive	**Soil**	**Killed Control**	**Control**	**Molasses**	**Corn steep liquor**	**Soybean oil crude**	**SoyClear 1500**	**SoyGold 1000**	**Safflower oil**	**Potato starch**	**Solulac**	**Corn starch**	**Unrefined chitin**
TNT	Contaminated (C)	0.4	1.0	0.6	0.5	2.6	2.0	2.0	2.6	1.1	0.5	1.3	1.1
	Clean (G)	0.5	2.0	3.8	2.1	3.1	-[a]	-	-	1.9	-	-	-
RDX	Contaminated (C)	0.3	2.6	1.2	1.7	1.1	0.8	0.7	0.9	1.9	0.9	1.6	1.4
	Clean (G)	0.4	2.1	30.5	6.2	39.3	-	-	-	6.2	-	-	-
HMX	Contaminated (C)	0.3	1.9	1.2	1.2	1.5	1.1	1.2	1.4	1.8	0.9	1.8	1.7
	Clean (G)	0.2	2.0	7.4	2.9	8.9	-	-	-	5.6	-	-	-

[a]Not determined

Figure 1. Comparison of TNT and RDX adsorption isotherms for the two MMR soils, sawdust, and peat moss.



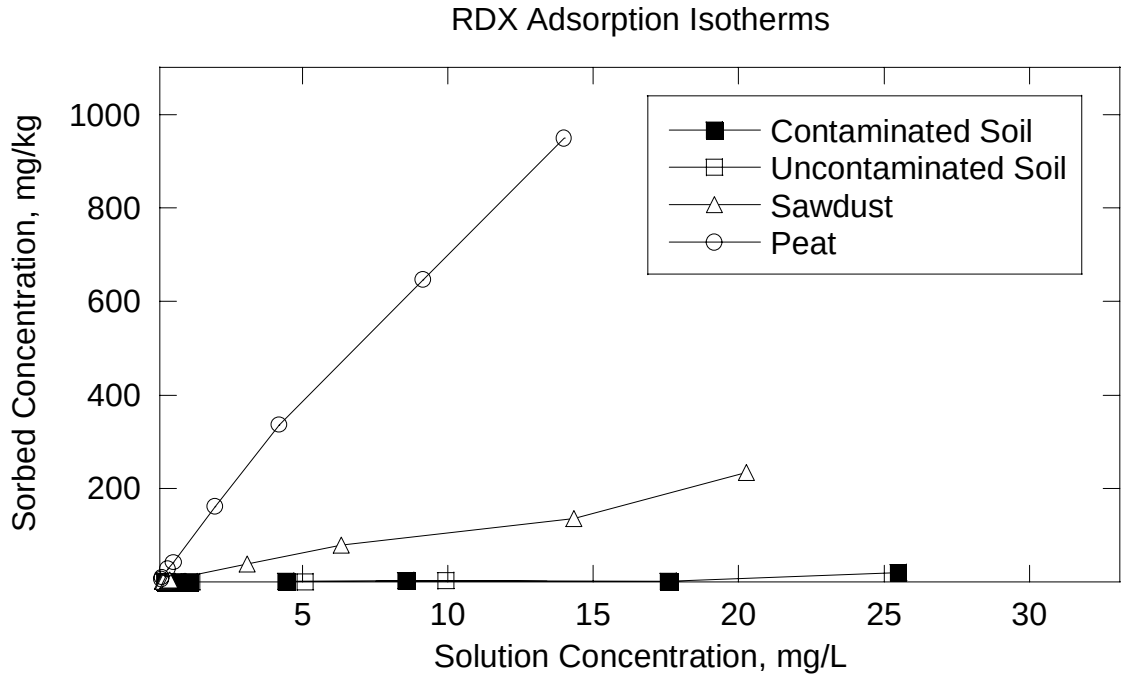


Figure 2. Mineralization of TNT, RDX, and HMX in contaminated MMR soil slurries amended with various cosubstrates.

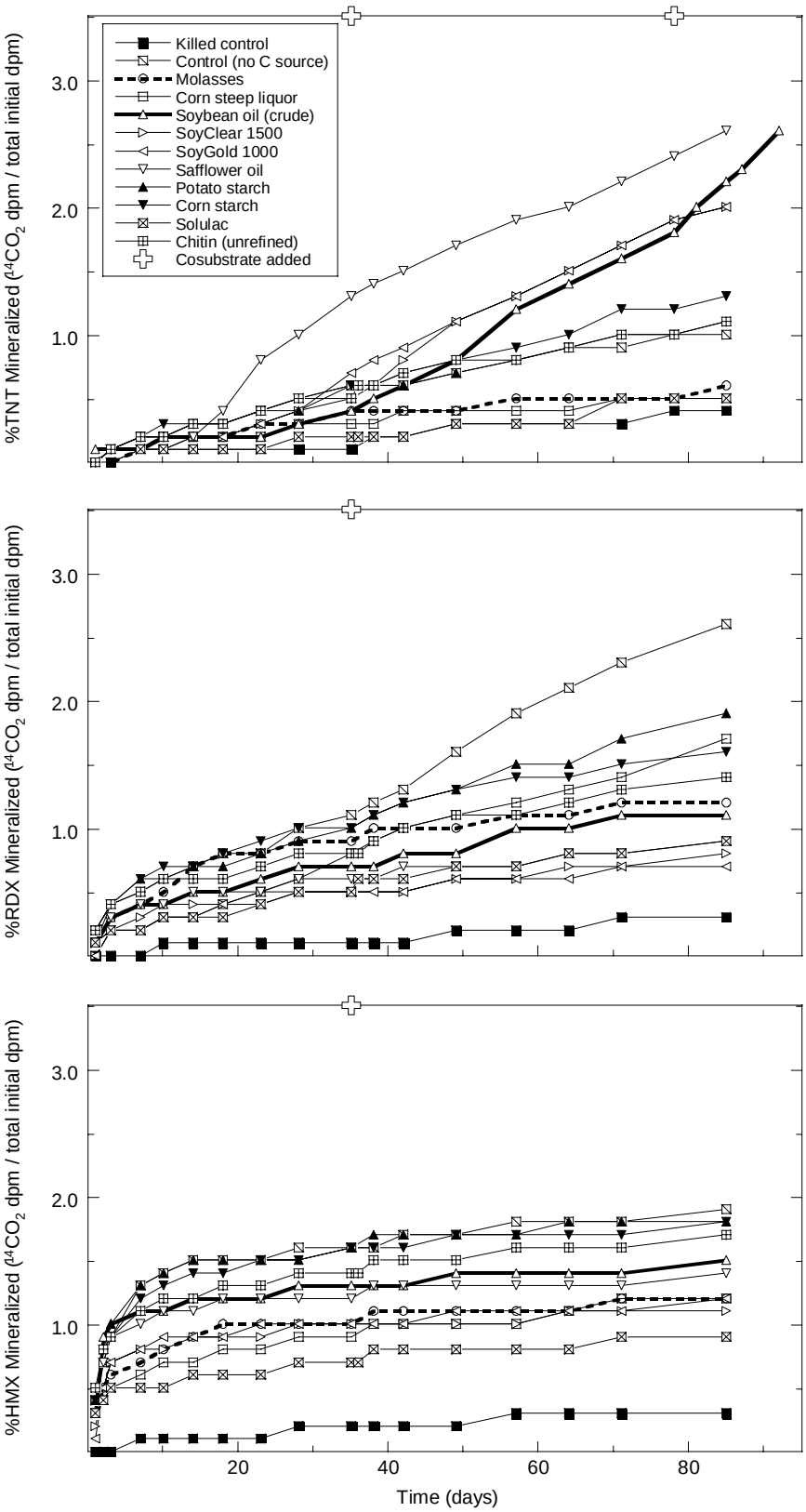


Figure 3. Mineralization of TNT, RDX, and HMX in uncontaminated MMR soil slurries amended with various cosubstrates.

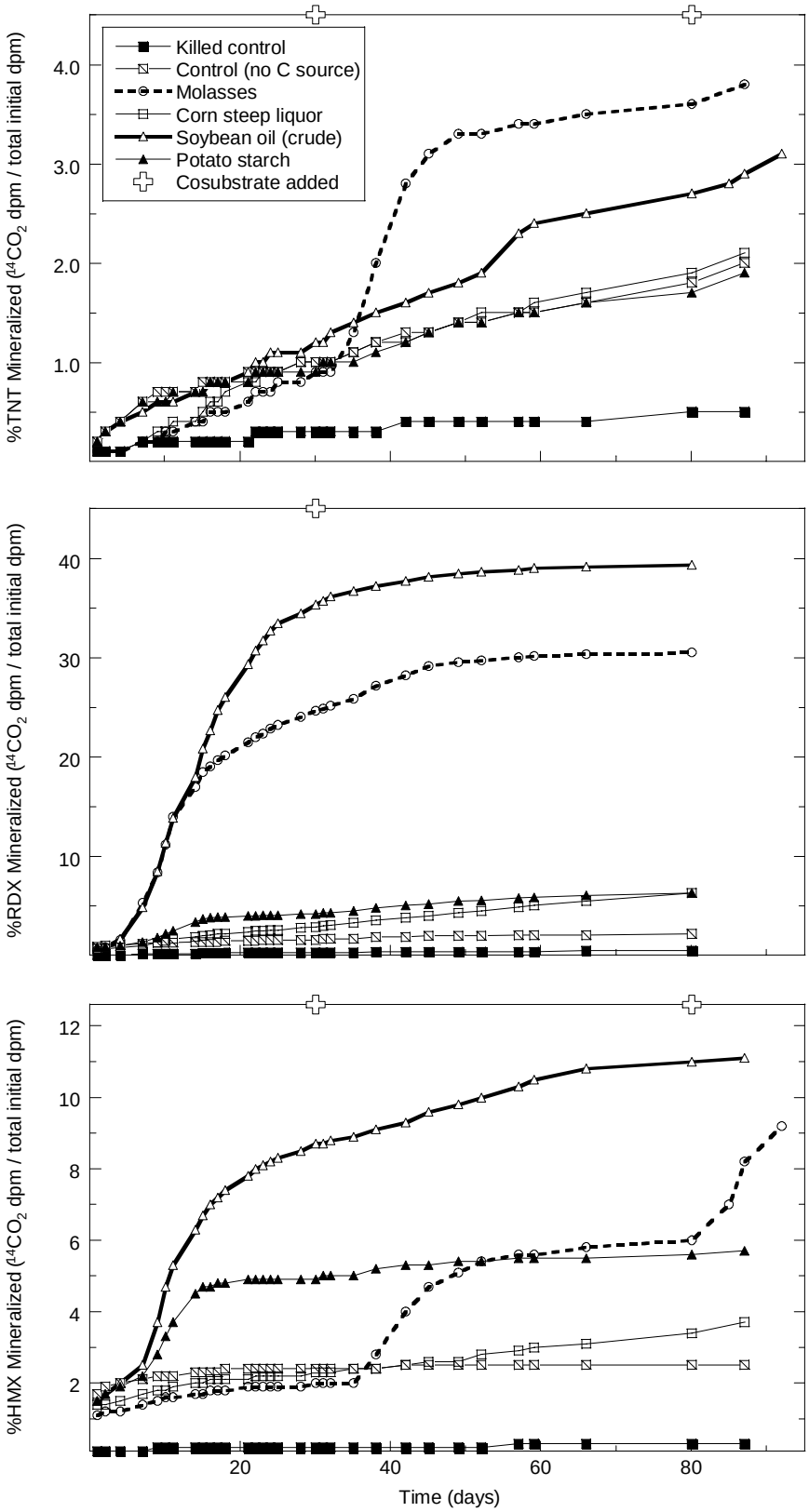
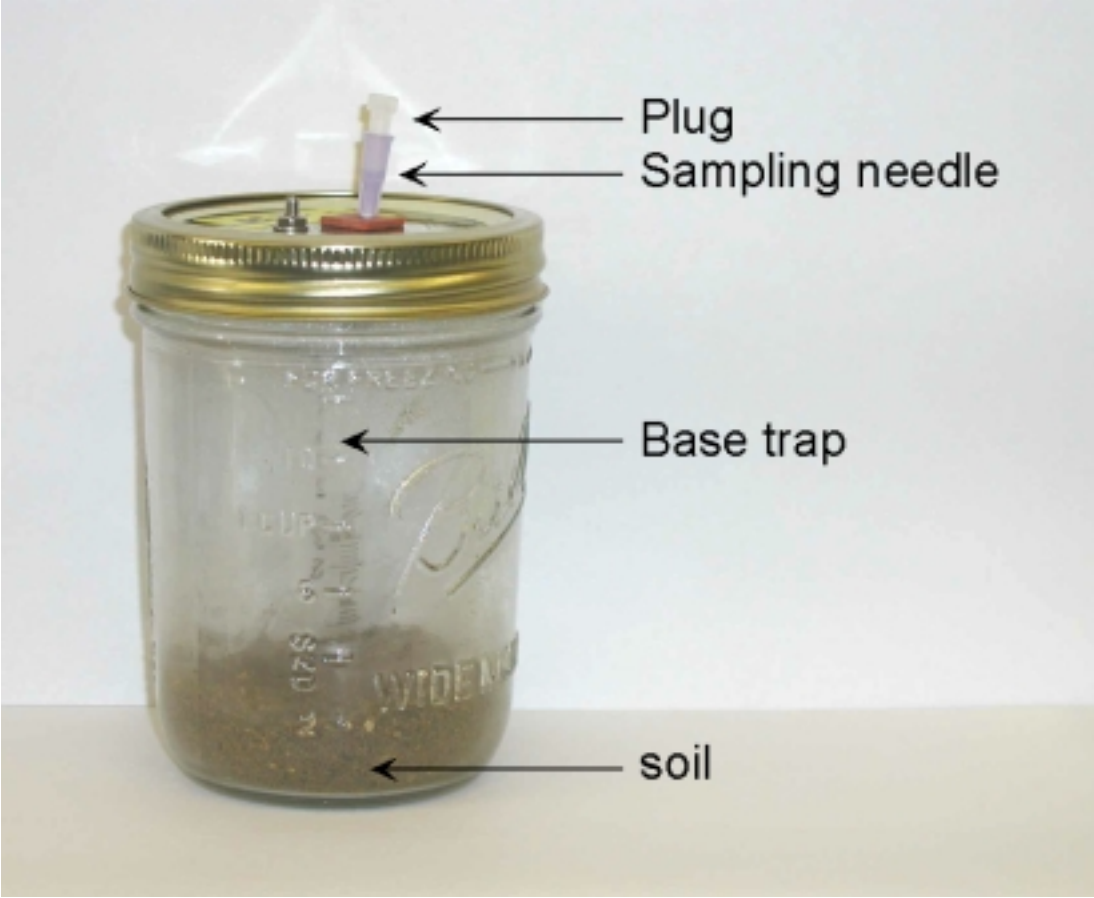


Figure 4. Unsaturated soil microcosm apparatus.



Quarterly Progress Report

SERDP Project 1229 - Immobilization of Energetics on Live Fire Ranges
Year 2002 – Second Quarter
July 15, 2002

The SERDP contract for FY 2001 was awarded to Envirogen on August 1, 2001. This report covers technical progress for SERDP Project 1229 from April 15, 2002 – July 15, 2002.

The objective of this project is to develop a cost-effective technology to immobilize energetic compounds (TNT, RDX, HMX, and breakdown products) released as residues at firing ranges to prevent their migration to groundwater. The goal is to develop an inexpensive soil treatment that can be readily applied over wide and remote areas prior to or immediately following firing range activities.

During the current quarter, activities have focused on completing all microcosm experiments used to evaluate sorbents and cosubstrates, and conducting experiments with repacked soil cores to evaluate explosive transport and biodegradation under unsaturated flow conditions.

II. MICROCOSM STUDIES.

During the past quarter, the screening of each of the potential cosubstrates for enhancing the biodegradation of TNT, RDX, and HMX was completed. The degradation of explosives in MMR soil after sorption to peat moss or sawdust with or without cosubstrate addition was also examined.

1. COSUBSTRATE SCREENING.

The initial objective of this task was to evaluate the ability of several organic materials to serve as cosubstrates for TNT, RDX and HMX biodegradation. The substrates were chosen based on cost, availability, and previous laboratory data. Screening assays were performed under optimal conditions in soil slurries. During this quarter, efforts were made to close the mass balance of the explosive compounds in the microcosms.

1.1. METHODS:

The methodology for screening the cosubstrates was described in previous QPRs for this project. Briefly, soil slurries were prepared using soils from MMR. The soils were spiked with radiolabelled TNT, RDX, or HMX, and amended with various cosubstrates and/or sorbent materials. The [^{14}C]CO_2 that was evolved over time was trapped and measured using liquid scintillation counting.

At the end of the incubation, the microcosms were deconstructed, and several different procedures were performed to assess the state of the residual [^{14}C] in each bottle or jar (see below). HPLC analyses were performed to determine the form of the [^{14}C] present after various extraction procedures.

For the slurry microcosms, the slurry was initially acidified by adding 0.2 ml of 6 N HCl to drive all dissolved carbon dioxide out of the aqueous phase. Microcosms were then incubated for 24 h with shaking, and the base trap was sampled and analyzed for $[^{14}C]CO_2$. Afterwards, the residual $[^{14}C]$ in the following fractions of the slurry was quantified:

a) WATER SOLUBLE (by centrifugation)- An aliquot (1.5 ml) of the slurry was placed in a microfuge tube, and centrifuged at 14,000 rpm for 20 minutes at 4°C. The cleared supernatant was removed, 0.5 ml was analyzed using liquid scintillation counting, and the remainder was analyzed using HPLC.
b) WATER SOLUBLE (by centrifugation+filtration)- Same as (a) above, except that the cleared supernatant was passed through a 0.45 µm glass fiber syringe filter prior to scintillation counting and HPLC analysis.
c) TOTAL ACETONITRILE EXTRACTABLE- An aliquot (3.0 ml) of the slurry was placed in a glass vial and dried at 60°C for at least 24 h. The dried slurry was ground with a glass pipet, and then extracted with 5.0 ml acetonitrile for 18 h in a cooled ultrasonic bath. The extract was allowed to settle, then 2.0 ml was filtered through a 0.2 µm nylon syringe filter. A 0.5 ml subsample was analyzed for dpm by scintillation counting, and the remainder was analyzed by HPLC for explosives and explosive metabolites.
d) TOTAL DICHLOROMETHANE EXTRACTABLE- Same as (c) above, except that the dried slurry was extracted with 5 ml hexane, and the solvent extract was filtered through a 0.2 µm teflon filter prior to scintillation counting. This procedure was only performed on the slurries prepared from the historically uncontaminated MMR soils.
e) TOTAL HEXANE EXTRACTABLE- Same as (c) above, except that the dried slurry was extracted with 5 ml dichloromethane, and the solvent extract was filtered through a 0.2 µm teflon filter prior to scintillation counting. This procedure was only performed on the slurries prepared from the historically uncontaminated MMR soils.

Mass balances were calculated by adding the cummulative dpm released as $[^{14}C]CO_2$ and the dpm recovered by the total acetonitrile extractable procedure. The deficit between the total dpm added to a given microcosm and the total recovered was assumed to be in a non-extractable, non-soluble state. The percentage of $[^{14}C]$ in the different fractions was calculated by dividing the dpm obtained for a given fraction by the total dpm added to the microcosm.

1.2. RESULTS & DISCUSSION:

The final graphs depicting explosives mineralization in contaminated and uncontaminated MMR soils are presented in Figures 1 and 2. None of the results stated in the previous progress report were significantly affected by an additional 50 days of incubation.

Mass balance results for the historically contaminated soil slurries are presented in Tables 1A to 1C, and those for the uncontaminated (grassy) soils are presented in Table 2 Mass balances for the slurries prepared from historically contaminated MMR soils were generally higher than those obtained for the slurries prepared from uncontaminated MMR soils. This may reflect a saturation of binding sites for the explosives and/or their breakdown products by the “older” explosives in the historically contaminated soil.

Since the extent of mineralization in the slurries prepared with historically contaminated MMR soils was so low, the focus of additional extractions and data analyses was for the slurry microcosms prepared with uncontaminated MMR soil.

The mass balance values based on the acetonitrile extractions were lower for [^{14}C]TNT spiked soils compared to [^{14}C]RDX and [^{14}C]HMX for any given treatment. There was a strong negative correlation between the percent of the initial [^{14}C] extracted with acetonitrile and the percent of the radioactivity recovered as [^{14}C]CO_2. This suggests as the biological activity increases more of the explosive is transformed into residues that are not extractable with acetonitrile

The amount of added [^{14}C] recovered in different fractions of the slurry microcosms exhibited some dependence on both the degree of mineralization and the cosubstrate added. The percent of initial dpm recovered as soluble dpm was in the order TNT << HMX < RDX. The exceptions were for molasses- and soybean oil-amended microcosms spiked with [^{14}C]RDX; these microcosms had very low soluble dpm values. Soluble dpm was strongly negatively correlated to [^{14}C]CO_2 production, which again likely reflects the conversion of the explosives to insoluble or soil-bound breakdown products in the more active microcosms. Soluble [^{14}C] dpm measured in samples that were both centrifuged and filtered were almost identical to samples that were only centrifuged (r^2 = 0.99), indicating that the centrifugation was sufficient at pelleting all material <0.2 µm.

The material extracted with dichloromethane (log K_{ow} = 1.25), which is less polar than acetonitrile (log K_{ow} = -0.34), was likely in the form of slightly hydrophobic compounds. The highest percent recovery of [^{14}C] was from the [^{14}C]RDX spiked slurries, while the lowest was from the [^{14}C]HMX spiked slurries. For [^{14}C]TNT and [^{14}C]HMX, the data indicate that dichloromethane extracted on the order of 50% of the dpm extracted by acetonitrile. However, dichloromethane extracted about 4-fold, 8-fold, and 10-fold more dpm than acetonitrile from the soybean oil amended treatments spiked with [^{14}C]TNT, [^{14}C]RDX and [^{14}C]HMX, respectively. Only the dichloromethane extractable dpm from the RDX spiked microcosms was moderately negatively correlated to the total [^{14}C]CO_2 produced (r^2 = 0.91).

The nonpolar solvent hexane (log K_{ow} = 4.11) extracted less than 3% of the added [^{14}C], and roughly 5-10% of the dpm extracted by acetonitrile. Hexane extractable dpm was not correlated to the percent of [^{14}C]CO_2 produced in a given microcosm, regardless of the explosives being examined. This dpm extracted by hexane was likely in the form of strongly hydrophobic compounds.

HPLC analysis of the acetonitrile extractions is currently underway to determine if the residual concentrations of the parent explosives and known breakdown products. Dr. Eugen Madsen at Cornell University has contacted us about the availablity of the subsamples from these microcosms for use in his SERDP project as well (CU-1233, Development and Application of a Flash Pyrolysis-GC/MS Assay for Documenting Natural and Engineered Attenuation of Nitroaromatic Compounds). We are hopeful that this collaboration will yield additional information on the fate of the explosives compounds in these slurry microcosms.

These results indicate that, in the soil slurry system, very little of the added TNT or RDX (5% and 1%, respectively), and only slightly more of the added HMX (25%), is present in a water soluble form when biological activity is stimulated using molasses or soybean oil. The residual explosives are in a form that is not effectively extracted with acetonitrile, dichloromethane, or hexane, with the exception of TNT and HMX in soybean oil-amended slurries. More work on how soybean oil is interacting with either the explosives or the solvents should should be pursued.

This supports the approach of using molasses and/or soybean oil to promote conversion of explosive residues into compounds that will be retained near the soil surface rather than migrate down into the groundwater.

2. COMBINED COSUBSTRATE-SORBENT EVALUATION.

To determine if an approach can be designed which leads to both immobilization and biodegradation of explosives residues at or near the soil surface, a treatment matrix consisting of the most effective cosubstrate and sorbent was applied to unsaturated soil microcosms. During this quarter, efforts were made to close the mass balance of the explosives compounds in the microcosms.

2.1. METHODS:

Microcosms were prepared as described in previous progress reports. Briefly, dry soil (uncontaminated MMR soil) was added to mason jars, amended with sorbents (sawdust or peat moss) and cosubstrates (molasses or soybean oil), and wetted to approximately 70% of water holding capacity with a solution spiked with [^{14}C]-labeled explosives. Jars were sealed and base was added to each trap. Jars were incubated at room temperature for approximately 2.5 months. The base was sampled periodically and analyzed using liquid scintillation counting to determine production of [^{14}C]CO_2.

At the end of the incubation, mass balance procedures for the unsaturated microcosms were performed by thoroughly homogenizing the soil, and removing samples to determine the amount of [^{14}C] in the following fractions:

a) TOTAL ACETONITRILE EXTRACTABLE- An aliquot (3 to 5 g wet weight) of the soil was placed in a glass vial and dried at 60°C for at least 24 h. The dried soil was ground with a glass pipet, and a 1.0 g subsample of the dried soil was then extracted with 5.0 ml acetonitrile for 18 h in a cooled ultrasonic bath. The extract was allowed to settle, then 2.0 ml was filtered through a 0.2 µm nylon syringe filter. A 0.5 ml subsample was analyzed for dpm by scintillation counting, and the remainder was analyzed by HPLC.
b) TOTAL DICHLOROMETHANE EXTRACTABLE- Same as (a) above, except that the dried slurry was extracted with 5 ml dichloromethane, and the solvent extract was filtered through a 0.2 µm teflon filter prior to scintillation counting.

c) TOTAL HEXANE EXTRACTABLE- Same as (a) above, except that the dried slurry was extracted with 5 ml hexane, and the solvent extract was filtered through a 0.2 µm teflon filter prior to scintillation counting.
d) MICROBIAL BIOMASS ASSOCIATED- A modification of the chloroform fumigation/extraction procedure widely used in agricultural research was used to determine the amount of [^{14}C] that had been incorporated into microbial biomass. Briefly, 2 subsamples (3 to 5 g wet weight) of the soil were placed in separate glass vials. One replicate was extracted immediately by adding 12 ml of 0.01 M KSO_4 and shaking horizontally for 1 h at room temperature. The extract was filtered through a 0.45 µm glass fiber filter, and the cleared solution was analyzed using scintillation counting. The other replicate was fumigated with chloroform vapors in a sealed chamber for 18-24 h at room temperature. This procedure is generally assumed to lyse >99% of the microorganisms present in the soil, making their carbonaceous cell components readily extractable. The fumigated soil was then extracted and analyzed as described above. The difference in extracted dpm between the fumigated and non-fumigated soil represents the [^{14}C] incorporated into microbial biomass.

Mass balances were calculated by adding the cummulative dpm released as [^{14}C]CO_2 and the dpm recovered by the acetonitrile extraction. The deficit between the total dpm added to a given microcosm and the total recovered was assumed to be in a non-extractable, non-soluble state. The percentage of [^{14}C] in the different fractions was calculated by dividing the dpm obtained for a given fraction by the total dpm added to the microcosm.

2.2. RESULTS & DISCUSSION:

The data showed that no significant [^{14}C]CO_2 was released during the incubation of the explosives with the sorbents prior to the addition of soil and cosubstrates (data not shown). Mineralization of radiolabelled explosives over the course of the incubation are presented in Figure 3.

Mineralization of [^{14}C]TNT was minimal, and the no substrate controls actually showed the greatest evolution of [^{14}C]CO_2 during 70 days of incubation. Soybean oil and molasses stimulated some TNT mineralization, which was reduced if the TNT had been pre-sorbed to peat.

Significant mineralization was observed for RDX (42%) in the presence of soybean oil. A slightly lower amount of RDX was mineralized (35%) if peat moss was also added to the soil, and a significantly smaller amount of RDX was converted to [^{14}C]CO_2 (17%) sawdust was present. Molasses also stimulated some RDX mineralization (32%), and the pattern of less conversion of RDX to carbon dioxide in the presence of peat (20%) or sawdust (2%) was also observed. Peat and sawdust alone stimulated some limited (~5%) mineralization of RDX, relative to the unamended control.

HMX mineralization in the unsaturated microcosms was essentially negligible except when the soil had been amended with molasses. Pre-adsorption of the HMX to peat reduced the [^{14}C]CO_2 by 4-fold compared to the treatment without peat.

HPLC analysis of the acetonitrile extractions is being performed to determine if the residual dpm is present as the parent explosives, known breakdown products, or some other unknown compounds. The results obtained thus far indicate the formation of 2-amino-4,6-DNT as the major metabolite from TNT, and MNX >>DNX>TNX from RDX. Dr. Eugen Madsen at Cornell University has also contacted us about the availablity of the subsamples from these microcosms for use in his SERDP project (CU-1233, Development and Application of a Flash Pyrolysis-GC/MS Assay for Documenting Natural and Engineered Attenuation of Nitroaromatic Compounds). We are hopeful that this collaboration will yield additional information on the fate of the explosives compounds in these slurry microcosms.

Mineralization of a single explosive in the presence and absence of the other two explosives is presented in Figure 4. The degree of mineralization was low for both TNT and HMX, but the trends appear to indicate that the mixture of explosives degraded similarly to the treatments with only a single explosive added. For RDX, mineralization was similar for RDX alone and in combination with TNT and HMX during the first 35 days in sawdust + soybean oil amended soil, at which time the treatment with the mixture leveled off. The final difference in RDX mineralization between the two treatments was less than 5%. There was also an approximate 5% difference in the sawdust + molasses amended soil, but the level of RDX mineralization was much lower than lower than observed with soybean oil.

Residual radioactivity in different fractions of the soil, and mass balances of the explosives ($[^{14}C]CO_2$ + acetonitrile extracted $[^{14}C]$) at the end of the incubation are presented in Tables 3A to 3C. There was a general trend for all three explosives of higher total mass balances in the microcosms amended with soybean oil compared to molasses, and several of the soybean oil amended treatments yielded mass balances of greater than 100%. Extractions with dichloromethane and hexane are currently underway, and results will be included in the project final report.

In general, there were relatively small amounts of $[^{14}C]$ associated with the microbial biomass in the soil as determined using the chloroform fumigation/extraction procedure (Tables 3A-3C). The least incorporation of $[^{14}C]$ into microorganisms was observed for TNT (0-3%), with slightly more incorporation of RDX (0-15%) and HMX (3-13%). There was no apparent correlation between the amount of $[^{14}C]$ incorporated into microbial biomass and degree of mineralization of the explosives.

These results provided the framework for the first of a series of repacked soil core experiments. Additional analyses of the soil in these microcosms is currently being planned. The remaining soil may also be used to do some simple leachability studies.

III. CONDUCT SOIL COLUMN STUDIES.

During the past quarter, a major effort has been directed at designing and constructing the apparatus needed to conduct experiments using repacked soil cores operated under unsaturated flow conditions. An experiment using three such soil cores was also initiated during the latter part of the quarter.

1. SOIL CORE DESIGN AND CONSTRUCTION

The use of soil cores, prepared at several scales, to mimic field conditions is a common experimental approach for conducting environmental research. However, the materials used and the design employed need to be carefully considered depending on which chemicals are being studied, what processes are being measured, and the conditions that are attempting to be replicated in the laboratory.

1.1 METHODS – DESIGN AND CONSTRUCTION

The design of the soil core apparatus was based on that of published reports and the experience of Envirogen personel during previous research. The final conceptual design is depicted in Figure 5. The objectives factored into this design were:

a) the ability to maintain the cores at less than 100% saturation, under a slight negative suction (head) so as to generate an unsaturated flow regime as occurs in surface soils.
b) the ability to collect samples at various depths below the soil surface in order to monitor the downward migration of explosive compounds from the soil surface.
c) the ability to contain and capture all radioactivity introduced into the system (including gaseous species such as $[^{14}C]CO_2$) in order to achieve mass balance.

It was desirable to also be able to see into the soil core to allow preferntial flow paths, water movement, etc. to be assessed.

Various parts and the final repacked soil core apparatus are shown in Figure 6, with descriptions below. The core casing was schedule 40 transparent PVC pipe, with schedule 40 white PVC end caps and couplers, and clear PVC drainage tubing. Although the affinity of the explosives for PVC is not known, the relative surface area of the casing is small compared to that of the soil. All tubing inside the core was teflon (or stainless steel in the case of that attached to the spray nozzle), and the fittings were made of polypropylene. These materials did not sorb appreciable amounts of TNT, RDX or HMX in a laboratory study using radiolabelled compounds (data not shown). The luer valves attached to each in-core sampler were made of polystyrene. High flow ceramic samplers (at 4” and 8” below the soil surface) were employed within the soil core. The dead volume of the lysimeter samplers was reduced to approximately 20 ml by filling the ceramic form with 4 mm glass balls. A high flow ceramic plate (1 bar rating) placed at the very bottom of the soil core (below a layer of clean silica sand) and above a water filled reservoir, served to regulate the water retention of the soil. The edge of the ceramic plate was wrapped with rubber gasket material and affixed using epoxy, then the ceramic:rubber junction was further sealed using black silicone. This constrained water flow through the plate in the vertical direction. Cup-type samplers were constructed using polypropylene syringes, teflon tubing, and polypropylene tubing fittings. Two cup-type samplers were placed at both 4” and 8” below the soil surface, and one was placed at the soil:sand interface at the bottom of the soil core (12” below the soil surface). Four threaded stainless steel rods were used to secure the PVC pipe to the lower section of the core apparatus, with sufficient pressure to achieve a water tight seal between the bottom edge of the PVC pipe and the ceramic plate.

A continuous flow of humidified air was supplied using a cross-flow humidifier. The humidified air was distributed using a simple PVC manifold to the very top of the core apparatus, and exited the core apparatus at a port positioned approximately 4" above the soil surface. Exiting air flowed through both an active purging and a passive KOH trap (25 ml per trap) designed to scrub CO_2 from the airstream.

A system for producing rain events was constructed using a spray nozzle inserted into the top of the core apparatus. Water from a reservoir was delivered to the spray nozzle using a peristaltic pump and a programmable timer through small diameter flexible tubing. At a flow rate of greater than 30 ml/min, the water exiting the spray nozzle was in the form of a very fine mist, although a few drops did form as the system pressurized and depressurized at the beginning and end of each rain event.

Water exiting the core through the bottom of the core apparatus was collected in a glass vessel, and the volume was determined gravimetrically (weight of vessel plus water – weight of empty vessel).

Water samples were obtained from the lysimeter samplers by slowly applying suction using a 20 ml polypropylene syringe. Holes were drilled in the plunger shaft to allow a small metal pin to maintain the syringe plunger at a given position without having to manually hold the syringe as it filled with water. During sampling, 20 ml was first collected to remove the dead volume (called the "flush"), followed by collection of the actual 5-10 ml volume sample. Samples were transferred to glass vials after collection. Actual volumes were determined gravimetrically (weight of vial plus water – weight of empty vial).

Water samples were only obtained from the cup-type samplers during and after rain events, since these samplers only collected free flowing water from the surrounding soil as it became saturated. Samples were collected in glass vials and volume was determined gravimetrically (weight of vial plus water – weight of empty vial).

2. SOIL CORE TRANSPORT AND FATE EXPERIMENTS

The fate of explosives residues which were deposited on top of a sorbent-cosubstrate barrier was determined in an experiment involving three identical repacked soil cores.

2.1 METHODS

Repacking of each soil core apparatus was performed as follows: The bottom ~4" of the core was packed with clean quartz to provide an inert but hydraulically conductive spacer between the soil and the ceramic pressure plate. A cup sampler was installed at the sand:soil interface. Dry uncontaminated MMR soil was then added to the apparatus in six 548 g aliquots, with careful attention given to assuring the soil remained unsorted as it was being added. A threaded steel rod was also used to mix the interface between different layers, to work the soil to fill any voids around the lysimeter and cup-type samplers, and to redistribute soil to fill any visible preferential

flow paths. An additional amount of soil (200-250 g) was added to bring the soil height within the apparatus to 12", yielding final soil masses of approximately 3.5 kg dry soil.

The soil was slowly saturated from the bottom with distilled water (dH_2O). A Moriata bottle was used as the water reservoir, which was sequentially raised in 4" intervals relative to the soil in the core apparatus over the course of at least three days. The amount of water needed to saturate the soil was determined, then the soil was progressively drained by placing the core drain tubing at greater and greater relative distances below the ceramic plate (0.5 to 1 ft intervals, 12+ h equilibration at each interval). The amount of water recovered for each interval was determined and recorded. . The final position of the drainage tubing outlet was approximately 4 ft (1.2 m) below the level of the ceramic plate, which represents a -0.1 bar suction (head) applied to the soil core. The moisture retention curves for the three soil cores are presented in Figure 7.

A test of the rain system was performed by applying 240 ml of dH_2O to the surface of each of the three soil cores. Free water was collected from the cup samplers at 4" below the soil surface and from the drainage tube at -4 ft. Water recovery was 90-93% (by volume), and indicated that 1" of rain applied to the surface infiltrated at a rate of approximately 2 ml/min. Based on the planned intensity of rain events for these experiments, collection of free water was expected to be possible only from the cup samplers positioned 4" below the soil surface. Water was also successfully recovered from the lysimeter samplers positioned 4" below the soil surface in all three soil cores, but only from two out of three of the cores from the lysimeters positioned 8" below the soil surface.

The cores were designated:
C1 Control (non-functioning 8" lysimeter)
C2 Peat
C3 Peat+soybean oil

Peat (25 g air dry) and peat+soybean oil (0.5 g oil per g peat, premixed) was added to the surface of the soil in cores C2 and C3, respectively. This resulted in a layer of material approximately 0.25" to 0.50" deep. A 75 g aliquot of dry soil (sieved to >70 µm and < 1 mm) was spiked with a solution of [^{14}C]RDX and unlabelled explosives in acetone to achieve final nominal explosive concentrations of 1100 mg TNT/kg, 900 mg RDX/kg, 110 mg HMX/kg, and 4.8 total µCi. The acetone solution was misted onto the soil in serial applications with frequent mixing between each application. The acetone was evaporated from the soil under nitrogen. The soil was thoroughly homogenized and distributed into three 24 g aliquots. The remainder of the spiked soil was extracted with acetonitrile to determine the actual starting explosives concentrations as well as the initial radioactivity. C1 received 24 g of spiked soil applied over the entire surface of the soil core, while C2 and C3 received the same amount applied evenly on top of the peat or peat+soybean oil layer. Two aliquots of 5 ml dH_2O were applied via the spray nozzle to wet the applied soil over the first 12 h.

The base traps were switched out approximately once per week. Samples from the lysimeters were collected several time per week, while water from the cup samplers was only collected during and after rain events as described above. The initial rain event (140 ml dH_2O) occurred

four days after adding the spiked soil. The simulated rainwater contained 100 mg/L bromide (Br) as a conservative tracer. The rain was applied as 0.5 minute pulses (40 ml/min misting rate) separated by 9.5 minutes intervals of no rain. Additional rain events (no Br) occurred on Day 13 (140 ml), Day 14 (160 ml), and Day 20 (160 ml). The cup samplers appeared to be clogged during the Day 14 rain event, and were backflushed with dH_2O prior to the Day 20 rain event, during which they operated correctly.

Samples of the KOH base traps were analyzed for $[^{14}C]CO_2$, and aqueous samples were analyzed for dpm using scintillation counting as described previously. Aqueous samples were also analyzed for Br using an ion selective probe. Selected aqueous samples were analyzed using HPLC to determine concentrations of TNT, RDX, HMX and their breakdown products. Some samples from the cup samplers were filtered through 0.45 µm glass fiber filters, and the dpm in the filtrate was compared to that in the unfiltered sample to determine if any of the dpm was associated with (i.e., sorbed to) colloidal material.

2.2 RESULTS

The repacked soil cores have been operating for approximately 25 days. Results to date are summarized in Figures 8 and 9 and Table 4. No significant $[^{14}C]CO_2$ has been captured in the KOH traps (0.22%, 0.09%, and 0.06% of initial dpm for C1, C2, and C3, respectively). Radioactivity reaching 4” below the soil surface has been greatest in Control core C1 (3.5% of initial dpm), and significantly less in Peat core C2 and Peat+soybean oil core C3 (1.7% and 1.1% of initial dpm, respectively). Bromide concentrations detected at 4” have been relatively similar for all three treatments. HPLC analysis of the most recent samples from the 4” cup samplers indicated the presence of all three of the explosives, as well as some metabolites of RDX and TNT.

Less than 0.05% of the added $[^{14}C]$ has been detected 8” or greater below the soil surface. Br concentrations have increased over time in C2 and C3 (no data available for C1 due to faulty lysimeter at 8” below the soil surface).

This particular experiment will continue for an additional few weeks. Real rain water will be used in place of dH_2O to more closely resemble natural conditions. Also, heavier/longer rain events will be simulated in order to determine the rate of water loading that the peat and peat+oil treatments can handle before they result in explosives leaching equivalent to that observed in the control treatment.

We believe the results to date indicate that the technology is very promising, but that further research and experiments at larger scales are needed to proceed with development. These issues will be addressed in a White Paper to be submitted by July 31, 2002.

IV. FIGURES AND TABLES

The tables and figures supporting this document have been provided to the SERDP Office as a separate attachment.

V. RESPONSES TO IN-PROGRESS REVIEW COMMENTS

Below are our responses to some of the comments that were raised during our IPR conducted on May 3, 2002.

1) Multiple criteria (i.e., mineralization and leachability) should be considered in selecting sorbents. Please discuss the means toward testing the leachable fraction of TNT from selected sorbents before proceeding with the column studies.

These comments were received by us after we had already initiated the design and construction of the unsaturated soil core apparatus, and because conducting soil core experiments was an actual task slated for our first year of research, we made the decision to move forward with initial core experiments before these multiple criteria for the selection of the best treatments were explicitly assessed.

Regarding the criteria themselves, at the simplest level, the more of an explosive compound that is mineralized, the less that is available to migrate into the groundwater. Many of our results to date support our selection of mineralization as the first screening criteria for the cosubstrates. For example, in the slurry microcosms strong negative correlations were observed between the percent of the explosives that were mineralized and the amount of the residual explosives (or their breakdown products) which were extractable using acetonitrile (i.e., the greater the degree of mineralization, the less acetonitrile extractable residue). TNT residues not extractable with acetonitrile are generally considered to be present as a bound residue (2) which is not likely to become solubilized and migrate with infiltrating water. Although extensive research involving bound residue formation from RDX and HMX has not been published, it seems reasonable to assume similar processes may occur with these explosives as well. This relationship was not observed in the unsaturated microcosms in which sorbents and cosubstrates were combined, but we do have sufficient material leftover from the unsaturated microcosm experiments to conduct a series of leachability studies to examine the solubility of the residual explosives directly. We believe more research is needed to directly examine the use of multiple criteria.

Finally, we believe that although simple microcosm-type studies examining multiple criteria for sorbent and cosubstrate selection are needed, larger-scale experiments such as the unsaturated soil cores are essential to more closely approximating field conditions. Multiple criteria are currently being assessed in our cores (both mineralization and downward migration of explosives residues during and after rain events).

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2) Please discuss differentiating ^{14}C that becomes associated with microorganisms versus soil.

This was an interesting comment. Since none of the three explosives we are examining are known to be used as sole carbon and energy sources by microorganisms, the amount of the compounds incorporated into microbial biomass is expected to be low. Previous work looking at incorporation of [^{14}C]TNT in a soil bioslurry reactor only showed 25-30% incorporation of [^{14}C] into biomass (1). Others have noted <10% incorporation of radiolabelled RDX and HMX (3).

Our application of a standard protocol for extracting microbial biomass carbon (chloroform fumigation/extraction) to assess [^{14}C] incorporation during the unsaturated microcosm experiments yielded mixed results. Extremely low percentages of [^{14}C]TNT (0-2%) were incorporated into microorganisms, while a wider range of incorporation values were observed for [^{14}C]RDX (0-15%) and HMX (3-13%). The form in which the [^{14}C] is incorporated may be as protein, lipid, nucleic acid, or carbohydrate, but was not determined during these analyses. Regardless, this material is not likely to exist in a form that is a potential toxicological threat even if it were to be released from microbial cells and migrate into the groundwater.

3) Please initiate communication with regulators to assess their concerns with this technology. For example, what will be the effect of topical applications of molasses or oil on ecological populations? What is the possibility of mobilizing contaminants through treatment?

A limited review of existing technologies that are similar to ours has been conducted. We have found reports of the use of peat moss as a soil amendment and even as a "green" bulk sorbent for treating chemical spills. Technologies in which vegetable oils are used as soil amendments or as biopesticides were also found. Extracts from an EPA document covering the use of vegetable and flower oils (EPA738-R-93-031) is included with the full progress report coming under separate cover.

Although the exact combination of materials we are examining was not specifically addressed, we believe the potential negative ecological effects of application of the most promising sorbents (peat moss, sawdust) and cosubstrates (soybean oil, molasses) to the land surface at live fire ranges will be minimal, especially in relation to the potential benefits of explosive residue remediation and groundwater protection.

We do intend to pursue the regulatory issues associated with this technology as the project progresses, and will deal with the subject in detail in the final report.

REFERENCES

1. Boopathy, R., and J. Manning. 1998. A laboratory treatability study on explosives-contaminated soils from the Iowa Army Ammunition Plant, Burlington, Iowa. U.S. Army Environmental Center, Environmental Technology Division. Report# SFIM-AEC-ET-98.

2. Hundal, L., P. Shea, S. Comfort, W. Powers, and J. Singh. 1997. Long-term TNT sorption and bound residue formation in soil. Journal of Environmental Quality 26:896-904.

3. Shen, C., J. Hawari, G. Ampleman, S. Thiboutot, and S. Guiot. 2000. Enhanced biodegradation and fate of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetraazocine (HMX) in anaerobic soil slurry bioprocess. Bioremediation Journal 4:27-39.

Table 1A. Percentage of [^{14}C] in different fractions for [^{14}C]TNT spiked slurries prepared using historically contaminated soil.

	Percent of initial dpm[a]			
TREATMENT	[^{14}C]CO_2	WATER SOLUBLE	ACETONITRILE EXTRACTABLE	MASS BALANCE[b]
Killed Control (HgCl2, Na Azide)	0 (0)	32 (1)	76 (4)	77 (4)
Control (no C source)	1 (0)	35 (9)	28 (13)	29 (13)
Molasses	1 (0)	23 (1)	22 (2)	22 (2)
Corn steep liquor	1 (0)	37 (1)	29 (2)	29 (2)
Soybean oil-crude	3 (0)	10 (1)	14 (1)	17 (1)
SoyClear 1500	2 (1)	9 (0)	40 (3)	42 (2)
SoyGold 1000	2 (0)	10 (1)	35 (6)	37 (6)
Safflower oil	3 (0)	11 (2)	16 (0)	19 (0)
Potato starch	1 (0)	28 (1)	21 (2)	23 (2)
Solulac	1 (0)	26 (2)	17 (5)	17 (5)
Corn starch	2 (0)	23 (0)	14 (4)	15 (4)
Unrefined chitin	1 (0)	20 (1)	14 (2)	15 (2)

[a]Results presented as average percent (± difference between duplicate bottles / 2).
[b]Mass balance is the sum of [^{14}C]CO_2 and acetonitrile extractable values.

Table 1B. Percentage of [^{14}C] in different fractions for [^{14}C]RDX spiked slurries prepared using historically contaminated soil.

	Percent of initial dpm[a]			
TREATMENT	[^{14}C]CO_2	WATER SOLUBLE	ACETONITRILE EXTRACTABLE	MASS BALANCE[b]
Killed Control (HgCl2, Na Azide)	0 (0)	30 (2)	108 (0)	109 (0)
Control (no C source)	4 (0)	41 (7)	97 (2)	102 (2)
Molasses	2 (0)	64 (0)	101 (0)	103 (0)
Corn steep liquor	4 (1)	62 (1)	83 (1)	87 (1)
Soybean oil-crude	1 (0)	15 (1)	93 (0)	94 (0)
SoyClear 1500	1 (0)	18 (5)	71 (20)	72 (20)
SoyGold 1000	1 (0)	15 (1)	46 (0)	47 (0)
Safflower oil	1 (0)	13 (6)	84 (19)	85 (19)
Potato starch	2 (0)	47 (2)	97 (2)	99 (2)
Solulac	2 (0)	60 (0)	90 (0)	92 (0)
Corn starch	2 (0)	56 (0)	101 (2)	103 (2)
Unrefined chitin	2 (0)	27 (0)	109 (9)	111 (8)

[a]Results presented as average percent (± difference between duplicate bottles / 2)
[b]Mass balance is the sum of [^{14}C]CO_2 and acetonitrile extractable values.

Table 1C. Percentage of [^{14}C] in different fractions for [^{14}C]HMX spiked slurries prepared using historically contaminated soil.

	Percent of initial dpm[a]			
TREATMENT	[^{14}C]CO_2	WATER SOLUBLE	ACETONITRILE EXTRACTABLE	MASS BALANCE[b]
Killed Control (HgCl2, Na Azide)	0 (0)	29 (2)	97 (8)	97 (8)
Control (no C source)	2 (0)	27 (0)	101 (3)	104 (3)
Molasses	1 (0)	38 (1)	79 (6)	80 (6)
Corn steep liquor	1 (0)	32 (1)	84 (1)	86 (1)
Soybean oil-crude	2 (0)	9 (3)	99 (7)	100 (7)
SoyClear 1500	1 (0)	18 (4)	81 (15)	82 (15)
SoyGold 1000	1 (0)	13 (3)	68 (15)	69 (15)
Safflower oil	1 (0)	20 (6)	71 (6)	73 (6)
Potato starch	2 (0)	28 (2)	91 (0)	92 (0)
Solulac	1 (0)	17 (4)	85 (1)	86 (1)
Corn starch	2 (0)	38 (1)	82 (2)	84 (2)
Unrefined chitin	2 (0)	12 (2)	93 (4)	95 (4)

[a]Results presented as average percent (± difference between duplicate bottles / 2)
[b]Mass balance is the sum of [^{14}C]CO_2 and acetonitrile extractable values.

Table 2. Percentage of [^{14}C] in different fractions for slurries prepared using uncontaminated soil.

	Percent of initial dpm[a]					
EXPLOSIVE & TREATMENT	[^{14}C]CO_2	WATER SOLUBLE	ACETONITRILE EXTRACTABLE	DICHLOROMETHANE EXTRACTABLE	HEXANE EXTRACTABLE	MASS BALANCE[b]
TNT						
Killed Control (HgCl2, Na Azide)	1 (0)	53 (1)	67 (5)	33 (1)	1 (1)	68 (5)
Control (no C source)	2 (0)	17 (0)	20 (4)	10 (0)	1 (0)	23 (4)
Molasses	4 (0)	6 (1)	9 (1)	4 (0)	1 (0)	13 (1)
Corn steep liquor	3 (0)	14 (2)	10 (2)	4 (1)	1 (1)	12 (2)
Soybean oil-crude	3 (0)	4 (1)	6 (0)	24 (8)	1 (0)	9 (1)
Potato starch	2 (0)	10 (2)	23 (3)	8 (3)	1 (0)	25 (3)
RDX						
Killed Control (HgCl2, Na Azide)	0 (0)	89 (0)	93 (6)	61 (6)	2 (0)	93 (6)
Control (no C source)	2 (0)	88 (1)	85 (3)	60 (2)	1 (0)	87 (3)
Molasses	31 (5)	1 (0)	1 (0)	0 (0)	0 (0)	32 (4)
Corn steep liquor	10 (5)	66 (7)	58 (11)	45 (12)	1 (0)	67 (7)
Soybean oil-crude	39 (3)	1 (0)	3 (1)	9 (5)	1 (1)	42 (4)
Potato starch	7 (0)	72 (1)	57 (3)	21 (1)	1 (1)	63 (3)
HMX						
Killed Control (HgCl2, Na Azide)	0 (0)	66 (2)	76 (2)	6 (0)	2 (-)	76 (2)
Control (no C source)	3 (0)	62 (1)	76 (3)	7 (2)	0 (0)	78 (3)
Molasses	10 (4)	30 (8)	40 (10)	1 (1)	1 (0)	50 (6)
Corn steep liquor	4 (1)	53 (0)	62 (3)	2 (0)	1 (0)	67 (4)
Soybean oil-crude	11 (3)	28 (1)	51 (1)	28 (8)	0 (0)	62 (4)
Potato starch	6 (0)	54 (0)	69 (6)	3 (0)	1 (0)	75 (5)

[a]Results presented as average percent (± difference between duplicate bottles / 2)
[b]Mass balance is the sum of [^{14}C]CO_2 and acetonitrile extractable values.

Table 3A. Percentage of [^{14}C] in different fractions for [^{14}C]TNT spiked uncontaminated soil microcosms.

	Percent of initial dpm[a]			
TREATMENT	[^{14}C]CO_2	ACETONITRILE EXTRACTABLE	MICROBIAL BIOMASS	MASS BALANCE[b]
Control (no sorbent or C source)	7 (0)	16 (7)	0 (0)	23 (7)
Soybean oil-crude	5 (0)	84 (3)	0 (0)	89 (3)
Molasses	4 (0)	17 (12)	2 (1)	21 (13)
Peat Control (no C source)	7 (0)	9 (2)	0 (0)	16 (3)
Peat + Soybean oil	4 (0)	56 (5)	0 (0)	60 (5)
Peat + Molasses	2 (0)	10 (9)	0 (0)	12 (9)
Sawdust Control (no C source)	1 (0)	37 (8)	0 (0)	38 (8)
Sawdust + Soybean oil	1 (0)	162 (9)	0 (0)	163 (9)
Sawdust + Molasses	0 (0)	14 (3)	2 (2)	15 (3)
[14C]TNT+RDX+HMX+Sawdust+Soybean oil	1 (0)	99 (3)	0 (0)	100 (3)
[14C]TNT+RDX+HMX+Sawdust+Molasses	0 (0)	17 (3)	0 (0)	17 (3)

[a]Results presented as average percent (± difference between duplicate bottles / 2).
[b]Mass balance is the sum of [^{14}C]CO_2 and acetonitrile extractable values.

Table 3B. Percentage of [^{14}C] in different fractions for [^{14}C]RDX spiked uncontaminated soil microcosms.

	Percent of initial dpm[a]			
TREATMENT	[^{14}C]CO_2	ACETONITRILE EXTRACTABLE	MICROBIAL BIOMASS	MASS BALANCE[b]
Control (no sorbent or C)	6 (0)	60 (1)	0 (0)	66 (2)
Soybean oil-crude	43 (0)	59 (3)	1 (1)	101 (3)
Molasses (2X)	12 (2)	31 (14)	15 (1)	43 (12)
Molasses	33 (5)	0 (0)	1 (1)	33 (5)
Molasses (2X, lower %H2O)	12 (3)	23 (14)	11 (5)	35 (11)
Peat Control (no C)	3 (0)	58 (11)	7 (7)	61 (11)
Peat + Soybean oil	36 (3)	26 (0)	0 (0)	62 (3)
Peat + Molasses (2X)	23 (0)	10 (6)	1 (1)	33 (6)
Sawdust Control (no C)	6 (1)	58 (1)	10 (2)	64 (0)
Sawdust + Soybean oil	19 (1)	132 (26)	3 (1)	151 (27)
Sawdust + Molasses (2X)	2 (0)	55 (0)	11 (0)	57 (0)
[14C]RDX+TNT+HMX+Sawdust+Soybean oil	13 (5)	95 (27)	1 (1)	108 (21)
[14C]RDX+TNT+HMX+Sawdust+Molasses (2X)	2 (1)	57 (18)	10 (10)	59 (17)

[b]Mass balance is the sum of [^{14}C]CO_2 and acetonitrile extractable values.

[b]ND, not determined.

[c]Mass balance is the sum of [^{14}C]CO_2 and acetonitrile extractable values.

Table 3C. Percentage of [^{14}C] in different fractions for [^{14}C]HMX spiked uncontaminated soil microcosms.

	Percent of initial dpm[a]			
TREATMENT	[^{14}C]CO_2	ACETONITRILE EXTRACTABLE	MICROBIAL BIOMASS	MASS BALANCE[b]
Control (no sorbent or C source)	1 (0)	65 (4)	6 (4)	67 (4)
Soybean oil-crude	1 (0)	113 (7)	11 (5)	115 (7)
Molasses	8 (1)	33 (7)	4 (0)	41 (8)
Peat Control (no C source)	1 (0)	64 (4)	3 (3)	65 (4)
Peat + Soybean oil	1 (0)	102 (5)	13 (0)	103 (5)
Peat + Molasses	2 (0)	55 (3)	3 (1)	57 (3)
Sawdust Control (no C source)	1 (0)	38 (8)	4 (4)	39 (8)
Sawdust + Soybean oil	1 (0)	131 (6)	7 (1)	132 (6)
Sawdust + Molasses	1 (0)	39 (9)	8 (0)	40 (9)
[14C]TNT+RDX+HMX+Sawdust+Soybean oil	1 (0)	83 (1)	12 (6)	84 (1)
[14C]TNT+RDX+HMX+Sawdust+Molasses	1 (0)	42 (5)	4 (0)	43 (5)

[a]Results presented as average percent (± difference between duplicate bottles / 2).
[b]Mass balance is the sum of [^{14}C]CO_2 and acetonitrile extractable values.

Table 4. Explosives concentrations detected at 4" below the soil surface on Day 20 of the unsaturated soil core experiment.

	Concentration, mg/L		
COMPOUND	Control	Peat	Peat+Oil
HMX	0.5	0.2	0.2
MNX	0.4	0.1	BD
DNX	BD[a]	BD	BD
TNX	0.1	BD	BD
RDX	9.9	4.6	2.4
TNT	0.1	BD	BD
2-Amino-DNT	2.9	BD	BD
4-Amino-DNT	0.1	BD	BD

[a]BD, below detection limit, <0.1 mg/L.

Figure 1. Mineralization of TNT, RDX, and HMX in contaminated MMR soil slurries amended with various cosubstrates.

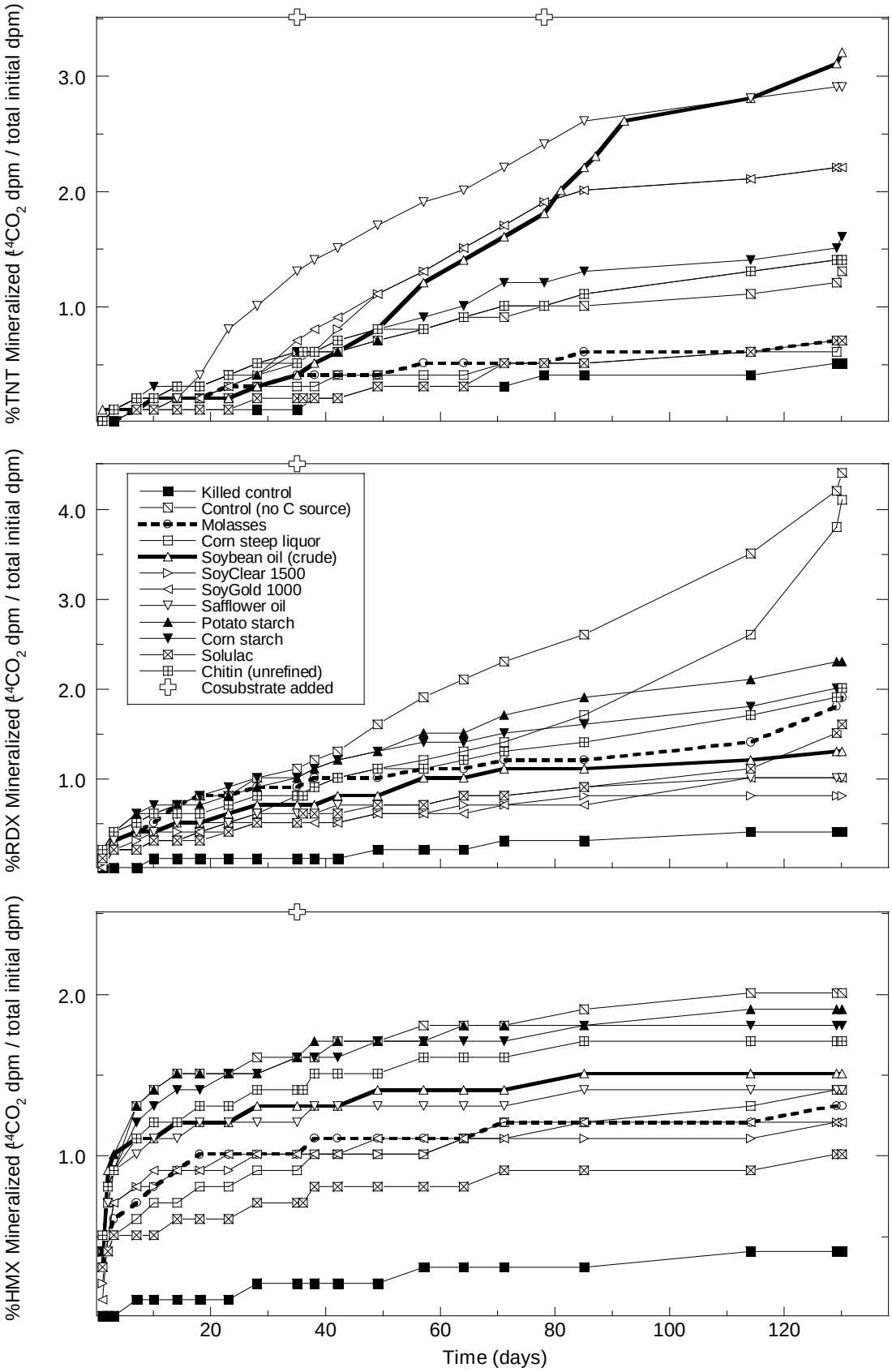


Figure 2. Mineralization of TNT, RDX, and HMX in uncontaminated MMR soil slurries amended with various cosubstrates.

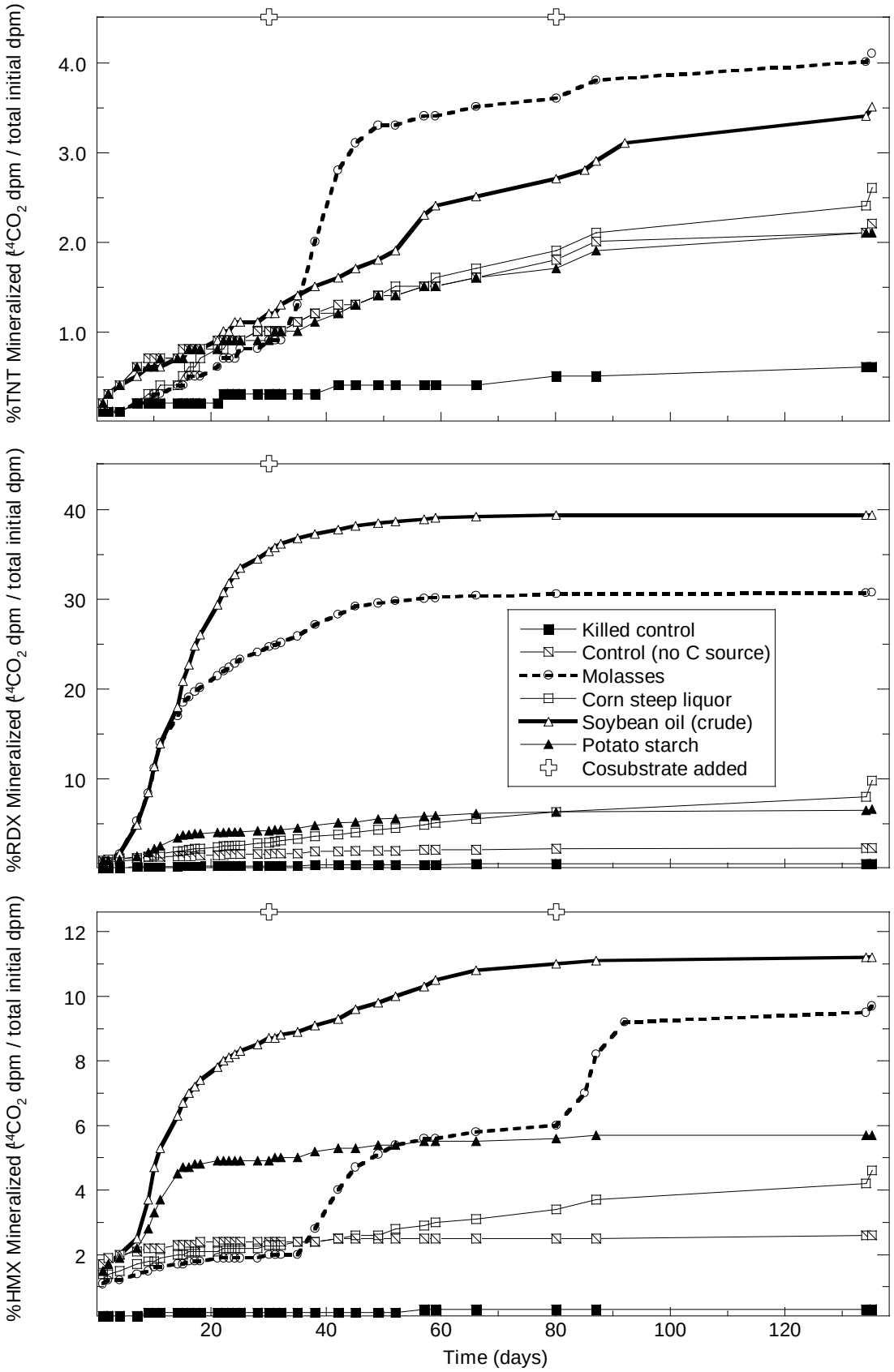


Figure 3. Mineralization of TNT, RDX, and HMX in uncontaminated MMR soil amended with molasses or soybean oil, and in the presence or absence of sawdust or peat moss.

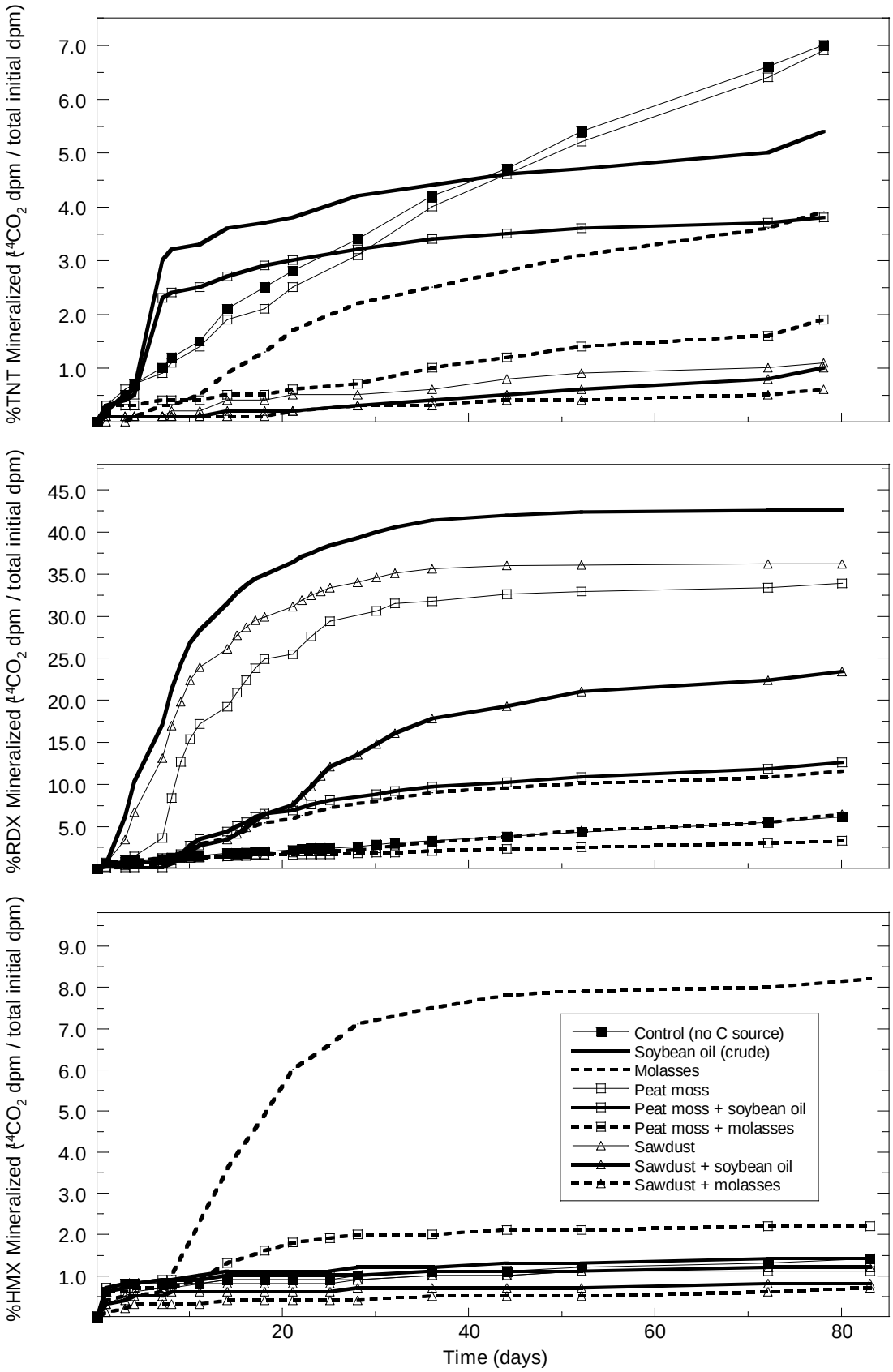


Figure 4. Effect of mixtures of TNT, RDX, and HMX on the mineralization of single explosive compounds in uncontaminated MMR soil amended with sawdust and soybean oil or molasses.

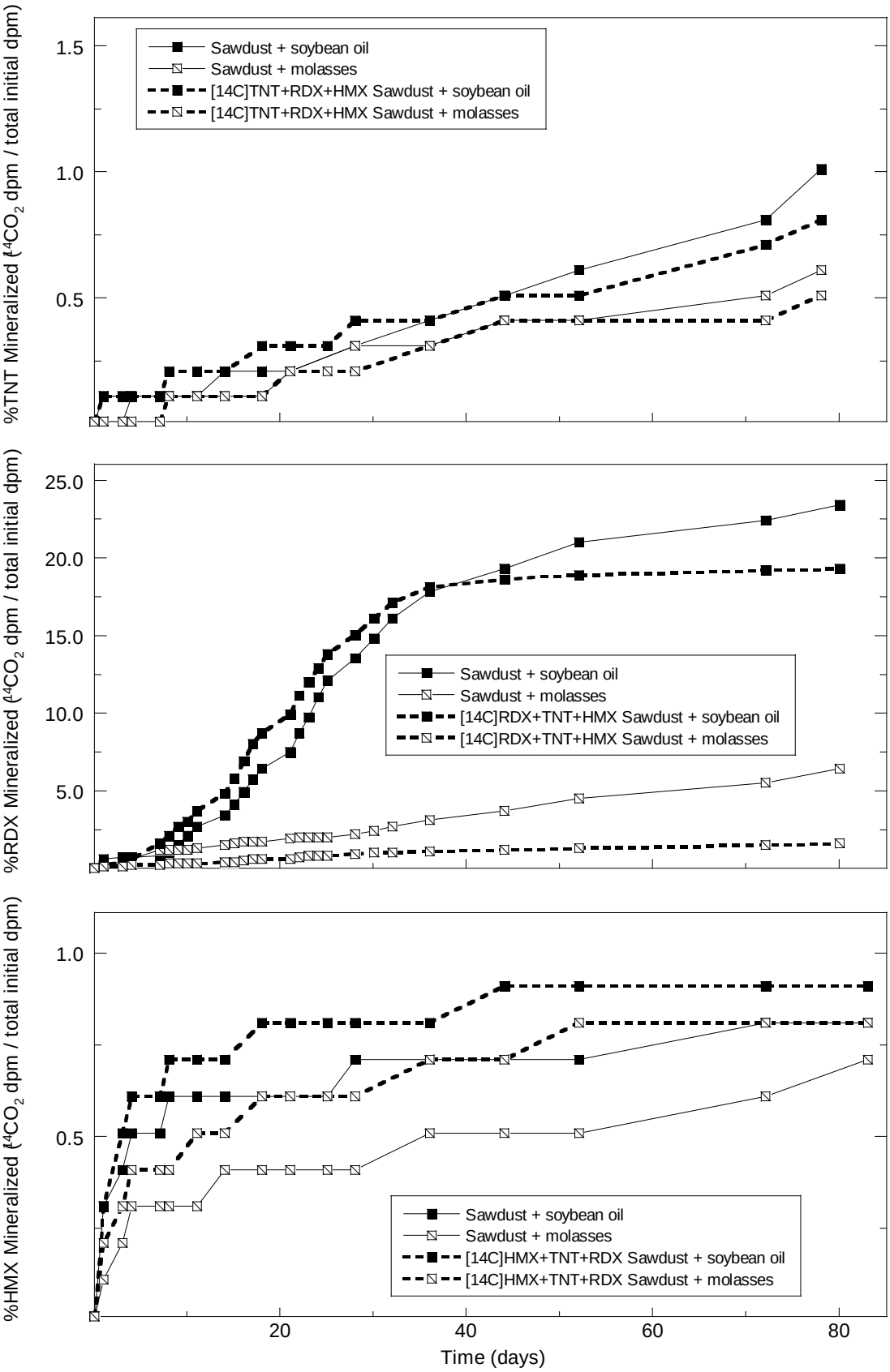


Figure 5. Conceptual design for the unsaturated soil core aparatus.

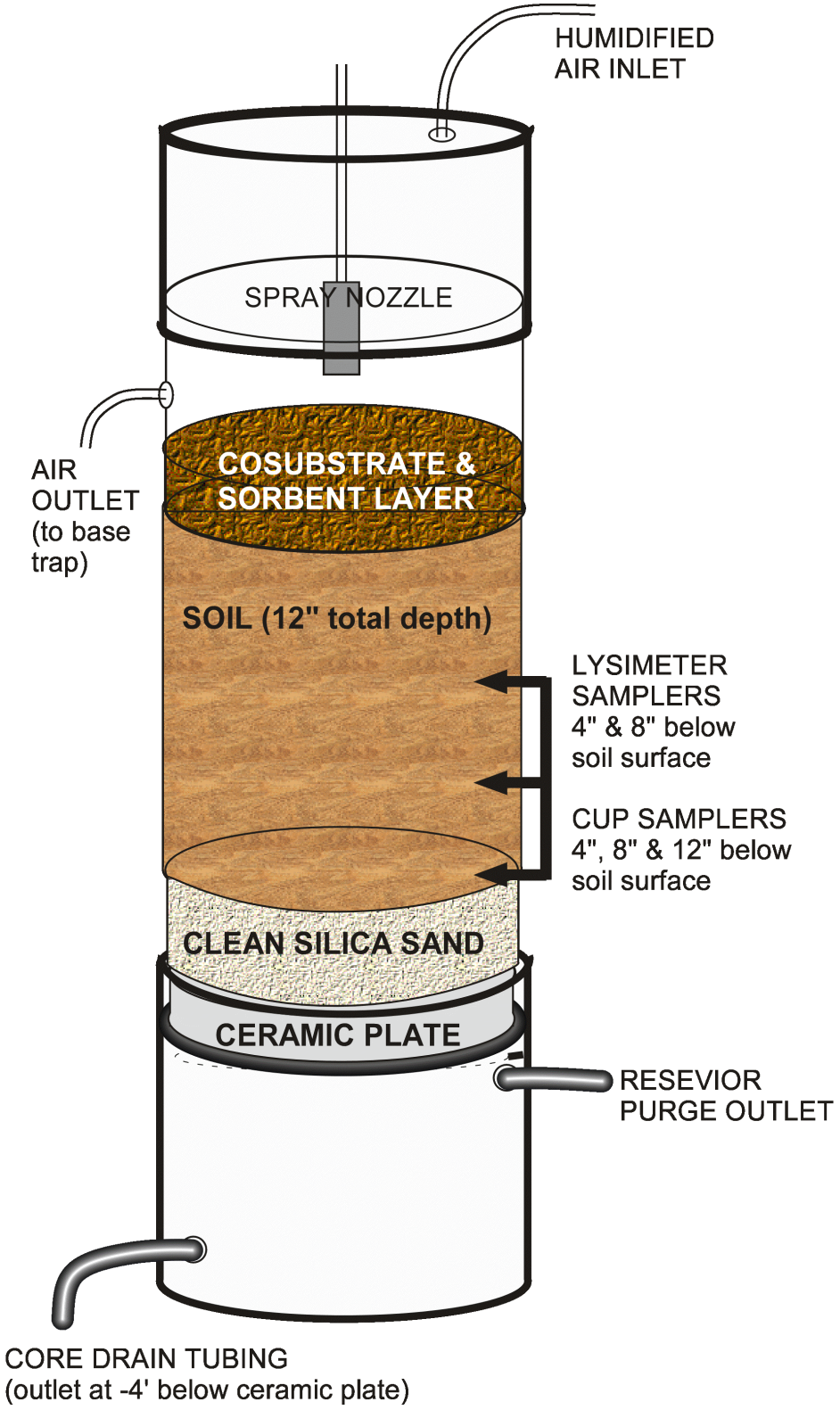


Figure 6. Photographs of the unsaturated soil core apparatus.

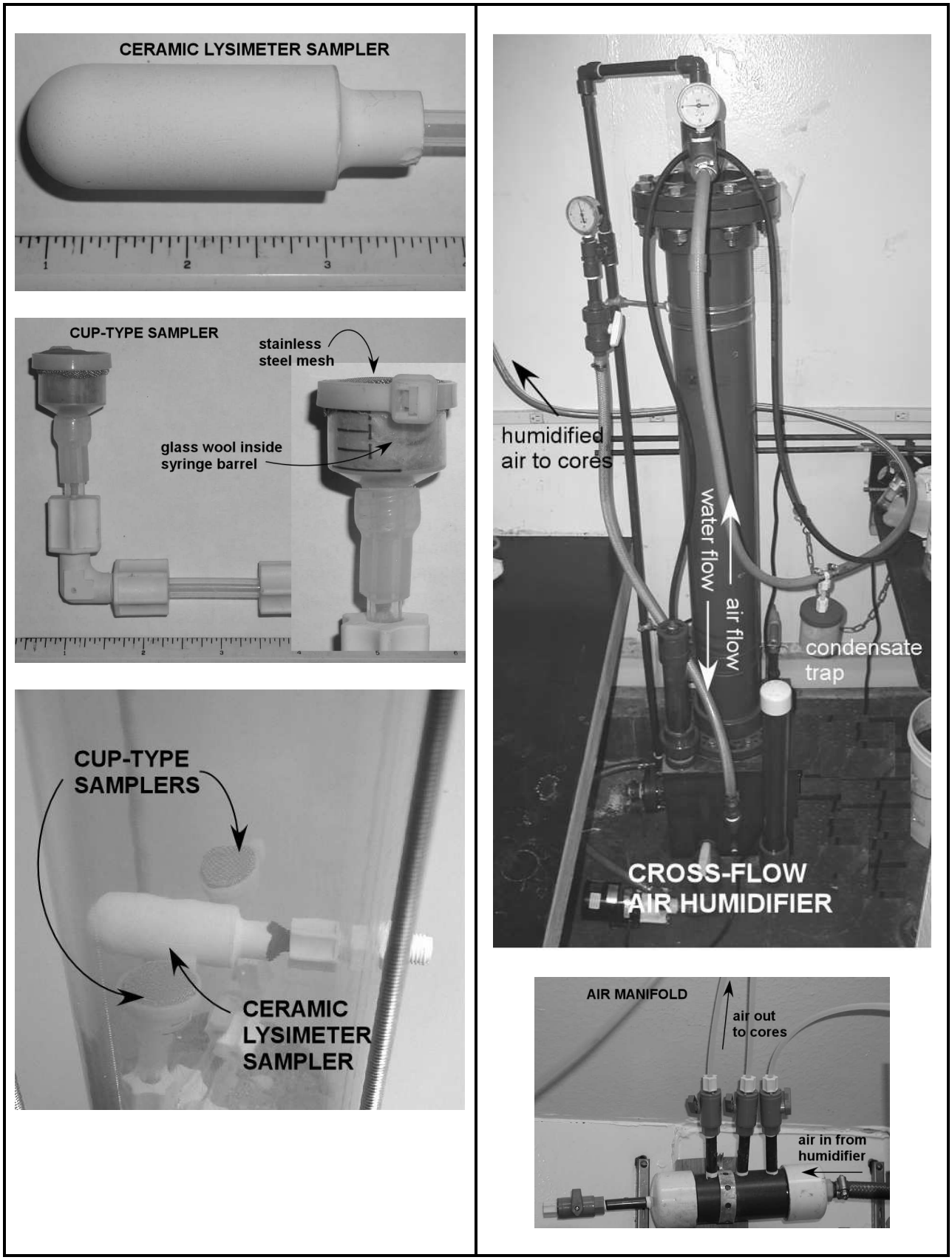


Figure 6. Photographs of the unsaturated soil core apparatus (cont.).

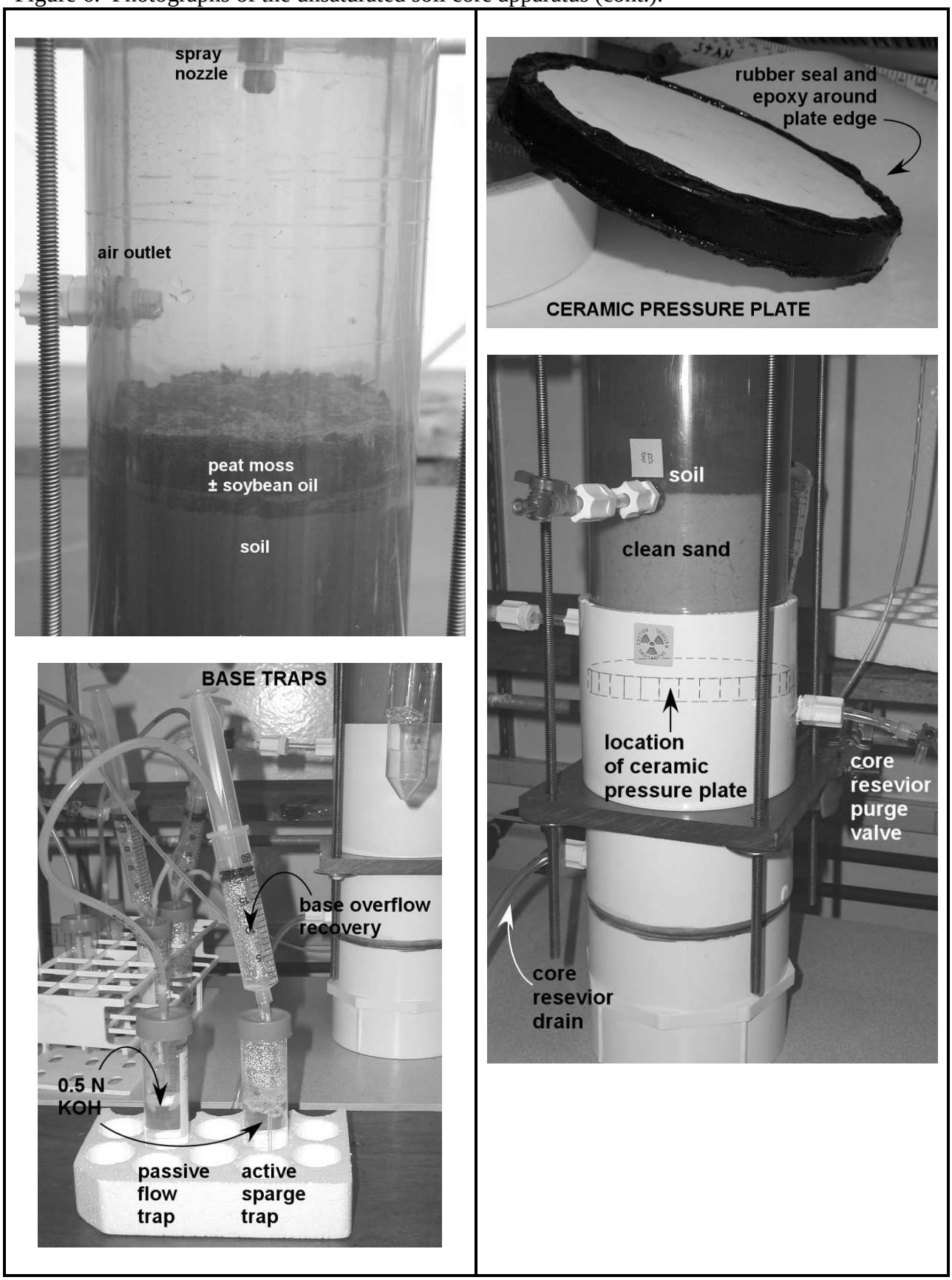


Figure 6. Photographs of the unsaturated soil core apparatus (cont.).

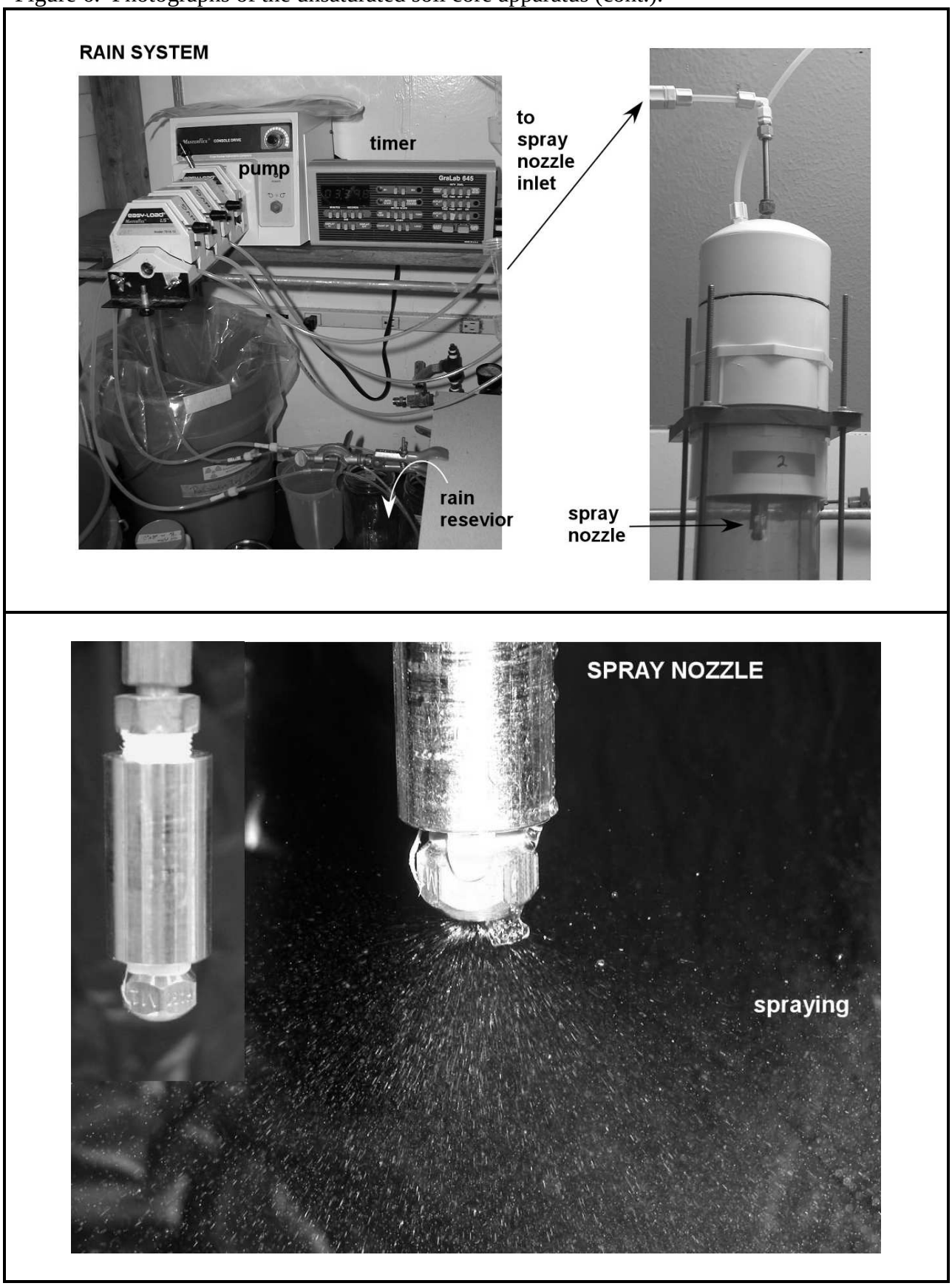


Figure 6. Photographs of the unsaturated soil core apparatus (cont.).

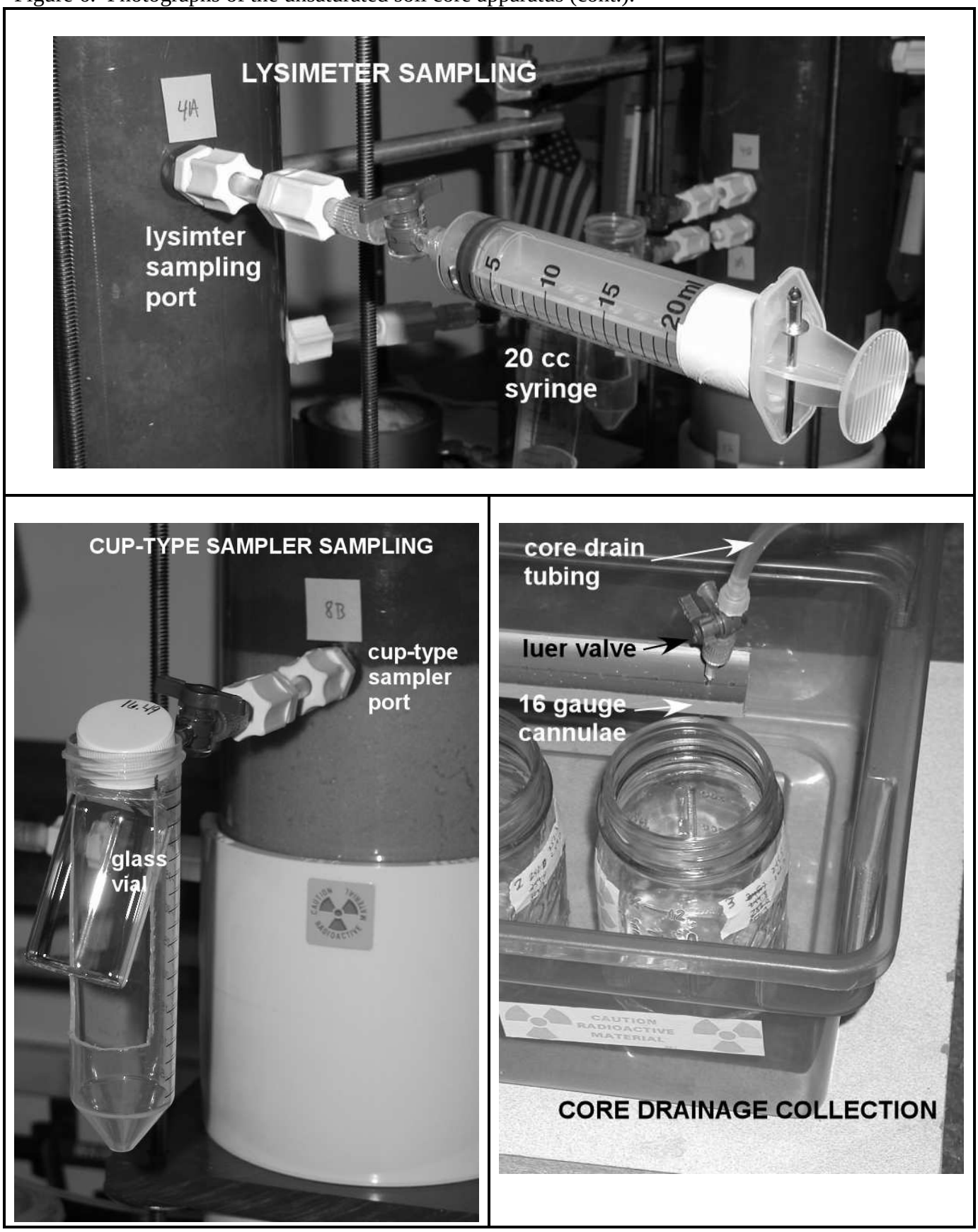


Figure 6. Photographs of the unsaturated soil core apparatus (cont.).

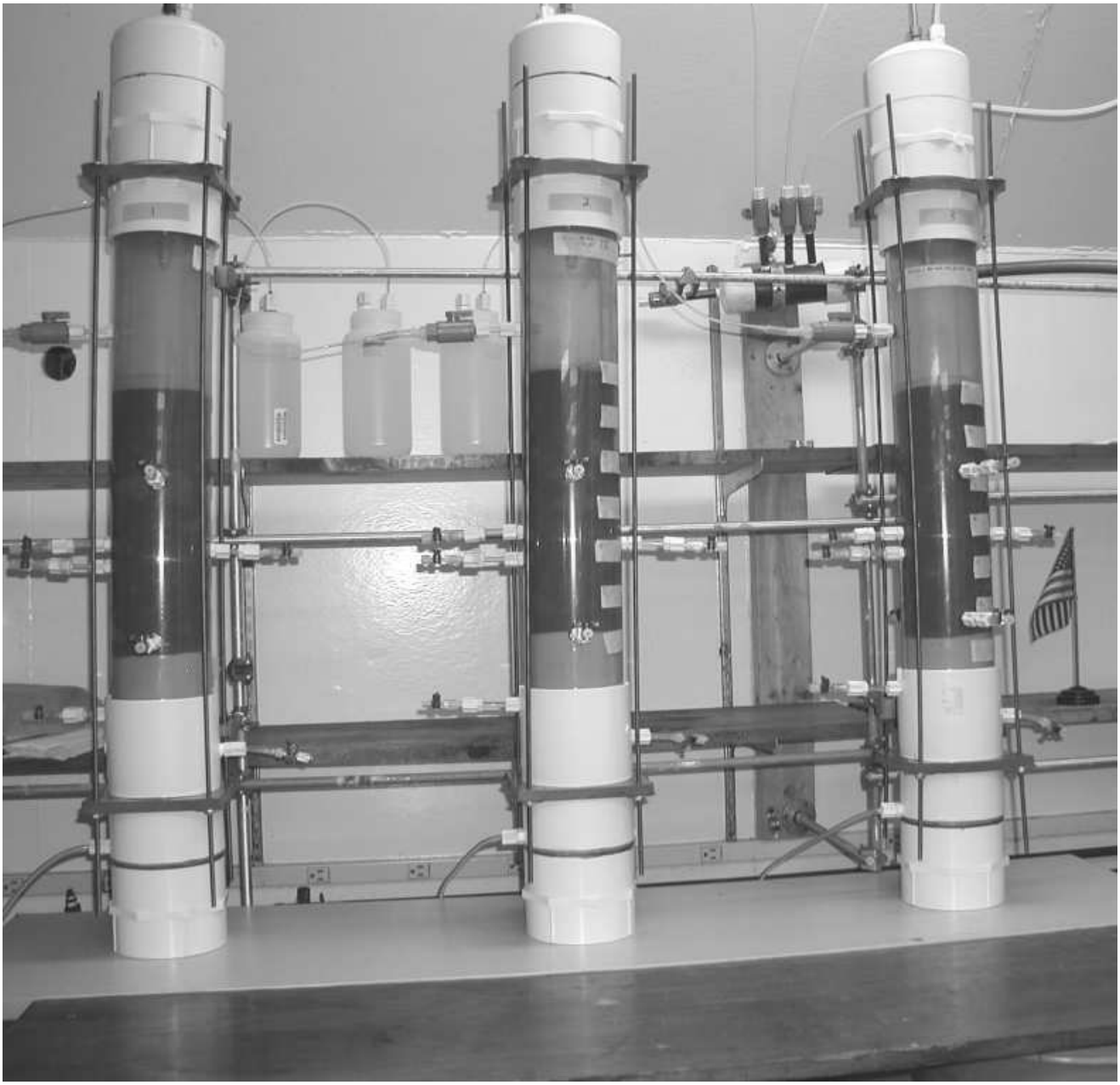


Figure 7. Moisture retention curves for unsaturated soil cores.

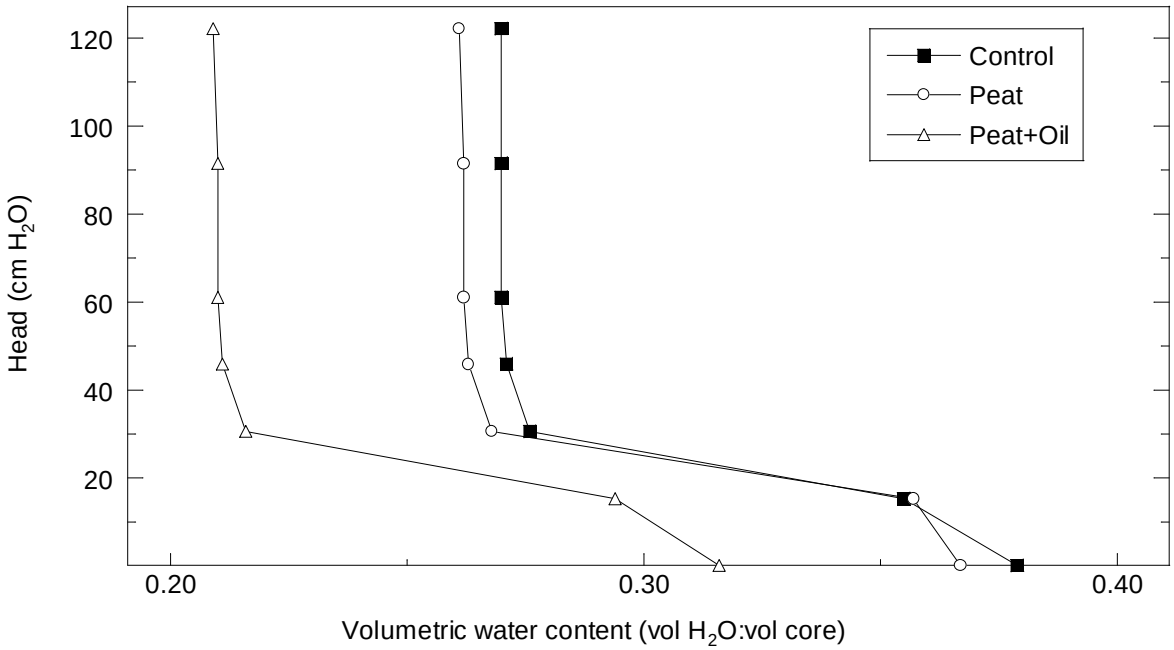


Figure 8. Time profile of bromide concentrations 4” and 8” below the surface of the soil in the repacked cores.

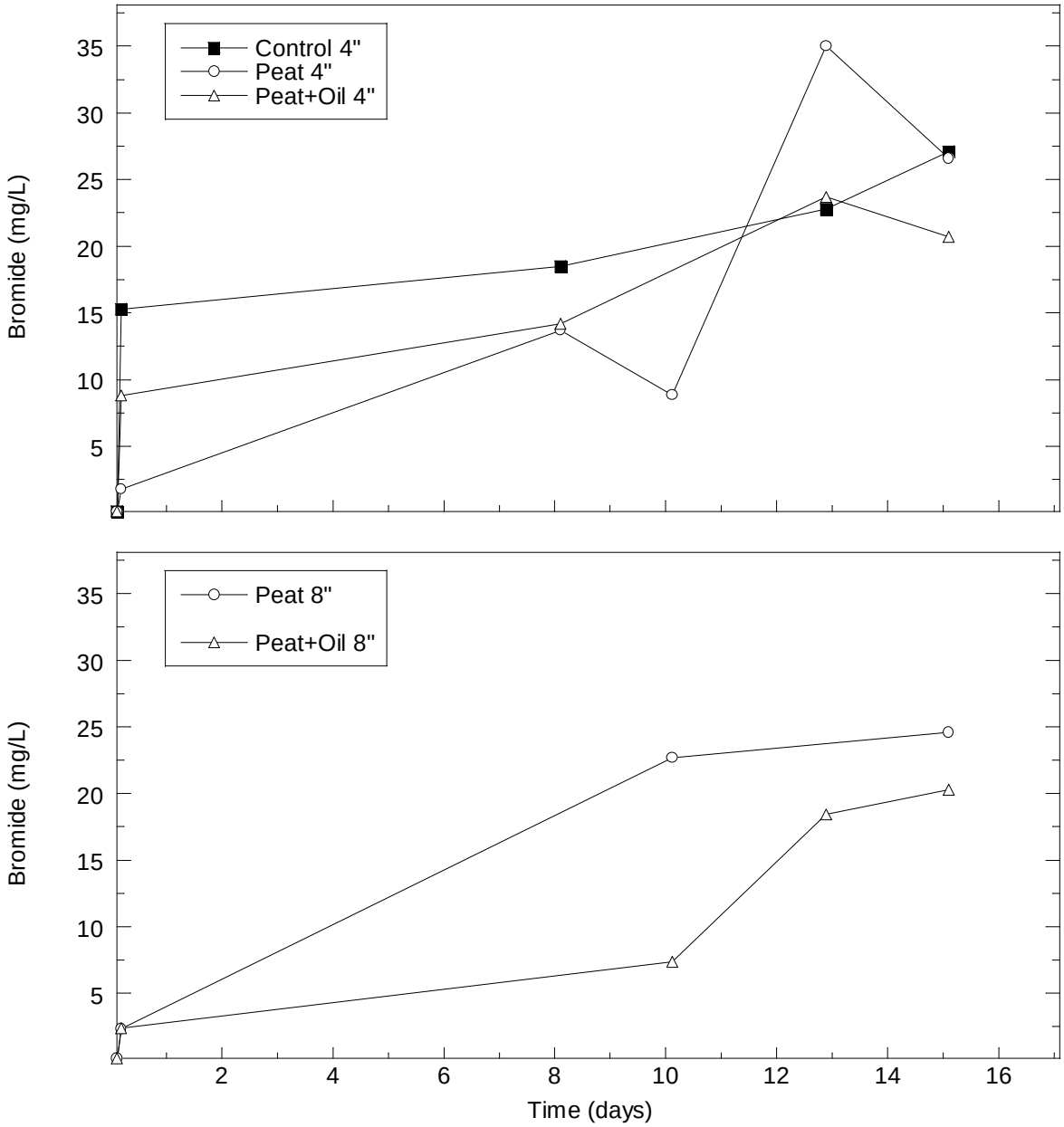
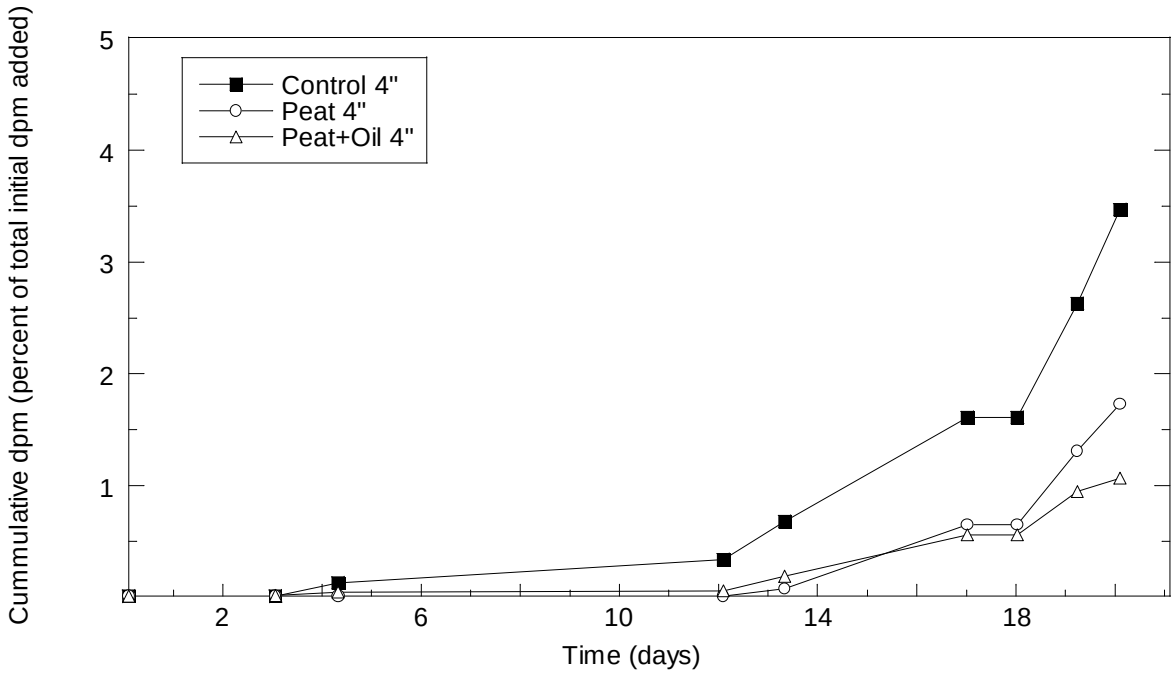


Figure 9. Time profile of cummulative [^{14}C] appearing at a depth of 4" below the soil surface in the repacked cores.



United States
Environmental Protection
Agency

Prevention, Pesticides
And Toxic Substances
(7508W)

EPA 738-R-93-031
December 1993

EPA

Reregistration Eligibility Decision (RED)

Flower and Vegetable Oils

Regulatory Conclusion

The use of currently registered pesticide products containing flower and vegetable oils in accordance with approved labeling will not pose unreasonable risks or adverse effects to humans or the environment. Therefore, all current uses of these products are eligible for reregistration except the use of essential oils in antimicrobial products, which is not eligible for reregistration. Essential oils must either be deleted from or converted to inert ingredients in antimicrobial products.

The eligible flower and vegetable oils products will be reregistered once the required physical chemistry studies, terrestrial ecological effects data for oil of mustard, product-specific data, revised Confidential Statements of Formula and revised labeling are received and accepted by EPA. Products also containing other active ingredients will be reregistered only when the other active ingredients are eligible for reregistration.

4. For Soybean Oil:

Type of Pesticide: acaricide and insecticide

Use Sites: citrus fruits, household/domestic dwellings outdoor premises, ornamental and/or shade trees, ornamental and/or shade trees, ornamental herbaceous plants, ornamental nonflowering plants, ornamental woody shrubs and vines, recreational areas.

Target Pests: citrus rust mite, clover mite, Texas citrus mite, citrus red mite, European red mite, two-spotted spider mite, thrips, anobiid beetles, cerambycid bark beetles, lyctid beetles, rose chafer, Japanese beetle, gnats, rose midge, midges, mosquitoes, homopterans, whiteflies, aphids, leafhoppers, mealybugs, roseslug, gypsy moth (eggs).

Formulation Types Registered: emulsifiable concentrate

Method and Rates of Application:

Equipment - high volume ground sprayer, sprayer,

Method and Rate - up to 10.67 gallons per acre or up to 0.5 fluid ounces in 1 gallon of water diluent.

Timing - when needed or foliar or potted

Use Practice Limitations: none

Soybean oil is obtained from soybeans, Glycine max., by solvent extraction using petroleum hydrocarbons or, to a lesser extent, by expression using continuous screw press operations. The oil is usually refined with alkali. It is a pale yellow to brownish-yellow oil. It is miscible with organic solvents (3).

Soybean oil is used in three currently registered products for use as an insecticide and miticide on citrus fruits and a variety of ornamentals. Soybean oil is considered to be GRAS by FDA (21 CFR 173.340 and 182.70). It is commonly used as a human food. It is widely ditributed in commerce and available to the general public throughout the United States for nonpesticidal uses. There are no incident reports on file for soybean oil. Soybean oil has a non-toxic mode of action for the target pests. It is an organic compound known to be rapidly degraded in the environment to elemental constituents by normal biological, physical and/or chemical processes that can be reasonable expected to exist where the pesticide is applied. The active ingredient soybean oil in the registered product is not likely to result in adverse human health effects, based upon available reports and information.

Generally, these substances have low acute toxicity, except for allyl isothiocyanate, as described below. Also, many have GRAS (Generally Recognized As Safe) status by FDA under 21 CFR 172, 173, and 182 and are used in food preparation. Exposure to humans and the environment from the individual ingredients is expected to be very low. For example these products are formulated in very low concentrations (most at 1 percent or less) and the products are used at low volumes in the United States. Only the three current soybean oil-containing products are formulated with significant amounts (40, 40 and 93 percent). Since people are exposed to this compound from food or other sources, the incremental exposure from the pesticide products is expected to be negligible.

First Quarterly Progress Report

SERDP Project 1229 - Immobilization of Energetics on Live Fire Ranges
January 15, 2002

The objective of this project is to develop a cost-effective technology to immobilize energetic compounds (TNT, RDX, HMX, and breakdown products) released as residues at firing ranges to prevent their migration to groundwater. The goal is to develop an inexpensive soil treatment that can be readily applied over wide and remote areas prior to or immediately following firing range activities.

We are currently awaiting the contract for Year 2 of the project to be finalized, and are making preparations to begin work. Technical reporting for this project will begin again in April 2003.

Quarterly Progress Report

SERDP Project 1229 - Immobilization of Energetics on Live Fire Ranges
Year 2003 – First Quarter
April 4, 2003

The SERDP contract for FY 2002 funding of this project arrived at Envirogen on January 15, 2003. This report covers technical progress for SERDP Project 1229 from January 15, 2003 – March 15, 2003.

The objective of this project is to develop a cost-effective technology to immobilize energetic compounds (TNT, RDX, HMX, and breakdown products) released as residues at firing ranges to prevent their migration to groundwater. The goal is to develop an inexpensive soil treatment that can be readily applied over wide and remote areas prior to or immediately following firing range activities.

During the current quarter, activities have focused on:

• finishing data analysis for completed experiments (slurry and soil microcosms);
• preparing and submitting manuscripts for publication;
• continuing sampling and analysis of repacked soil core experiments;
• designing apparatus for planned laboratory experiments;
• identifying and contacting sources of explosives for planned experiments;
• design of planned meso-cosms scale experiments, and;
• estrablishing contacts with DoD facilities that may be interested in field testing this technology.

II. MICROCOSM STUDIES.

(Sorption-desorption isotherms, Cosubstrate screening in slurries, Combined cosubstrate-sorbent evaluation in unsaturated soil)

All microcosms studies have been completed. The results have been thoroughly analyzed, and two manuscripts have been written and submitted to *Environmental Toxicology and Chemistry* for publication. The manuscripts are entitled:

> Enhancing adsorption and biodegradation of TNT, RDX, and HMX in surface soils at military impact ranges: Sorption-desorption isotherms. P. Hatzinger, M. Fuller, D. Rungkamol, R. Schuster and R. Steffan.

> Enhancing adsorption and biodegradation of TNT, RDX, and HMX in surface soils at military impact ranges: Combined sorption and biodegradation. M. Fuller, P. Hatzinger, D. Rungkamol, R. Schuster and R. Steffan.

III. CONDUCT SOIL COLUMN STUDIES.
2. SOIL CORE TRANSPORT AND FATE EXPERIMENTS.
2.1 METHODS.
The methodology for these experiments were described in detail in the Year 2002 Second Quarter QPRs for this project. Briefly, MMR soil was repacked into three clear PVC tubes and instrumented to allow sampling of the aqueous phase of the soil. One column served as a control (no treatment), one received a layer of peat moss, and the third received peat moss plus soybean oil. Soil spiked with unlabeled and radiolabelled TNT, RDX, or HMX was added to the top of the columns. The columns were sealed, airflow was initiated, and precipitation events were begun. The $[^{14}C]CO_2$ that was evolved over time was trapped and measured using liquid scintillation counting. Liquid samples were collected at 4, 8, and 12 inches below the soil surface and analyzed using liquid scintillation counting and HPLC. The ambient temperature of the room the columns were located in was 65-75°F.

The cores were designated:
C1 Control (non-functioning 8" lysimeter)
C2 Peat
C3 Peat plus soybean oil

These experiments have been operating for approximately nine months. This includes an unfunded period from September 1, 2002 until January 15, 2003 before Year 2 funds arrived at Envirogen. During this period, the columns were maintained, but minimal experimental work was performed.

Precipitation added to the columns was changed from distilled water to actual rainwater on Day 27. The rainwater had a pH range of 4 to 5.5 S.U., and a conductivity of 15 to 50 µS/cm. Rainwater was collected and stored at 4°C. Prior to use, rainwater was passed through a 4.5 µm pore size glass fiber filter to remove large particulates. Precipitation was added as larger rain events, as well as smaller volumes meant to simulate a typical morning dew deposition. A graph depicting precipitation events is presented in Figure 1. Bromide (100 mg/L) was added as a conservative tracer to the precipitation event on Day 4 and Day 171.

2.1 RESULTS.
RDX MINERALIZATION.
Results to date are summarized in Figure 2. Mineralization was not very evident during the first month after the experiment began. It was suspected that the continuous flow of humidified air into the cores was not allowing the slightly anoxic conditions needed for RDX biotransformation to occur. Therefore, humidified air flow into the columns was changed approximately 40 days after the start of the experiment from a continuous flow to 0.5 h of flow six times per day (total of 3 h per day). This flow regime allows the headspace in the columns to be replaced approximately 30 times per day, but would still allow some local areas of low oxygen to occur within the peat moss and/or slightly below the soil surface.

The greatest amount of [^{14}C]RDX was converted to [^{14}C]CO_2 in the core with peat moss plus soybean oil (C3), and the onset of mineralization in C3 coincided with the change in the humidified air flow regime. The mineralization exhibited a rapid initial rate for 150 days followed by a much slower rate after Day 180. A slower but steady mineralization of RDX was observed in the unamended control (C1), which did not seem to coincide with the change in air flow. Essentially no production of [^{14}C]CO_2 was observed in the peat moss only treatment (C2).

The overall low degree of mineralization in any of the columns relative to the amount of [^{14}C]RDX converted to [^{14}C]CO_2 during the previous microcosm experiments probably reflects a combination of factors, all of which have some bearing on the assessment of this technology:

1) As mentioned above, the aeration status of the soil and soil amendment layers may have been too high, or the flow of air may have been too fast, to allow low oxygen areas to be maintained. Some modeling of air flow above the ground surface may be possible to see if low oxygen areas can be established and maintained under typical environmental conditions. It seems likely, however, that at the interface between the peat moss ± soybean oil and the soil that the needed conditions could occur.

2) The dissolution and migration of the of the explosives from the spiked soil applied at the top of the columns may be very slow, thereby limiting the amount of [^{14}C]RDX that is available for mineralization.

3) Related to dissolution, the majority of the [^{14}C]RDX that does dissolve and begin to migrate into the biologically "active" zone in the peat or peat plus soybean oil becomes sorbed and unavailable to the microorganisms.

In general, the mixing of the [^{14}C]RDX into the soil and amendments was much less than in the microcosms, which also may have reduced the total mineralization. These columns are being operated under conditions more relevant to actual field contamination scenarios, so the mineralization data may simply not be the best basis for evaluating the technology. Rather, it may be more important to assess the downward migration (leaching) of explosives.

EXPLOSIVES LEACHING.
NOTE: Fewer samples at 8" below the soil surface were obtained from the control column (C1) due to a malfunctioning sampling device.

Bromide and dpm concentrations reaching 4" and 8" below the soil surface in the three columns are depicted in Figures 3 and 4. The bromide concentrations detected at 4" were similar in both the control and treatment, and at 8" in both the treatment columns. This indicates that the water movement down through the soil was comparable in all three columns.

After 260 days, the cummulative amount of [^{14}C] dpm reaching 4" below the soil surface in C3 was only 40% that detected at the same depth in control column C1. Peat moss alone in C2 resulted in [^{14}C] dpm at 4" below the soil surface that were approximately 60% those observed in the control column. These results indicate that both peat moss and peat moss plus soybean oil

treatment significantly reduced the downward leaching of [^{14}C]RDX and/or its metabolites. This pattern (C1 > C2 > C3) in the amount of radioactivity detected was also observed for the other sampling depths within the columns (Table 1).

Soluble concentrations of TNT, RDX, and HMX and the RDX metabolites MNX, DNX, TNX, as determined by HPLC analysis, at 4" and 8" below the soil surface are presented in Figures 5, 6, 7, and 8. A summary of the maximum concentrations of all the explosives for the whole columns is given in Table 2. The sharp decreases in soluble explosive concentratrions observed around Day 45 followed the large precipitation event on Day 35, indicating that soluble explosives were likely flushed out of the soil. Except for a few sporadic points, the concentrations of TNT, RDX, and HMX, and of the RDX metabolites was always lower in C3 than in either C1 or C2.

TNT was not detected at all in C3 at a depth of 4" below the soil surface, and has not been detected at that depth in C2 since approximately Day 80. TNT metabolites (2A-DNT, 2,4-DNT, 4A-DNT, and 2,6-DNT) were only detected a few times in any of the cores, and concentrations were higher in C1 that in C2 or C3. This reflects the likelihood that TNT and its metabolites have been rendered immobile by being strongly sorbed to the peat moss. Results for samples from 8" below the soil surface were similar

After 260 days, the RDX concentration 4" below the soil surface in C3 were approximately 33% of those in C1. The concentration of RDX in C2 was slightly higher than in C1 (4 vs. 3 mg/L). The concentrations of the RDX metabolites MNX and TNX were generally higher than those of DNX. These metabolites were detected in all the columns, and there was no clear indication that the treatments increased the concentrations of these compounds at 4". RDX concentrations in C3 at 8" was approximately 50% of that in C2 at the same depth, and the concentrations of RDX metabolites were lower in the column amended with peat moss plus soybean oil (C3) than in the one amended with peat moss only (C2).

The concentrations of HMX detected at 4" and 8" showed a similar patter, in that a jump in concentrations was observed after the large precipitation event on Day 171. This possibly reflects the increase dissolution of the relatively insoluble HMX by the added rainwater. However, since a corresponding increase in TNT and RDX was not observed, the reason for the increase in HMX concentrations is not completely understood. In any case, by Day 260, the concentrations of HMX in the C2 and C3 at a depth of 4" below the soil surface were approximately 60% and 50%, respectively, of those in C1.

Taken together, these results indicate the potential for this soil amendment technology to greatly reduce the leaching of explosive residues through soils similar to those at MMR. They also indicate that the application of a combination of a sorbent and a cosubstrate (i.e., peat moss plus soybena oil) yields better results than the application of a sorbent alone.

$^{14}CO_2$ TRAPPING EFFICIENCY.

An experiment was also performed to evaluate the efficiency of KOH traps for [^{14}C]CO_2. This experiment was performed using the control column (C1). A 5 mL aliquot of deionized distilled

water (ddH_2O) was made alkaline (pH >11 S.U.) by adding 5 µL of 10 N NaOH. An aliquot of $[^{14}C]NaCO_3$ was mixed into the basic solution, and the initial dpm/mL in triplicate subsamples was measured using liquid scintillation counting. A total of 5 mL of this solution (2.8×10^6 dpm) was then frozen into a small glass jar overnight at -20°C. Alkaline ddH_2O was sequentially frozen on top of the radioactive ice until an additional 7 mL had been applied.

Column C1 was prepared for use by placing an plastic-wrapped cardboard circle on the surface of the soil. This circle prevented $[^{14}C]CO_2$ from the soil from being released into the headspace, and also prevented acid vapors from entering into the soil. The jar of frozen $[^{14}C]NaCO_3$ was placed on the circle, and 1.0 mL of 6 N HCl was added. This amount of acid was shown to reduced the pH of the alkaline ddH_2O to <0.05. As the alkaline ice melted, and the acid mixed with the solution, the $[^{14}C]NaCO_3$ was converted to $[^{14}C]CO_2$, which was released into the column headspace and flushed into the KOH base traps. The acidified solution and base traps were sampled after 2 days, and the radioactivity was determined by using liquid scintillation counting.

The results indicated that no residual $[^{14}C]$ remained in the jar. The overall trapping efficiency of the active and passive KOH traps was determined to be 66% for this high concentration burst of $[^{14}C]CO_2$. The trapping efficiency of the KOH for the $[^{14}C]CO_2$ produced from the $[^{14}C]$RDX in the columns can therefore assumed to be at least 66%. However, the actual trapping efficiency for the columns may be higher, because 1) the $[^{14}C]CO_2$ is released slowly over time rather than in a huge burst; 2) the traps usually scrubbed the air exiting the columns for seven to fourteen days or more, rather than just two days, and; 3) the $[^{14}C]CO_2$ concentrations in the KOH traps from $[^{14}C]$RDX were typically 100-fold lower than those seen during the efficiency test, reducing the chances that the base traps would become saturated.

3. CONDUCT EXPERIMENTS WITH CRYSTALLINE EXPLOSIVES AND/OR ACTUAL EXPLOSIVE COMPOSITIONS.

Due to the delay in receiving SERDP funds for Year 2, there has been a delay in obtaining materials, setting up, and running these experiments.

Obtaining the types of materials needed for these experiments has become more difficult due to the heightened level of security after the September 11, 2001 attacks. We are currently in contact with people at the Naval Surface Warfare Center (IHNSWC) in Indian Head, MD about receiving samples from their milling facilities.

We have initiated discussions with Dr. Thomas Jenkins and Ms. Marianne Walsh a the U.S. Army Corp of Engineers, and with the UXO experts at Shaw Environmental & Infrastructure, about obtaining actual explosives residues from controlled detonations. This latter material is relatively limited, and it may not be possible to obtain enough of it to perform the experiments. Therefore, we are planning on using contaminated MMR soil as the source of explosive residues for most of these experiments.

Also, in light of the results to date, we are adjusting the research plan for this Task from what was described in the White Paper submitted at the end of Year 1. This White Paper described a series of additional microcosm and unsaturated soil column experiments looking at soil amendment application procedures, additional soil amendment combinations. We believed that using a test system somewhere intermediate between microcosms and the large unsaturated soil columns is more appropriate. These test systems are illustrated in Figure 9, and are currently being constructed.

The basic system will be a short repacked core of clean MMR soil in a polypropylene bottle. The soil column will receive precipitation at the surface, and the soil moisture will be maintained at below water holding capacity by constant application of a vacuum at the bottom of the bottle. Leachate will be collected and analyzed as in previous experiments. They will allow us to examine more variables at once, while still being run under more field-relevant unsaturated flow conditions. The combination of less soil depth (4" vs. 12" in the columns), and larger and more frequent precipitation will allow the timeframe of the experiments to be accelerated compared to the larger columns

Some of the variables to be examined using these soil columns will include
• Application rate (depth of peat plus soybean oil)
• Application method (surface vs. below a shallow layer of soil)
• Mobilization and leaching of actual explosives formulations (i.e., Comp B, Octol)
• Potential for non-aqueous phase transport of explosives in the soybean oil

These experiments are expected to be underway within one month, and continue for several months.

V. MESO-SCALE SOIL CORE EXPERIMENTS.
1. DESIGN AND CONSTRUCT CORES.
2. COLLECT INTACT /VEGETATED CORES FROM MMR
The conceptual basis for these tasks is illustrated in Figure 10. The funding delay has resulted in a delay in both of these Tasks as well. However, we have done a significant amount of locating materials, planning core collection, and design of the experiment. Several issues arose during the design of these meso-scale core experiments that are currently being addressed:

1) Collect the intact cores at MMR and transport them to New Jersey, or collect and set-up the intact cores on site at MMR. We are currently planning on the latter option, because this will reduce the chance of damage (i.e., fracturing) the cores during transport, and also allow the experiments to be performed under actual MMR environmental conditions. Envirogen's Canton, MA office has determined that MMR personnel are open to this option, and arrangements are being worked out.

2) Operation of the cores under unsaturated conditions. As with the unsaturated soil columns, a constant negative head needs to be applied to the meso-scale intact soil cores in order to keep the

water in the soil below saturation (or water holding capacity). There are basically two ways of doing this, both with advantages and disadvantages:

a] First, the cores can be elevated above the ground on a platform, with a capillary water column attached to the bottom of each core. This option presents some safety issues, and a large diameter porous ceramic plate must be placed the bottom of the soil core. However, this option does not require any electricity, so the cores can be kept relatively close to the area of the field from which they were taken.

b] Secondly, the cores can be placed on a platform with a lower portion that is maintained at less than atmospheric pressure using a vacuum pump. This is similar to what will be done with the smaller soil cores that are being constructed. Alternatively, the vacuum can be applied individually to each core. Electricity is required for the pump, which means the cores cannot be kept far out in the field unless a battery or solar powered pump is used. However, safety issues are less of a concern.

Both options (a) and (b) are being evaluated, but placing the cores on a platform with a vacuum source is currently preferred.

Once these issues are settled, this work will move forward. It is expected that the experiments will be up and running by June 2003.

VI. EVALUATE TECHNOLOGY PERFORMANCE AND PLAN FOR YEAR 3 FIELD-SCALE DEMONSTRATION

In advance of completing the work for Year 2 of this project, we have established contacts at several DoD facilities where a field demonstration of this technology could be performed. The potential sites identified at this time for a field demonstration include:

1) The Massachusetts Military Reservation. MMR is a primary site, given that a substantial amount of the previous research has been conducted using soil from this facility. Envirogen has established very good working relationships and has earned a good reputation with the military administrators and other private contractors at MMR.

2) Westover Air Reserve Base (ARB) in Chicopee, MA. We have established contact with personnel in the Base Civil Engineering Office regarding a field demonstration of this technology, and they seem very optimistic about the proposed project. Westover ARB is currently in the planning/design phase for a new EOD proficiency training area, and officials there have expressed great interest in testing and implementing the proposed technology as part of their effort to achieve best management practices and sustainability of the new training area. Envirogen personnel from our Canton, MA office have also visited the site.

3) Picatinny Arsenal in Rockland, NJ. Picatinny Arsenal is currently exploring the option of establishing a new munitions test site at their facility. The test site would be self-contained with an integrated leachate collection sytem. This type of test site presents an ideal location for assessing the applicability of the soil amendment technology for mitagation of explosives residues over small areas.

4) The Naval Surface Warfare Center (IHNSWC) in Indian Head, MD. Indian Head has several areas where experimental plots could be established. Envirogen has developed contacts in several divisions at Indian Head while conducting laboratory-scale research (SERDP CU-1163) and a field-scale demonstration of *in situ* perchlorate bioremediation technology at Indian Head for the past three years.

Envirogen, Inc. was recently acquired by The Shaw Group, and Envirogen's Technology Development Group will become part of Shaw Environmental & Infrastructure, Inc.'s (Shaw E&I) Science and Technology Group. The merger of Envirogen and Shaw E&I is expected to provide access to additional sites for developing and testing the proposed technology, as well as provide ready outlets for technology transfer and commercialization of the technology should positive results be obtained during this project.

FIGURES AND TABLES

The tables and figures supporting this document have been provided to the SERDP Office as a separate attachment.

Table 1. Cummulative radioactivity at all points within the columns expressed as a percent of the amount applied at the surface .

Depth (inches)	%Initial dpm recovered at each depths		
	C1	C2	C3
4	19.4	12.2	8.3
8[a]	ND	4.8	3.3
12[b]	ND	0.4	0.2
16[c]	0.5	0.3	0.1

[a]No samples from C1 were able to be collected.
[b]No sample from C1, and only one sample from C2 and C3, from this depth was able to be collected, on Day 171 after the very large precipitation event.
[c]These samples were collected below the ceraminc plate at the bottom of each of the cores.

Table 2. Maximum concentrations of explosives and explosive metabolites detected at all points within the unsaturated soil columns.

Concentration	Depth below the soil surface											
	4”			8”			12”[a]			16”[b]		
(mg/L)	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3
HMX	3.2	3.3	3.2	5.8	3.7	2.3	-	2.8	1.0	1.9	0.7	0.3
TNX	3.6	2.3	1.4	-	2.0	1.5	-	-	-	-	0.1	-
DNX	0.6	0.3	0.3	-	0.3	0.2	-	-	-	-	0.1	0.1
MNX	0.5	5.2	4.8	-	4.3	2.8	-	-	-	8.9	-	-
RDX	12.7	8.8	7.1	-	5.6	3.8	-	-	-	0.2	0.9	0.5
TNB	-	-	-	7.2	-	-	-	-	-	-	-	-
TNT	0.4	0.1	-	0.2	1.0	-	-	-	-	-	-	-
NB	-	0.1	-	-	-	-	-	-	-	11.2	-	-
2A-DNT	2.9	0.4	-	-	-	-	-	-	-	1.9	-	0.3
2,4-DNT	-	-	-	-	-	-	-	-	-	11.2	-	-
4A-DNT	0.2	-	-	-	-	-	-	-	-	-	-	-
2,6-DNT	-	-	-	-	-	-	-	-	-	-	-	-
2-NT	0.2	-	-	-	0.1	-	-	-	-	-	-	-
4-NT	-	-	-	-	-	-	-	-	-	-	0.1	-
3-NT	0.2	-	-	-	-	-	-	-	-	-	-	-

[a]No sample from C1, and only one sample from C2 and C3, from this depth was able to be collected, on Day 171 after the very large precipitation event.
[b]These samples were collected below the ceraminc plate at the bottom of each of the cores.
[c]Below the analytical detection limit of 25 µg/L.

Figure 1. Precipitation history of the repacked unsaturated soil column experiments.

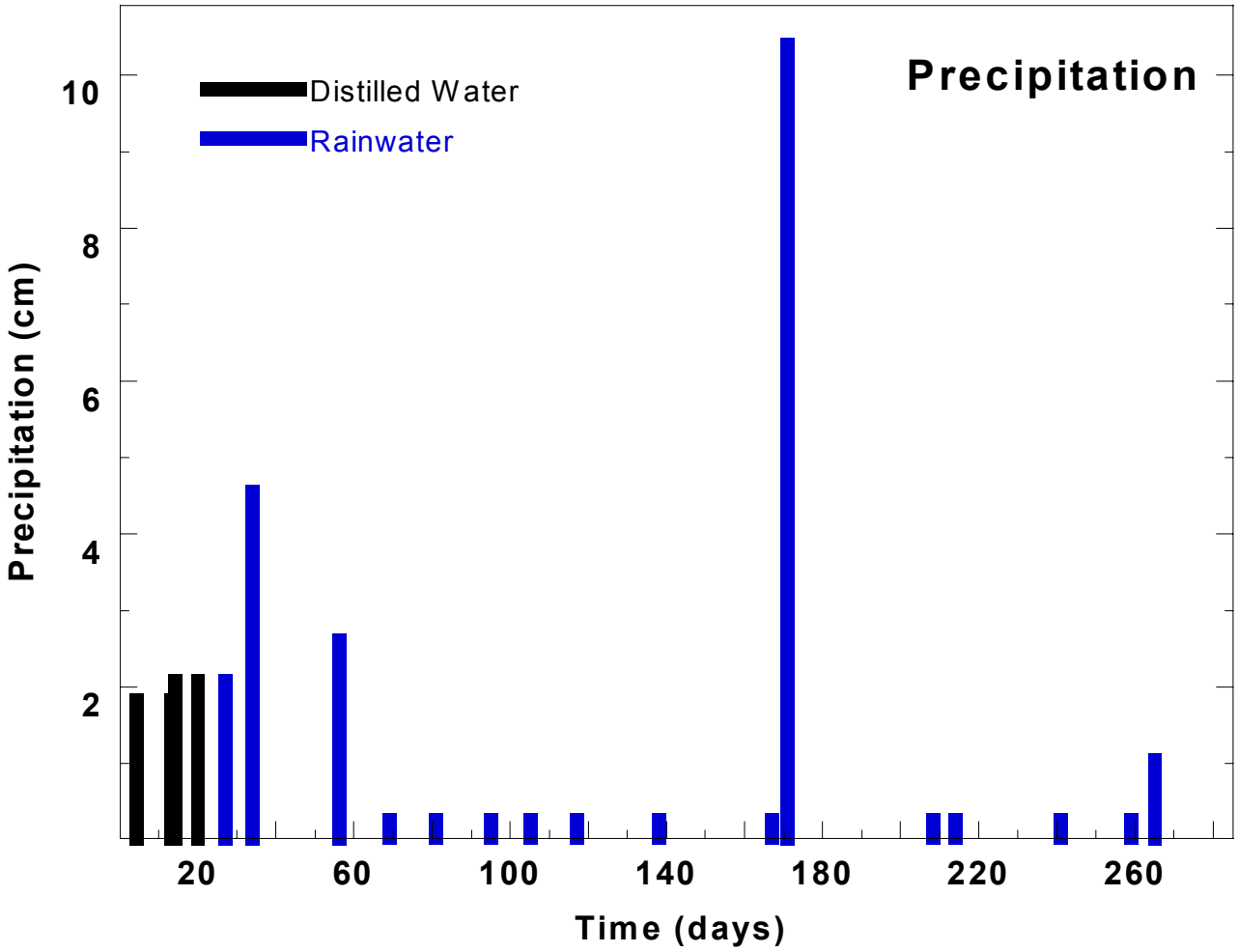


Figure 2. Mineralization of RDX over the course of the repacked unsaturated soil column experiments.

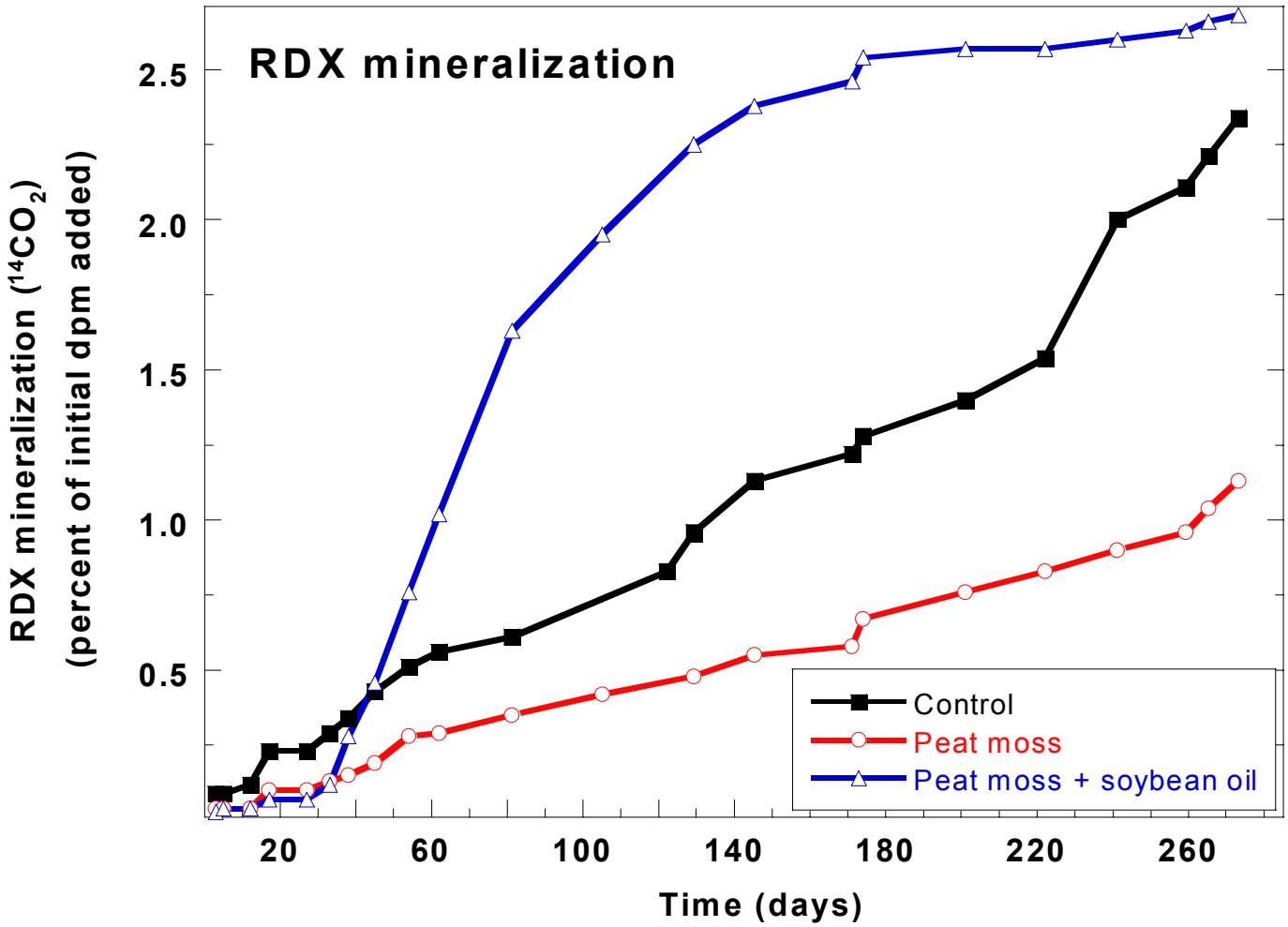


Figure 3. Concentrations of bromide and radioactivity (dpm) 4" below the soil surface over the course of the repacked unsaturated soil column experiments.

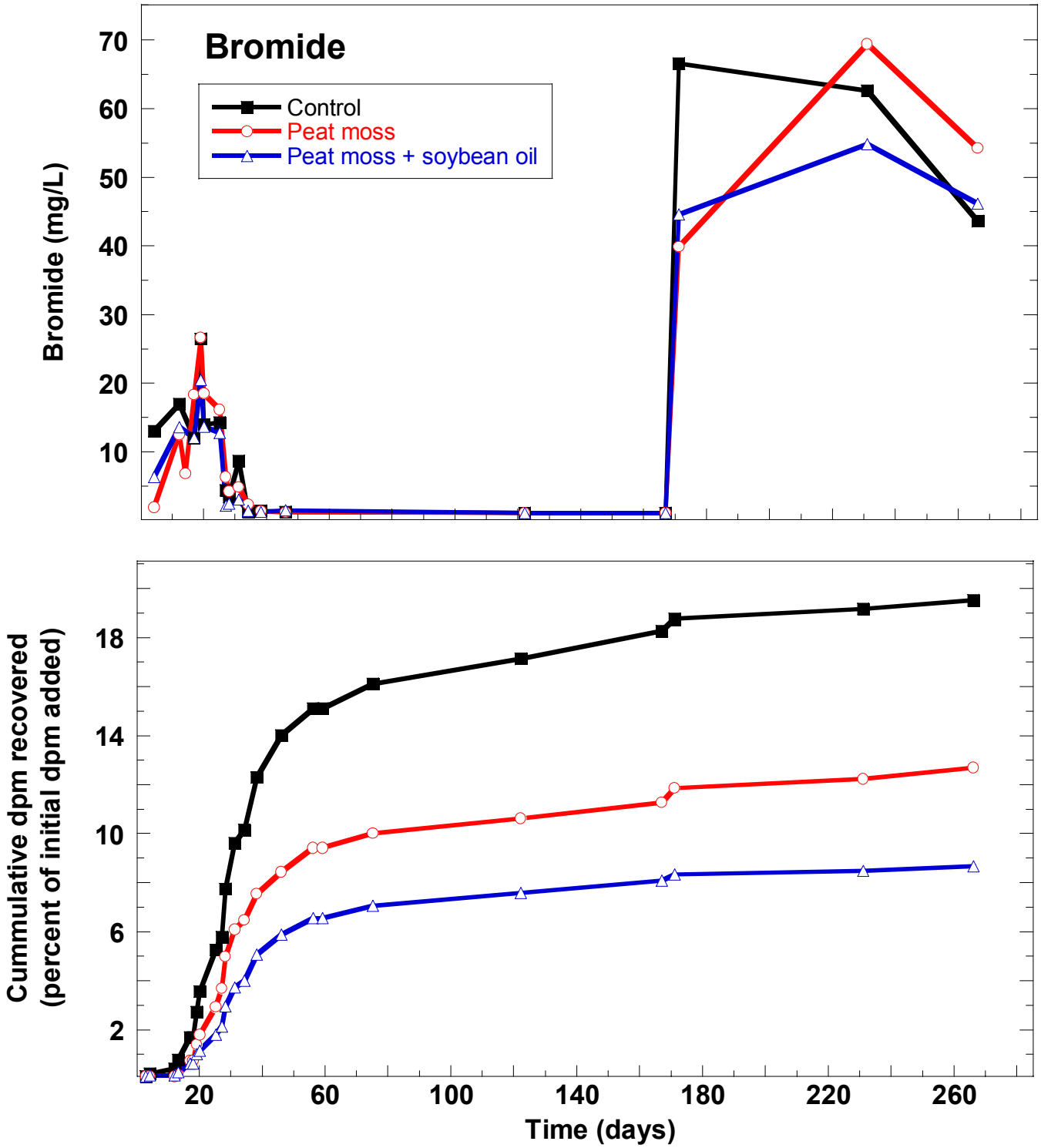


Figure 4. Concentrations of bromide and radioactivity (dpm) 8” below the soil surface over the course of the repacked unsaturated soil column experiments.

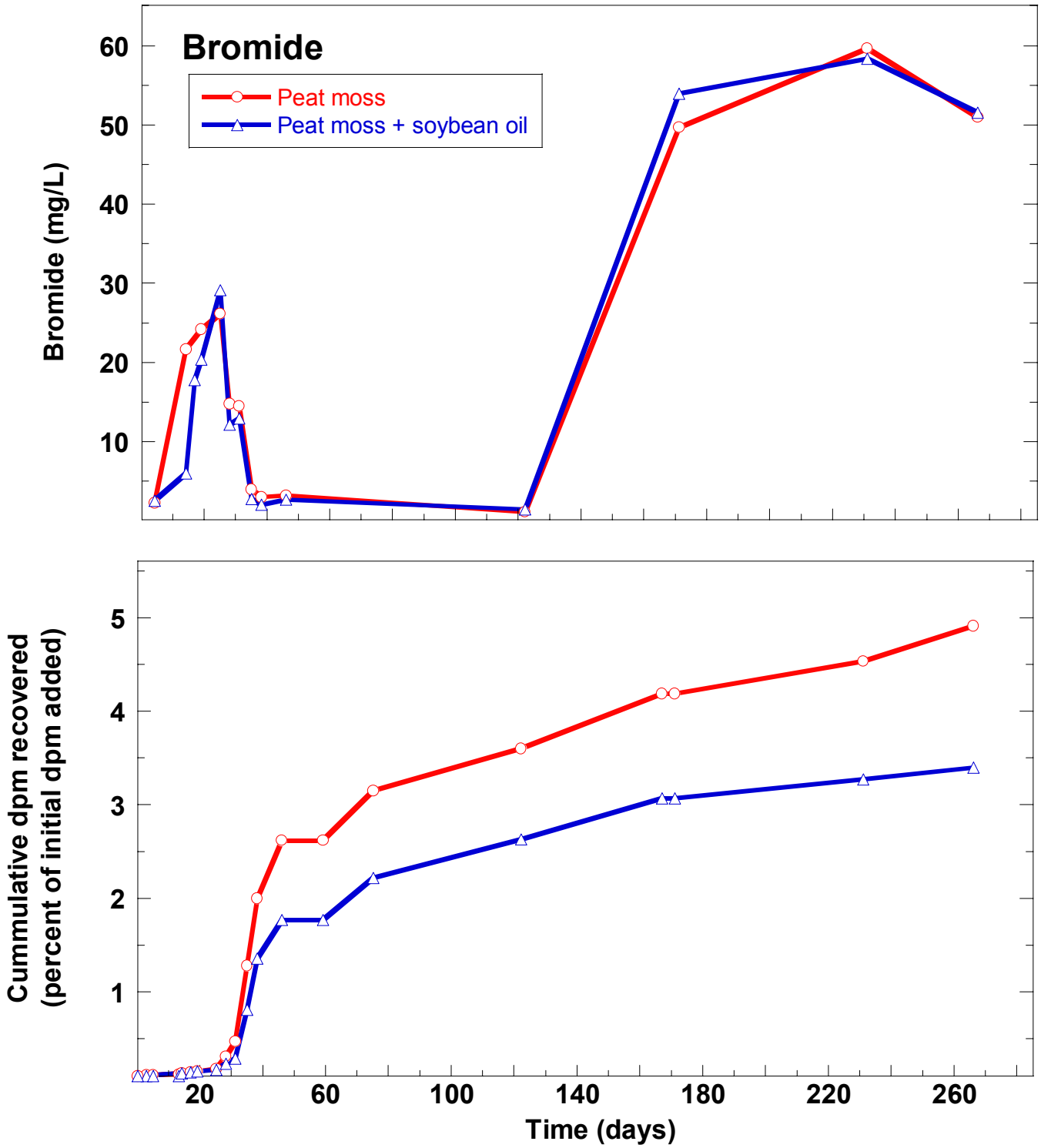


Figure 5. Concentrations of explosives 4" below the soil surface over the course of the repacked unsaturated soil column experiments.

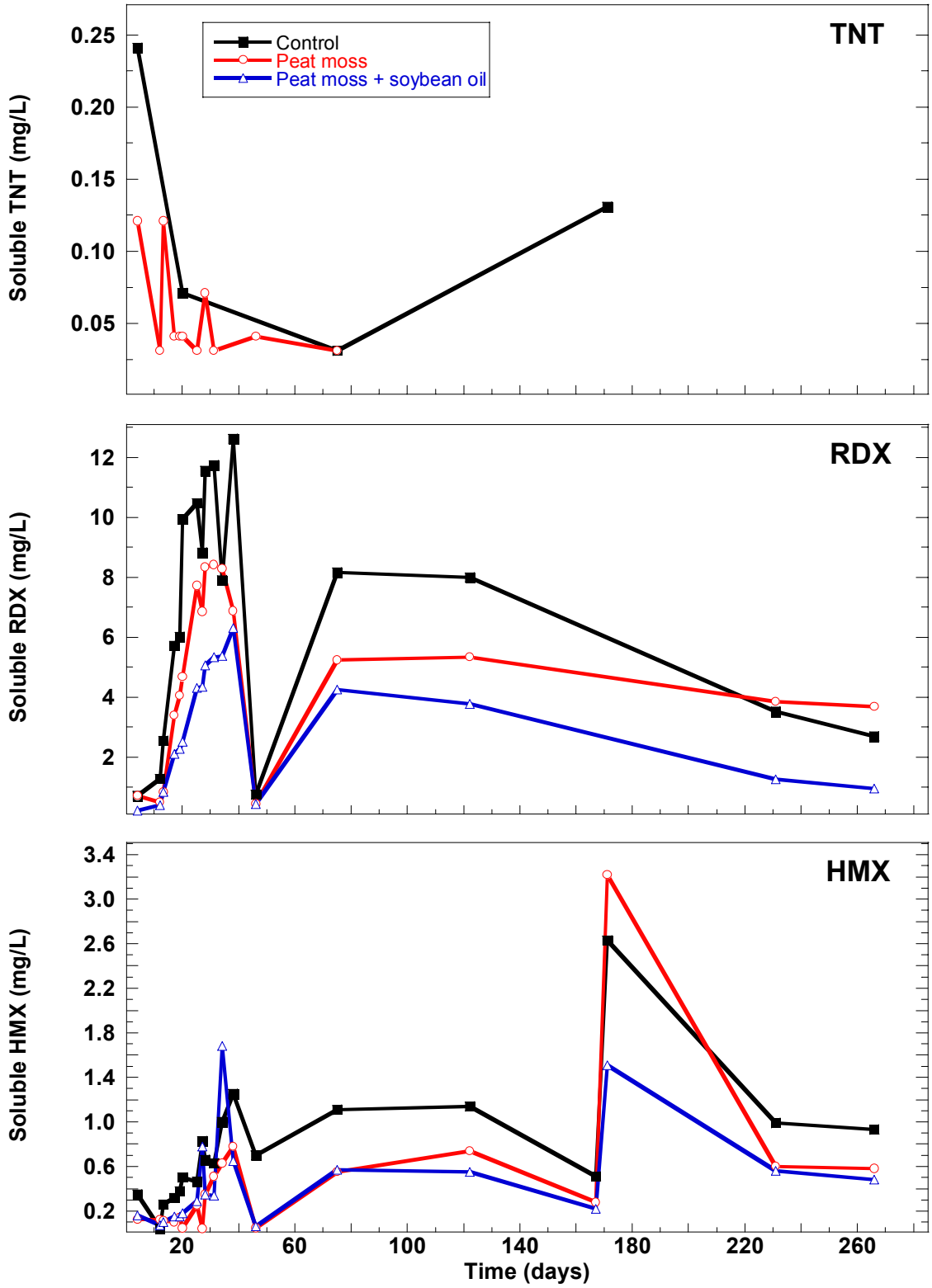


Figure 6. Concentrations of RDX metabolites 4" below the soil surface over the course of the repacked unsaturated soil column experiments.

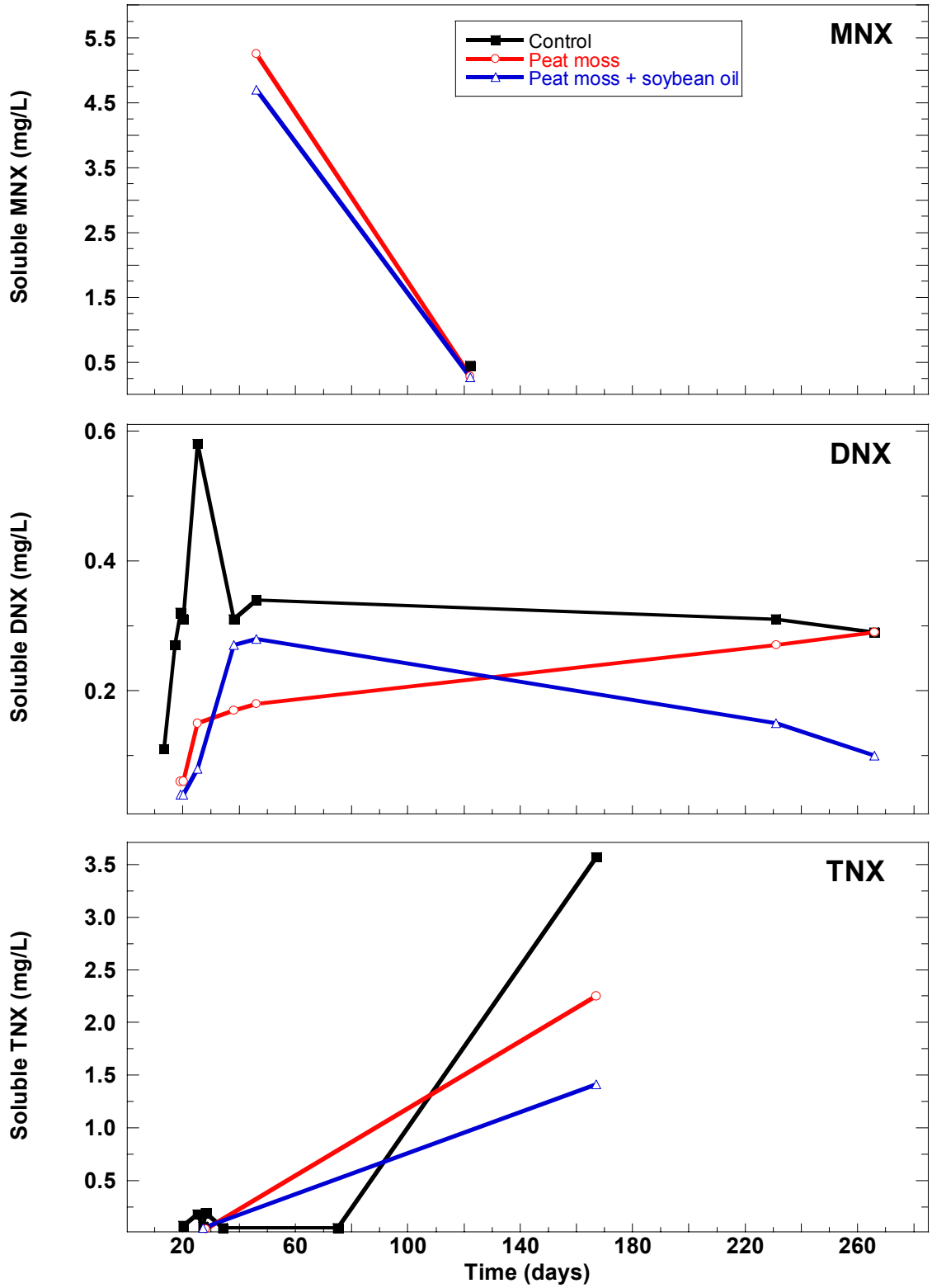


Figure 7. Concentrations of explosives 8" below the soil surface over the course of the repacked unsaturated soil column experiments.

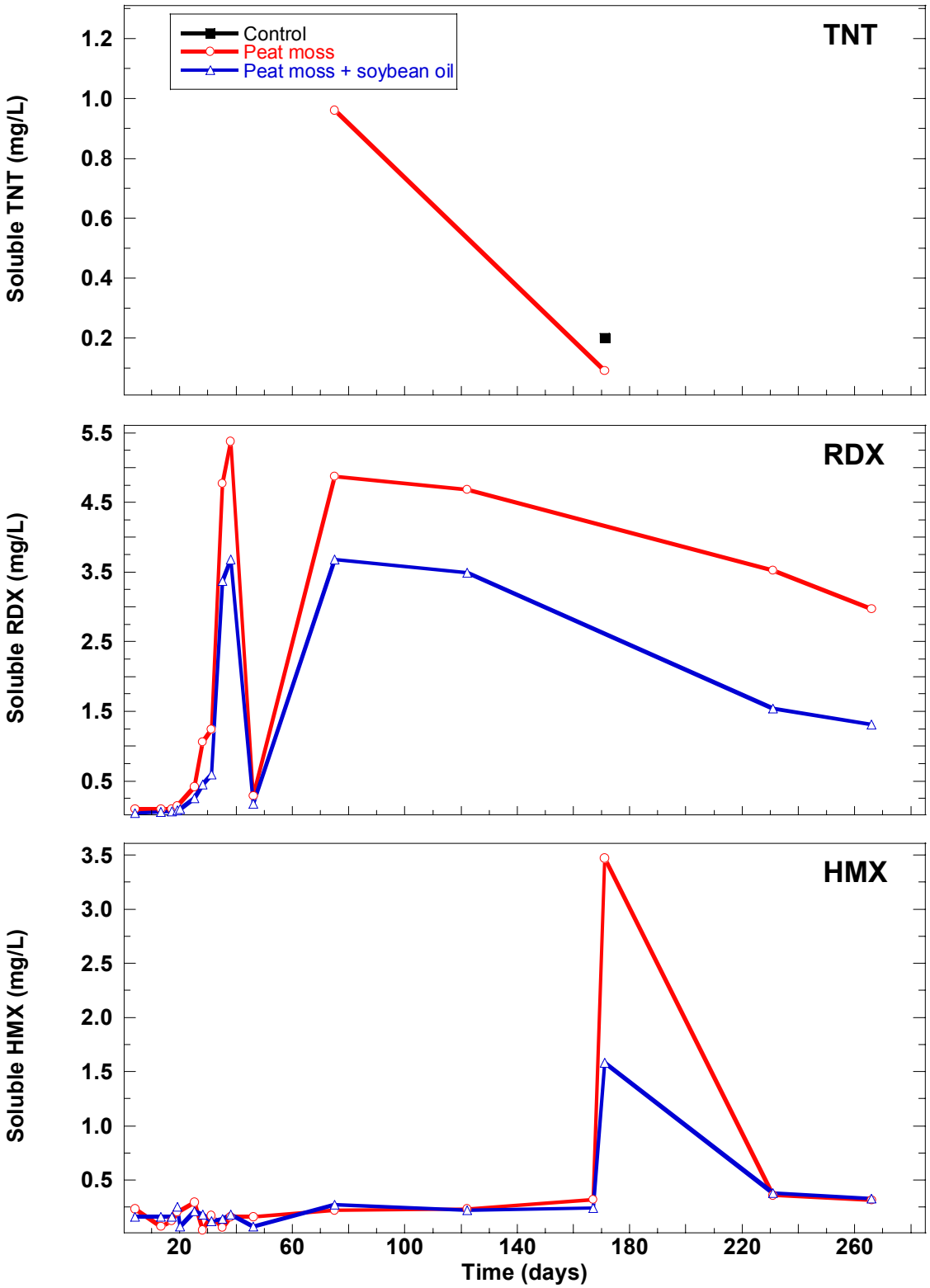


Figure 8. Concentrations of RDX metabolites 8" below the soil surface over the course of the repacked unsaturated soil column experiments.

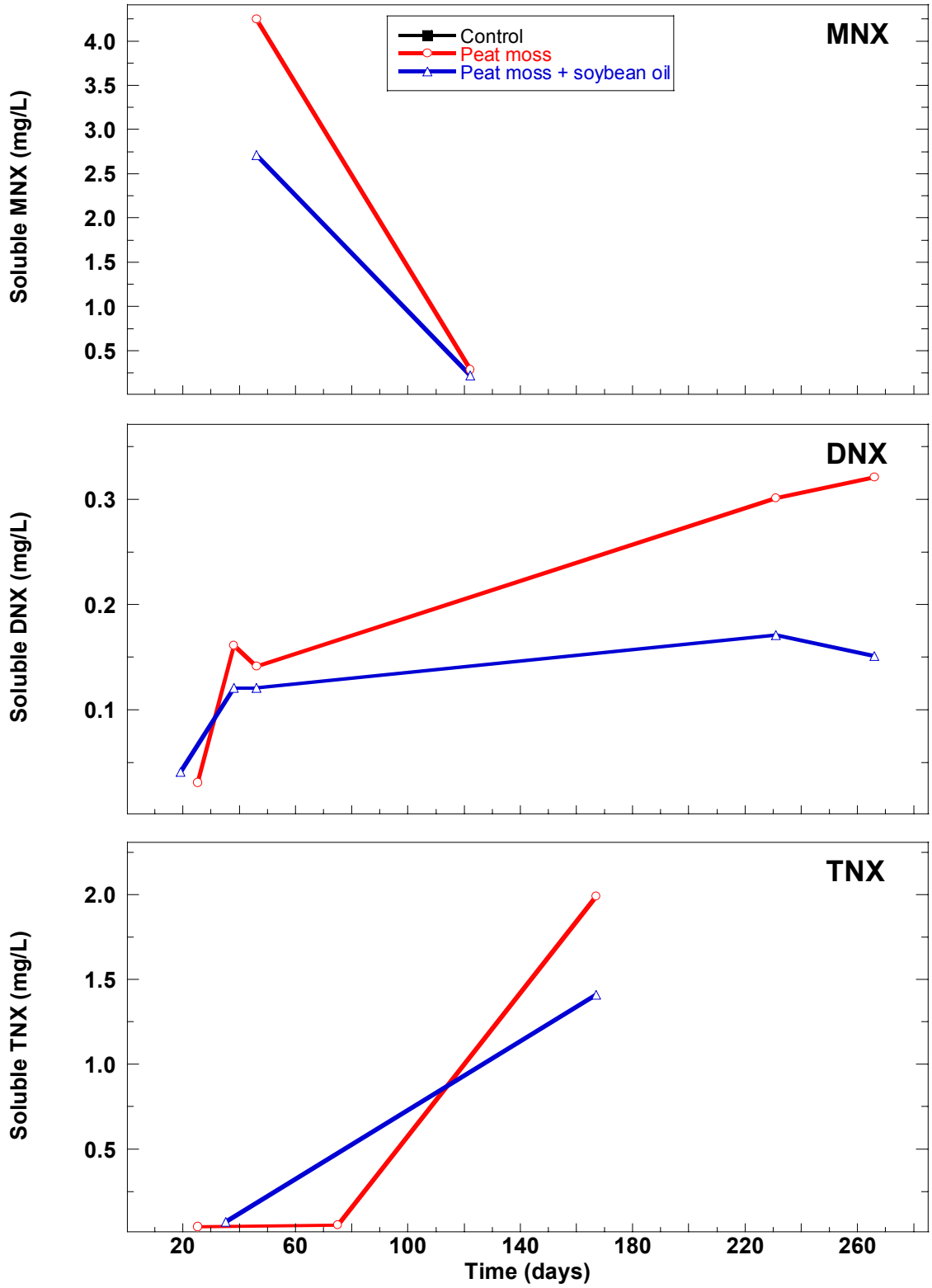


Figure 9. Illustration of the shorter soil columns to be used for Year 2 experiments.

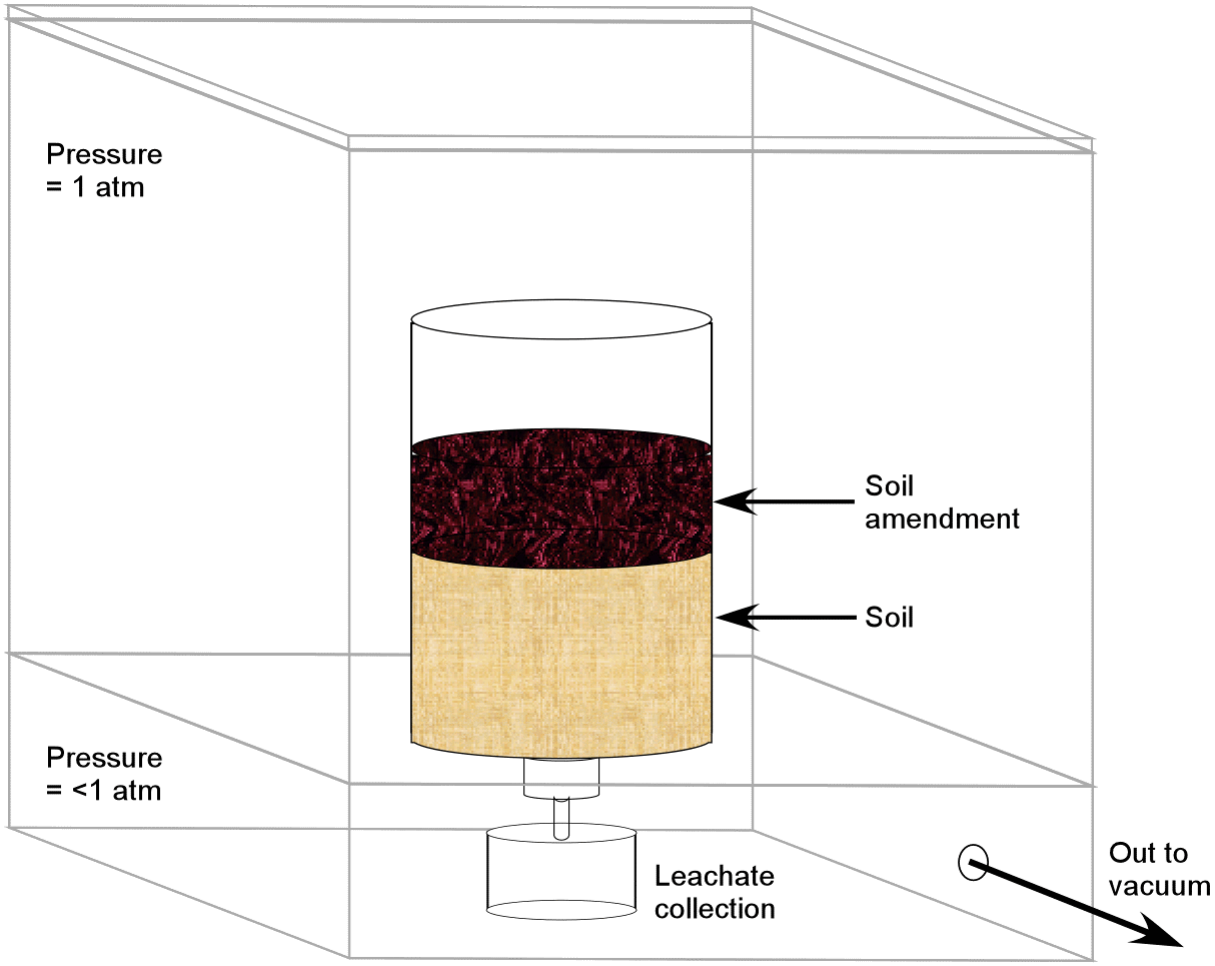
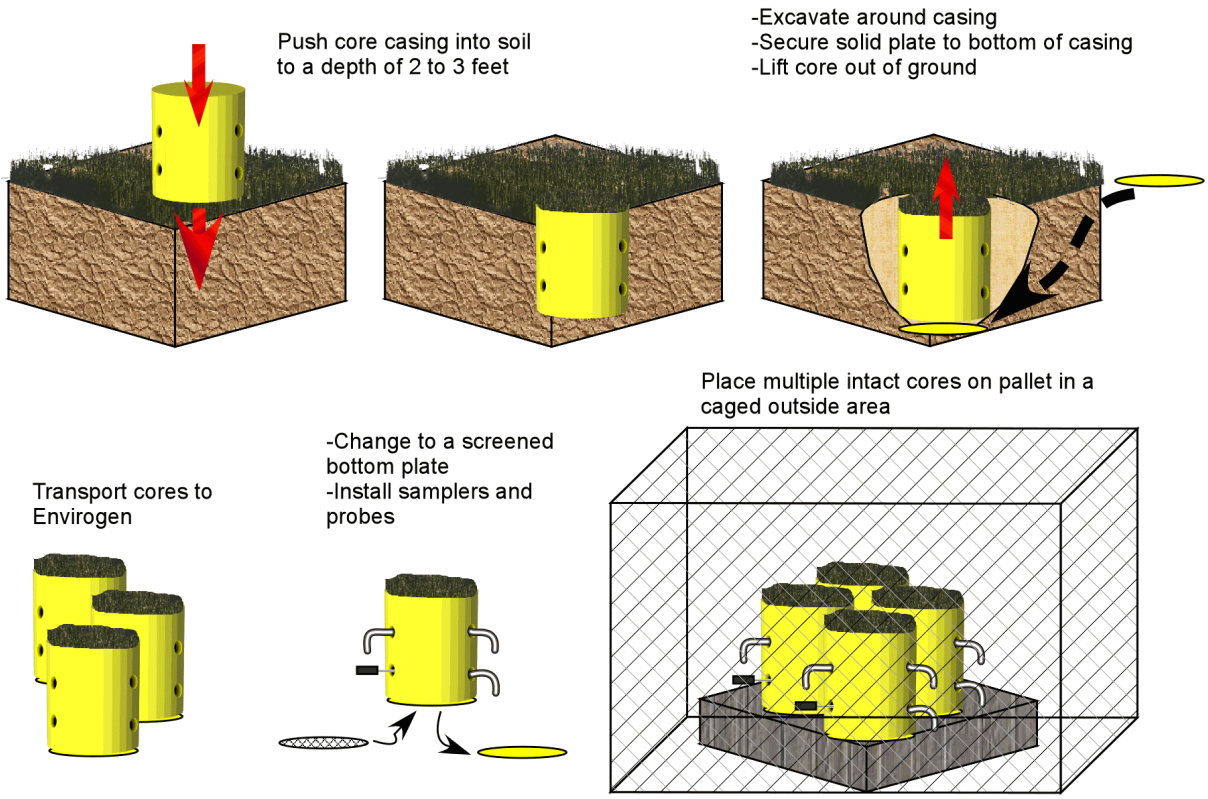


Figure 10. Conceptual design of the meso-scale intact soil core experiments.



Quarterly Progress Report

SERDP Project 1229 - Immobilization of Energetics on Live Fire Ranges
Year 2003 – First Quarter
July 15, 2003

The SERDP contract for FY 2002 funding of this project arrived at Envirogen on January 15, 2003. This report covers technical progress for SERDP Project 1229 from March 15, 2003 – June 15, 2003.

The objective of this project is to develop a cost-effective technology to immobilize energetic compounds (TNT, RDX, HMX, and breakdown products) released as residues at firing ranges to prevent their migration to groundwater. The goal is to develop an inexpensive soil treatment that can be readily applied over wide and remote areas prior to or immediately following firing range activities.

During the current quarter, activities have focused on:

• revising 2 manuscripts accepted for publication;
• continuing sampling and analysis of repacked soil core experiments;
• addressing issues discussed during the Spring IPR meeting, including
  - multiphase transport
- conceptualizing and modeling contaminant flux
  - effects of pH on contaminant migration/retention

II. MICROCOSM STUDIES.
(Sorption-desorption isotherms, Cosubstrate screening in slurries, Combined cosubstrate-sorbent evaluation in unsaturated soil)
All microcosms studies have been completed. The results have been thoroughly analyzed, and two manuscripts have been submitted and accepted for publication in *Environmental Toxicology and Chemistry*. The manuscripts are entitled:

1. Fuller, M. E., D. Rungmakol, R. L. Schuster, P. B. Hatzinger, and R. J. Steffan. Enhancing the attenuation of explosives in surface soils at military facilities: Combined sorption and biodegradation. Environmental Toxicology and Chemistry (Accepted/In revision).

2. Hatzinger, P. B., M. E. Fuller, D. Rungmakol, R. L. Schuster, and R. J. Steffan. Enhancing the attenuation of explosives in surface soils at military facilities: Sorption-desorption isotherms. Environmental Toxicology and Chemistry (Accepted/In revision).

Copies of these manuscripts will be forwarded to the SERDP office when they are published.

III. CONDUCT SOIL COLUMN STUDIES.
2. SOIL CORE TRANSPORT AND FATE EXPERIMENTS.
2.1 METHODS.
The methodology for these experiments were described in detail in the Year 2002 Second Quarter QPR's for this project. Briefly, MMR soil was repacked into three clear PVC tubes and instrumented to allow sampling of the aqueous phase of the soil. One column served as a control (no treatment), one received a layer of peat moss, and the third received peat moss plus soybean oil. Soil spiked with unlabeled and radiolabelled TNT, RDX, or HMX was added to the top of the columns. The columns were sealed, airflow was initiated, and precipitation events were begun. The $[^{14}C]CO_2$ that was evolved over time was trapped and measured using liquid scintillation counting. Liquid samples were collected at 4, 8, and 12 inches below the soil surface and analyzed using liquid scintillation counting and HPLC. The ambient temperature of the room the columns were located in was 65-75°F.

The cores were designated:
C1 Control (non-functioning 8" lysimeter)
C2 Peat
C3 Peat plus soybean oil

These experiments have been operating for approximately twelve months. These experiments are being terminated within a few weeks. The soil within the columns will be analyzed for HMX, RDX, TNT, explosive compound metabolites, and residual $[^{14}C]$-labeled material. This will allow calculation of mass balances.

3. CONDUCT EXPERIMENTS WITH CRYSTALLINE EXPLOSIVES AND/OR ACTUAL EXPLOSIVE COMPOSITIONS.
We are still working to get small amounts of actual explosives compositions for selected experiments.

ADDITIONAL EXPERIMENTS
During Year 1, isotherm experiments were performed with pure sorbent phases (i.e, peat moss, sawdust, etc.). The results were very promising, and indicated that peat moss was a very effective sorbent for HMX, RDX, and TNT. A question was raised during the Spring IPR meeting about how the soybean oil would potentially affect the sorption-desorption of the explosives, as well as how the explosives might partition into, and move with, the crude soybean oil. Several experiments were designed and conducted this quarter to address these issues.

4. ASSESS MULTIPHASE TRANPORT OF EXPLOSIVES - PARTITION COEFFICIENTS
These experiments were performed to assess to what degree the explosives compounds TNT, RDX, and HMX may partition from the aqueous phase into the hydrophobic crude soybean oil phase. All three compounds have relatively low water solubilities, but TNT has an elevated octanol-water partition coefficient (K_{OW}), as indicated in the table below.
Compound // Solubility (mg/L, 25°C) // K_{OW}

TNT // 130 // 72.4
RDX // 40 // 7.4
HMX // 5 // 1.8

From these physical-chemical parameters, it was expected that the partitioning of explosives into the hydrophobic oil phase would be in the order TNT >> RDX > HMX.

4.1 METHODS

An initial experiment was performed to estimate the magnitude of potential partitioning of individual explosives into the oil phase of the crude soybean oil. Solutions of each explosive were prepared in sterile 3 mM $CaCl_2$ with nominal concentrations of 40, 5 and 1 mg/L of TNT, RDX, and HMX, respectively. [^{14}C]Labelled compounds (~3.0 µCi) of each explosive was also added to the solution. Actual concentrations of explosives were determined by HPLC analysis, and total radioactivity was determined using scintillation counting, as described in previous QPR's. Three treatments were prepared: control (no soybean oil), low soybean oil, and high soybean oil. Each treatment was prepared in duplicate. Nominal ratios of soybean oil:$CaCl_2$ were 0.01 and 0.10 (vol:vol) for the low and high treatments, respectively; the actual weight of oil added to each vial was measured and recorded, and volume was calculated based on the oil density of 0.91 g/ml. The purpose for examining two different soybean oil:$CaCl_2$ ratios was to evaluate the potential impact of water-soluble oil components on the aqueous phase activity (visual observations showed that the aqueous solution in contact with the soybean oil became slightly "cloudy", indicating that some fraction of the soybean oil was hydrophilic/water soluble.

After oil was added to sterilized serum vials (20 ml) with teflon-lined rubber septa, 10 ml of the explosives solutions were added, the vials were closed with crimp seal caps, and shaken vigorously (250 rpm) for 24 h at room temperature. Vials were then inverted, allowing the oil and water phases to separate. Duplicate 2 ml samples of the aqueous phase (1.0 ml) were removed, and the radioactivity in an aliquot (0.5 ml) was counted. The soluble fraction of the soybean oil did not affect the efficiency of scintillation counting based on a simple quench curve test. The concentration of either TNT, RDX, or HMX in the oil phase was determined via mass balance (controls indicated that mass losses in the serum vials were negligible).

The initial experiment was followed by a partition isotherm using multiple concentrations of TNT, RDX, and HMX. The set-up and procedure was as described above, except that the experiment with TNT compared the partitioning at two oil:$CaCl_2$ ratios (0.005 and 0.01, vol:vol), while those with RDX and HMX were done with only one ratio (0.01 vol:vol). Each treatment was prepared in triplicate, and duplicate samples of the aqueous phase in each vial were counted for radioactivity after 24 h.

4.2 RESULTS

The initial experiment yielded the partition coefficients presented in Table 1. The TNT soybean oil-water partition coefficient (K) averaged 100, which is approximately an order of magnitude higher than for RDX or HMX (K = 9 and 7, respectively). Also, There was no apparent relationship between the oil:$CaCl_2$ ratio and the measured K for RDX and HMX, while the data for TNT were somewhat inconclusive at high (< 40 mg/L) aqueous concentrations.

The subsequent multipart partition isotherm indicated that a single soybean oil:$CaCl_2$ partition coefficient would be applicable over a range of explosives concentrations for all three compounds (Figure 2). The average *K* for TNT, RDX, and HMX were 116, 7.8, and 6.6, respectively (slope of the lines). These data are consistent with the expected results based on the K_{OW} values for the three explosives.

These results indicate that only TNT is expected to partition to any large degree into free soybean oil phases in the treatment layer. However, with a peat moss to soybean oil ratio similar to that being used in these experiments, the soybean oil phase is not expected to be mobile within the layer (see Section 5 below), and may provide an additional mechanisms by which TNT can be immobilized at the soil surface.

5. ASSESS MULTIPHASE TRANPORT OF EXPLOSIVES - SORPTION EFFECTS

In addition to the possibility of the explosives partitioning into the soybean oil, it also became apparent that the ability of the soybean oil to affect the sorption of the explosives onto peat moss needed to be assessed. Affects could potentially arise due to 1) the soluble fraction of the soybean oil affecting the ionic strength of the aqueous phase; 2) the soluble fraction of the soybean oil acting as another sorbent phase for the explosives, or; 3) competition between the soybean oil (soluble and oil phases) and the explosives for the available sorption sites on and in the peat moss.

5.1 METHODS

These experiments were set-up similarly to the oil partition coefficient experiments, except that peat moss (0.25 g, dry wt) was added as third (sorbent) phase. Treatments with no sorbents, peat moss only, and peat moss plus soybean oil were prepared in duplicate serum vials. The soybean oil:$CaCl_2$ ratio was 0.05. Individual single concentration solutions of unlabeled and [^{14}C]labeled TNT, RDX, and HMX (81, 16, and 3.6 mg/L, respectively) in $CaCl_2$ were added, and the vials were sealed and incubated. After shaking for 24 h, duplicate samples were removed from each vial and analyzed using liquid scintillation counting. The mass of contaminant in the oil phase was calculated using the regressed partition coefficients shown in Figure 1 (0.01 oil to water ratio).

5.2 RESULTS

The results are presented in Table 2. The presence of the oil did not affect the sorption of any of the explosives to the peat moss. Also, the elevated oil:$CaCl_2$ ratio did not appear to impact the oil-water partitioning.

6. ASSESS MULTIPHASE TRANPORT OF EXPLOSIVES - SMALL-SCALE COLUMNS

These experiments were performed to examine the kinetics of the sorption-desorption of TNT, RDX, and HMX on the peat moss and determine how the soybean oil affects the sorption behavior under flow conditions.

6.1 METHODS

Small columns were constructed using 60 ml polypropylene syringe barrels (Figure 2). Soil was omitted from the columns to simplify the experiment (i.e., remove the variable of sorption of explosives and metabolites to the soil matrix). Each column was mounted over a leachate collection vessel constructed from a 50 ml polypropylene centrifuge tube. Polypropylene, teflon, and stainless steel was used for all tubing and fittings, except the small length of tubing used in the pump heads of the peristaltic pump.

Two sorbent/cosubstrate treatments were tested: peat moss (2.5 g) and peat moss plus soybean oil (1.25 ml). Each treatment was evaluated with two different contaminant loading regimes: 1) defined/constant = influent of constant soluble concentration of TNT, RDX, and HMX in 3 mM $CaCl_2$ plus 50 mg/L bromide; 2) variable = dissolution of a solid TNT, RDX, and HMX crystallized onto a glass fiber filter (Figure 2) in the 3 mM $CaCl_2$ plus 50 mg/L bromide that was applied to the top of the columns. Peat moss or peat moss plus soybean oil was wetted with $CaCl_2$, mixed well, and loaded into the bottom of the syringes. The height of the material was approximately 3 cm (1.2"). A glass fiber filter was placed at the surface to promote cross-sectional distribution of the artificial precipitation that was applied dropwise to the columns. For the variable loading regime, the solid crystalline explosives were placed on top of the filter paper, and a second glass fiber filter was placed over the explosives, forming a filter paper "sandwich" with the crystalline explosives in the middle. A summary of the treatments in the four columns were as follows:

Column	Treatment	Loading Regime
A	peat moss	constant
B	peat moss + oil	constant
C	peat moss	variable
D	peat moss + oil	variable

One peristaltic pump equipped with four pump heads delivered the artificial precipitation to the columns, while another pump and pump heads transferred the leachate to a fraction collector. Selected tubes were amended with 0.1 ml of 1 N HCl to preserve the leachate as it was collected.

The flow rate to the columns was initially adjusted to 15 ml/hr, with fractions collected at timed intervals ranging from 10 minutes to 1.5 h. These flow rates translate to an approximate rainfall rate of 1 inch per hour, or that that could be expected during a heavy rainstorm. After three days, the flow was stopped to simulate increased contaminant residence time in the treatment zone due to variable whether (i.e. rainfall) conditions expected in the field. Longer residence would be expected to affect both the abiotic sorption and the biological degradation of the explosives. Flow was restarted after 77 hours at a lower flow rate of 2 ml/h, with collection intervals up to 4 hr. This lower flow rate translates to a rainfall of approximately 0.15 inches per hour.

After an additional 42 hours at the 2 ml/h flowrate, the influent to columns A and B was switched to a solution of $CaCl_2$ plus bromide only, to allow desorption of the TNT, RDX, and HMX into the effluent. The flow rate was kept at 2 ml/h for this phase of the experiment.

The explosive-dosed filters were removed from columns C and D and placed in clean syringe barrels. A flow of $CaCl_2$ plus bromide (2 ml/h) was initiated to determine the dissolution rate of the crystalline TNT, RDX, and HMX, which provided an estimate for the loading rate of these compounds into the treatment zone of columns C and D. After 56 hours, the flow rate was increased to 15 ml/h. The influent was switched to $CaCl_2$ with no bromide after an additional 24 hours, followed by a final decrease in the flow rate back to 2 ml/h.

An additional small column experiment was performed to determine the extent to which water would displace the soybean oil in the treatment layer, thus mobilizing the soybean oil and increasing the potential for multiphase transport of explosives that had partitioned into the soybean oil (especially TNT). Peat moss was completely saturated with water (2.5 g peat plus 5.7 ml H_2O) in a 60 ml syringe barrel, and water was passed through the column at 9.75 ml/h. The column height was 3 cm A 5 ml volume of soybean oil was then applied to the top of the saturated peat moss and allowed to infiltrate. Water was then pumped through the column, using the syringe plunger to apply additional displacement pressure to the column. The water and oil that eluted was collected and measured volumetrically.

Fractions were removed and processed daily (excluding weekends), as follows:

1) Fraction weights were measured and recorded (for volume determination).
2) 0.2 to 1.0 ml subsamples from unpreserved fractions were taken for heterotrophic plate counts and stored at 4°C until analysis.
4) 0.75 ml subsamples from were removed and passed through 0.45 µm glass fiber syringe filters into HPLC vials, sealed and stored at 4°C until analysis.
3) 2.0 ml susbsamples of unpreserved fractions were transferred to 15 ml polypropylene centrifuge tubes and capped for pH measurement, followed by bromide measurement.
5) 2.0 ml subsamples of preserved fractions were transferred to 15 ml polypropylene centrifuge tubes and capped for measurement bromide concentrations.
6) The remainder of the samples was sealed with parafilm, wrapped with aluminum foil, and stored at 4°C.
7) Selected unpreserved fractions were pooled for DOC/TOC analyses.

6.2 RESULTS

The leaching of TNT, RDX, and HMX through the four columns, as well as the elution of the conservative bromide tracer and the pH of the effluent, is presented in Figures 3 to 7.

The mean residence time of the bromide is approximately equal to the water saturated pore volume divided by the volumetric flow rate, indicating that saturated flow was occurring with no apparent short circuiting within the treatment layer. The pH of the effluent from all four columns was lower than that of the influent (~6 S.U.), indicating that the peat moss was making the percolating water acidic, probably by dissolution of humic acids. This is expected to also occur if the technology were deployed in the field, since natural rainwater does not have a high buffering capacity.

Comparison of the TNT breakthrough curves to that of the RDX and HMX breakthrough curves indicated that TNT transport through the peat/oil system is more retarded (i.e., longer mean residence time) than RDX or HMX. This is in qualitative agreement with the batch partitioning and sorption studies that show TNT uptake in the peat moss and soybean oil is significantly greater than RDX or HMX uptake in either of these sorbents. In addition, a difference in contaminant transport between treatments with and without soybean oil is only observed for the TNT. This result is expected due to the relatively low partitioning of RDX and HMX into soybean oil compared with TNT partitioning into soybean oil (Table 1).

The small oil displacement experiment indicated that aqueous $CaCl_2$ solution displaced only 60% of the oil, with a residual 40% remaining in the peat moss. During the actual small column experiments, the oil pore saturation was only 12%, which is well below the 40% oil saturation that the peat moss could potentially hold. This indicates that the soybean oil is not likely to be mobilized and move as a separate phase at the peat moss to soybean oil ratios that are being used for these experiments (or under field conditions).

Additional data evaluation and conceptual/numerical modeling are currently underway in order to identify the physical, chemical, and biological processes affecting contaminant transport through the peat/oil systems. However, preliminary evaluation indicates that rate-limited adsorption and desorption processes may be impacting overall contaminant flux. If confirmed, this finding would suggest that mass transfer processes, as well as biodegradation and phase partitioning, will need to be evaluated in order to select the optimum “barrier” to explosives migration to the subsurface. Additional sorbents that proved effective during the previous year's research (e.g., sawdust) may therefore be studied in column experiments to evaluate overall effectiveness of the barrier media.

V. MESO-SCALE SOIL CORE EXPERIMENTS.

1. DESIGN AND CONSTRUCT CORES.

2. COLLECT INTACT /VEGETATED CORES FROM MMR

We are continuing to assess whether these experiments will provide the relevant data needed for transport and fate modeling, as well as to move the technology forward to field testing. At this time, we believe it is important to continue with the smaller, well-controlled column experiments in order to obtain the needed data.

FIGURES AND TABLES

The tables and figures supporting this document have been provided to the SERDP Office as a separate attachment.

V. RESPONSES TO IN-PROGRESS REVIEW COMMENTS

Below are our responses to the comments that were raised during our IPR conducted on May 3, 2003.

It is critical that the focus of this project be on technically sound column and soil core experiments to answer some of the fundamental questions that remain for the technology prior to its transition to the field. Please address the following issues in the next Quarterly Progress Report

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1) The effect of pH on sorption/desorption of RDX and its breakdown products needs to be isolated and monitored. Please comment on the ease to incorporate this analysis during all experiments (e.g., consider measuring the pH of water percolating through the column).

We have initiated efforts to monitor the pH of both the influent (artificial "rain") and the effluent (leachate) for all current and future studies. Our current results indicate that the peat moss will tend to auto-adjust the percolating water to around pH 4.5 regardless of the starting pH of the influent solution. Natural rainwater has a very low buffering capacity, so the peat moss will also result in a pH 4.5 leachate going through the system.

We are not planning on performing a series of additional experiments over a range of pH's as part of this project. The effects of pH on the dissolution of the explosives has been or is being examined by other investigators, and should be able to be incorporated into any transport and fate models that we develop should pH become an issue at a given site.

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2) To ensure statistical validity of the results, it is critical that an appropriate number of replicates be included in all studies. Please provide a summary of replicates for all experiments planned in Year 2.

We have attempted to include replicates in all the smaller-scale experiments we have conducted to date. For instance, the isotherm experiments were performed with triplicates of each treatment, and the slurry and unsaturated microcosms were performed in duplicate. For the 12" unsaturated soil columns, we only set up single replicates of each treatment due to the complexity of the experiments.

During the remainder of this project, we will attempt to continue to either 1) employ duplicates of different treatments, or; 2) run the specific experiment at least twice to assess reproducibility. The method of replication used will depend on the scale of the experiment.

---

3) Concerns remain about the potential for partitioning of RDX/HMX into the soybean oil and migration of the contaminants as a separate phase. This is important in terms of transport into the saturated zone and creation of a long-term source of contamination. Please elaborate on plans to address this concern in the experimental design and modeling.

We have address this issue in depth during the past quarter of research. Please see sections 4, 5, and 6 above for details.

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4) Outline plans to investigate the reversibility of adsorption/partitioning into amendments following completion of the column studies.

This aspect of the research has been address to some extent by the work performed this quarter (see section XXX above). We intend to continue to examine this aspect of the technology directly using our smaller column experiments, as well as during the efforts to close the mass balances of TNT, RDX, and HMX (and breakdown products) for all the experiments we are conducting.

---

5) For field application, it is critical to calculate and plot mean flux as a function of unsaturated zone depth (i.e., loading factor to groundwater as opposed to concentrations). Please provide an initial analysis and plan to present information in the future according to this logic.

Initial flux (or mass loading) calculations are one of the specific results from this quarter's work. We have rethought our work plan, and made changes to the experimental design to make sure that the data needed to model the fate and transport of the explosives is generated. This will allow the mass loading to be calculated as a function of precipitation rate, time, etc.

At this point, it is not clear if the flux as a function of unsaturated zone depth is the best way to present the data. The processes occurring in the unsaturated zone, and their effects on the flux of explosive compounds to groundwater, will likely be very similar for both areas that receive the amendments (i.e., peat moss plus soybean oil) and those that do not. The only exception may be that the very top zone of the soil underlying the amendments may be more biologically active due to the infusion of nutrients and retention of moisture. Therefore, the controlling factor on mass loading to groundwater will likely not be the depth of the unsaturated zone, but the ability of the amendments to reduce the flux of contaminants to the soil surface. However, future results will be presented in multiple ways to best express different aspects of how this technology will help reduce contamination of groundwater with explosive compounds.

It is also likely more relevant to think of the flux or mass loading of contaminants as a function of rainfall rate, which will vary considerably at different locations. We believe our results from the smaller-scale experiments we are conducting will allow this to be taken into consideration, but larger scale, "real world" experiments will also be needed.

Table 1. Soybean oil:$CaCl_2$ partition coefficents for TNT, RDX, and HMX.

	K	
Compound	**Oil:$CaCl_2$ = 0.01**	**Oil:$CaCl_2$ = 0.001**
TNT	117	84
RDX	10	8
HMX	6	7

Table 2. Peat moss partition coefficents for TNT, RDX, and HMX.

	K	
Compound	**No oil**	**With oil**
TNT	174	174
RDX	41	42
HMX	96	85

Figure 1. Soybean oil:$CaCl_2$ partition isotherms for TNT, RDX, and HMX.

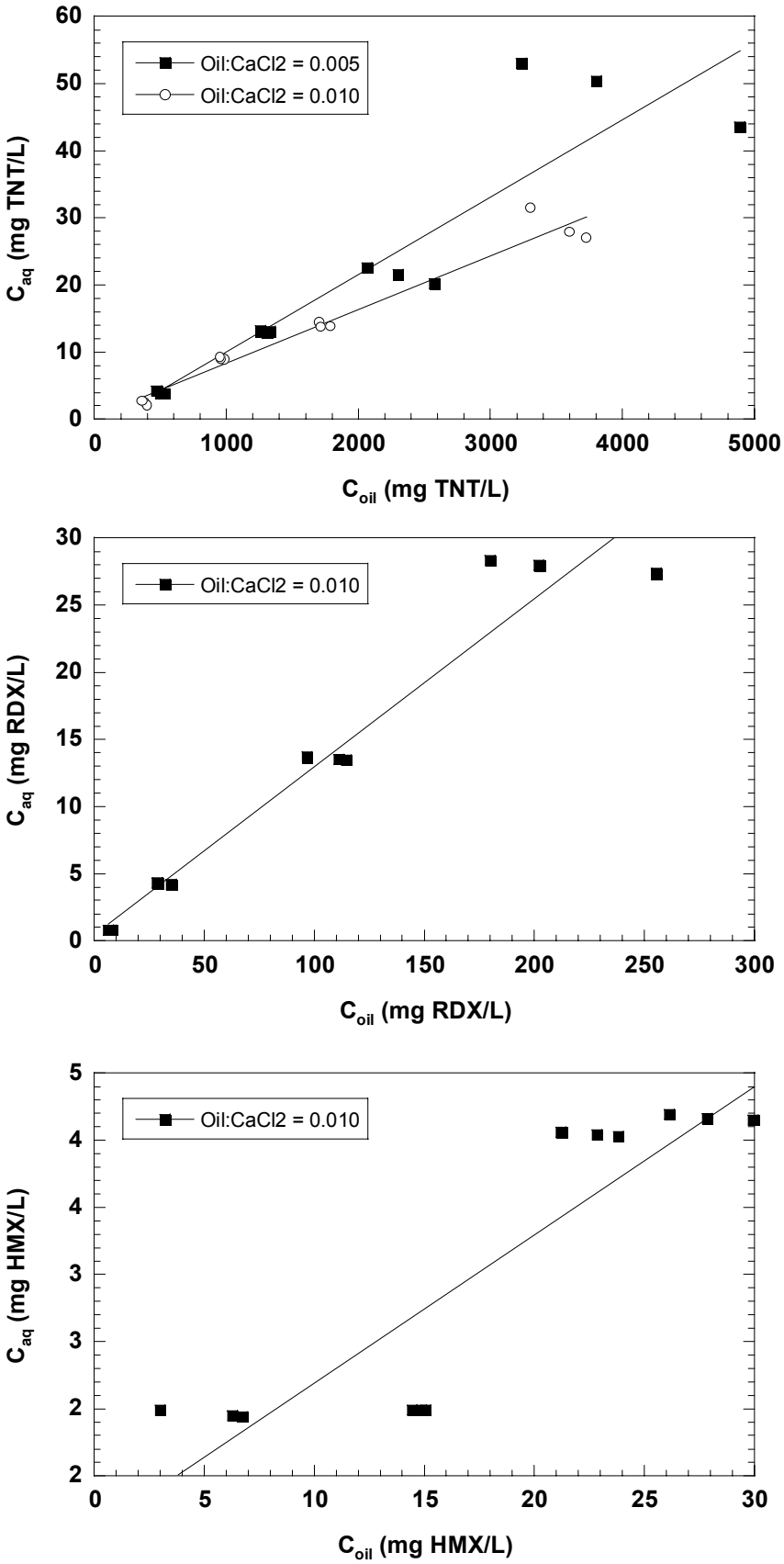
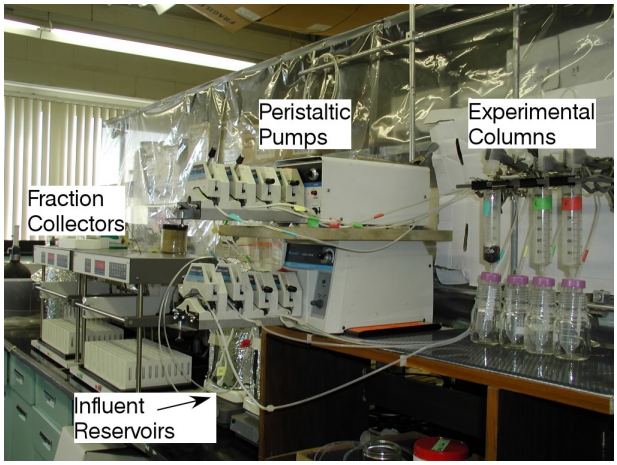
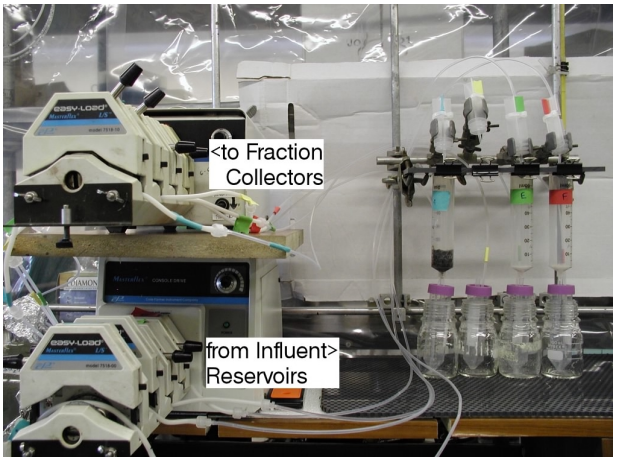


Figure 2. Photographs of the experimental set-up for the small-scale columns.

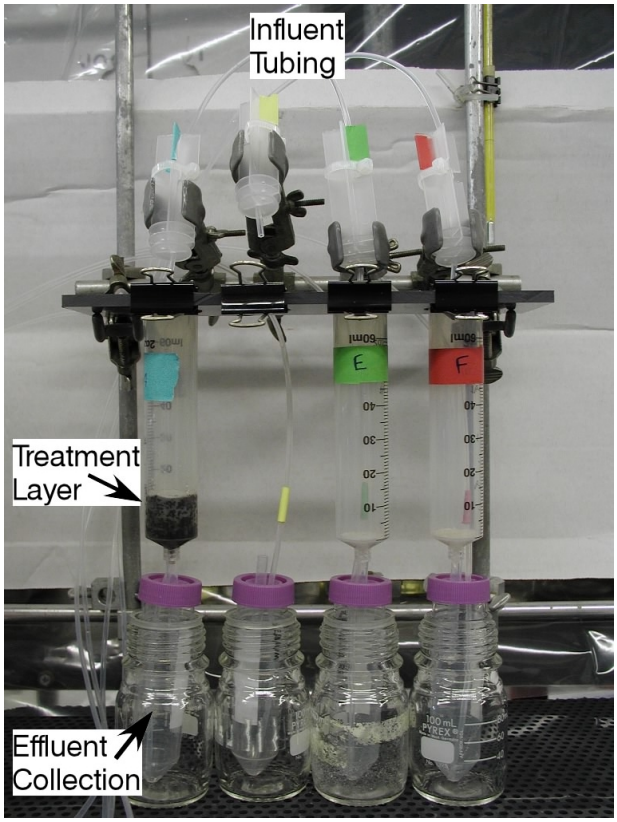
Experimental set-up



Pumps and columns



Close-up of experimental columns



Close-up of the explosives-impregnated filter disks used in the variable loading columns

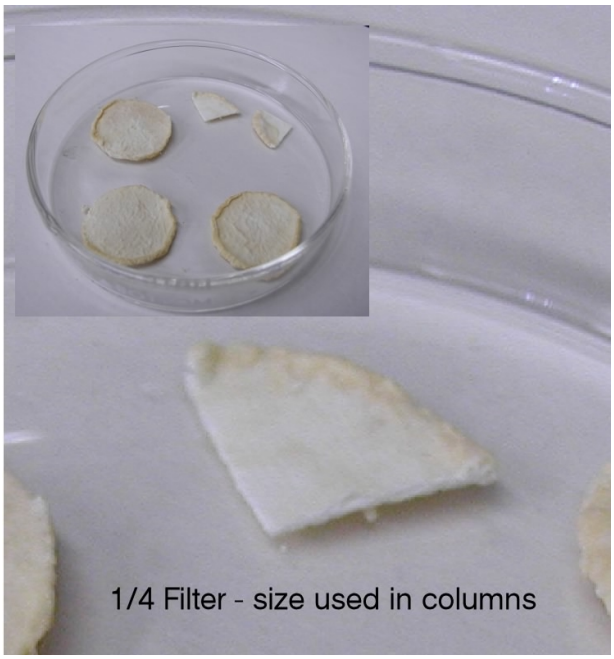


Figure 3. Concentrations of bromide in the influent and effluent of peat moss and peat moss plus soybean oil columns under the constant and variable loading regime.

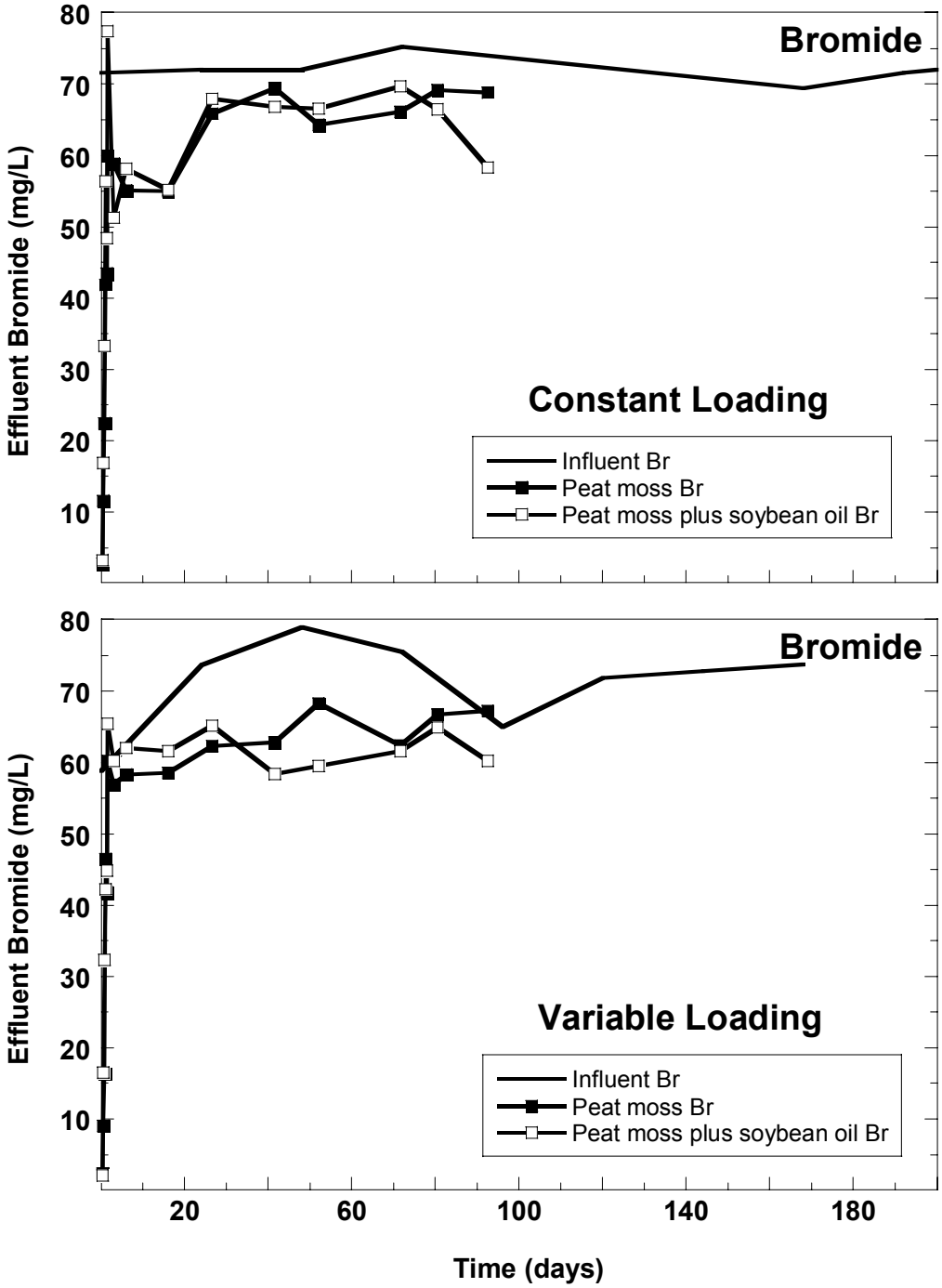


Figure 4. pH of the influent and effluent of peat moss and peat moss plus soybean oil columns under the constant and variable loading regime.

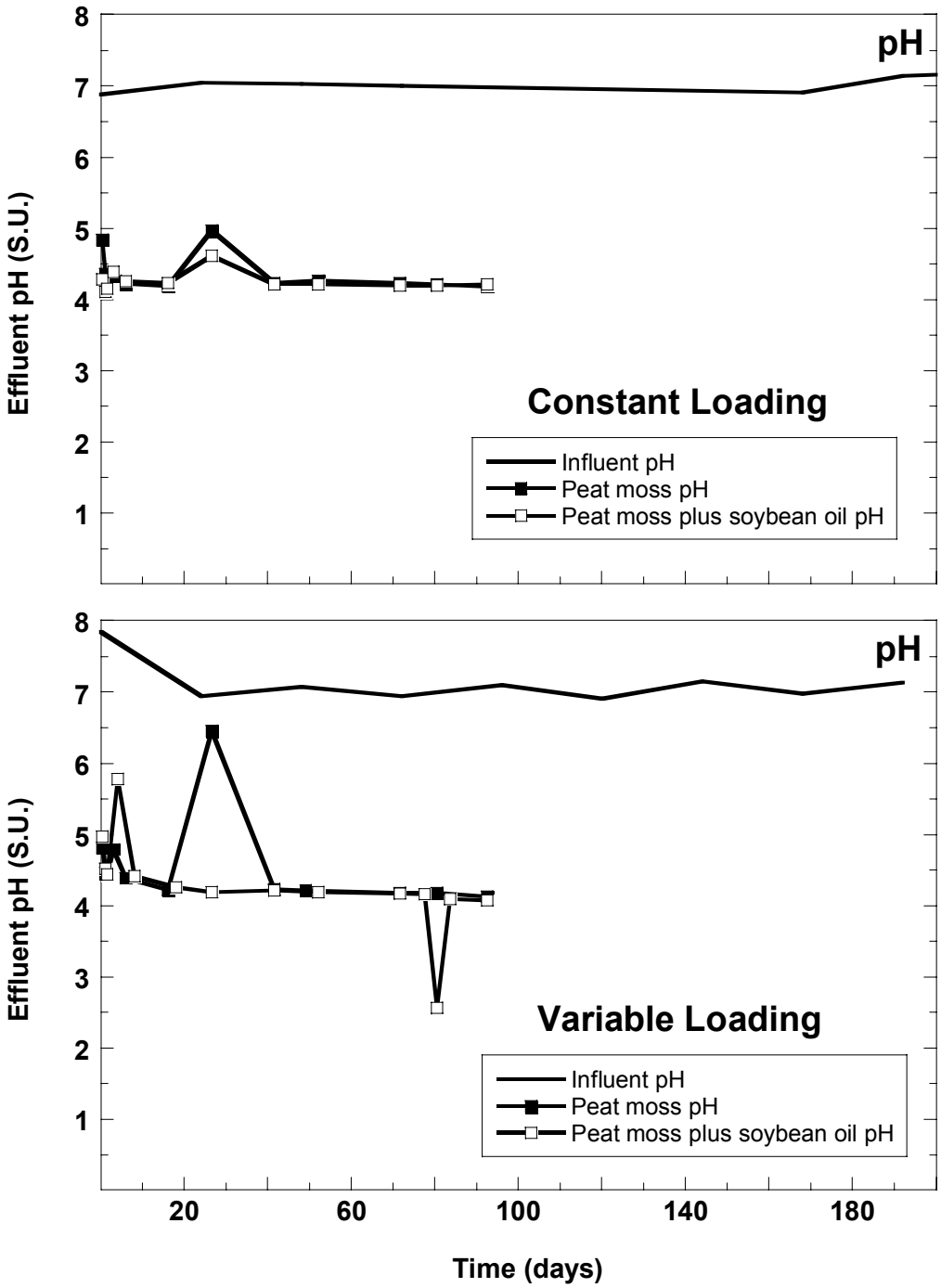


Figure 5. Concentrations of TNT in the peat moss and peat moss plus soybean oil columns under the constant and variable loading regimes. Desorption was initiated when the influent was changed to a solution without explosives.

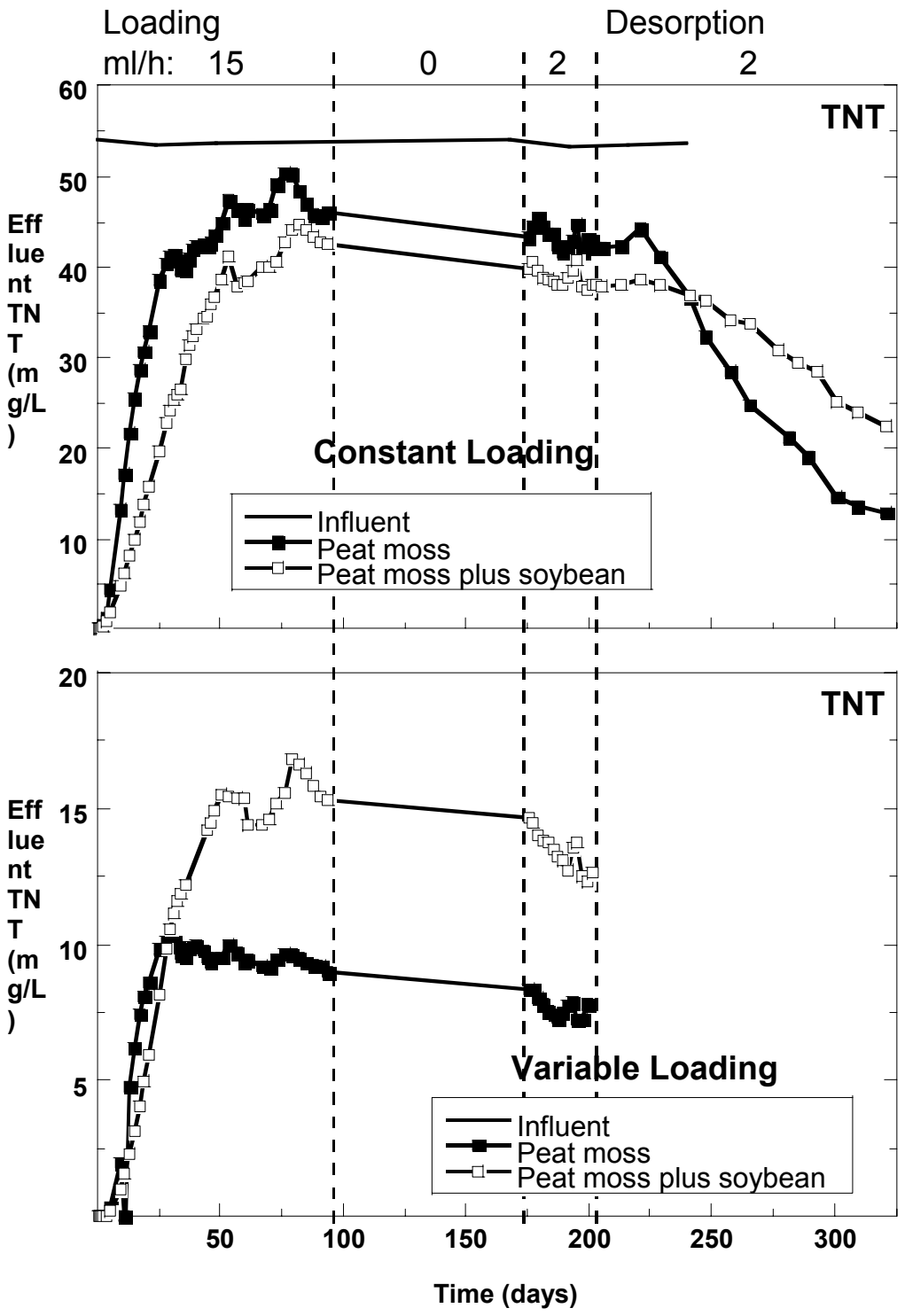


Figure 6. Concentrations of RDX in the peat moss and peat moss plus soybean oil columns under the constant and variable loading regimes. Desorption was initiated when the influent was changed to a solution without explosives.

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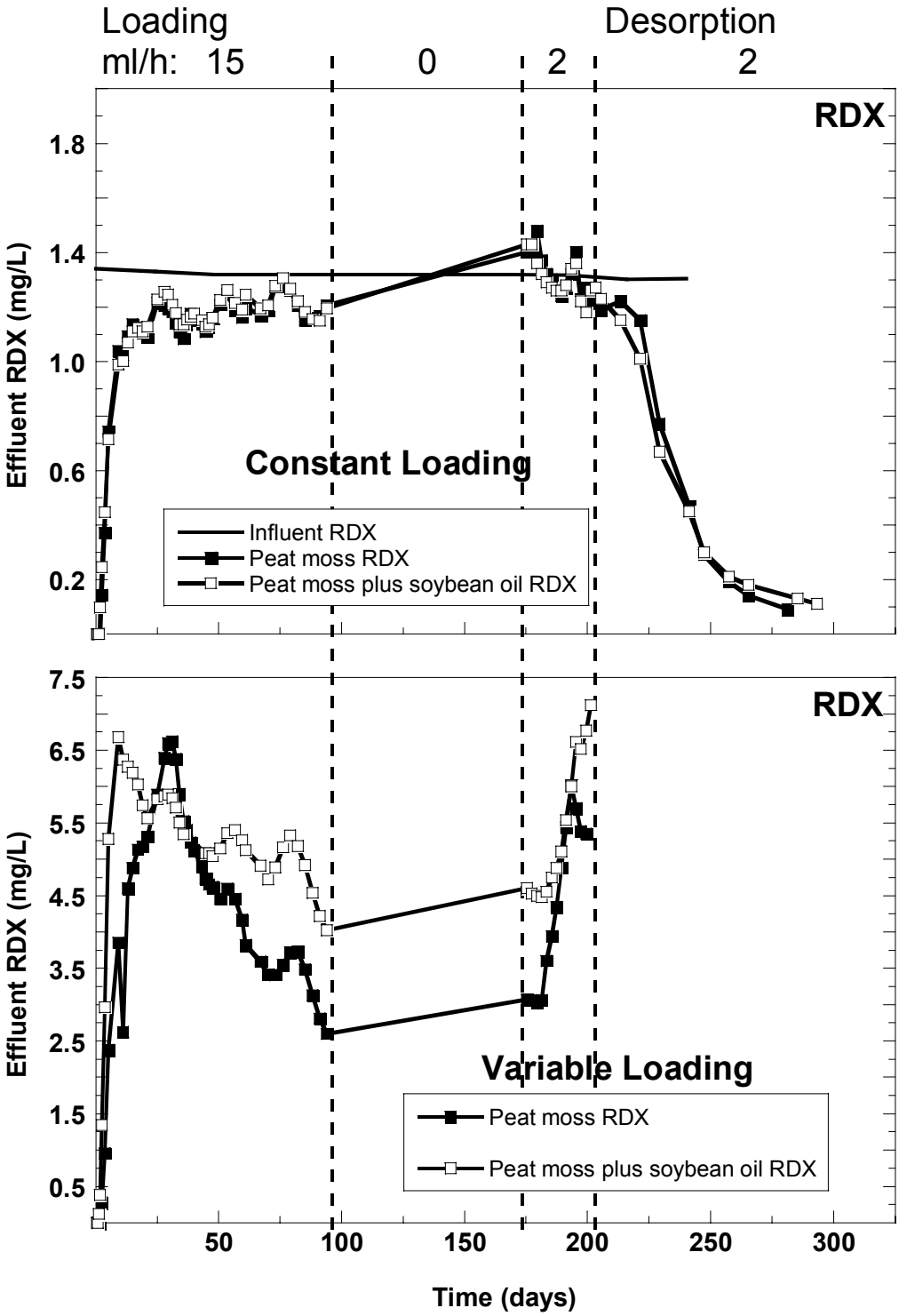

Figure 7. Concentrations of HMX in the peat moss and peat moss plus soybean oil columns under the constant and variable loading regimes. Desorption was initiated when the influent was changed to a solution without explosives.

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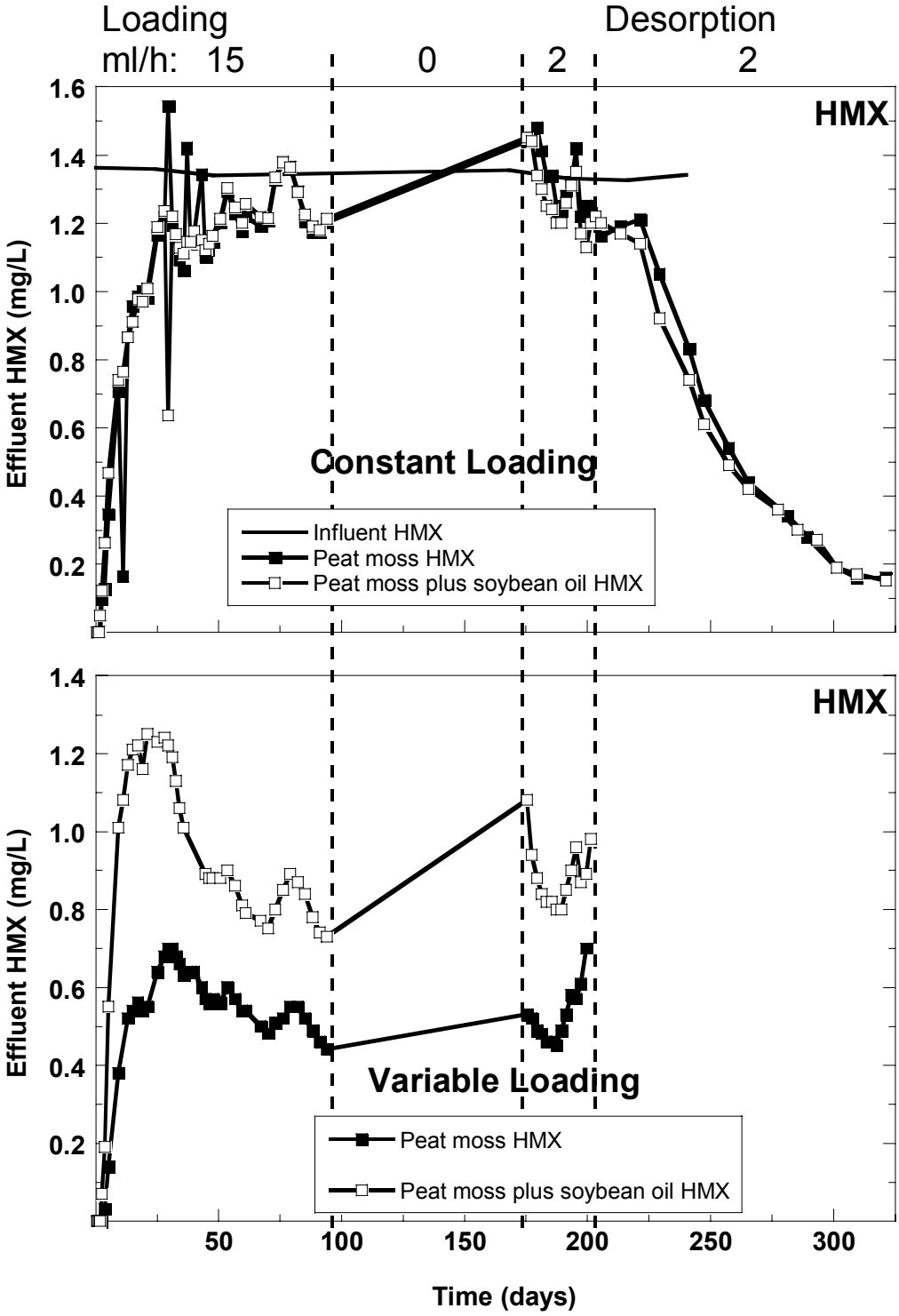


Quarterly Progress Report

SERDP Project 1229 - Immobilization of Energetics on Live Fire Ranges
Year 2003 – Third Quarter
October 15, 2003

This report covers technical progress for SERDP Project 1229 from June 15, 2003 – September 15, 2003.

The objective of this project is to develop a cost-effective technology to immobilize energetic compounds (TNT, RDX, HMX, and breakdown products) released as residues at firing ranges to prevent their migration to groundwater. The goal is to develop an inexpensive soil treatment that can be readily applied over wide and remote areas prior to or immediately following firing range activities.

During the current quarter, activities have focused on:

• completing initial repacked soil core experiments;
• performing experiments to determine fate and transport of explosives in the peat moss plus soybean oil treatment layer;
• development and evaluation of a model to predict and help explain the explosives fate and transport in the peat moss plus soybean oil treatment layer

III. CONDUCT SOIL COLUMN STUDIES.
2. SOIL CORE TRANSPORT AND FATE EXPERIMENTS.
2.1 METHODS.
The methodology for these experiments were described in detail in the Year 2002 Second Quarter QPR's for this project. Briefly, MMR soil was repacked into three clear PVC tubes and instrumented to allow sampling of the aqueous phase of the soil. One column served as a control (no treatment), one received a layer of peat moss, and the third received peat moss plus soybean oil. Soil spiked with unlabeled and radiolabelled TNT, RDX, or HMX was added to the top of the columns. The columns were sealed, airflow was initiated, and precipitation events were begun. The $[^{14}C]CO_2$ that was evolved over time was trapped and measured using liquid scintillation counting. Liquid samples were collected at 4, 8, and 12 inches below the soil surface and analyzed using liquid scintillation counting and HPLC. The ambient temperature of the room the columns were located in was 65-75°F.

The cores were designated:
C1 Control
C2 Peat
C3 Peat plus soybean oil

These experiments were terminated after 430 days. The peat moss ± soybean oil and soil within the columns were carefully removed in 1 inch increments from the top to the bottom of the

profile. Subsamples of each layer were removed, dried, extracted and analyzed for residual [^{14}C] and explosives residues (TNT, RDX, HMX, metabolites). Additional procedures using samples of peat moss ± soybean oil layer and the first 1" layer of soil from each column were performed to allow the mass balance to be closed. The first procedure was to grind dried material with a mortar and pestle, extract with acetonitrile, and analyze using scintillation counting and HPLC. A second approach was to mix the dried and ground material with distilled water, sonicate for 1 h, and analyze the whole sample using scintillation counting. The third procedure was to first sieve the dried material into several size fractions (<70 µm, 70-250 µm, 250-500 µm, 500 µm - 1 mm, >1 mm), extract each fraction with acetonitrile, and analyze using scintillation counting and HPLC. The fourth procedure involved complete combustion of subsamples in a furnace with capture of the liberated [14C]carbon dioxide in 0.5 N KOH, and scintillation counting of the base.

2.2 RESULTS

The cumulative recovery of [14C] as carbon dioxide, representing mineralization of RDX, is presented in Figure 1. The mineralization was minimal during these experiments, which may have been due to several factors: 1) very slow dissolution and movement of the RDX into the bioactive treatment layer and underlying soil; 2) insufficient biological activity in the treatment layer or underlying soil, or; 3) lack of formation of anoxic zones within the treatment layer and underlying soil which allow/promote RDX transformation.

The aqueous concentrations of bromide, radioactivity, and explosives in the columns is shown in Figures 2 to 5. The concentrations of bromide were similar, indicating that water moved through the columns similarly (Figure 2). The aqueous cumulative recovery of radioactivity (derived from [14C]RDX) was lower from the treatment columns compared to the control column, and the peat moss plus soybean oil treatment resulted in lower cumulative recovery compared to the peat moss only treatment (Figure 3). Aqueous explosives concentrations at a depth of 4" varied as a function of the precipitation applied, with lower concentrations of TNT, RDX, and HMX in the samples from the peat moss plus soybean oil treatment column than samples from the control column (Figure 4). RDX in samples from the peat moss only treatment column were similar to the control, while HMX concentrations were lower. At a depth of 8", aqueous concentrations of TNT were below detection in the peat moss plus soybean oil column, and only sporadic in the peat moss only column (Figure 5). RDX concentrations were reduced by approximately 50% by the peat moss plus soybean oil treatment compared to the peat moss only treatment, whereas HMX concentrations were similar in both treatment columns at this depth. [No 8" data was available from the control column because of a malfunctioning sampler.]

The distribution of radioactivity through the soil profile in the three cores is presented in Figure 6. The recovery of radioactivity in the soil columns was greatest in the top layers of the columns (i.e., top layer of soil in the control column, and in the peat moss ± soybean oil layers in the treatment columns). The procedures used to recover the radioactivity from the samples yielded varying results (Table 1), with acetonitrile extraction yielding approximately the same radioactivity on a per gram basis as complete combustion for the samples analyzed so far. While these results are preliminary, it appears that formation of unextractable bound residues of [14C]RDX did not occur to any great degree.

The radioactivity in different size fractions of the treatment layer from the peat moss only column is presented in Table 3. Interesting, although the radioactivity was initially added to each of the columns on 24 g of soil with a size range between 70 µm to 1 mm, the greatest radioactivity was observed on the >1 mm fraction. This seems to suggest that the radioactivity redistributed itself after it was added. However, chemicals usually partition into the smaller size fractions due to their higher surface areas relative to larger size fractions.

The final concentrations of explosives in the column soil as a function of depth are presented in Figures 7 and 8. As with the aqueous concentrations, these data indicated that the downward migration of the TNT, RDX, HMX, and metabolites was the least in the column receiving the peat moss and peat moss plus soybean oil treatment compared to the control column. TNT concentrations were relatively low (Figure 7), and moderate levels of 2,6-DNT (but not other TNT metabolites) were observed through the soil (Figure 8). The highest concentrations of RDX were observed in the top layers of the column receiving peat moss only, with significantly lower concentrations on the control column and the column receiving peat moss plus soybean oil. Concentrations of RDX below the treatment layers appeared to be similar in the treated and the control columns. High HMX concentrations were observed in the peat moss plus soybean oil layer, but were much lower in the soil layers of the treatment columns than in the control column.

The initial mass balances are presented in Table 2. The greatest total recovery was achieved in the control column (66%), with lesser recoveries in the treatment columns. Work is continuing on these analyses, including combustion of additional column samples, and results will be included in the next report.

ADDITIONAL EXPERIMENTS

6. ASSESS MULTIPHASE TRANPORT OF EXPLOSIVES - SMALL-SCALE COLUMNS

These experiments were performed to examine the kinetics of the sorption-desorption of TNT, RDX, and HMX on the peat moss and determine how the soybean oil affects the sorption behavior under flow conditions.

6.1 METHODS

Methods were as described in the Second Quarterly Report. Briefly, peat moss with an without soybean oil was packed into 60 ml syringe barrels, and the following treatments were applied

Experiment 1
Column: Treatment (Loading Regime)
A: peat moss (constant-dissolved explosives)
B: peat moss + oil (constant-dissolved explosives)
C: peat moss (variable-crystalline compound dissolution)
D: peat moss + oil (variable-crystalline compound dissolution)

Fractions were removed and processed daily (excluding weekends), as follows:

1) Fraction weights were measured and recorded (for volume determination).
2) 0.2 to 1.0 ml subsamples from unpreserved fractions were taken for heterotrophic plate counts and stored at 4°C until analysis.
4) 0.75 ml subsamples from were removed and passed through 0.45 µm glass fiber syringe filters into HPLC vials, sealed and stored at 4°C until analysis.
3) 2.0 ml subsamples of unpreserved fractions were transferred to 15 ml polypropylene centrifuge tubes and capped for pH measurement, followed by bromide measurement.
5) 2.0 ml subsamples of preserved fractions were transferred to 15 ml polypropylene centrifuge tubes and capped for measurement bromide concentrations.
6) The remainder of the samples was sealed with parafilm, wrapped with aluminum foil, and stored at 4°C.
7) Selected unpreserved fractions were pooled for DOC/TOC analyses.

At the end of the experiment, portions of the peat moss/soybean oil layers were removed, extracted, and analyzed for explosives concentrations, allowing mass balance analysis to be performed.

A second similar experiment was performed, except that double the amount of oil was added (2.5 ml soybean oil per 2.5 g peat moss), with the following treatments:

Experiment 2
Column: Treatment (Loading Regime)
A: peat moss + oil (constant-dissolved explosives)
B: peat moss + oil (constant-dissolved explosives)
C: peat moss (constant-dissolved explosives)
D: autoclaved peat moss (constant-dissolved explosives)

Autoclaved versus nonsterile peat moss was compared to look at differences in explosive compound fate and transport through the peat moss when the biological activity is minimized. The flow rate during this second experiment was 2 ml/h. The flow regime with a influent solution with a constant concentration of TNT, HMX, and RDX was: continuous flow for 168 h; no flow for 168 h; continuous flow for an additional 48 h. The influent solution was then changed to an explosives-free 3mM $CaCl_2$ to allow desorption of the explosives from the peat moss. This experiment is still in progress after 454 h of desorption.

Laboratory column data was evaluated using SOLUTRANS three-dimensional modeling software (Fitts Geosolutions, 1999). The SOLUTRANS model is based on the analytical solution developed by Leij et al. (1993), and is based on the coupled differential equations presented in Figure 9, Equation 1. First-order biodegradation is assumed to occur in the aqueous phase. The model also assumes that sorption sites are divided into two regimes: an equilibrium regime where sorption occurs instantaneously, and a kinetic regime where the rate of contaminant uptake by the sorbet is controlled by mass transfer. For the peat and peat-oil

sorbents, the kinetic sites are assumed to be located in intraparticle hydrophobic pore spaces where mass transfer is controlled by diffusion to organic carbon sites.

The water flow rate (v), water-filled porosity (θ), and bulk density (ρ) were directly measured in the column experiments. The linear sorption coefficient (K) was measured in parallel batch sorption studies for each compound for sorbents consisting of both peat and peat with soybean oil. The dispersion coefficient was estimated based on the elution curve of the bromide tracer (bromide tracer analysis was performed in each column prior to the start of the experiments). The biodegradation rate constants (μ) were estimated by measuring the decrease in dissolved contaminant concentration during the stagnation period ($v = 0$) of the column experiments using Equation 2.

The fraction of sorption sites that are within the kinetically controlled regime (f) is a function of both the sorbent (i.e., peat) and the sorbate. Different contaminants will have varying affinities for the hydrophobic sorption sites located in the kinetic regime. For the simulation results presented in Figures 10 and 11, f was estimated for each contaminant based on a fit to the data (visually regressed to the data).

The remaining term in Equation 1 is the rate coefficient between the equilibrium and kinetic regimes. This term is derived from the mass transfer coefficient using a shape factor that relates the area of mass transfer to the overall pore volume of the peat (Equation 3). It is noted that the shape factor is not a function of the contaminant, but rather is a function of the peat alone. For the peat sorbent, the mass transfer coefficient (k) between the kinetic and equilibrium regimes is calculated assuming that mass transfer is controlled via diffusion through stagnant pore water adjacent to hydrophobic kinetic sorption sites. This relationship is described in Equation 4. The aqueous diffusion coefficient for TNT is 1.1 x 10E-5 cm^2/sec (Wu, 2001); RDX and HMX have similar structures and molar volumes to that of TNT, thus it is assumed that RDX and HMX also have an aqueous diffusion coefficient equal to that of TNT. The average peat particle radius (0.014 cm) is used as an estimate for the diffusion length.

The expression for the mass transfer coefficient for the peat-oil sorbent is shown in Equation 5. When soybean oil is added to the peat, it is assumed that hydrophobic pores located within the peat particles become oil-filled. Thus, diffusion to kinetic sites located within these pores requires diffusion through oil instead of diffusion through pore water. In addition, the presence of the soybean oil within pores located in the kinetic regime causes the concentration driving force between the kinetic and equilibrium regimes to increase, as hydrophobic organic contaminants partition into the oil.

Desorption hysteresis was observed in the previously performed sorption isotherms. It is assumed that this hysteresis was due to phase transition phenomena associated with organic carbon sites located in the kinetic sorption domain. To account for this hysteresis in the model, the mass transfer coefficient (k) was multiplied by the slope of the desorption isotherm and divided by the slope of the adsorption isotherm. This ratio was used to estimate the average decrease in mass transfer (due to a decrease in concentration driving force) between the peat and fluid phases.

6.2 RESULTS

Experiment 1

The observed and predicted transport of TNT, RDX, and HMX through the minicolumns receiving explosives in the aqueous phase (Col A & B) is presented in Figure 10. The experimental data as points and the predicted results as solid lines. The mass balance calculations for the first experiment resulted in values of 85-100%.

Experiment 2

The observed and predicted transport of TNT, RDX, and HMX through the minicolumns receiving explosives in the aqueous phase (Col A & B) is presented in Figure 11. The experimental data as points and the predicted results as solid lines.

Preliminary analyses of the experimental data and model simulations have shown the following:

• The presence of small volume of soybean oil (< 12% of the pore volume) does not have any measurable affects of water flow through the pore space, as indicated by the bromide tracer elution curves. Thus, the presence of low volumes of oil will not reduce the water residence time within the peat barrier and decrease treatment efficiency.

• Biodegradation of TNT and RDX were observed in the column experiments, as indicated by the reduction in contaminant concentrations after restarting water flow following the stagnation period. This decrease was likely not due to sorption, as the columns were at or very near to equilibrium conditions prior to the stagnation period. Furthermore, TNT biodegradation daughter products were observed after restart of water flow for several pore volumes. No measurable biodegradation of HMX was observed in the peat columns.

• The presence of oil has a negligible effect on RDX and HMX transport (excluding effects of biodegradation during stagnation periods), but has a significant effect on TNT transport (TNT flux was reduced when oil was present). The reason for this difference is that the oil-water partition coefficient (K_{OW}) for TNT is greater than 10x the K_{OW} value for either RDX or TNT, allowing the oil to accelerate sorption processes in the kinetic domain for TNT. It should be noted that the difference between the peat and peat/oil simulations for TNT can not be explained by simply adjusting the linear partition coefficient to account for sorption into the oil, but rather the primary effect of oil addition on TNT transport is on the mass transfer coefficient (Equation 5).

• Desorption hysteresis reduces the flux of contaminants desorbing from the peat. This will increase residence time within the peat and will likely increase the mass of contaminants that become biodegraded.

• Contaminant transport through peat during moderate to heavy rainfall events will likely be kinetically controlled, as mass transfer limitations will control the overall contaminant flux to the subsurface

• The model was validated by the experimental observations. A calculation based on the model parameters indicated that a 1.25" layer of peat moss plus soybean oil would reduce the mass loading to soil of TNT by more than 95%, and RDX by around 80%, relative to soil with no treatment (Figure 12).

The results from Columns C & D from Experiment 1 and Experiment 2 will be presented and discussed in the next report.

V. MESO-SCALE SOIL CORE EXPERIMENTS.

1. DESIGN AND CONSTRUCT CORES.

2. COLLECT INTACT /VEGETATED CORES FROM MMR

We are continuing to assess whether these experiments will provide the relevant data needed for transport and fate modeling, as well as to move the technology forward to field testing. At this time, we believe it is important to continue with the smaller, well-controlled column experiments in order to obtain the needed data.

FIGURES AND TABLES

The tables and figures supporting this document have been provided to the SERDP Office as a separate attachment.

Table 1. Recovery of radioactivity in the topmost layers of the soil columns after various procedures.

	[14C], dpm/g			
Column	**AcNit Extract**	**Pulverize & AcNit Extract**	**Sonicate**	**Pulverize & Combustion**
C1 Soil	200	450	--	1700
C2 Peat moss	8000	9300	5300	8300
C3 Peat moss + soybean oil	7000	--	--	6900

Table 2. Preliminary mass balance of [^{14}C] dpm in soil column experiments.

	Percent of radioactivity added recovered in				
Column	**[^{14}C]CO_2**	**Aqueous samples**	**Treatment layer**	**Soil samples**	**TOTAL**
C1 Soil	3	35	--	27	66
C2 Peat moss	1	25	12	10	48
C3 Peat moss + soybean oil	3	16	10	5	34

Table 3. Distribution of [^{14}C] dpm in different size fractions of the peat moss treatment layer.

	Percent of radioactivity	
Size fraction	**Weight percent**	**Percent of radioactivity**
>1mm	22	66
500 µm - 1 mm	20	7
250 µm - 500 µm	24	4
70 µm - 250 µm	8	10
<70 µm	25	12

Figure 1. Cumulative recovery of radioactivity as [^{14}C]CO_2 from [^{14}C]RDX] in columns with different treatments.

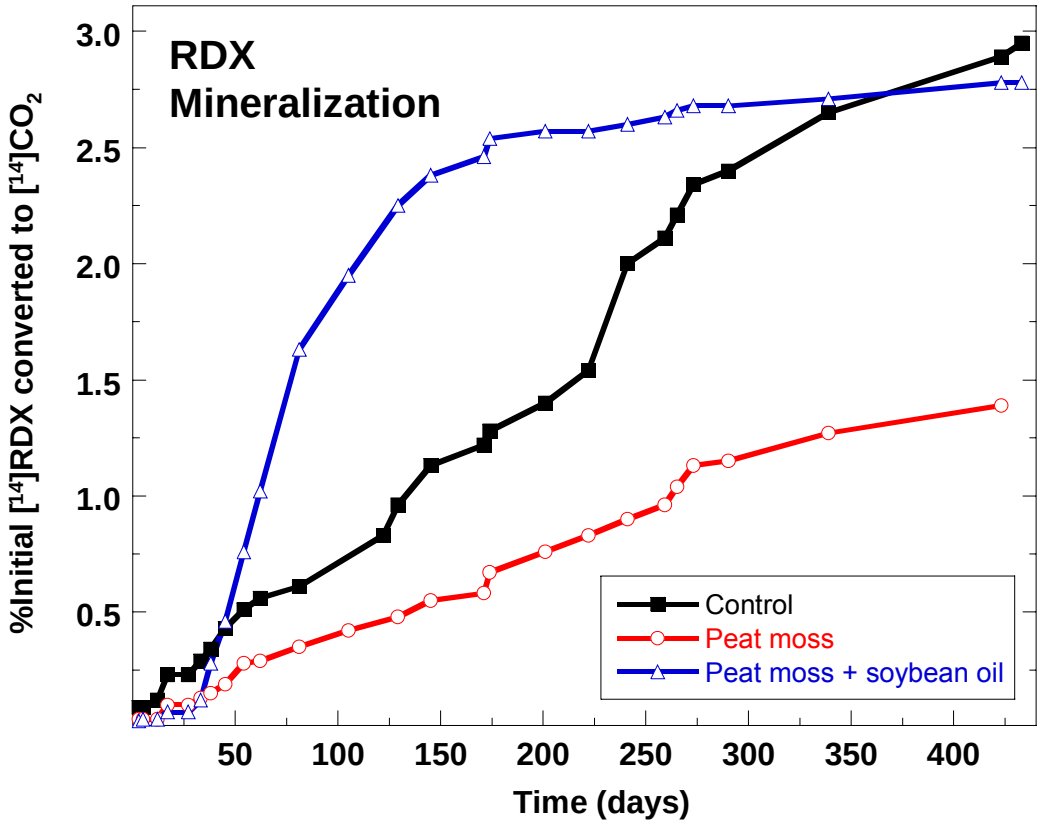


Figure 2. Concentration of bromide in aqueous samples at depths of 4”and 8” below the soil surface in soil columns with different treatments.

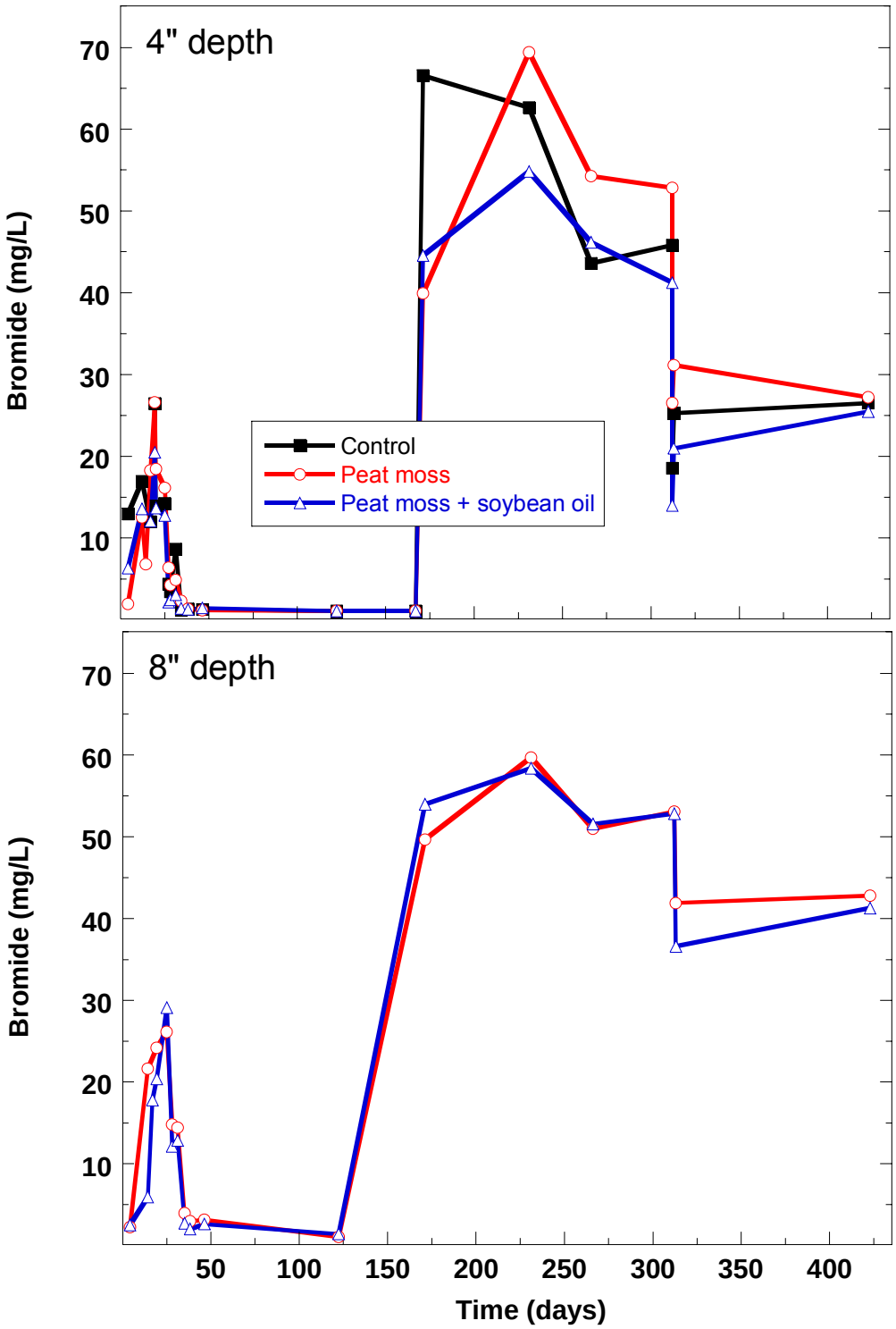


Figure 3. Concentration of [^{14}C] dpm in aqueous samples at depths of 4” and 8” below the soil surface in soil columns with different treatments.

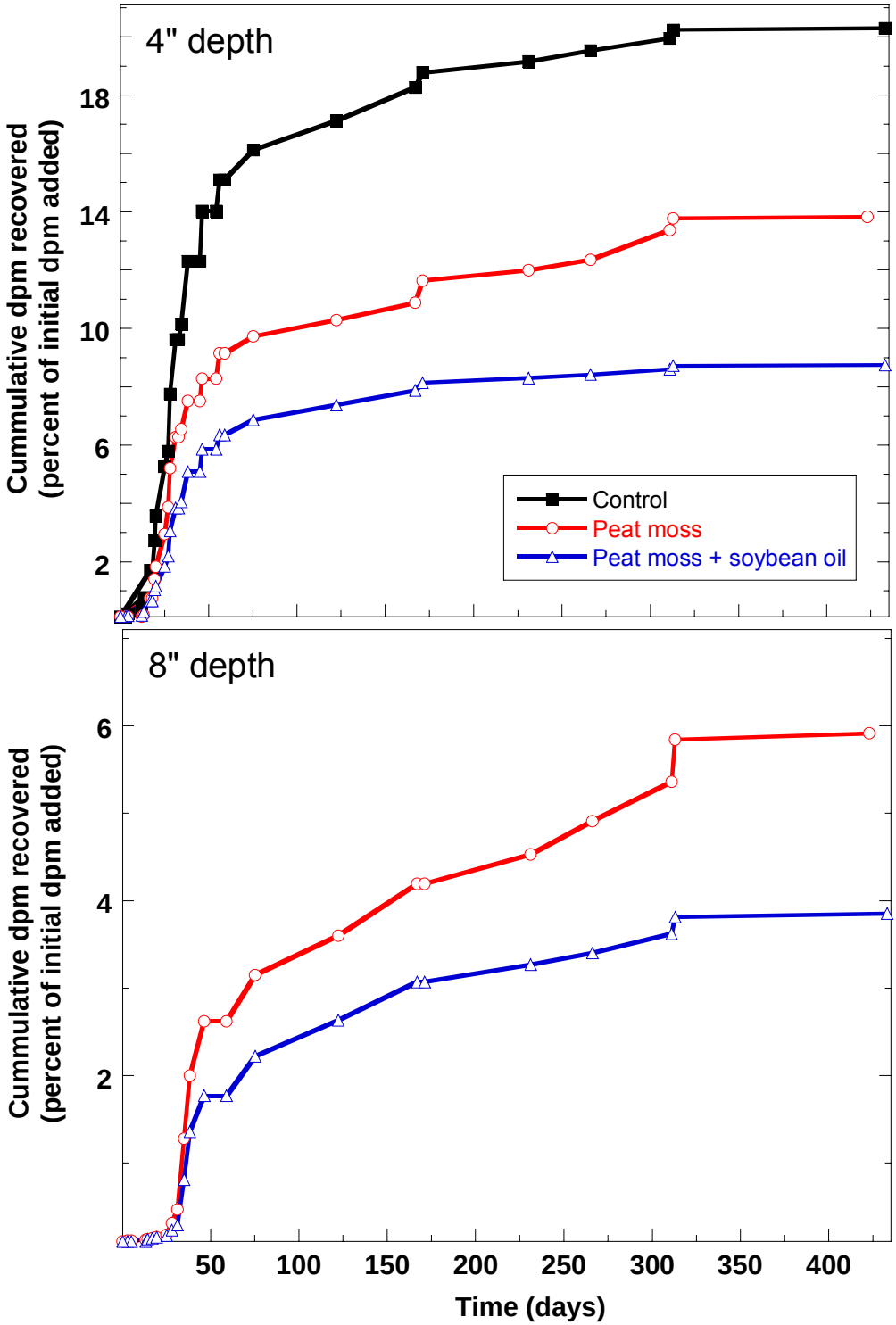


Figure 4. Concentrations of TNT, RDX, and HMX in aqueous samples at a depth of 4" below the soil surface in soil columns with different treatments

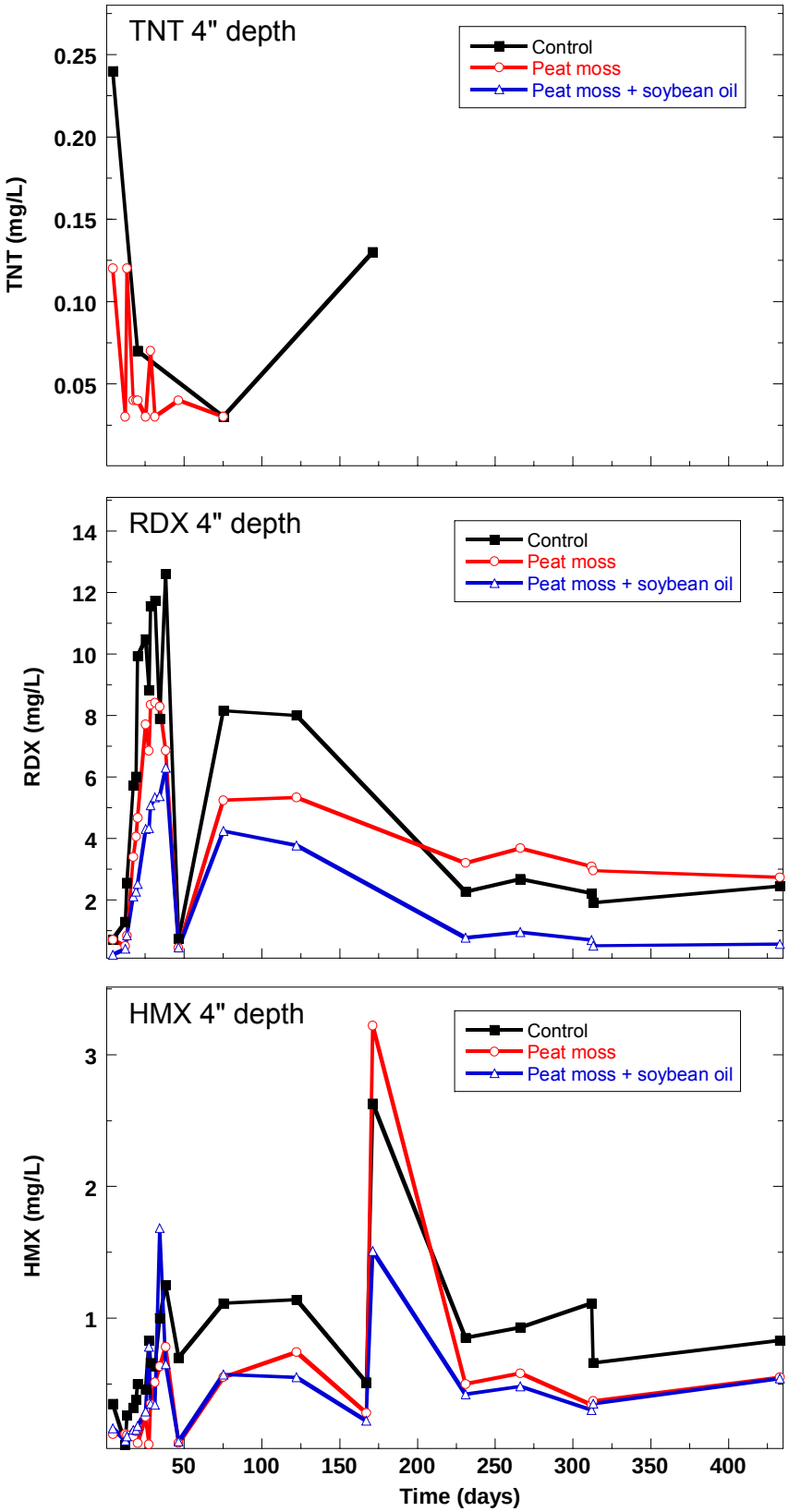


Figure 5. Concentrations of TNT, RDX, and HMX in aqueous samples at a depth of 8" below the soil surface in soil columns with different treatments.

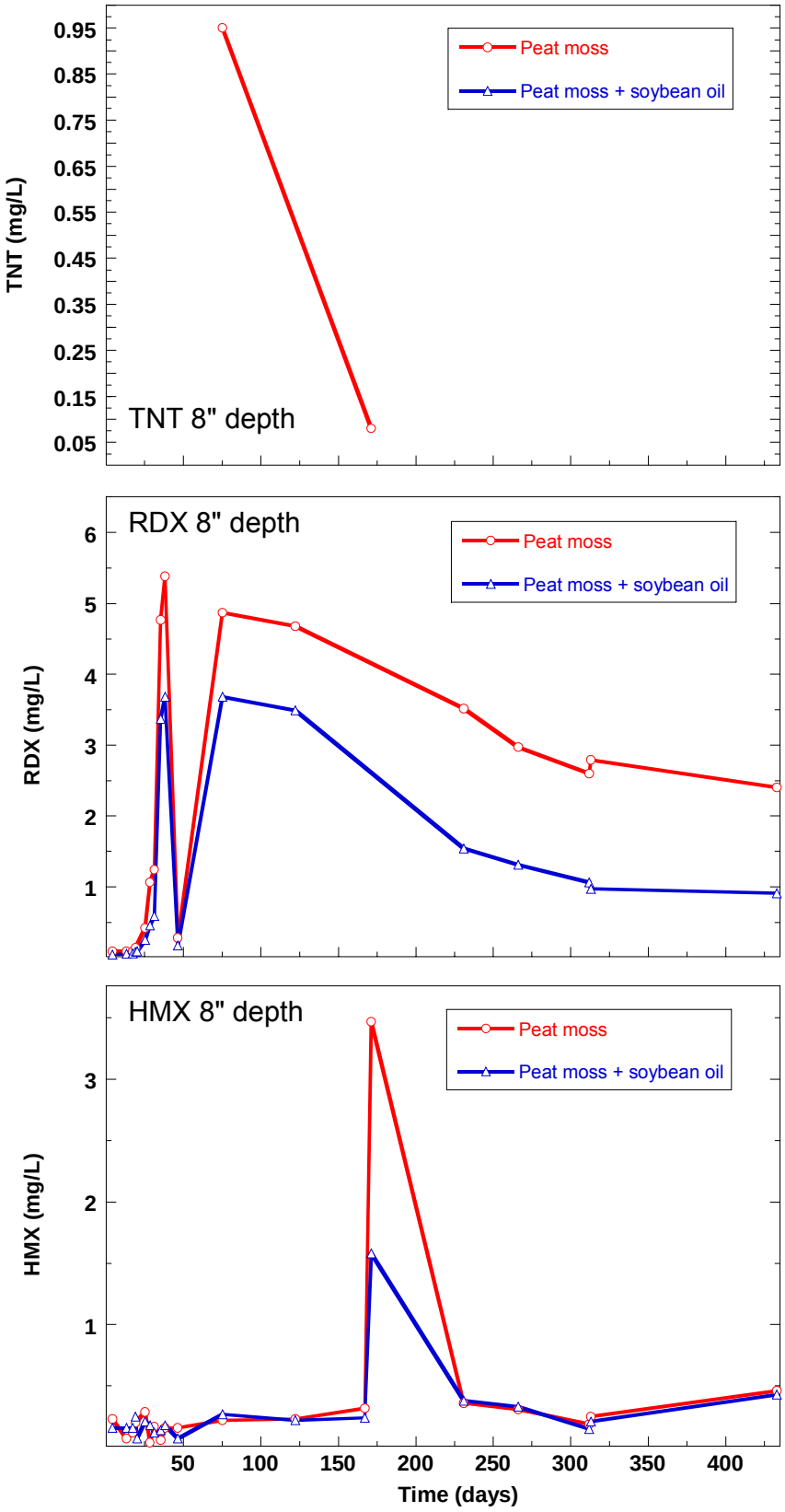


Figure 6. Distribution of radioactivity through the profiles of the soil columns at the termination of the experiment.

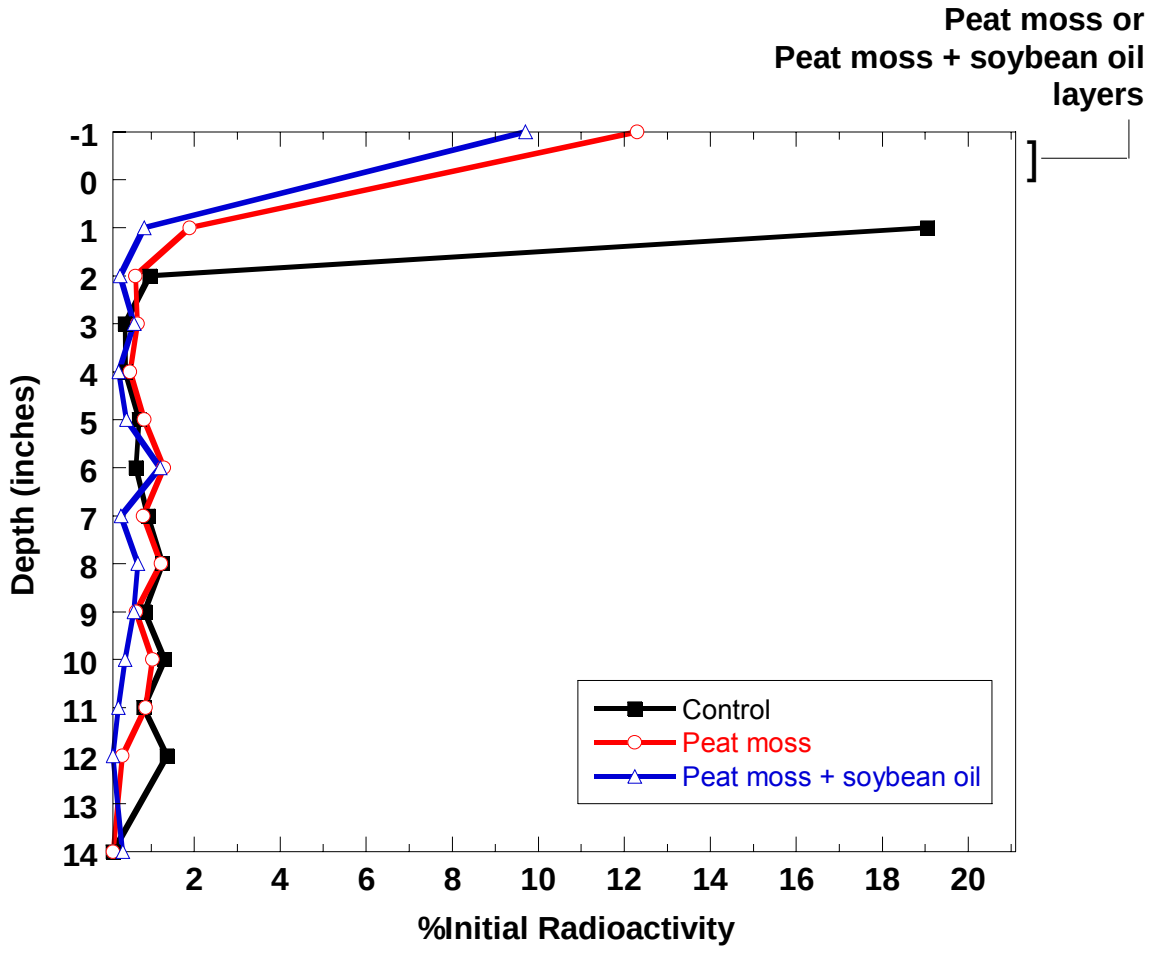


Figure 7. Distribution of TNT, RDX, and HMX through the profiles of the soil columns at the termination of the experiment.

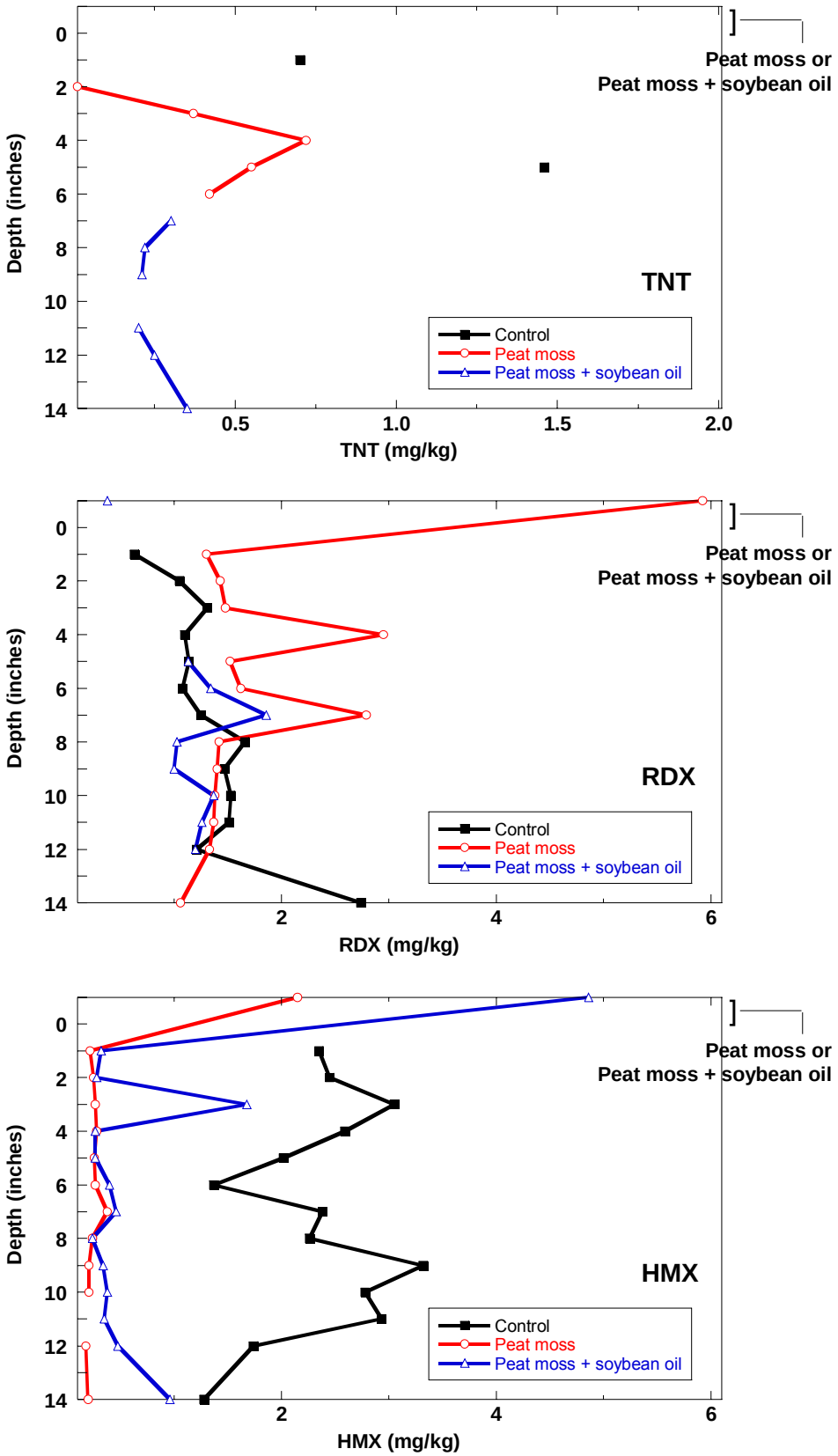


Figure 8. Distribution of the TNT metabolite 2,6-DNT through the profiles of the soil columns at the termination of the experiment.

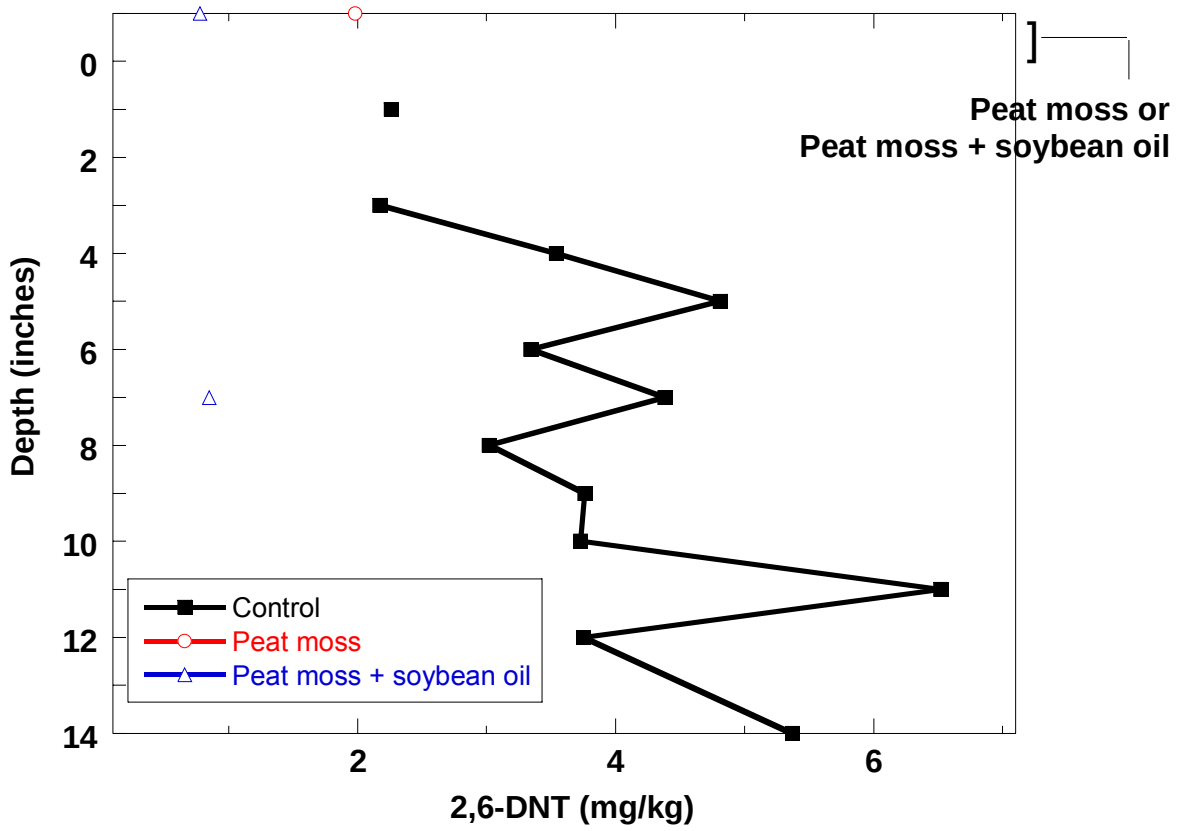


Figure 9. Modeling equations, definitions, and assumptions.

EQUATION 1

$$\left(1+\frac{f\rho K}{\theta}\right)\frac{\partial C}{\partial t}=D_x\frac{\partial^2 C}{\partial x^2}-v\frac{\partial C}{\partial x}+D_y\frac{\partial^2 C}{\partial y^2}+D_z\frac{\partial^2 C}{\partial z^2}-\frac{\alpha\rho}{\theta}\left[(1-f)KC-S\right]-\mu C$$

$$\frac{\partial S}{\partial t}=\alpha\left[(1-f)KC-S\right]$$

where,
C = aqueous phase concentration
S = solid phase concentration in the kinetically controlled domain
ν = water linear flow rate
ρ = density
θ = porosity
K = linear sorption constant (peat + oil)
μ = first order biodegradation rate constant
α= rate coefficient between equilibrium and kinetic domains
D = dispersion coefficient
f = fraction of peat sorption sites in the equilibrium domain

EQUATION 2

$$\mu=\frac{-\ln\left(C_2/C_1\right)}{t}$$

where,
C_2 = concentration prior to stopping
C_1 = concentration after re-starting
t = elapsed stop time (“stagnation”)

EQUATION 3

$$\alpha=kF$$

where,
F = shape factor (cm^2/cm^3)
k = mass transfer coefficient (cm/sec)

EQUATION 4

$$k = \frac{D_{aq}}{L}$$

where,
D_{aq} = aqueous phase diffusion coefficient
L = diffusion length

EQUATION 5

$$k = \frac{\left[\frac{\mu_{oil}}{\mu_{water}}\right]^{2/3} D_{aq}}{L} K_{OW}$$

where,
μ = viscosity
K_{OW} = measured linear soybean oil – water partition coefficient
L = diffusion length

NOTE: The viscosity ratio to the 2/3 power is an empirical relationship describing the effects of solvent viscosity on liquid-phase diffusion coefficients.

Figure 10. Predicted and observed TNT, RDX, and HMX concentrations in effluent of the constant loading mini-columns during Experiment 1.

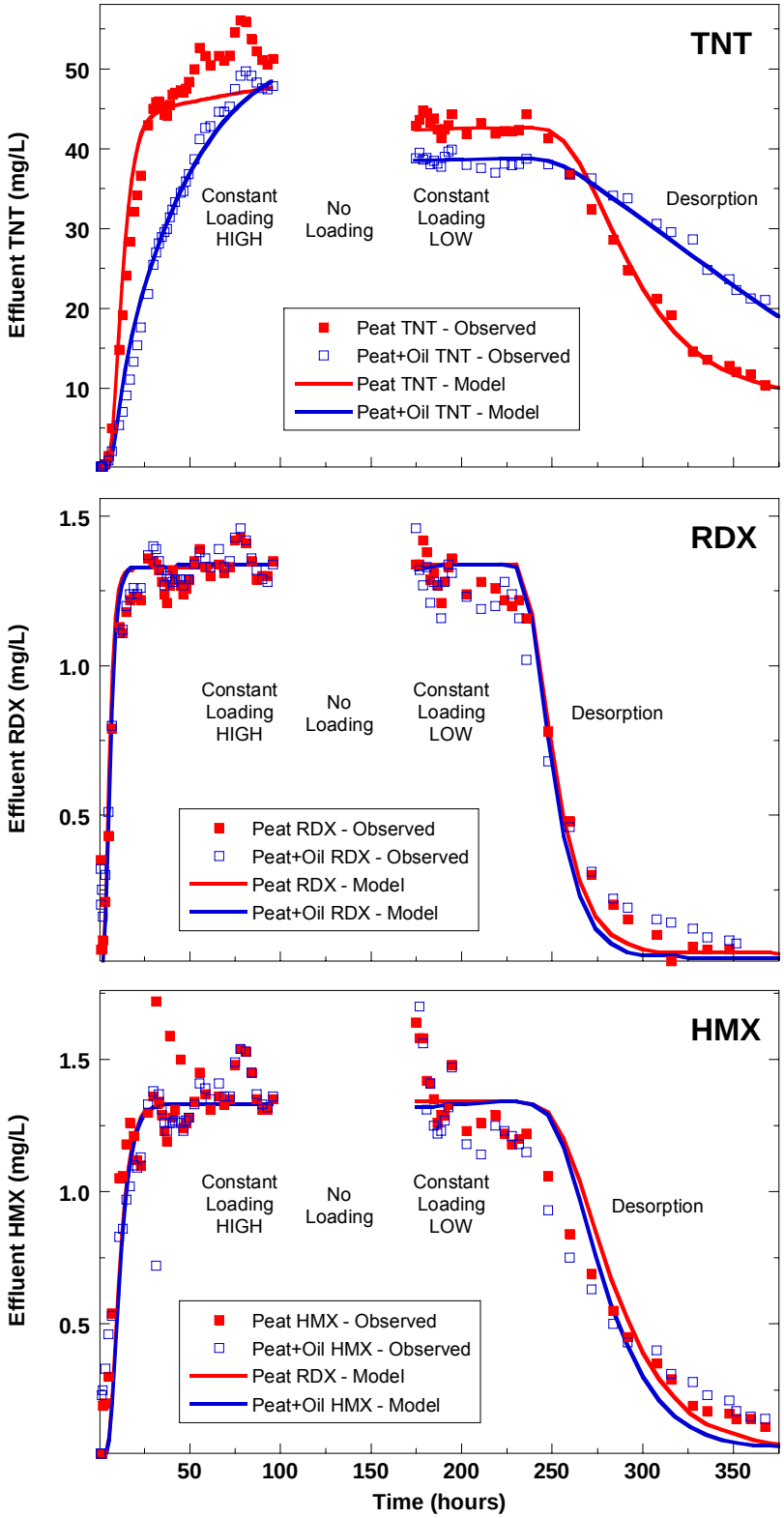


Figure 11. Predicted and observed TNT, RDX, and HMX concentrations in effluent of mini-columns during Experiment 2.

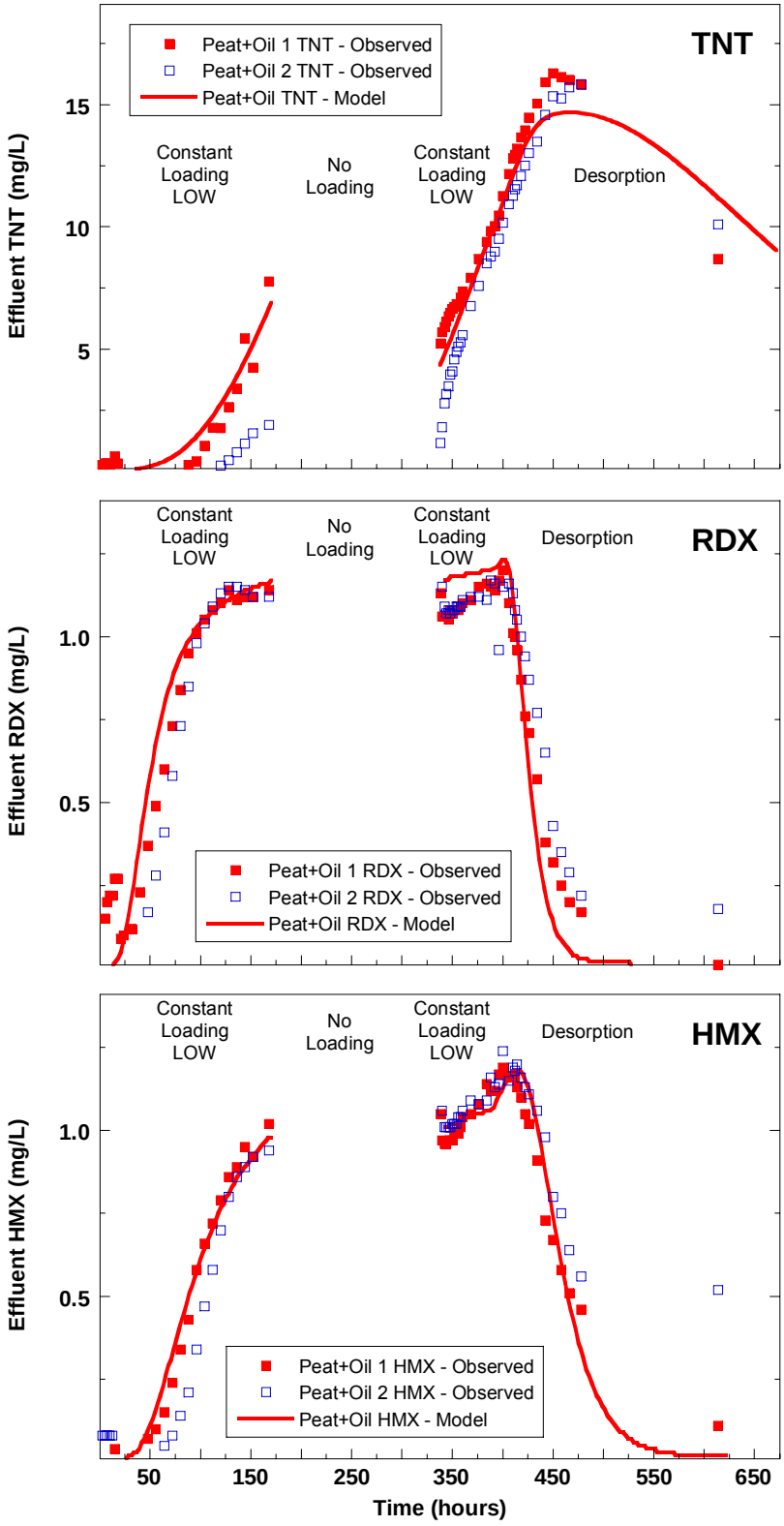
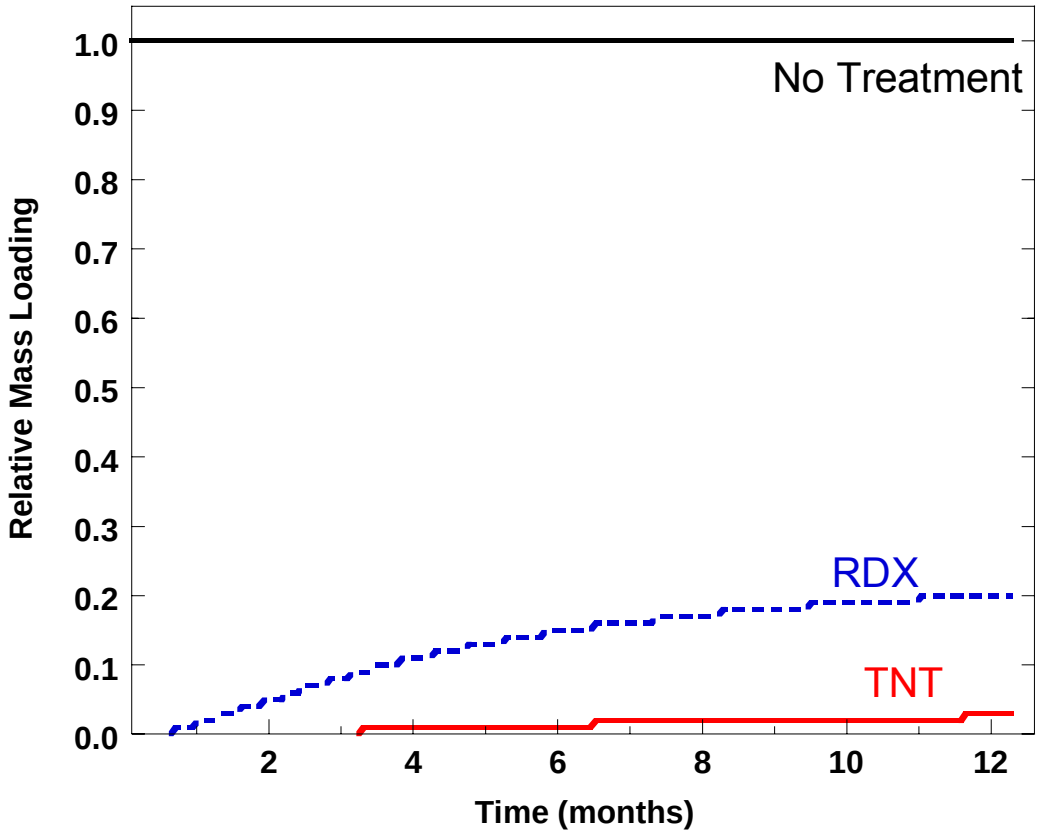


Figure 12. Predicted loading of TNT and RDX residues to range soil in the absence and presence of a 1.25" layer of peat moss plus soybean oil (0.5 ml oil/g peat) cover treatment. Loading of HMX would be similar to HMX.



Quarterly Progress Report

SERDP Project 1229 - Immobilization of Energetics on Live Fire Ranges
Year 2004 – First Quarter
April 15, 2004

This report covers technical progress for SERDP Project 1229 from December 15, 2003 – March 15, 2004.

The objective of this project is to develop a cost-effective technology to immobilize energetic compounds (TNT, RDX, HMX, and breakdown products) released as residues at firing ranges to prevent their migration to groundwater. The goal is to develop an inexpensive soil treatment that can be readily applied over wide and remote areas prior to or immediately following firing range activities.

During the current quarter, activities have focused on:

-continuing analyses of samples from the unsaturated soil column studies;
-performing a second experiment to determine fate and transport of explosives in the peat moss plus soybean oil treatment layer;
-further development and evaluation of a model to predict and help explain the explosives fate and transport in the peat moss plus soybean oil treatment layer.
-completing publication of 2 manuscripts, and preparing an additional manuscript for publication, detailing the results of our initial research.

ADDITIONAL EXPERIMENTS

6. ASSESS MULTIPHASE TRANSPORT OF EXPLOSIVES - SMALL-SCALE COLUMNS

Several additional experiments were performed to examine adsorption and desorption kinetics of TNT, RDX, and HMX on the peat moss and determine how the soybean oil affects the sorption behavior under flow conditions.

6.1 METHODS

General methods were as described in the Second and Third Quarter Reports. Briefly, peat moss with and without soybean oil was packed into 60 ml syringe barrels, and a constant flow of aqueous explosives-containing solution was passed through the material. Laboratory column data was evaluated using SOLUTRANS three-dimensional modeling software.

6.2 RESULTS

The results from these experiments are the basis of the Schaefer et al. paper. Themost significant findings were as follows:

-Uptake of explosives into the peat was mass transfer limited.

-Addition of the soybean oil had a negligible effect on the transport of RDX and HMX, but had a significant impact on the transport of TNT through the columns. The relatively large impact of soybean oil on TNT transport was due, in part, to enhanced biotransformation of TNT.
-The TNT biotransformation rate constant in the presence of soybean oil was approximately 10-times greater than in the presence of peat alone, and at least 10-times greater than the biotransformation rate constant of RDX or HMX.
-The presence of the soybean oil also increased the rate of TNT mass transfer to kinetically-controlled sorption sites, resulting in an overall decrease in TNT flux from the peat + soybean oil columns.

A diffusion model incorporating the effects of liquid viscosity and soybean oil-water contaminant partitioning was shown to accurately describe the impact of soybean oil addition on the transport of energetic compounds, suggesting that mass transfer limitations in peat moss are the result of liquid phase diffusion, and that the presence of non-aqueous phase liquids can affect sorption kinetics.

7. MISCELLANEOUS EXPERIMENTS

7.1 SORPTION ISOTHERMS WITH SINGLE AND MIXTURES OF EXPLOSIVES

7.1.1 METHODS

Single-point sorption experiments onto peat moss were carried out to evaluate the effects of competitive sorption among TNT, RDX, and HMX. The basic method was as described in previous reports. Experiments containing only one of these sorbates were compared to an experiment in which all three sorbates were present as a mixture.

7.1.2 RESULTS

Measured linear partition coefficients (K^s_d) for both the single-compound and mixture experiments are shown in Table 1. Results indicate that the presence of co-contaminants had a negligible effect (i.e., <10% change in the value of K^s_d) on uptake of TNT and RDX onto the peat. However, about a 25% decrease, relative to the single sorbate experiment, in HMX uptake onto the peat was observed for the mixture. This reduction in HMX sorption indicates that HMX sorption is non-ideal when TNT and RDX are present at the concentrations shown in Table 1. This non-ideal behavior is likely due to competition for organic carbon sorption sites within the peat.

7.2 ABIOTIC VS. BIOTIC LOSSES OF EXLOSIVES

Experiments were carried out to measure observed transformation rates of TNT, RDX, and HMX in peat moss and in peat moss amended with soybean oil.

7.2.1 METHODS

Experiments were carried out with and without formaldehyde addition (0.1% and 1% wt:v) to evaluate the relative effects of biotic versus abiotic transformation. Methods were as described

in previous reports. Biotic transformation rate constants were determined by measuring the decrease in aqueous phase concentration after the initial 24-hour sorption equilibrium period, adjusting these concentrations so only decreases in concentration to the abiotic (formaldehyde) controls were plotted, then regressing an exponential equation to the data to calculate the first-order biotransformation rate constant.

7.2.2 RESULTS

Results for the peat-only experiments are shown in Figure 1. No measureable decrease in RDX or HMX concentrations were observed, thus the biotransformation rate constants for these compounds were effectively zero. The observed biotransformation rate constant for TNT was 0.0008/hr. No significant decreasing trend in the RDX and HMX abiotic (formaldehyde) controls were observed, indicating that abiotic transformation was negligible. However, TNT concentrations decreased in the abiotic controls. The decreases in TNT concentration due to biotic and abiotic losses are also plotted in Figure 1, and the total transformation rate was calculated at 0.0017/hr.

Figure 2 shows the change in concentration after 24 hours due to total (biotic and abiotic) transformation in the peat plus oil system. No transformation of RDX or HMX were observed during this time period. However, the observed TNT total transformation rate constant was approximately four time greater (0.0069/hr) than the total transformation rate constant for the peat-only case. This is likely due to increase microbial activity resulting from the presence of the soybean oil. Addition of formaldehyde to the peat plus oil experiment did not appear to serve as an effective microbial kill agent, as losses in the abiotic controls were nearly identical to the losses in the biotic samples. This may be due to partitioning of the formaldehyde into the oil phase, thus negating its effectiveness as an aqueous kill agent. Assuming that the "real" abiotic losses for TNT were similar to those obtained in the peat-only experiments, the "real" TNT abiotic losses in the peat plus oil experiments were negligible.

7.3 SORPTION ISOTHERMS WITH DIFFERENT SIZE FRACTIONS OF PEAT MOSS

All work to date has been performed using peat moss in the size range 1-4 mm, or <1 mm. While we believe the results obtained using these size fractions are relevant to bulk peat, it was decided to confirm this by sieving peat into several size fractions, and evaluating sorption and desorption of TNT, RDX, and HMX onto the different size fractions.

7.3.1 METHODS

Bulk peat moss was separated into size fractions <1 mm, 1-4 mm, < 4 mm, and >4 mm using standard metal sieves. Fractions were dried at 50°C and weighed to determine the mass in each size fraction as a percentage of the total weight of bulk peat sieved.

Isotherms were conducted with three different concentrations of a mixture of TNT, RDX and HMX. Portions of the dried peat size fractions (0.15 g for <1 mm, 1-4 mm, <4 mm; 0.25 g for >4 mm and whole peat) were weighed into 40 ml clear glass vials. The explosive solution (20 ml) was added and the vial was closed with a teflon-lined silicon rubber stopper. Vials were

incubated at room temperature with horizontal shaking at 200 rpm. Aqueous samples were removed after 24 and 196 h and analyzed for explosives concentrations using HPLC.

7.3.2 RESULTS

The weight distribution of bulk peat fractions is presented in Table 2. A full 80% of the bulk peat was <4 mm in size, and about 75% of this material was <1 mm. It is likely that this large percentage of small sized peat particles makes this material such a good sorbent because of its high surface area.

The results of the sorption isotherms are given in Table 3, and illustrated graphically in Figure 3. The K^s_d was lowest for the >4 mm size fraction, while the K^s_d for the <1 mm size fraction was essentially the same as that for the whole peat. The K^s_d for the <4 mm and 1-4 mm size fractions fell between the lowest and highest K^s_d. Regardless of this variation of the K^s_d with size fraction, the absolute differences between the size fractions was small (~15%).

PUBLICATIONS

The following manuscripts are being prepared for submission. A full text PDF version of the Schaefer et al draft manuscript is attached.

Schaefer, C. E., M. E. Fuller, J. M. Lowey, and R. J. Steffan. Use of peat moss amended with soybean oil for mitigation of dissolved explosive compounds leaching into the subsurface: Insight into mass transfer mechanisms. Environmental Engineering Science (In preparation).

Fuller, M. E., J. M. Lowey, C. E. Schaefer, and R. J. Steffan. Evaluation of a peat moss-based technology for treating residues of the explosives TNT, RDX, and HMX. Science of the Total Environment (In preparation).

FIGURES AND TABLES

The tables and figures supporting this document have been provided to the SERDP Office as a separate attachment.

Table 1. Sorption to peat moss with single solutions and mixtures of TNT, RDX, and HMX.

	TNT K^s_d	RDX K^s_d	HMX K^s_d
Single compound	164	36	96
Mixture of TNT/RDX/HMX	156	33	74
%Decrease (single vs mixture)	-4.9	-9.7	-30.8

Table 2. Size fractionation of bulk peat moss

Peat size fraction	Weight%	Cumulative%
>4 mm	20	20
1 mm - 4 mm	26	46
<1 mm	54	100

Table 3. Sorption of explosives to different size fractions of peat moss

	K^s_d				
	Whole Peat	>4 mm	<4 mm	1-4 mm	<1 mm
TNT	205	138	171	162	196
RDX	43	33	39	38	43
HMX	101	64	80	82	96

Figure 1.

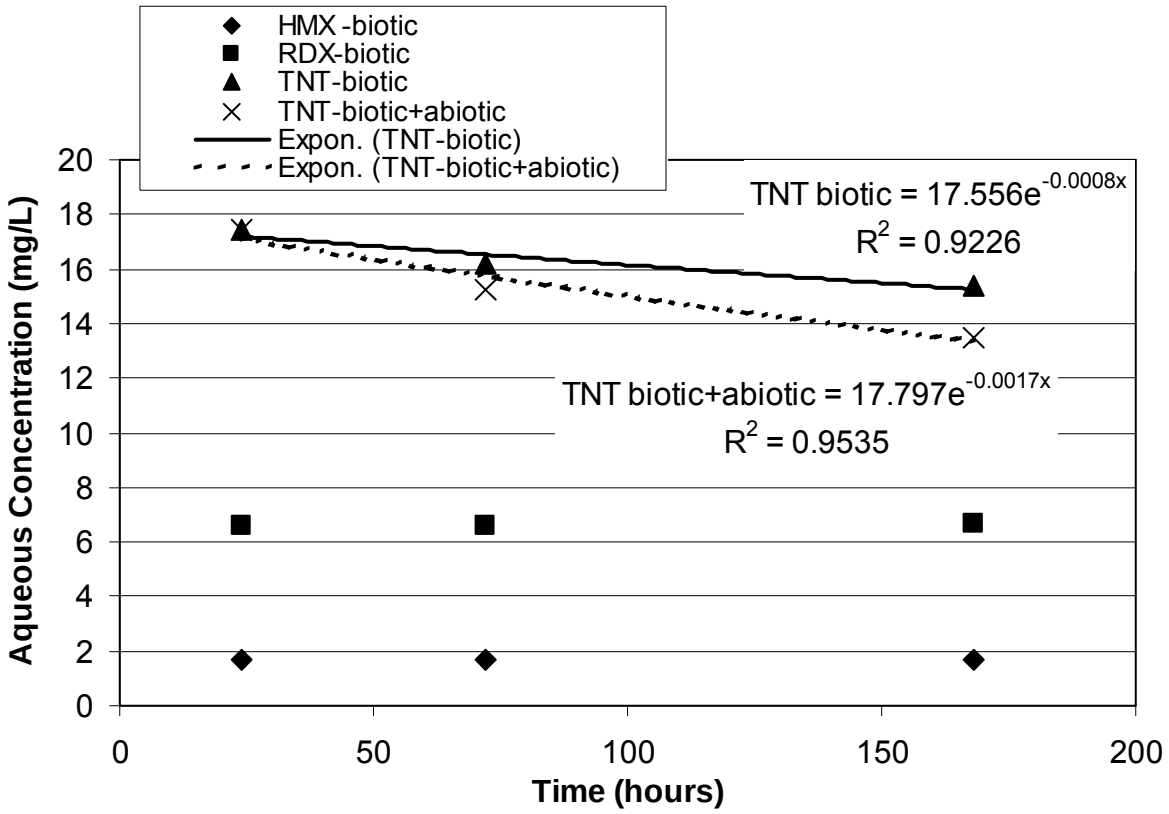

HMX -biotic
RDX-biotic
TNT-biotic
TNT-biotic+abiotic
Expon. (TNT-biotic)
Expon. (TNT-biotic+abiotic)
TNT biotic = 17.556e-0.0008x
R2 = 0.9226
TNT biotic+abiotic = 17.797e-0.0017x
R2 = 0.9535
Aqueous Concentration (mg/L)
Time (hours)
0
2
4
6
8
10
12
14
16
18
20
0
50
100
150
200

Figure 2.

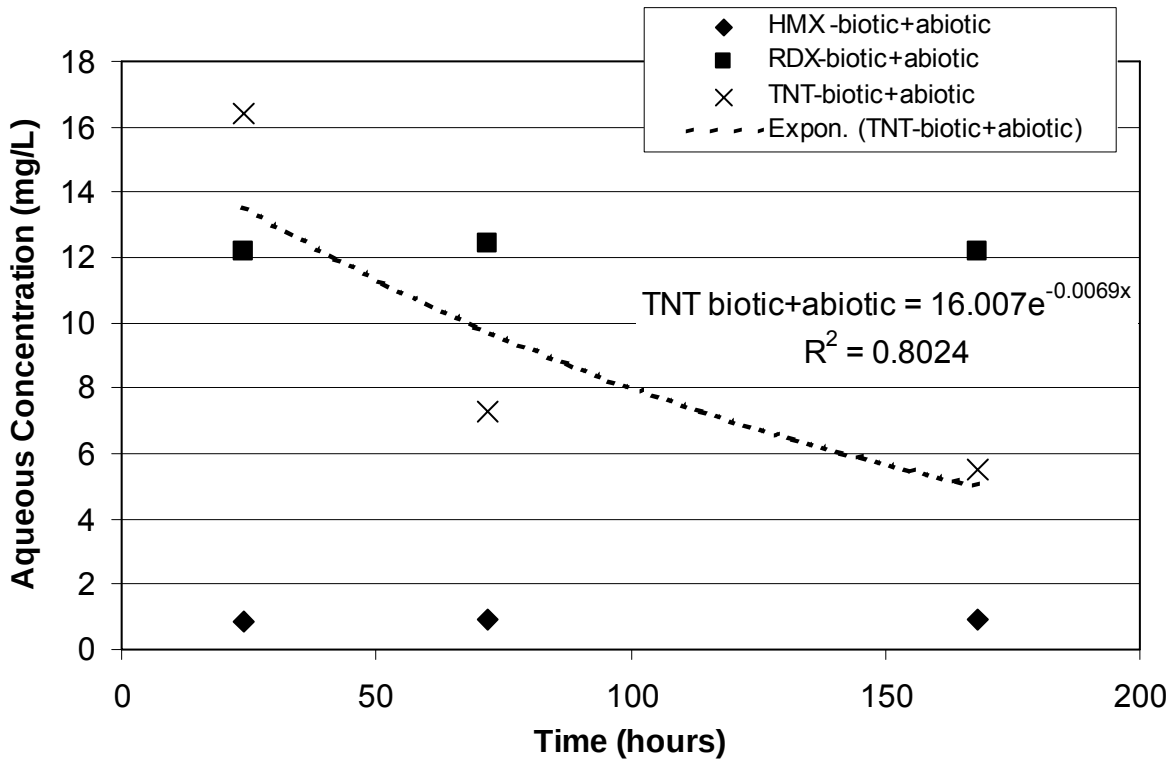
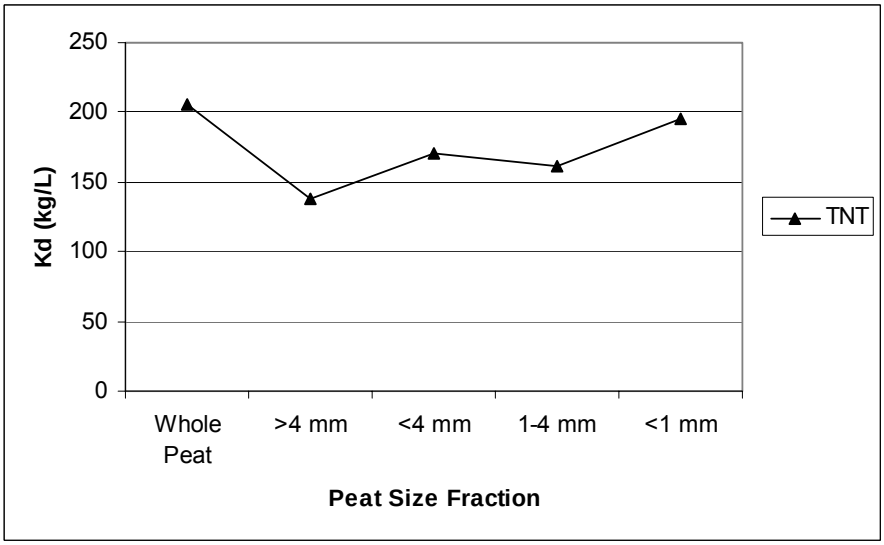
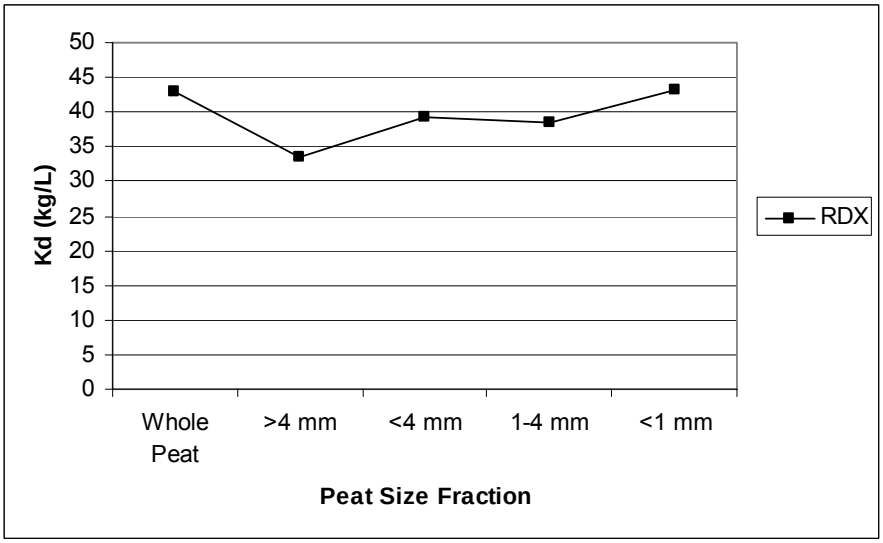
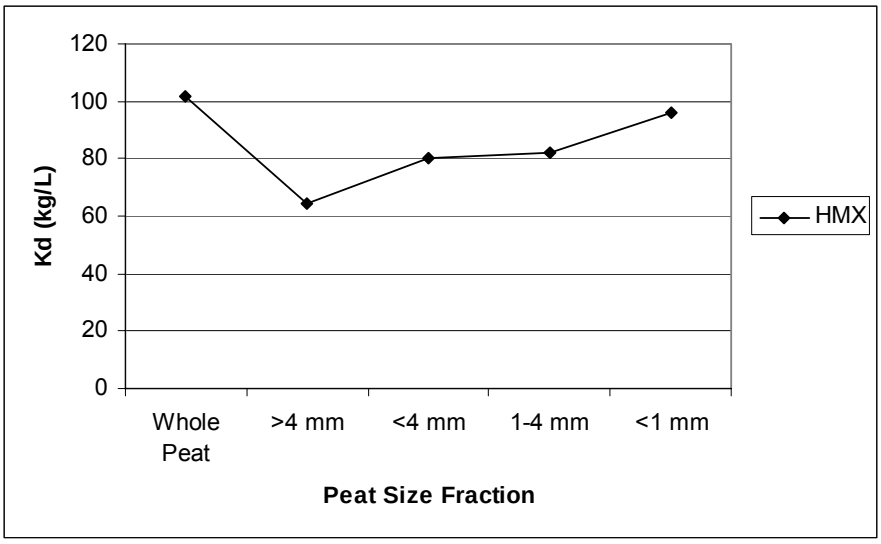

HMX -biotic+abiotic
RDX-biotic+abiotic
TNT-biotic+abiotic
Expon. (TNT-biotic+abiotic)
TNT biotic+abiotic = 16.007e-0.0069x
R2 = 0.8024
Aqueous Concentration (mg/L)
Time (hours)
0
2
4
6
8
10
12
14
16
18
0
50
100
150
200

Figure 3. Sorption coefficients for TNT, RDX, and HMX as a function of peat moss size fraction.







Quarterly Progress Report

SERDP Project 1229 - Immobilization of Energetics on Live Fire Ranges
Year 2004 – Second Quarter
July 1, 2004

This report covers technical progress for SERDP Project 1229 from March 15, 2003 – June 15, 2004.

The objective of this project is to develop a cost-effective technology to immobilize energetic compounds (TNT, RDX, HMX, and breakdown products) released as residues at firing ranges to prevent their migration to groundwater. The goal is to develop an inexpensive soil treatment that can be readily applied over wide and remote areas prior to or immediately following firing range activities.

During the current quarter, activities have focused on:

-completing all laboratory work and sample analyses
-continuing data analysis
-continuing preparation and submission of manuscripts for publication

RESULTS

The significant results of this work will be more fully reported in the Final Report for this project, which will be submitted at the end of September 2004.

PUBLICATIONS

The following manuscripts have been submitted or are being prepared for submission. A PDF version of the draft Fuller et al. manuscript is attached.

Schaefer, C. E., M. E. Fuller, J. M. Lowey, and R. J. Steffan. Use of peat moss amended with soybean oil for mitigation of dissolved explosive compounds leaching into the subsurface: Insight into mass transfer mechanisms. Environmental Engineering Science (Submitted).

Fuller, M. E., J. M. Lowey, C. E. Schaefer, and R. J. Steffan. Evaluation of a peat moss-based technology for treating residues of the explosives TNT, RDX, and HMX. Science of the Total Environment (In preparation).

FIGURES AND TABLES

A hardcopy of Fuller et al. manuscript has been provided to the SERDP Office as a separate attachment.

LIFE-CYCLE COST ESTIMATE

I. INTRODUCTION

A request was made during the 2002 In-Progress Review meeting to provide a life-cycle cost estimate for this technology if it were to be applied in the field. We have performed the analysis to calculate this estimate based on laboratory data generated during Year 1 of this project.

The preliminary results indicated that 0.5 inches of a combination of peat moss and soybean oil resulted in a decrease in the amount of leaching of explosives compounds and their breakdown products into the soil, compared to both the control and peat moss only treatments. Also, more RDX was mineralized in the soil column treated with peat moss plus soybean oil compared to the other two soil columns.

Based on these results, the life-cycle cost estimate was prepared with a starting case of a 0.5 inch application of peat moss plus soybean oil, and including 1 inch and 2 inch application rates for comparison. A per acre cost for a single application was calculated for each application rate. Due to the nature of the technology, new applications would be required prior to each live fire training activity in a given area.

II. CALCULATIONS

Life-Cylce Cost Estimate
SERDP CU-1229, Immobilization of Energetics on Live Fire Ranges

Assumptions
1) Calculations are on a per acre basis.
2) Site contains no UXO (workers & equipment can navigate through area).

Permitting	TOTAL
None expected due to innocuous nature of materials	0

Equipment Rental	$/day	#	Total/day	Days	TOTAL
Mixer	400	2	$800	2	$1,600
Mulch spreader/blower	500	1	500	2	1,000

NOTES: Costs is a best estimate based on several sources, and includes a 2X conservative multiplier

Labor	$/hr	#	Total/hr	Hours	TOTAL
Supervisor/Landscape Engineer	65	1	$65	16	$1,040
Field Tedchnicians	40	3	120	24	2,880

NOTES: Costs do not reflect Travel and Food & Lodging costs if coming from out of town.

Other	TOTAL
Demobilization	0
Waste disposal	0
SUBTOTAL	$6,520

Materials	Depth of applied peat moss plus soybean oil layer (inches)	Peat moss	Soybean oil	Materials TOTAL	**GRAND TOTAL**
	0.50	$1,650	$459	$2,109	**$8,629**
	1.00	$3,300	$919	4,218	**10,738**
	2.00	$6,599	$1,837	8,436	**14,956**

NOTES: A depth of 0.5 inches was used for the initial soil column experiments.
Costs for peat moss do not reflect bulk pricing discounts or shipping costs.
Costs for soybean oil are for bulk purchase, excluding transport.
Application rate of soybean oil based on initial soil column experimental conditions.

Appendix B

List of Technical Publications

1. Articles in peer-reviewed journals

Fuller M. E., Hatzinger P. B., Rungkamol D., Schuster R. L., and Steffan R. J. (2004) Enhancing the attenuation of explosives in surface soils at military facilities: Combined sorption and biodegradation. *Environmental Toxicology and Chemistry* 23: 313-324.
[Reprint Attached]

Fuller M. E., Lowey J. M., Schaefer C. E., and Steffan R. J. Evaluation of a peat moss-based technology for treating residues of the explosives TNT, RDX, and HMX. *Soil & Sediment Contamination: an International Juornal* (Submitted):
[Draft Attached]

Hatzinger P. B., Fuller M. E., Rungkamol D., Schuster R. L., and Steffan R. J. (2004) Enhancing the attenuation of explosives in surface soils at military facilities: Sorption-desorption isotherms. *Environmental Toxicology and Chemistry* 23: 306-312.
[Reprint Attached]

Schaefer C. E., Fuller M. E., Lowey J. M., and Steffan R. J. Use of peat moss amended with soybean oil for mitigation of dissolved explosive compounds leaching into the subsurface: Insight into mass transfer mechanisms. *Environmental Engineering Science* (Submitted):
[Draft Attached]

2. Published technical abstracts.

Fuller M., Rungkamol D., Hatzinger P., and Steffan R. (2002) New approaches to protecting groundwater resources at live fire ranges (Invited Platform Session). The 2002 Partners in Environmental Technology Technical Symposium & Workshop. Washington, D.C., USA.

Fuller M. E., Rungkamol D., Hatzinger P. B., and Steffan R. J. (2002) Immobilization and biodegradation of energetic compounds at live fire ranges. The 2002 Partners in

Environmental Technology Technical Symposium & Workshop. Washington, D.C., USA.

Fuller M. E., Schaefer C. E., Lowey J. M., Hatzinger P. B., and Steffan R. J. (2003) Enhancing the immobilization and biodegradation of energetic compounds at live fire ranges. The 2003 Partners in Environmental Technology Technical Symposium & Workshop. Washington, D.C., USA.

Fuller M., Hatzinger P., Schuster R., and Steffan R. (2003) Enhanced biodegradation of explosive compounds in soil. ASM 103rd General Meeting. Washington, D.C., USA.

Fuller M. E., Rungkamol D., Hatzinger P. B., and Steffan R. J. (2001) Biodegradation and immobilization of high energy materials at live fire ranges and arsenals. The 2001 Partners in Environmental Technology Technical Symposium & Workshop. Washington, D.C., USA.

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ENHANCING THE ATTENUATION OF EXPLOSIVES IN SURFACE SOILS AT MILITARY FACILITIES: SORPTION–DESORPTION ISOTHERMS

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(*Received* 28 *March* 2003; *Accepted* 9 *July* 2003)

Abstract—The primary objective of the present study was to develop inexpensive soil amendments that can be applied to enhance the adsorption of energetic compounds on military training ranges, thus limiting the potential for these compounds to migrate to groundwater. Adsorption and desorption isotherms were determined for 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine with a wide variety of natural and man-made adsorbents, including wheat straw, sawdust, peat moss, ground rubber tires, and clays. Among the various adsorbents tested, peat moss proved to be the most effective sorbent for the three explosives. The adsorption coefficients (K_d^s) for TNT and RDX with peat (310 and 87 L/kg, respectively) were at least two orders of magnitude higher than that determined for adsorption of these energetics with two surface soils. The adsorption–desorption isotherms for the explosives showed considerable hysteresis ($K_d^s < K_d^d$) with some of the solid adsorbents, suggesting that the sorption process is not readily reversible but, rather, that some fraction of the adsorbed contaminant is either irreversibly bound or present as a slowly desorbed fraction. The data indicate that the application of specific adsorbents to soils at military impact ranges may significantly improve the protection of local groundwater resources.

Keywords—Isotherm Explosives Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine Hexahydro-1,3,5-trinitro-1,3,5-triazine 2,4,6-Trinitrotoluene

INTRODUCTION

There has been increasing interest regarding the fate of explosives and other energetic compounds in the environment as reports of soil and groundwater contamination with these materials at military installations have become more frequent [1–3]. Several common explosives, including 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), have been observed to be recalcitrant in many environments, leading to the potential for long-term contamination at facilities where they are released [4]. Impact ranges, which are used by the U.S. Department of Defense for testing new ordnance and for training personnel to use mortars, rockets, and other munitions, are common sites for environmental contamination with these compounds.

Although no federal drinking water standards currently exist for the aforementioned explosives, the U.S. Environmental Protection Agency has established health advisory levels for TNT, HMX, RDX, 2,4-dinitrotoluene (2,4-DNT), and 2,6-dinitrotoluene (2,6-DNT) in drinking water (http://www.epa.gov/waterscience/drinking/standards/dwstandards.pdf). Both 2,4-DNT and 2,6-DNT have also been listed on the Unregulated Contaminant Monitoring Regulation List, and RDX will be added when appropriate analytical methods have been established (http://www.epa.gov/safewater/standard/ucmr/ucmrfact.html#list). The drinking water health advisory levels for lifetime exposure to HMX, RDX, and TNT are 400, 2, and 2 μg/L, respectively. Based on standards for other drinking water contaminants, the eventual maximum contaminant levels for these compounds likely will be similar values. The low health advisory levels for RDX and TNT reflect the potential threat that these compounds pose to humans and other organisms. Most of the energetic compounds examined are toxic and/or mutagenic at concentrations considerably less than their respective solubility limits [5–7]. These data provide the impetus for research efforts focused on preventing new groundwater contamination with explosives and on treating existing contamination.

Nitroaromatic and nitramine explosives generally have low octanol–water partition coefficients (e.g., log K_{ow} values for RDX and TNT are 0.87 and 1.86, respectively) and, subsequently, a high potential for mobility in the environment [8]. The TNT has been observed to adsorb to specific clay minerals and to humic acids (a key component of soil organic matter), but the extent of sorption is highly dependent on environmental factors, such as redox potential, pH, ionic strength, and the exchangeable cations associated with the clays [8–10]. The sorption characteristics of the nitramine explosives RDX and HMX to specific soil components have not been extensively investigated, although RDX has been shown to exhibit a low sorption coefficient (K_d^s of 0.83 L/kg) in topsoil [1] and to be relatively unaffected by the exchangeable-cation composition during adsorption to clay minerals [11]. In sandy soils with little organic matter or clay content, such as those at the Massachusetts Military Reservation (MMR; Cape Cod, MA, USA), transport of explosives from contaminated soils in training areas to groundwater is likely. Recent reports of groundwater contamination at MMR with RDX confirm this assumption (http://www.groundwaterprogram.org/iagwsp.htm).

The objective of the present research effort was to develop a technology that can be applied to the soil surface to prevent the migration of explosive residues at impact ranges and other military training facilities. If adsorption of explosives can be enhanced in surface soils, groundwater resources will be protected. Because of the large expanse of many military ranges,

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Table 1. Characteristics of the Massachusetts Military Reservation soils[a]

	Concentration (mg/kg)[b]					Concentration (mg/kg)[c]						
Soil	TNT	2ADNT	TNB	HMX	RDX	NO_2-N	NO_3-N	TKN[c]	NH_3-N	Total P	TOC	log CFU/g
Soil C	284	12	4	124	1296	5	<4	640	340	390	2900	4.8
Soil G	<1	<1	<1	<1	<1	<4	<4	380	70	190	6400	5.6

[a] TNT = 2,4,6-trinitrotoluene; 2ADNT = 2-amino-4,6-dinitrotoluene; TNB = 1,3,5-trinitrobenzene; HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine; TKN = total Kjeldahl nitrogen; TOC = total organic carbon; CFU = colony-forming units.
[b] 60°C dried soil basis.
[c] 105°C dried soil basis.

the focus of this project was on the application of inexpensive sorbents that can be applied effectively over large, targeted areas. This report describes the results of studies performed to quantify the adsorption of RDX, HMX, and TNT to a wide variety of natural and man-made adsorbent materials, including peat moss, sawdust, ground rubber tires, straw, and clays. These materials were chosen based on availability, cost, and differences in physical characteristics and sorptive properties. The most effective adsorbents were subsequently combined with microbial cosubstrates to promote biological reduction of the adsorbed energetic compounds. The results of those experiments are reported in a companion paper [12].

MATERIALS AND METHODS

Chemicals, media, and soil

All solvents were of high-performance liquid chromatography (HPLC) grade, and chemicals were of reagent grade or better. Sources for the materials tested as sorbents were as follows: Kaolin clay, montmorillonite clay KSF, and montmorillonite clay K10 were purchased from Aldrich Chemical (Milwaukee, WI, USA). Both KSF and K10 clays differed in terms of bulk density (300–370 g/L and 800–850 g/L, respectively) and surface area (220–270 m^2/g and 20–40 m^2/g, respectively). Ground rubber tires in two sizes (no. 30 mesh and no. 30–40 mesh) were obtained from an Oklahoma (USA) rubber-recycling plant. Sawdust (conifer/deciduous mix), seaweed, lobster shells, and clam shells were obtained from local New England (USA) sources. Sphagnum peat moss was purchased from a home and garden store, and wheat straw was obtained from a New Jersey (USA) farmer. Rice hulls were obtained from an rice-processing plant in Arkansas (USA), and vermiculite was of standard packing material grade. The three clays, ground rubber tires no. 30 and no. 30 to 40, and rice hulls were used without further processing. The peat moss, sawdust, and vermiculite were sieved, and particles ranging from 1 to 4 mm were collected for experiments. The wheat straw and seaweed were cut and sieved, and the 1- to 4-mm size fraction was collected. The clam and lobster shells were thoroughly washed, dried, crushed using a rubber mallet, and then sieved as described above.

Crystalline TNT (30% H_2O, wt:wt) and analytical standard–grade solutions of RDX and HMX were purchased from ChemService (West Chester, PA, USA). Analytical standards for the U.S. Environmental Protection Agency Method 8330 were purchased from Restek (Bellefonte, PA, USA). Small quantities of the three nitroso-containing metabolites (hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine; hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine; and hexahydro-1,3,5-trinitroso-1,3,5-triazine) of RDX were purchased from SRI International (Menlo Park, CA, USA). Research quantities of RDX (~7% HMX as a manufacturing impurity) were a gift from James Phelan at Sandia National Laboratories (Albuquerque, NM, USA). Research quantities of HMX and TNT (specific activity = 26.3 mCi/mmol) were a gift from Herb Fredrickson at the U.S. Army Engineer Research and Development Center (Environmental Laboratory, Vicksburg, MS, USA). The [^{14}C]RDX (specific activity = 60.0 mCi/mmol) and [^{14}C]HMX (specific activity = 6.8 mCi/mmol) were purchased from Perkin-Elmer Life Sciences (Boston, MA, USA). Radiolabeled compounds were uniformly ring-labeled and had a radiochemical and chemical purity of >99%.

Soils were obtained from two places in demolition area 1 at the MMR. These soils were used as surrogates to represent those found on the impact ranges at MMR. The presence of unexploded ordnance in the impact area prevented sample collection from this region. One soil sample (soil C) was collected from within the most contaminated zone of demolition area 1, and one noncontaminated soil sample (soil G) was taken from a grassy area adjacent to the explosive-dumping region. Explosive concentrations and other relevant soil properties for the two soils are listed in Table 1.

Sorption kinetics and capacity

An initial experiment was performed to determine the kinetics of explosive adsorption to and desorption from the various sorbents and to select those materials that exhibited the greatest sorption capacity. All of the sorbent materials were evaluated for TNT and HMX adsorption and desorption. For RDX, montmorillonite clay KSF, peat moss, ground rubber tires no. 30 and no. 30 to 40, sawdust, rice hulls, and wheat straw were evaluated. Air-dry sorbents (~1 g) were weighed into duplicate Teflon® Oak Ridge centrifuge tubes (30-ml volume; Fisher Scientific, Pittsburgh, PA, USA) and autoclaved (1 h, 121°C, 20 psi) to inhibit biological activity (i.e., explosive transformation) during the experiments. Tubes without sorbent were prepared to control for abiotic losses and sorption onto the inside of the tubes. A single concentration of each explosive was prepared in an aqueous solution of $CaCl_2$ (3 mM) by adding a known volume of a concentrated explosive stock (in acetonitrile) to sterile glass bottles, evaporating the solvent under a stream of nitrogen, adding a known volume of $CaCl_2$, and then sonicating until all the explosive had dissolved. The concentrations of TNT, RDX, and HMX used for these experiments were approximately 80, 37, and 5 mg/L, respectively. The ^{14}C-labeled explosive was then added to the solution to achieve approximately 100,000 dpm/ml of each compound. The actual concentration of the unlabeled explosive was determined by HPLC as described below. The ^{14}C-labeled explosives were quantified by mixing an aliquot of the explosive solution with 5 ml of liquiscint scintillation cocktail (Na-

tional Diagnostics, Atlanta, GA, USA) followed by analysis using a Pharmacia LKB Model 1209 Rackbeta scintillation counter (Pharmacia LKB Nuclear, Gaithersburg, MD, USA).

Fifteen milliliters of the explosive solution were added to the Oak Ridge tubes containing the sorbents. The tubes were sealed and shaken horizontally (250 rpm) at room temperature, and small (0.5-ml) subsamples were removed periodically during a 24-h period. The samples were centrifuged, and 100 μl of the supernatant were analyzed by scintillation counting. After the radioactivity in the supernatant reached apparent equilibrium, the entire tube was centrifuged (10,000 *g*, 4°C, 20 min) to pellet the solid sorbents, and all the liquid was removed and analyzed for residual radioactivity. To determine desorption kinetics, a fresh, 15-ml aliquot of $CaCl_2$ solution was added to each tube used in the initial adsorption studies. The tubes were shaken, and subsamples were periodically analyzed for radioactivity as described above. When apparent equilibrium was achieved, this procedure was repeated a second time for selected sorbents.

Sorption and desorption isotherms

Sorption and desorption isotherms for each explosive were determined using the four solids that exhibited the highest percentage adsorption during the kinetic studies. Isotherms were also determined for the two MMR soils. The experiments were performed as described for the kinetic studies, except that several concentrations of each explosive were tested in triplicate and the aqueous concentration of each was measured only after apparent equilibrium had been achieved (24 h). The aqueous concentration of the various explosives in each treatment was determined by HPLC (see below) or calculated from the specific activities of the ^{14}C-labeled explosive compounds (for those concentrations less than the detection limit of the HPLC). The nominal concentration ranges used for these experiments were as follows: TNT, 0.050 to 70 mg/L; RDX, 0.025 to 30 mg/L; HMX, 0.025 to 3 mg/L. Actual explosive concentrations were measured using HPLC.

The distribution coefficient (K_d) for each explosive between the aqueous and adsorbed phases was determined for each of the adsorbents tested. The adsorption coefficient (K_d^s) and the desorption coefficient (K_d^d) were determined by modeling the data from the above experiments using the Freundlich equation [13,14]:

$$C_s = K(C_e)^n$$

where C_s is the concentration of the explosive sorbed to the solid phase (mg/kg), C_e is the aqueous concentration (mg/L) of the explosive at equilibrium with the sorbed phase, K is the distribution coefficient, and n is a constant. Goodness of fit was based on the coefficient of determination (r^2).

Analysis of explosives

Analyses of RDX, HMX, and TNT were performed according to U.S. Environmental Protection Agency Method 8330. The analysis was conducted using a Hewlett-Packard Model 1050 high-performance liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) fitted with an autosampler, quaternary pump, Pinnacle Octyl C_{18} reverse-phase column (Restek), and diode-array detector (peak detection at 230 nm). The mobile phase was 1:1 methanol:water (vol:vol), with a flow rate of 0.85 ml/min. Effective detection limits for the analytes were determined to be approximately 0.025 mg/L.

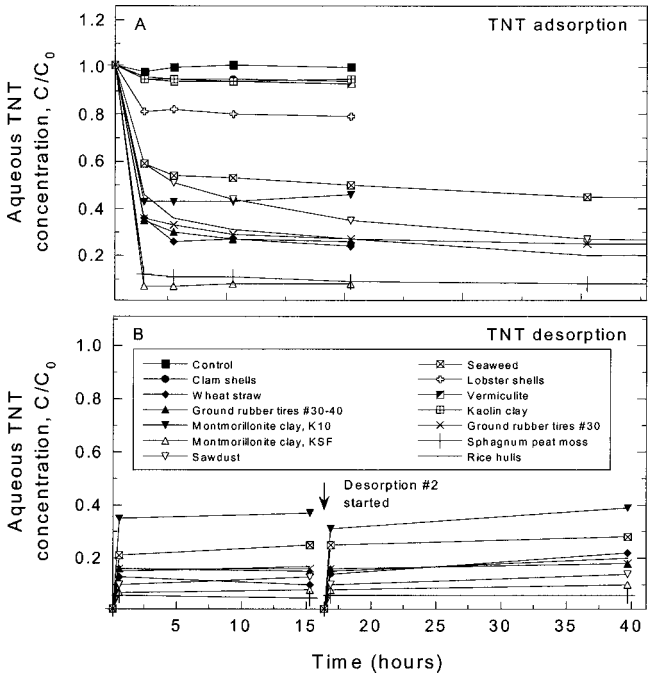

Fig. 1. Kinetics of 2,4,6-trinitrotoluene (TNT) sorption to (**A**) and desorption from (**B**) test sorbents. C = aqueous TNT concentration at time of sampling; C_0 = initial aqueous TNT concentration.

RESULTS

Sorption capacity and kinetics

The TNT adsorption to and desorption from a variety of different solids as a function of time is presented in Figure 1. The rates of adsorption for RDX and HMX were similar to those of TNT (data not shown). In general, an apparent equilibrium between the adsorbed and aqueous phases of each explosive was achieved within 5 h. Desorption kinetics were equally rapid. The percentage of each explosive adsorbed to and desorbed from the different solids at equilibrium (24 h) is presented in Table 2. Sphagnum peat moss was the most effective sorbent for each explosive, adsorbing 94, 87, and 88% of the added TNT, RDX, and HMX, respectively. Sawdust, rice hulls, and wheat straw were also effective adsorbents of the three explosives, removing greater than 77% of the added TNT, 50% of the RDX, and 58% of the HMX from solution. Greater than 90% of the TNT adsorbed to montmorillonite clay KSF, although sorption of RDX and HMX to this material was much lower (26 and 64%, respectively). Both TNT and HMX exhibited relatively low adsorption to kaolin, lobster shells, vermiculite, and clam shells. Therefore, these materials were not examined for RDX adsorption or used in further experiments.

Sorption and desorption isotherms

Representative sorption and desorption isotherms for TNT, RDX, and HMX are provided in Figures 2, 3, and 4, respectively. The Freundlich model parameters for all three compounds with different sorbent materials, and for TNT and RDX with the two MMR soils, are given in Table 3. Although some exceptions were found, the adsorption and desorption isotherms for the explosives with the different sorbents were reasonably linear across the concentrations tested. This observation is evidenced by the n values for the Freundlich curve fits being near one. The adsorption coefficients for TNT and

Table 2. Sorption and desorption of single concentrations of explosives to different adsorbent materials after equilibration

Material	TNT[a]		RDX[a]		HMX[a]	
	% Sorbed[b]	% Desorbed[c]	% Sorbed	% Desorbed	% Sorbed	% Desorbed
Montmorillonite clay KSF	94	14	26	NP[d]	64	46
Montmorillonite clay K10	58	35	ND[e]	NP	48	30[f]
Kaolin clay	7	NP	ND	NP	18	NP
Sphagnum peat moss	94	9	87	18	88	20
Ground rubber tires no. 30–40	75	22	ND	NP	13	NP
Ground rubber tires no. 30	78	23	21	NP	19	NP
Sawdust	80	19	52	32	58	33
Rice hulls	81	26	50	35	63	36
Wheat straw	77	23	55	54	66	NP
Seaweed	59	26	ND	NP	46	15[f]
Lobster shells	22	NP	ND	NP	29	NP
Vermiculite	8	NP	ND	NP	14	NP
Clam shells	7	NP	ND	NP	5	NP

[a] Initial explosive concentrations: TNT, 80 mg/L; RDX, 37 mg/L; HMX, 5 mg/L. See Table 1 for definitions.
[b] Amount of explosive removed from aqueous solution after 24 h of incubation, normalized to the mass of dry material used.
[c] Cumulative release of sorbed explosive after two sequential desorption steps.
[d] NP = desorption was not performed because the amount of explosive sorbed was too low.
[e] ND = not determined.
[f] Only a single desorption step was performed.

RDX with each of the sorbents tested were appreciably higher than those with either of the MMR soils. The K_d^s value for these two explosives with peat moss was at least two orders of magnitude higher than that with either of the soils. These data indicate that the explosives adsorbed much more extensively to the solid-phase adsorbents than to the sandy MMR soils. This observation suggests that addition of these materials to such soils has the potential to retard the migration of explosives to groundwater.

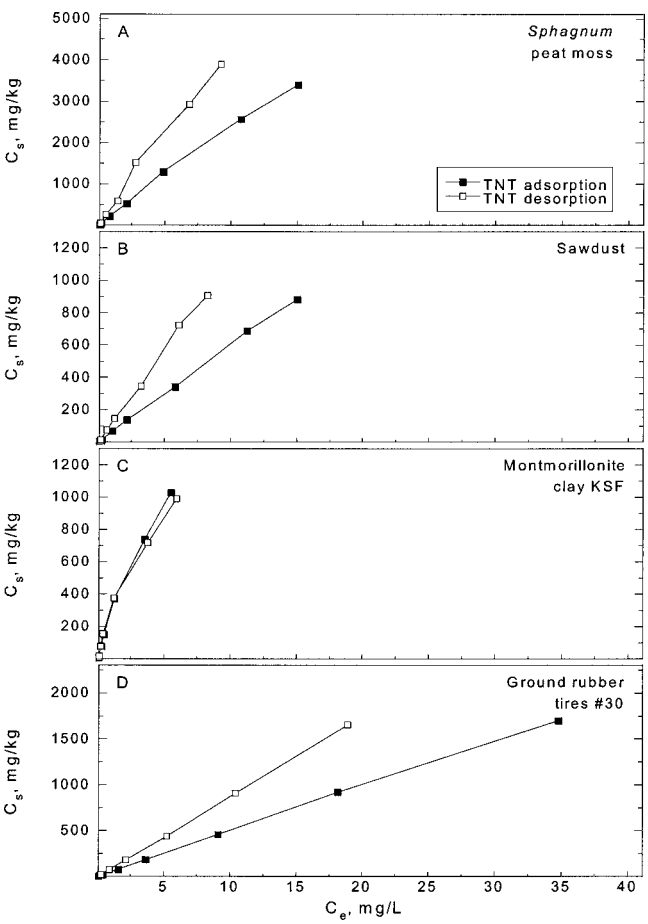

Fig. 2. 2,4,6-Trinitrotoluene (TNT) sorption and desorption isotherms for peat moss (**A**), sawdust (**B**), montmorillonite clay KSF (**C**), and ground rubber tires no. 30 (**D**). C_e = aqueous concentration of TNT at equilibrium C_s = concentration of sorbed TNT.

The adsorption and desorption isotherms for TNT, as well as for RDX and HMX, with specific adsorbents exhibited appreciable hysteresis such that K_d^d was often significantly greater than K_d^s (Table 3). Hysteresis was also apparent for the adsorption and desorption of TNT and RDX to the two MMR soils. For instance, in Figure 2A, B, and D, the slope of the desorption isotherm is appreciably greater than that of the adsorption isotherm. This observation suggests that the sorption of explosives to these materials is not readily reversible but, rather, that some fraction of the adsorbed contaminant is either irreversibly bound or present as a slowly desorbed fraction. In either case, the binding of the explosives in this state likely will help to prevent or delay their desorption and migration to groundwater.

DISCUSSION

The two surface soils collected from MMR showed a poor adsorptive capacity for RDX and TNT. Adsorption coefficients (K_d^s) for RDX varied from 0.22 to 0.52 L/kg, and those for TNT ranged from 1.9 to 3.1 L/kg. Although these values are low, they appear to be consistent with other adsorption data reported for these compounds in surface soils (0.21–0.83 L/kg for RDX and 0.58–11 L/kg for TNT) [1,15]. It should be noted that soil C has existing contamination with TNT (284 mg/kg) and RDX (1296 mg/kg), which may have influenced the adsorption of the freshly added explosives (e.g., by occupying binding sites). To improve adsorption of explosives at the soil surface and, thus, reduce the potential for migration to groundwater, the sorption capacity of TNT, RDX, and HMX by a variety of readily available, inexpensive solids was evaluated. These solids included agricultural and forest byproducts (e.g., rice hulls, wheat straw, and sawdust), various clay minerals, ground rubber tires, Sphagnum peat moss, and several materials that are readily available near MMR (e.g., seaweed and lobster shells). These materials were chosen based on cost,

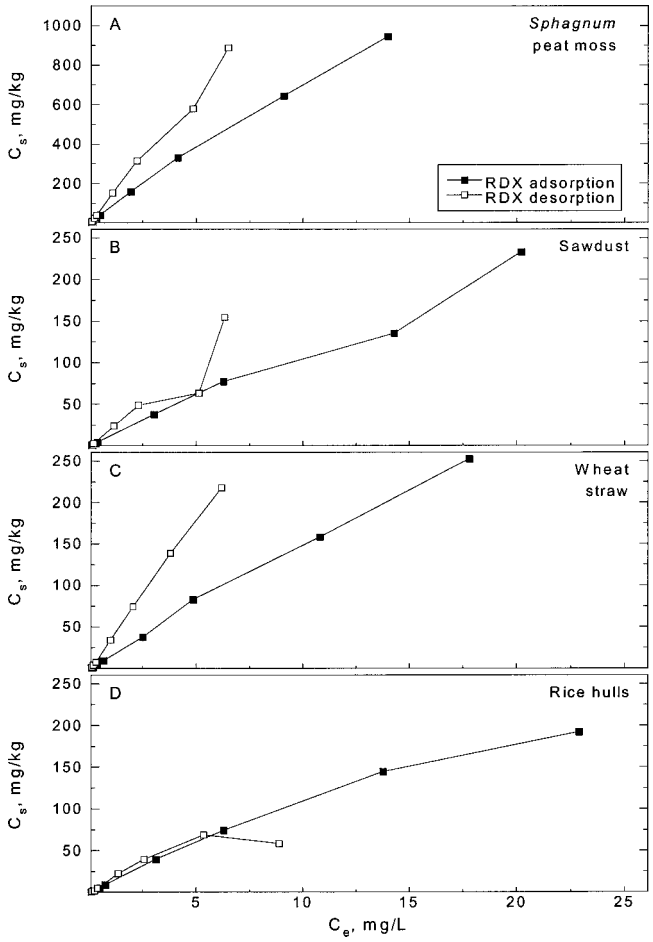

Fig. 3. Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) sorption and desorption isotherms for peat moss (**A**), sawdust (**B**), wheat straw (**C**), and rice hulls (**D**). C_e = aqueous concentration of RDX at equilibrium; C_s = concentration of sorbed RDX.

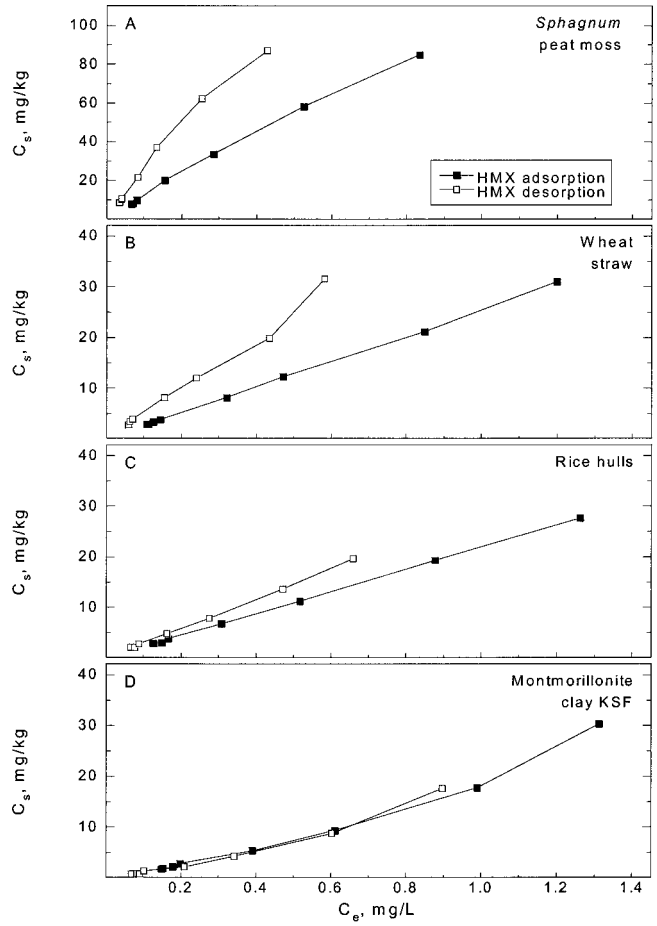

Fig. 4. Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) sorption and desorption isotherms for peat moss (**A**), wheat straw (**B**), rice hulls (**C**), and montmorillonite clay KSF (**D**). C_e = aqueous concentration of HMX at equilibrium; C_s = concentration of sorbed HMX.

Table 3. Freundlich equation fit parameters to sorption-desorption isotherm data

	TNT[a]			RDX[a]			HMX[a]		
Sorption[b]	K_d^s (L/kg)	n	r^2	K_d^s (L/kg)	n	r^2	K_d^s (L/kg)	n	r^2
MMR soil C	1.88	0.93	1.00	0.521	0.96	0.97	ND[c]	ND	ND
MMR soil G	3.13	0.82	1.00	0.223	0.78	0.83	ND	ND	ND
Sphagnum peat moss	310	0.88	1.00	87.3	0.92	1.00	111	0.84	0.99
Montmorillonite clay KSF	291	0.83	1.00	ND	ND	ND	9.29	2.05	0.97
Sawdust	66.5	0.96	1.00	12.7	0.95	1.00	ND	ND	ND
Ground rubber tires no. 30	53.1	0.98	1.00	ND	ND	ND	ND	ND	ND
Wheat straw	ND	ND	ND	16.0	0.98	1.00	25.9	0.97	1.00
Rice hulls	ND	ND	ND	12.2	0.94	1.00	21.7	1.01	0.98
Desorption	K_d^d (L/kg)	n	r^2	K_d^d (L/kg)	n	r^2	K_d^d (L/kg)	n	r^2
MMR soil C	32.6	0.95	0.99	18.2	0.78	0.96	ND	ND	ND
MMR soil G	42.0	1.20	0.99	2.48	0.67	0.61	ND	ND	ND
Sphagnum peat moss	538	0.88	1.00	149	0.93	1.00	203	0.79	0.96
Montmorillonite clay KSF	298	0.77	0.99	ND	ND	ND	13.4	1.68	0.95
Sawdust[d]	122	0.96	1.00	22.9	0.99	1.00	ND	ND	ND
Ground rubber tires no. 30	90.9	0.98	1.00	ND	ND	ND	ND	ND	ND
Wheat straw	ND	ND	ND	36.3	0.99	1.00	49.6	0.98	0.92
Rice hulls[e]	ND	ND	ND	14.8	0.96	0.99	29.0	0.98	0.95

[a] See Table 1 for abbreviations.
[b] MMR = Massachusetts Military Reservation.
[c] ND = not determined.
[d] Calculations for RDX desorption ignored the apparent outlier 7th datapoint.
[e] Calculation for RDX desorption ignored the apparent outlier 8th datapoint.

bulk availability, physical and chemical characteristics, and in some cases, previous research concerning the sorption of explosives or similar compounds. For example, Haderlein et al. [8] showed that TNT is strongly adsorbed by clays, and Steffan [16] used ground rubber to adsorb and enhance the biodegradation of nitroaromatics in aqueous solution.

Based on initial screening studies, several adsorbents, including montmorillonite, Sphagnum peat moss, ground rubber tires, sawdust, rice hulls, and wheat straw, were used in isotherm studies to better quantify the adsorption of TNT, RDX, and/or HMX. Among the various adsorbents tested, Sphagnum peat moss proved to be the most effective sorbent for the three explosive compounds. The adsorption coefficient for TNT and RDX with peat was at least two orders of magnitude higher than that calculated for adsorption of these explosives to the two MMR soils. The peat moss, which is composed of fibrous organic materials, may also serve as a slow-release, organic substrate for enhancing microbial reduction of explosive compounds. The adsorption studies reported herein were performed with autoclaved solids under sterile conditions to prevent any biological degradation of the parent explosives, which could confuse the sorption data. However, the influence of combined biodegradation and sorption processes on the fate of each explosive is described in a companion paper [12].

Sawdust, wheat straw, rice hulls, and ground rubber tires also adsorbed appreciably more RDX and/or TNT than the two MMR soils, although the extent of adsorption was less than that for peat moss. Montmorillonite clay adsorbed TNT nearly as effectively as peat moss. The strong affinity of montmorillonite, an expanding-layer smectite clay, for TNT confirms the results of previous studies, which suggested that various clay minerals may play an important role in the adsorption of TNT and other nitroaromatics in soils [8,9]. Based on the isotherm data for HMX, however, the adsorption of nitramine explosives to clay minerals, such as montmorillonite, appears to be less important to their environmental fate. The data suggest that even in the absence of biodegradation, the migration of TNT, RDX, and HMX may be inhibited if selected sorbent materials are applied to impact ranges before training activities.

Significant hysteresis (i.e., $K_d^s < K_d^d$) was observed when comparing the adsorption and desorption isotherms for each explosive with Sphagnum peat moss. Hysteresis was also apparent for both HMX and RDX with wheat straw, for TNT and RDX with sawdust, and for TNT with ground rubber tires. When the adsorption process is readily reversible, sorption and desorption isotherms for a given compound should be similar. This appears to be the case with the adsorption of TNT to montmorillonite clay in the present study. Nitroaromatic compounds are hypothesized to adsorb to clay minerals by forming a readily reversible electron donor–acceptor complex at the siloxane surface of such minerals [8]. Thus, adsorption and desorption isotherms are expected to be similar for these contaminants with clays.

Conversely, when the K_d calculated from a desorption isotherm is appreciably higher than that from the adsorption isotherm, the data suggest that the desorption process is not quickly reversible but, rather, that some fraction of the adsorbed contaminant is either irreversibly bound or present as a slowly desorbed fraction. This resistance to desorption may reflect dissolution of the contaminant within an organic solid phase, movement of the chemical into tortuous micropores, or possibly, covalent binding to a chemical component of the solid [17–19]. The two initial processes are most likely to account for the hysteresis observed in the present study. If the studies had been conducted under nonsterile conditions, biological reduction of the nitro groups of TNT might have yielded products (e.g., amino derivatives of TNT) that are highly reactive [20]. In this case, covalent interactions would likely contribute to the hysteretic isotherms.

The data from the present studies show that the adsorption capacity of a variety of inexpensive organic solids for explosives greatly exceeds that of surface soils. The application of these materials to the surface of selected locations on military impact ranges or similar testing facilities may enhance the adsorption of explosive residues and, ultimately, prevent the movement of these compounds to groundwater or surface water bodies. Additional studies are underway to assess the cost and effectiveness of adsorbent application and the combined application of adsorbents and microbial cosubstrates for explosives treatment at the field scale.

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ENHANCING THE ATTENUATION OF EXPLOSIVES IN SURFACE SOILS AT MILITARY FACILITIES: COMBINED SORPTION AND BIODEGRADATION

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Abstract—This research evaluated soil amendments designed to enhance the adsorption and biodegradation of explosives at military training facilities, thus minimizing their potential for transport to subsurface environments. Several carbon cosubstrates were tested in soil slurries for their ability to stimulate the biodegradation of 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (royal demolition exposive [RDX]), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (high-melting explosive [HMX]) by indigenous soil microorganisms. Crude soybean oil and molasses stimulated mineralization of RDX (30–40%) and HMX (~10%). The TNT was not significantly mineralized in any of the treatments, but high-performance liquid chromatography (HPLC) analysis indicated extensive transformation of TNT to amino-containing compounds. The biodegradation of explosives was then examined in unsaturated soil microcosms amended with crude soybean oil and molasses combined with sphagnum peat moss and sawdust. Minimal TNT mineralization was observed, and HMX mineralization was only observed with molasses addition. In contrast, RDX mineralization was extensive in microcosms amended with soybean oil or molasses. The presence of peat moss decreased soybean oil–stimulated RDX mineralization by approximately 5%, but resulted in about 5% greater RDX mineralization compared with molasses only. Sawdust markedly decreased mineralization regardless of cosubstrate type. Mass balance results indicated that the formation of bound residues likely was occurring, especially for TNT. These results indicate that the application of inexpensive adsorbents and cosubstrates to soils may significantly improve the protection of groundwater resources underlying live fire ranges.

Keywords—Explosives Biodegradation Sorption Hexahydro-1,3,5-trinitro-1,3,5-triazine 2,4,6-Trinitrotoluene

INTRODUCTION

The munitions that are employed at U.S. Department of Defense impact ranges and other facilities contain a number of different explosive compounds. For example, a 60-mm mortar round contains 2,4,6-trinitrotoluene (TNT) in the primer, 2,4- and 2,6-dinitrotoluene (2,4-DNT and 2,6-DNT, respectively) in the propellant charge, TNT and hexahydro-1,3,5-trinitro-1,3,5-triazine (royal demolition explosive [RDX]) in the filler, and RDX and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (high-melting explosive [HMX]) in the fuse [1]. After partial detonation of a high-explosive munition, residues of these materials can remain in the impact area, and these compounds have proved to be recalcitrant under a range of normal environmental conditions. In addition, munitions that fail to detonate during training (i.e., unexploded ordnance) also are a potential long-term source for the release of explosive compounds into soils. The breakdown products of RDX include hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX). Although RDX poses a clear health concern (http://www.epa.gov/waterscience/drinking/standards/dwstandards.pdf), these nitroso-containing breakdown products, or other metabolites (i.e., hydrazines and formaldehyde) may actually be more toxic [2] (http://www.pu.org/main/reports/anrc9802.pdf). Therefore, remediation measures that promote complete degradation of explosives residues, as opposed to partial transformation, are preferable. In sandy soils with little organic matter or clay content, transport of TNT, RDX, and HMX to the vadose zone and ultimately to groundwater is possible. Contamination of groundwater with these compounds has been observed in groundwater collected from several military installations [3,4] (www.wes.army.mil/el/elpubs/pdf/tr02-8.pdf).

Based on extensive research that has been done on the biodegradation and biotransformation of explosive compounds by bacteria (see references [5–8] for reviews) and fungi [9–12], ex situ approaches for remediating explosive compounds–contaminated soils have been developed for the ex situ treatment of soil, including composting [12], bioslurry reactors [13–17], and land-farming [18]. In situ bioremediation also has been examined for subsurface explosives contamination [19,20]. The presence of unexploded ordnance on impact ranges, and the large expanse of the potentially contaminated areas, makes the application of these technologies impractical and prohibitively expensive. In addition, these technologies are retroactive rather than proactive, and are unable to prevent groundwater contamination from current and future military training activities.

The objective of this research was to develop a technology that could be applied to the soil surface to prevent or slow the migration and promote the biodegradation of explosives residues at impact ranges and other military training facilities. In the companion paper also in this issue [21], the adsorption to and desorption from several different materials (i.e., peat moss and sawdust) of TNT, RDX, and HMX was examined. The research reported here focused on stimulation of explosives biodegradation by various cosubstrates, and determination if explosives sorbed to peat moss and sawdust could be biodegraded when effective cosubstrates are present. Because of the large expanse of many military ranges, the focus of this re-

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Table 1. Mass balance and percentage of initial disintegrations per minute (dpm) in different fractions and extractions for Massachusetts Military Reservation (MMR) soil C slurries amended with different cosubstrates[a]

Treatment	Addition to microcosms (mg)		Percent of initial [^{14}C]TNT dpm[b]					Percent of initial [^{14}C]RDX dpm[b]	
	TOC	BOD	[^{14}C]CO_2	Acetonitrile-extractable	Mass balance[c]	Slurry	Water-soluble	[^{14}C]CO_2	Acetonitrile-extractable
Killed control ($HgCl_2$, Na azide)	0	0	0 (0)	76 (4)	77 (4)	110 (8)	32 (1)	0 (0)	108 (0)
Control (no C source)	0	0	1 (0)	28 (13)	29 (13)	102 (3)	35 (9)	4 (0)	97 (2)
Molasses	43	341	1 (0)	22 (2)	22 (2)	93 (3)	23 (1)	2 (0)	101 (0)
Corn-steep liquor	59	333	1 (0)	29 (2)	29 (2)	101 (0)	37 (1)	4 (1)	83 (1)
Soybean oil–crude	207	218	3 (0)	14 (1)	17 (1)	55 (2)	10 (1)	1 (0)	93 (0)
SoyClear 1500	207	0	2 (1)	40 (3)	42 (2)	79 (2)	9 (0)	1 (0)	71 (20)
SoyGold 1000	196	1	2 (0)	35 (6)	37 (6)	75 (2)	10 (1)	1 (0)	46 (0)
Safflower oil	207	1	3 (0)	16 (0)	19 (0)	66 (5)	11 (2)	1 (0)	84 (19)
Potato starch	98	1	1 (0)	21 (2)	23 (2)	98 (5)	28 (1)	2 (0)	97 (2)
Solulac	140	44	1 (0)	17 (5)	17 (5)	94 (1)	26 (2)	2 (0)	90 (0)
Corn starch	76	1	2 (0)	14 (4)	15 (4)	100 (8)	23 (0)	2 (0)	101 (2)
Unrefined chitin	52	3	1 (0)	14 (2)	15 (2)	79 (9)	20 (1)	2 (0)	109 (9)

[a] TNT = 2,4,6-trinitrotoluene; RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine; HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; TOC = total organic carbon; BOD = biological oxygen demand.
[b] Results presented as average percent (± difference between duplicate bottles/2).
[c] Mass balance is the sum of [^{14}C]CO_2 and acetonitrile-extractable values.

search was on inexpensive amendments (i.e., sorbents and microbial cosubstrates) that could be applied effectively over large areas. If both adsorption and degradation of explosives can be enhanced in surface soils, groundwater resources will be protected from contamination with these compounds.

MATERIALS AND METHODS

Chemicals, media, and soils

All solvents were of high-performance liquid chromatography (HPLC) grade and chemicals were reagent grade or better. Sawdust was obtained from a sawmill on Cape Cod (MA, USA). Sphagnum peat moss was purchased from a home and garden store. Sources of the materials tested as microbial cosubstrates were as follows: molasses (blackstrap, feedlot grade) was purchased from Zook Molasses (Honey Brook, PA, USA). Corn-steep liquor, solulac (distillers grain solids), and corn starch were obtained from Grain Processing (Muscatine, IA, USA). Crude soybean oil was obtained from Cargill (Minneapolis, MN, USA), and crude safflower oil was obtained from California Oils (Richmond, CA, USA). Two soybean oil–based green solvents, SoyClear 1500 and SoyGold 1000, were obtained from AG Environmental Products (Lenexa, KS, USA). Potato starch was obtained from Lance Products (Shore Acres, NJ, USA), and unrefined, practical-grade chitin was purchased from Sigma Chemical (St. Louis, MO, USA). Sources of explosives and [^{14}C]-radiolabeled compounds were as described in Hatzinger et al. [21].

Historically contaminated soils were obtained from Demolition Area 1 at the Massachusetts Military Reservation (MMR) on Cape Cod. The area was used for repeated explosives dumping and disposal operations typical at many military training facilities. A highly contaminated soil collected from this area was designated as soil C. An uncontaminated soil sample, designated soil G, was taken from a grassy area adjacent to the explosives dumping region. Explosives concentrations and other relevant properties of the two soils are presented in the companion paper [21].

Explosives biodegradation with cosubstrates

The ability of microbial cosubstrates to enhance the biodegradation of the three explosives was tested in microcosms. The microcosms consisted of 10 g (wet wt) of MMR soil (either soil C or soil G) made into a slurry with 25 ml of nutrient solution, which consisted of deionized water containing $(NH_4)_2HPO_4$ at 40 g/L and $(NH_4)H_2PO_4$ at 20 g/L. This resulted in final concentrations of nitrogen-N and phosphorus-P of approximately 80 and 100 mg/kg, respectively. Three aliquots of the nutrient solution were amended with unlabeled and [^{14}C]-labeled TNT, RDX, or HMX, resulting in final concentrations of explosives of 51, 21, and 3 mg/L, respectively. The killed control treatment received the same solution amended with mercuric chloride and sodium azide to achieve final concentrations of each biocide of 2,500 mg/kg (or 1,000 mg/L) in each microcosm. The TNT, RDX, and HMX in these microcosms were present at concentrations of 42, 21, and 5 mg/L, respectively. The total radioactivity added to each microcosm was approximately 100,000 dpm. Solid cosubstrates (2% cosubstrate:dry soil, w/w) were added to the microcosms before the soils were slurried, whereas liquid cosubstrates (0.5% cosubstrate:slurry, v/v) were added after the nutrient solution. Molasses and corn-steep liquor were added as 25% (v/v) solutions prepared in distilled water. The calculated total organic carbon and biological oxygen demand of the cosubstrates added to each bottle are presented in Table 1. The control and killed control treatments received no cosubstrate addition. Duplicate bottles of each treatment were prepared. After sealing the bottles, 0.5 ml of base (0.5 N potassium hydroxide [KOH]) was added to the base trap in each bottle. Bottles were incubated in the dark at room temperature with gentle shaking (150 rpm). Subsequent additions of cosubstrates, when performed, were made through the septa (for liquids) or under a nitrogen atmosphere (for solids); corn starch and potato starch were prepared as thick suspensions (0.5 g/ml) for these additions.

A second experiment was conducted to compare the biostimulation of explosives degradation in slurries of soil G by using crude soybean oil, a commercial food-grade soybean oil, and a combination of crude soybean oil and molasses. The microcosms were prepared as described above, except that water instead of nutrient solution was added to prepare the slurries.

To quantify the mineralization of each explosive, the KOH

Table 1. Extended.

Percent of initial [^{14}C]RDX dpm[b]			Percent of initial [^{14}C]HMX dpm[b]				
Mass balance[c]	Slurry	Water-soluble	[^{14}C]CO_2	Acetonitrile extractable	Mass balance[c]	Slurry	Water-soluble
109 (0)	116 (14)	30 (2)	0 (0)	97 (8)	97 (8)	103 (0)	29 (2)
102 (2)	103 (3)	41 (7)	2 (0)	101 (3)	104 (3)	99 (2)	27 (0)
103 (0)	97 (5)	64 (0)	1 (0)	79 (6)	80 (6)	103 (1)	38 (1)
87 (1)	100 (14)	62 (1)	1 (0)	84 (1)	86 (1)	102 (3)	32 (1)
94 (0)	92 (1)	15 (1)	2 (0)	99 (7)	100 (7)	106 (6)	9 (3)
72 (20)	46 (4)	18 (5)	1 (0)	81 (15)	82 (15)	91 (11)	18 (4)
47 (0)	47 (16)	15 (1)	1 (0)	68 (15)	69 (15)	66 (10)	13 (3)
85 (19)	93 (19)	13 (6)	1 (0)	71 (6)	73 (6)	52 (14)	20 (6)
99 (2)	109 (7)	47 (2)	2 (0)	91 (0)	92 (0)	99 (7)	28 (2)
92 (0)	90 (4)	60 (0)	1 (0)	85 (1)	86 (1)	102 (5)	17 (4)
103 (2)	107 (3)	56 (0)	2 (0)	82 (2)	84 (2)	101 (2)	38 (1)
111 (8)	106 (4)	27 (0)	2 (0)	93 (4)	95 (4)	101 (5)	12 (2)

in each internal trap was removed with a 1-ml syringe and added to a 20-ml vial containing 4.5 ml of OptiPhase HiSafe 3 liquid scintillation cocktail (National Diagnostics, Atlanta, GA, USA). After sampling, a fresh aliquot of KOH was placed in each base trap, and the incubation was continued. The 20-ml vials all were sealed, mixed well, and analyzed for radioactivity by using liquid scintillation counting (Model 1209 Rackbeta scintillation counter, Pharmacia LKB Nuclear, Gaithersburg, MD, USA). The scintillation data were used to calculate the cumulative mineralization of the explosive compounds in each microcosm, expressed as the disintegrations per minute (dpm), collected as $^{14}CO_2$ in the base trap divided by the total dpm added to the bottle. Mineralization of each explosive was calculated as the cumulative radioactivity recovered in the KOH traps for each treatment. Chemiluminescence and quench effects were monitored and corrected by using the Rackbeta's built-in functions. Anomalous readings and specific errors in the scintillation data were addressed on a case by case basis, and usually were easily resolved, except where noted below.

When the incubations were complete, the mass balance of each of the explosives was quantified by performing a series of extractions. Each bottle was initially amended with 0.2 ml of 6 N HCl to acidify the solution and liberate dissolved $^{14}CO_2$. The pH was reduced to <5.5 for the soil C slurries and to <3 for the soil G slurries with this addition. The bottles were incubated with shaking at room temperature for 24 h, at which time the base in the traps was removed and analyzed for radioactivity. After acidification, microcosms were stored at 4°C until samples were taken for extraction. Two milliliters of well-mixed slurry was removed and centrifuged for 10 min at 14,000 rpm at 4°C. An aliquot (0.5 ml) of the cleared supernatant was analyzed for residual soluble radioactivity by scintillation counting, and the remainder of the supernatant was analyzed by HPLC for soluble explosives and metabolites (see below). Another 3 ml of the slurry was transferred to a 20-ml glass vial and dried at 60°C for at least 24 h. The dried material was pulverized, 5 ml of acetonitrile was added, and the vial was sealed, mixed well, and placed in a water-cooled ultrasonic cleaning bath for 18 h. The extract was allowed to settle for about 1 h, then 2 ml of the extract was passed through a nylon syringe filter (0.2 μm). The filtrate was collected in a 2-ml HPLC autosampler crimp seal vial. A 0.5-ml sample of the filtrate was analyzed by scintillation counting, and the remainder was analyzed by HPLC for explosives and metabolites (see below). After the extractions were complete, the remainder of the slurry was mixed vigorously with a small stir bar while 0.1 ml was transferred to a glass vial containing 1 ml of distilled H_2O. The vial was placed in a sonic water bath for 1 h, 5 ml of scintillation cocktail was added, and the sample was analyzed for radioactivity. This amount of slurry added to the scintillation cocktail minimized any chemiluminescence and quench effects.

Explosives degradation with sorbent and cosubstrate combinations

In our companion paper, sphagnum peat moss and sawdust were found to have very high sorption capacities for all three explosives [21]. These two materials were combined pair-wise with the most effective cosubstrates, soybean oil and molasses, in unsaturated soil microcosms. These tests were conducted to evaluate whether the adsorption of TNT, RDX, or HMX, affected the enhanced mineralization of the compounds by cosubstrate addition. These microcosms were prepared in 1-pint (500-ml) mason jars. The lids were equipped with a base trap assembly for capturing the [^{14}C]CO_2. Solutions of single [^{14}C]-labeled and cold explosives were prepared in distilled water to achieve nominal TNT, RDX, or HMX concentrations of 50, 25, and 3 mg/L. A total of 30,000 to 40,000 dpm of [^{14}C]radiolabel and TNT at approximately 5 mg/kg, RDX at approximately 2 mg/kg, and HMX at approximately 0.3 mg/kg were added to microcosms. Microcosms also were prepared with solutions that contained a mixture of all three cold explosives, plus one of the radiolabeled explosives (i.e., [^{14}C]TNT + cold TNT, RDX, and HMX). Actual concentrations and radioactivities were determined by HPLC and scintillation counting, respectively, as described previously.

Peat moss and sawdust (2 g/jar, or 0.05 g/g soil) initially were added to the jars along with the explosives solutions (3.2 ml/jar). The sorbent and solution were thoroughly mixed, and the jars were sealed and incubated at room temperature (22–25°C) so that equilibrium would be achieved. After 72 h, the base traps were sampled and the radioactivity in the KOH solution was quantified as described previously. Soil G (40 g) then was added to each jar followed by soybean oil (0.05 ml/g soil) or molasses (25% [v/v] solution; 0.025 ml/g soil; 0.050 ml/g soil for some RDX treatments). The soil, sorbents, and cosubstrates were mixed together thoroughly. Controls sam-

ples received no cosubstrate. The jars were sealed and the microcosms were incubated at room temperature, and mineralization of each explosive was quantified by collecting and counting the KOH in each base trap with time, as described previously.

When the incubations were complete, several procedures were undertaken to assess the mass balance of the explosives. Microcosms remained at room temperature, and the base was sampled and replaced each time the jars were opened to remove subsamples of soil. Duplicate 0.25-g (wet wt) subsamples were transferred to preweighed glass scintillation vials and dried at 60°C overnight. The weight of the vial plus dry sample was recorded, 2 ml of distilled water was added, and the vials were sealed and mixed well. The vials were then placed in a cooled ultrasonic bath for 4 h. Scintillation cocktail (18 ml) was added to each sample, and the contents were analyzed by scintillation counting. Extraction and analysis of dried samples (1 g) with acetonitrile was performed as described for soil slurries.

The amount of radioactivity incorporated into the microbial biomass was determined by using a modification of the chloroform fumigation–extraction procedure widely used in agricultural research [22]. Briefly, two subsamples (30–5 g wet wt) of the soil were placed in separate glass vials. One replicate was extracted immediately by adding 12 ml of 0.01 M KSO_4 and shaking horizontally for 1 h at room temperature. The extract was filtered through a 0.45-μm glass fiber filter, and 5 ml of the cleared solution was combined with 10 ml of scintillation cocktail and was analyzed for radioactivity. The other replicate was fumigated with chloroform vapors in a sealed chamber for 18 to 24 h at room temperature. This procedure is generally assumed to lyse >99% of the microorganisms present in the soil, making their cell components readily extractable. The fumigated soil was then extracted and analyzed for radioactivity as described above. The difference in extracted radiolabel between the fumigated and nonfumigated soil represented the [^{14}C] incorporated into microbial biomass.

Analytical

Analyses of RDX, HMX, and TNT were performed according to U.S. Environmental Protection Agency Method 8330, by using a Dionex DX600 IC/LC (Dionex, Sunnyvale, CA, USA) with an autosampler, quatenary pump, Allure C_{18} reverse phase column (Restek, Bellefonte, PA, USA), and an ultraviolet detector (peak detection at 254 nm). The mobile phase was 1:1 methanol:water (v/v), with a flow rate of 0.90 ml/min. Effective detection limits for the analytes were determined to be approximately 0.025 mg/L.

RESULTS AND DISCUSSION

Explosives biodegradation in the presence of cosubstrates

The various cosubstrates added to the soil slurries stimulated mineralization of explosives, and the degree of mineralization varied for the three explosives and for the two different soils used in studies. On a percentage basis, very little mineralization of explosives was stimulated by any of the cosubstrates added to slurries of the highly contaminated soil, soil C (Fig. 1). In soil G slurries, molasses and soybean oil stimulated a significant degree of mineralization of [^{14}C]RDX and [^{14}C]HMX (i.e., >30% for RDX and >9% for HMX. Mineralization of RDX and HMX also was higher in samples receiving corn-steep liquor and potato starch than in unamended controls (Fig. 2). In contrast to RDX and HMX, only a

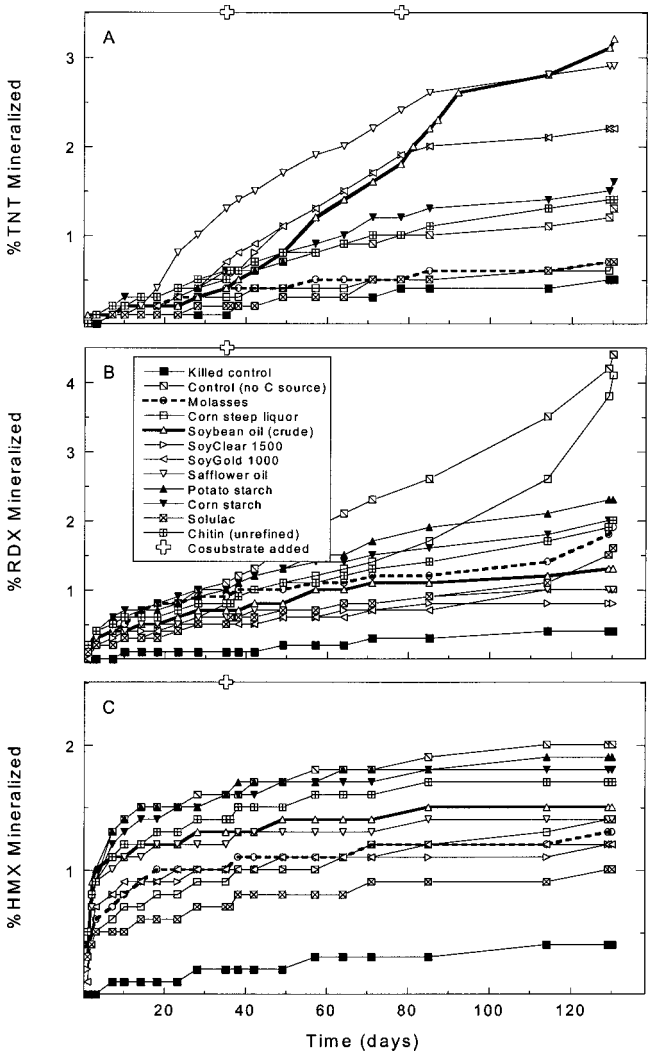

Fig. 1. Mineralization of 2,4,6-trinitrotoluene (TNT) (**A**); hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (**B**); and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) (**C**) in Massachusetts Military Reservation (MA, USA) soil C slurries in the presence of different cosubstrates.

small percentage of the [^{14}C]TNT was recovered as [^{14}C]CO_2 (i.e., <5%) in the soil G slurries, irrespective of the cosubstrate added. Mineralization of TNT was slightly enhanced in samples treated with molasses and soybean oil compared to the unamended control.

To more fairly compare the results observed in the unamended, molasses, and crude soybean oil treatments obtained with soil C (historically contaminated) and soil G (uncontaminated), the mineralization data also were analyzed in terms of the moles of the initial explosives in the soils. The radiolabeled explosives used in this research are uniformly labeled such that, on average, one molecule of [^{14}C]CO_2 is produced from each molecule of [^{14}C]-labeled explosive when it is mineralized. Therefore, to determine the total moles of each explosive mineralized, the percent [^{14}C]CO_2 captured was multiplied by the total initial micrograms of that explosive in the soil. This value was then divided by the molecular weight of the explosive. The results of converting the mineralization data from percent of radioactivity recovered as [^{14}C]CO_2 to total nanomoles of each explosive mineralized are illustrated in Figure 3. In the unamended slurries, the amount of TNT, RDX,

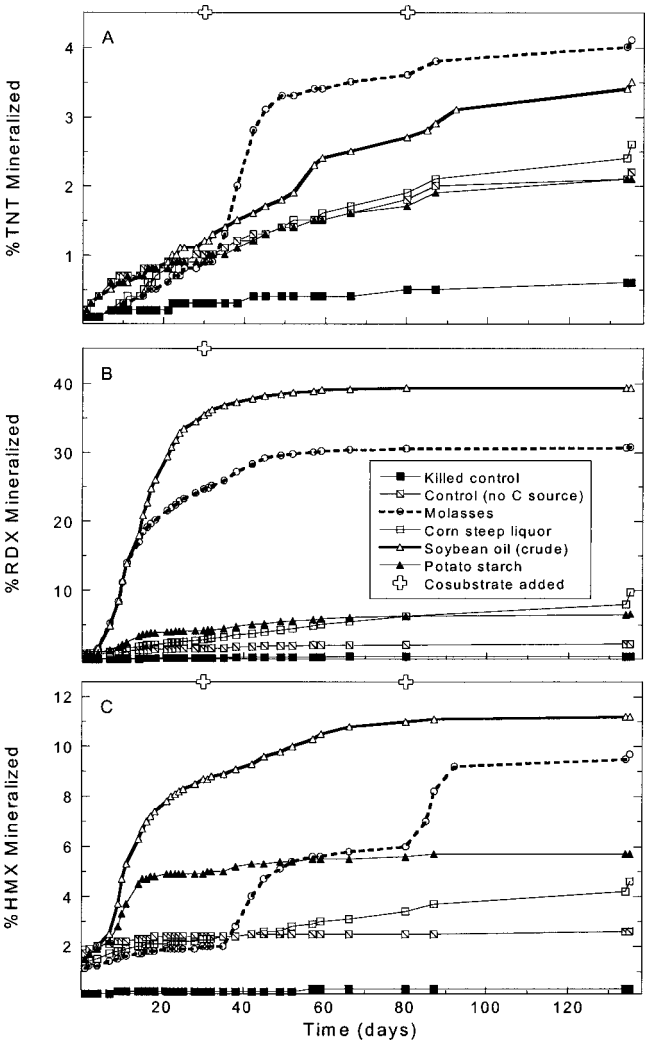

Fig. 2. Mineralization of 2,4,6-trinitrotoluene (TNT) (**A**); hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (**B**); and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) (**C**) in Massachusetts Military Reservation (MA, USA) soil G slurries in the presence of different co-substrates.

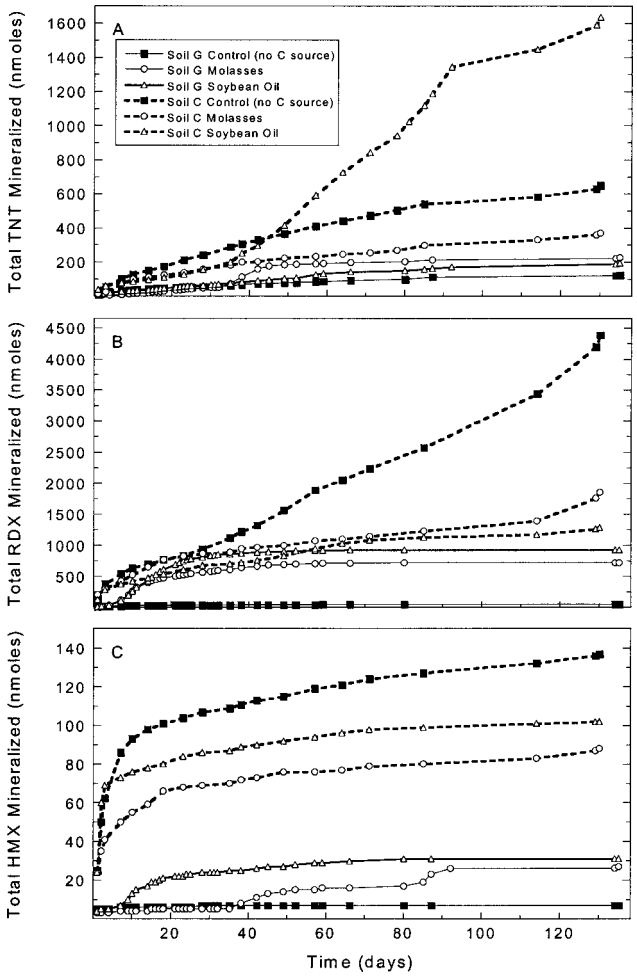

Fig. 3. Mineralization of 2,4,6-trinitrotoluene (TNT) (**A**); hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (**B**); and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) (**C**) in Massachusetts Military Reservation (MA, USA) soil C and soil G slurries for selected treatments expressed in terms of total nanomoles of each explosive mineralized.

and HMX mineralized in soil C compared to soil G was greater by 4-fold, 60-fold, and 20-fold, respectively. Molasses stimulated fourfold more HMX mineralization in soil C compared to soil G, but only 20% and 60% greater mineralization of TNT and RDX, respectively. Mineralization of TNT and HMX in slurries of soil C amended with crude soybean oil was sevenfold and fourfold greater, respectively, than in slurries of soil G, but RDX mineralization was only 25% greater in slurried soil C compared to slurried soil G. These results indicate that the potential for biodegradation of explosives is at least as great, if not greater, in historically contaminated soils than in uncontaminated soils.

The assumption was made that the freshly added radiolabeled and unlabeled explosives and the explosives already present in the soils were equivalent with respect to bioavailability and biodegradability. This assumption has been proven invalid for some classes of pollutants (i.e., pesticides and polychlorinated biphenyls [23,24]), and several researchers have noted the formation of sorbed and less or nonbioavailable pools of aged TNT in soil [25–27]. However, our research and other published literature indicate that RDX and HMX sorb to a much lower degree than does TNT [21,28]. Extractability of RDX also has been observed to remain greater over time than that of TNT [27,29]. Old and new explosives likely also behave more similarly in soils with low organic carbon and clay content, like the MMR soils used in this research. Therefore, it is reasonable to assume that the [^{14}C]-labeled and unlabeled explosives added to the microcosms and the explosives already in soil C were chemically and biologically equivalent.

Stimulation of explosives biodegradation in different environments with molasses has been reported previously by several investigators [14,15,30–34], so its effectiveness in these studies was not surprising. Potato starch and corn starch also have been observed to stimulate significant RDX biodegradation in anaerobic soil–water slurries under anaerobic conditions [35]. However, the results obtained with the addition of soybean oil are believed to be the first report of explosives biodegradation supported by a vegetable oil. Edible vegetable oils have proven effective as electron donors for stimulating reductive dechlorination [36–38], so the current results are reasonable considering the apparent nonspecific reductive processes involved in explosives degradation. However, given the rather widespread nature of this reductive process, it is interesting that the other substrates tested with soil G, corn-steep liquor and potato starch, were much less effective at promoting biological explosives degradation than the

Table 2. Mass balance and fate of ^{14}C-labeled explosives in Massachusetts Military Reservation soil G slurries amended with different cosubstrates[a]

	Percent of initial [^{14}C] dpm[b]				
	[^{14}C]CO_2	Acetonitrile-extractable	Mass balance[c]	Slurry	Water-soluble
TNT					
Killed control ($HgCl_2$, Na azide)	1 (0)	67 (5)	68 (5)	100 (6)	53 (1)
Control (no C source)	2 (0)	20 (4)	23 (4)	102 (3)	17 (0)
Molasses	4 (0)	9 (1)	13 (1)	81 (1)	6 (1)
Corn-steep liquor	3 (0)	10 (2)	12 (2)	90 (2)	14 (2)
Soybean oil–crude	3 (0)	6 (0)	9 (1)	61 (0)	4 (1)
Potato starch	2 (0)	23 (3)	25 (3)	123 (2)	10 (2)
RDX					
Killed control ($HgCl_2$, Na azide)	0 (0)	93 (6)	93 (6)	116 (11)	89 (0)
Control (no C source)	2 (0)	85 (3)	87 (3)	95 (5)	88 (1)
Molasses	31 (5)	1 (0)	32 (4)	4 (3)	1 (0)
Corn-steep liquor	10 (5)	58 (11)	67 (7)	78 (3)	66 (7)
Soybean oil–crude	39 (3)	3 (1)	42 (4)	8 (1)	1 (0)
Potato starch	7 (0)	57 (3)	63 (3)	79 (8)	72 (1)
HMX					
Killed control ($HgCl_2$, Na azide)	0 (0)	76 (2)	76 (2)	102 (4)	66 (2)
Control (no C source)	3 (0)	76 (3)	78 (3)	102 (8)	62 (1)
Molasses	10 (4)	40 (10)	50 (6)	57 (10)	30 (8)
Corn-steep liquor	4 (1)	62 (3)	67 (4)	89 (2)	53 (0)
Soybean oil–crude	11 (3)	44 (6)	55 (3)	45 (1)	28 (1)
Potato starch	6 (0)	69 (6)	75 (5)	94 (4)	54 (0)

[a] dpm = disintegrations per minute; TNT = 2,4,6-trinitrotoluene; RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine; HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine.
[b] Results presented as average percent (± difference between duplicate bottles/2).
[c] Mass balance is the sum of [^{14}C]CO_2 and acetonitrile-extractable values.

aforementioned two. This difference may reflect qualitative differences that influence the suitability of the cosubstrates to act as electron donors; affect the bioavailability or solubility of the labile fraction of the cosubstrate; or apply selective pressure on the microbial community, resulting in dominant bacterial populations that are not able to biotransform the explosives.

Additional slurry microcosm experiments, in which crude soybean oil, food-grade soybean oil, and crude soybean oil plus molasses were added to soil G slurries without additional nitrogen and phosphorus, indicated that crude soybean oil stimulated somewhat more mineralization of RDX than the more purified food-grade soybean oil (29% vs 21%). The combination of crude soybean oil and molasses did not greatly increase RDX mineralization compared to crude soybean oil only (31% vs 29%). None of the substrates used in this experiment stimulated mineralization of TNT, in agreement with the results presented above. Conversion of [^{14}C]HMX to $^{14}CO_2$ was stimulated by crude soybean oil plus molasses, but not by crude soybean oil or food-grade soybean oil (data not shown), in contrast to the microcosm data presented above (Fig. 2), which showed 10+% mineralization of HMX. These results suggest that crude soybean oil possessed enough noncarbonaceous nutrients (i.e., N and P) to support RDX mineralization, but not HMX mineralization. Based on the published pathways for RDX biodegradation [39,40], it is likely that sufficient nitrogen is released during RDX biodegradation to foster microbial growth and drive the process forward. Although nitrogen-containing compounds are also observed during HMX biodegradation [41], perhaps the amount or rate of nitrogen releases is not sufficient to promote extensive mineralization, and an external source is needed (as would be supplied in the molasses).

The mass balance for each compound was defined as the total radiolabel recovered as $^{14}CO_2$ plus the radiolabel extracted from the slurry by acetonitrile at the end of the incubation. The mass balance ranges for TNT, RDX, and HMX in soil C were 15 to 77%, 47 to 111%, and 69 to 104%, respectively (Table 1). The low mass balances for TNT reflect the tendency for this explosive to be converted into compounds that can form bound residues that are difficult to extract [42]. The lower mass balances occurred primarily in bottles amended with various soybean oils or safflower oil, but the low recoveries were not consistent among the three explosives. For example, after incubation of slurries with crude soybean oil, only 17% of the initial radiolabel was recovered from samples amended with [^{14}C]TNT, whereas average recoveries of 94 and 100% were calculated for [^{14}C]RDX and [^{14}C]HMX, respectively. These lower recoveries in some of these samples may reflect partitioning of the explosive into the nonaqueous oily phase combined with the difficulty of consistently sampling microcosms with multiple phases. When the slurries were directly analyzed for residual radioactivity, higher recoveries were observed for all the treatments, and the mass balances (alternatively defined as the sum of the [^{14}C]CO_2 plus [^{14}C] in the slurry) averaged 86 ± 14%, 91 ± 30%, and 94 ± 24% for TNT, RDX, and HMX, respectively.

In slurries prepared from soil G, mass balance ranges were 9 to 68% for TNT, 32 to 93% for RDX, and 50 to 78% for HMX (Table 2). As noted with soil C, lower mass balances occurred in the samples receiving soybean oil. However, in this case, the lower recoveries were reasonably consistent among the three explosives. In addition, the recovery of radiolabel in the samples with [^{14}C]RDX that received molasses as a cosubstrate was also low. Using the alternative mass balance formula ([^{14}C]CO_2 plus slurry radioactivity), the average

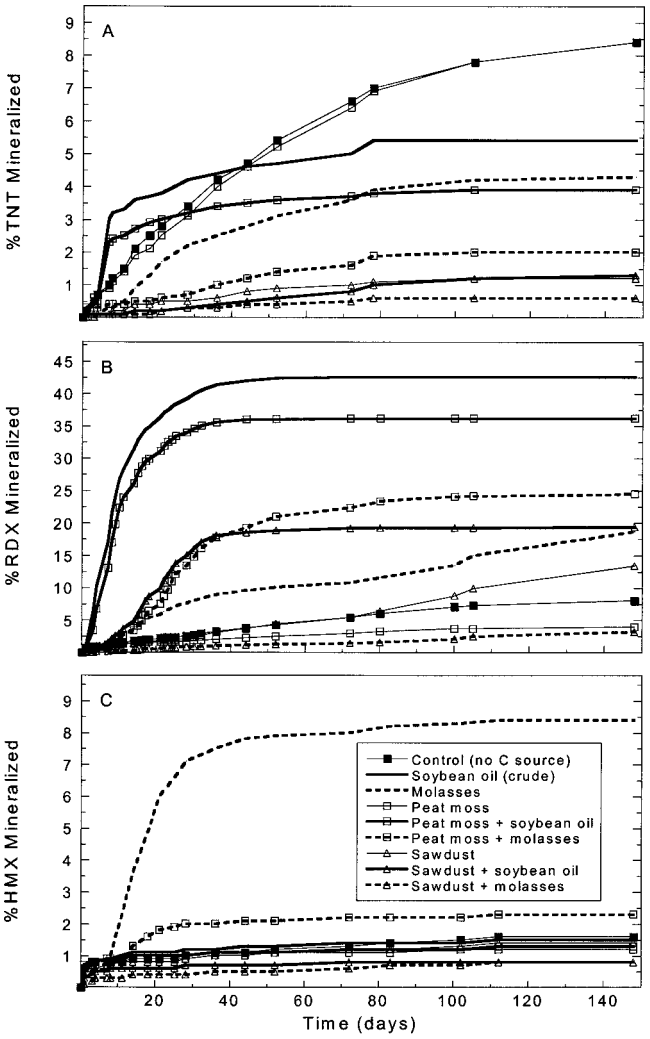

Fig. 4. Mineralization of 2,4,6-trinitrotoluene (TNT) (**A**); hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (**B**); and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) (**C**) in isolation in unsaturated Massachusetts Military Reservation (MA, USA) soil G microcosms amended with sorbents and cosubstrates.

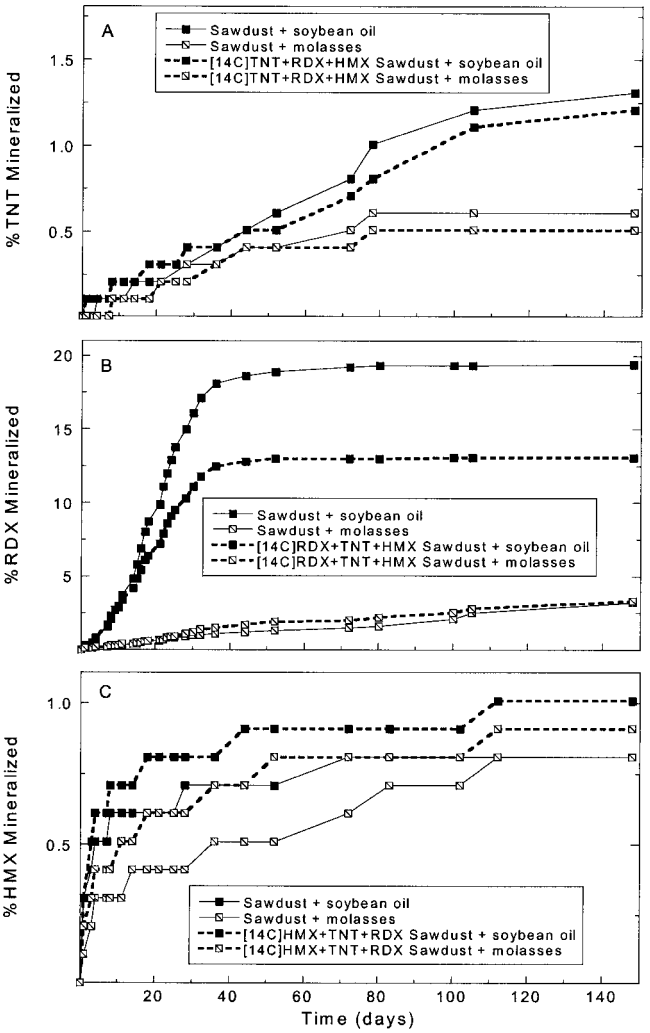

Fig. 5. Mineralization of 2,4,6-trinitrotoluene (TNT) (**A**); hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (**B**); and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) (**C**) singly and in combination in unsaturated in Massachusetts Military Reservation (MA, USA) soil G amended with soybean oil or molasses, and in the presence and absence of sawdust.

recoveries of TNT, RDX, and HMX were 94 ± 4%, 70 ± 13%, and 84 ± 11%, respectively. Mass balances for the slurries prepared from highly contaminated MMR soil C were generally higher than those obtained for the slurries prepared from uncontaminated MMR soil G. This may reflect a greater saturation of binding sites for the explosives or their breakdown products by the older explosives in soil C compared to soil G.

The amount of [^{14}C] recovered as soluble radioactivity exhibited some dependence on the explosive being examined and the cosubstrate added to the slurry. For the historically contaminated soil C, no clear relationships or patterns in water-soluble [^{14}C] derived from explosives were observed. However, for soil G, the percent of initial radiolabel recovered as a water-soluble fraction for each explosive was in the order RDX > HMX ≫ TNT. The exceptions were for soybean oil–amended microcosms spiked with [^{14}C]RDX; the water-soluble radioactivity in these microcosms was very low. In addition, in soil G samples receiving molasses as a cosubstrate, very little radiolabeled material was recovered as water-soluble products.

Results from the analysis of the acetonitrile extracts of the slurry microcosms are also presented in Tables 1 and 2. Acetonitrile was used to extract moderately polar explosives residues. The radiolabel recovered by the acetonitrile extractions was lower for [^{14}C]TNT spiked soils compared to [^{14}C]RDX and [^{14}C]HMX for any given treatment.

For each of the fractions examined (i.e., water soluble and solvent extractable), an effort was made to determine if a relationship existed between the percent of the residual radioactivity in that fraction (= (dpm in fraction)/(initial dpm − dpm recovered as $^{14}CO_2$)) and the percent of the initial radioactivity recovered as $^{14}CO_2$ (= (dpm recovered as $^{14}CO_2$)/(initial dpm)) These calculations indicated that water-soluble radioactivity was negatively correlated to [^{14}C]CO_2 production, which again likely reflects the conversion of the explosives to insoluble or soil-bound breakdown products in the more active microcosms. Soluble [^{14}C] dpm measured in samples that were both centrifuged and filtered were almost identical to samples that were only centrifuged (r^2 = 0.99), indicating that the centrifugation was sufficient to pellet all material <0.2 μm. A strong negative correlation also was found between the percent of the initial [^{14}C] extracted with acetonitrile and the percent of the radioactivity recovered as [^{14}C]CO_2. This sug-

Table 3. Mass balance and fate of ^{14}C-labeled explosives in unsaturated Massachusetts Military Reservation soil C soil amended with different sorbents and cosubstrates[a]

Treatment	Addition to microcosms (sorbent + cosubstrate) (mg)		Percent of initial [^{14}C]TNT dpm[b]				
	TOC	BOD	[^{14}C]CO_2	Acetonitrile extractable	Mass balance[c]	Soil	Microbial biomass
Control (no sorbent or C source)	0	0	8 (0)	16 (7)	24 (7)	53 (4)	0 (0)
Soybean oil	1,656	1,748	5 (0)	84 (3)	89 (3)	65 (2)	0 (0)
Molasses[d]	87	681	4 (0)	17 (12)	21 (13)	35 (1)	4 (2)
Peat control (no C source)	2,000	3	8 (0)	9 (2)	17 (3)	37 (9)	1 (0)
Peat + soybean oil	3,656	1,751	4 (0)	56 (5)	60 (5)	59 (10)	0 (0)
Peat + molasses	2,087	684	2 (1)	10 (9)	12 (9)	33 (2)	1 (0)
Sawdust control (no C source)	1,520	15	1 (0)	37 (8)	38 (8)	54 (3)	0 (0)
Sawdust + soybean oil	3,176	1,763	1 (0)	162 (9)	163 (9)	86 (18)	2 (0)
Sawdust + molasses	1,607	696	1 (0)	14 (3)	15 (3)	50 (1)	3 (3)
TNT/RDX/HMX + sawdust + soybean oil	3,176	1,763	1 (0)	99 (3)	101 (3)	77 (3)	2 (1)
TNT/RDX/HMX + sawdust + molasses	1,607	696	1 (0)	17 (3)	17 (3)	60 (2)	1 (1)

[a] TNT = 2,4,6-trinitrotoluene; dpm = disintegrations per minute; RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine; HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; TOC = total organic carbon; BOD = biological oxygen demand.
[b] Results presented as average percent (± difference between duplicate bottles/2).
[c] Mass balance is the sum of [^{14}C]CO_2 and acetonitrile-extractable values.
[d] Twice as much molasses was added to the [^{14}C]RDX-amended microcosms.

gests that, in samples where mineralization was extensive, few degradation intermediates remained.

Although this study relied primarily on the radiolabeled compounds to assess the fate of the explosives, soluble concentrations of explosives and metabolites in the slurries also were determined by HPLC analysis. The concentrations of these analytes as determined by HPLC varied depending on the treatment and evidenced a relatively wide variability among duplicate microcosms (data not shown). In the slurry microcosms prepared with soil C, the highest concentrations of soluble TNT (~40–60 mg/L) were observed in the killed and unamended slurries, followed by samples receiving corn starch and unrefined chitin as cosubstrates. The other treatments had relatively low (<1–5 mg/L) concentrations of TNT. Production of the TNT metabolite 2-amino-4,6-DNT appeared to be favored over its isomer, 4-amino-2,6-DNT, and traces of 2,4-DNT (~1–3 mg/L), but not 2,6-DNT, were also observed. Soluble RDX (10–60 mg/L) and HMX (1–20 mg/L) were observed in all the treatments. The RDX metabolites TNX and DNX also were observed at levels ranging to 20 mg/L. However, MNX was not detected in any of the treatments.

Interestingly, the soluble concentrations of HMX in some treatments were higher than the literature water solubility values [43]. Elevated soluble RDX and HMX concentrations were similarly observed in soil bioslurry reactors receiving molasses during previous experiments in our laboratory. Molasses and other cosubstrates may have a cosolvent effect, subsequently increasing the amount of RDX and HMX that can dissolve in the aqueous phase.

In the slurries prepared from the uncontaminated soil (soil G), the soluble concentrations of all the explosives and breakdown products were much lower than in soil C, and these materials were often times below the detection limit of the HPLC (0.025 mg/L). Soluble TNT was detected at the highest concentration in the killed control samples (22 mg/L), and at 2 mg/L in the unamended sample. 2,4,6-Trinitrotoluene was below detection in all of the cosubstrate-amended slurries. Measurable RDX concentrations (10–19 mg/L) were observed in all of the slurries except for the molasses and crude soybean oil treatments, each of which had <0.2 mg/L. Soluble HMX concentrations did not exhibit any clear relationship with the different cosubstrates, and ranged from 2 to 8 mg/L in samples.

High-performance liquid chromatography analysis was performed on the acetonitrile extracts to quantify the total parent compound remaining in the soil slurries at the end of the study. The data were somewhat variable among duplicate samples (data not shown). However, some clear differences between the treatments were observed. Approximately 30 to 50% of the TNT in soil C was transformed in unamended treatments (normalized to the residual TNT in the killed samples). Amendment with potato starch and corn starch resulted in less than 90% TNT transformation, with all other substrates promoting 95+% conversion of the TNT to other products. In contrast to TNT, neither RDX nor HMX was transformed in the unamended soil C slurries, and only minimal losses of the parent RDX and HMX were observed in the presence of the starches or chitin. In the presence of the vegetable oils and soybean oil–based solvents, 50 to 75% of the RDX, and up to 60% of the HMX was biotransformed. Surprisingly, molasses stimulated minimal biodegradation of RDX and HMX in these soil C slurry experiments.

For soil G, TNT, RDX, and HMX were present only at the relatively low concentrations added at the start of the experiments. High-performance liquid chromatograohy analysis revealed that no parent TNT remained (normalized to the residual TNT in the killed samples) in the soils amended with cosubstrates, irrespective of the substrate type. Approximately 9% of the parent compound remained (i.e., 91% loss) in the unamended control. This indicates extensive TNT transformation (both biotic and abiotic) in soil even without added carbon sources, as observed by others [44–46]. Molasses and crude soybean oil resulted in 100% reductions in extractable RDX from soil slurries. Amendment of soils with corn-steep liquor and potato starch resulted in an approximately 40% reduction in RDX levels, whereas most of the added RDX (89%) remained in the unamended control sample. In these RDX-spiked slurries, about 90% of the HMX added as an impurity also was degraded, both in the presence and absence of cosubstrates. Acetonitrile-extractable HMX concentrations were reduced by an average of 70% by all the cosubstrates and in the

Table 3. Extended.

Percent of initial [^{14}C]RDX dpm[b]					Percent of initial [^{14}C]HMX dpm[b]				
[^{14}C]CO_2	Acetonitrile-extractable	Mass balance[c]	Soil	Microbial biomass	[^{14}C]CO_2	Acetonitrile-extractable	Mass balance[c]	Soil	Microbial biomass
8 (0)	60 (1)	68 (2)	61 (5)	0 (0)	2 (0)	65 (4)	67 (4)	62 (5)	6 (4)
43 (0)	59 (3)	102 (3)	13 (2)	1 (1)	2 (0)	113 (7)	115 (7)	68 (16)	11 (5)
19 (0)	31 (14)	50 (14)	0 (0)	15 (1)	9 (0)	33 (7)	41 (8)	40 (0)	4 (0)
4 (0)	58 (11)	62 (11)	58 (12)	7 (7)	1 (0)	64 (4)	66 (4)	92 (9)	3 (3)
36 (3)	26 (0)	63 (3)	7 (2)	0 (0)	1 (0)	102 (5)	103 (5)	101 (1)	13 (0)
25 (0)	10 (6)	35 (6)	0 (0)	1 (1)	2 (0)	55 (3)	57 (3)	57 (5)	3 (1)
13 (1)	58 (1)	72 (0)	11 (3)	10 (2)	1 (0)	38 (8)	40 (8)	95 (21)	4 (4)
20 (1)	132 (26)	152 (27)	38 (7)	3 (1)	1 (0)	131 (6)	132 (6)	92 (7)	7 (1)
3 (1)	55 (0)	59 (0)	47 (11)	11 (0)	1 (0)	39 (9)	40 (9)	111 (35)	8 (0)
13 (5)	95 (27)	108 (21)	47 (11)	1 (1)	1 (0)	83 (1)	84 (1)	89 (10)	12 (6)
3 (1)	57 (18)	60 (17)	50 (4)	10 (10)	1 (0)	42 (5)	43 (5)	84 (12)	4 (0)

unamended control. Corn-steep liquor and molasses were only slightly more effective at stimulating HMX transformation than was crude soybean oil (75% vs 60%).

In summary, the slurry experiments indicated that out of all the cosubstrates examined, molasses and crude soybean oil were the most effective cosubstrates for supporting microbial biotransformation of TNT, RDX, and HMX in soil. These are two of the least expensive materials examined (bulk costs of approximately \$0.05–\$0.10/kg), and they are liquid in form, so their application at the field-scale over large areas would be feasible from an economic point of view.

Given that a large amount of organic carbon was added to the serum bottles, and the fact that the bottles were sealed, it is likely that anoxic or anaerobic conditions were prevalent in the slurries. These conditions are not likely to be relevant in the field, so the slurry results should be looked at in terms of the biostimulation potential of each cosubstrate, rather than an absolute value. Furthermore, the effectiveness of molasses and soybean oil for stimulating biotransformation of explosives compounds when combined with sawdust or peat moss in unsaturated soils needs to be assessed under more field-relevant conditions (i.e., unsaturated soil), as described in the next section.

Explosives biodegradation in the presence of sorbent–cosubstrate combinations

The decision was made to presorb the explosives to the peat moss and sawdust before adding the soils and cosubstrates to more closely simulate the likely occurrence of events if this technology is applied in the field. After a detonation, a large portion of the explosives residues would likely sorb to the applied material (sorbent). These residues would then desorb, especially during precipitation events, and become bioavailable to the indigenous microflora. This presorbing procedure is probably the worst-case scenario in terms of assessing the ability of the cosubstrates to stimulate biodegradation of the explosives in unsaturated soil, so the results are likely underestimates of the potential effectiveness of molasses and soybean oil. Additionally, during an actual field application, it is likely that the sorbent and cosubstrate would be coapplied to assure a uniform distribution of both over the surface of the soil. Future experiments are planned to address these issues more closely.

The mineralization of individual explosive compounds in unsaturated microcosms prepared with soil G is presented in Figure 4. Mineralization of [^{14}C]TNT was minimal, and the unamended controls (i.e., no sorbent or cosubstrate) and the peat moss–amended treatment actually showed the greatest evolution of [^{14}C]CO_2 during the incubation. The fact that peat moss–amended soil behaved similarly to unamended soil is intriguing, but the underlying mechanism has not been studied further. Soybean oil and molasses stimulated some TNT mineralization compared to the killed control sample, but only about 50% of the level observed in the unamended and peat moss–amended treatments. The percent mineralization in the presence of molasses and soybean oil was somewhat reduced in samples where the TNT was initially presorbed to either peat moss or sawdust.

In unamended controls, approximately 6% of the [^{14}C]RDX was mineralized. This value was slightly lower (3%) in samples with peat moss only and sawdust plus molasses. In contrast, marked RDX mineralization was observed in samples amended with soybean oil (43%), and a slightly lower amount of RDX was mineralized (36%) if peat moss also was added to the soil as a sorbent. These results confirm the previous data from the slurry microcosms showing that soybean oil is an effective cosubstrate to promote explosives biodegradation. The difference in mineralization in the presence and absence of peat moss may reflect a slight reduction in the bioavailable fraction of parent RDX. More RDX mineralization was observed in microcosms amended with sawdust compared to the unamended control, but still only about 15% of the RDX was converted to [^{14}C]CO_2. Molasses alone also stimulated RDX mineralization, but only 16% of the parent compound was collected as [^{14}C]CO_2 compared to 43% for crude soybean oil. Interestingly, mineralization of RDX was 23% in samples in which the RDX was initially added with peat moss as an adsorbent, indicating that peat moss itself may have stimulated the additional RDX mineralization in this case. Conversely, only 2% of the parent RDX presorbed to sawdust was mineralized in samples receiving molasses. Thus, with both molasses and soybean oil as cosubstrates, sawdust reduced RDX mineralization significantly.

Mineralization of HMX in the unsaturated soil was negligible except in samples receiving molasses. Preadsorption of the HMX to peat moss reduced the [^{14}C]CO_2 production in samples with molasses by fourfold compared to the treatment without peat moss as an adsorbent (i.e., 2% vs 8%).

The mineralization of each of the three [^{14}C]-labeled explosives in a mixture with the other two explosives in unsat-

urated soil G is shown in Figure 5. For RDX, the addition of HMX and TNT resulted in only a slightly lower extent of mineralization (12% vs 19%) in soil receiving sawdust with soybean oil as a cosubstrate. This difference was observed only after 40 d of incubation. No real differences in RDX mineralization were observed in soils amended with sawdust and molasses regardless of the presence or absence of TNT and HMX.

The very low percent mineralization of TNT and HMX precludes firm conclusions from studies in which these compounds were added in the presence or absence of other explosives, but gross differences were not observed between treatments with TNT or HMX alone and in the presence of the other two explosives.

The mass balances (sum of $[^{14}C]CO_2$ plus radioactivity recovered in the acetonitrile extract), for each of the explosives in the unsaturated soil G microcosms (Table 3) were generally much lower than those for the slurry microcosms (Table 2). The difference appears to reflect poor recovery of explosives residues from the microcosm soil. The soil samples yielded similar recovery of radiolabel (average percent difference between duplicates was 15 $\pm$ 10%), despite the difficulty in obtaining representative 0.25-g replicates, and the likely heterogeneous distribution of the radioactivity within the soil matrix. Therefore, the low residual dpm values detected in the soil likely reflects inefficiencies in the scintillation counting procedure. It also appeared that some treatments resulted in drastically lower soil dpm values. This may have been due to irreversible binding of $[^{14}C]$-labeled explosives or metabolites to soil particles and sorbents, or diffusion of the same deep within particles, effectively shielding the radioactivity and preventing its detection. Alternatively, some of the treatments may have directly interfered with the scintillation process. However, this latter possibility seems unlikely, given that the only errors reported during scintillation counting were due to chemiluminescence, which would have increased, rather than decreased, the apparent dpm in the sample.

The amount of the added ^{14}C in the soil, in acetonitrile-extractable residues, and in the microbial biomass are listed in Table 3. For these experiments, no relationships were observed between the percent of the residual radioactivity extracted by acetonitrile and the percent of initial radioactivity recovered as $^{14}CO_2$. This is likely a reflection that abiotic (i.e., sorption and mass transfer) and biological processes are different in slurried versus unsaturated soil, thereby affecting the final disposition of the explosive compounds and their metabolites. The unsaturated microcosms more closely approximate actual field conditions, and should therefore be given more weight in terms of the real-world relevance and implications of the results.

Interestingly, for these unsaturated microcosms, the radioactivity recovered by the acetonitrile extraction was usually higher in all the treatments that received soybean oil, in contrast to the results observed with the slurry microcosms. The reasons for this difference are not entirely clear. 2,4,6-Trinitrotoluene, RDX, and HMX all have relatively low octanol–water partition coefficients (log K_{OW} = 1.86, 0.87, and 0.26, respectively) [43], indicating that they are not extremely hydrophobic. However, if the explosives partition into soybean oil more than into octanol, and become unextractable by acetonitrile, then the partitioning may have been more extensive in the continuously shaking slurries than in the static unsaturated microcosms. Alternatively, the soybean oil added to the unsaturated microcosms may have bound to the soil matrix more in the unsaturated microcosms than in the slurries, leaving less free oil phase into which the explosives could partition and become less extractable. More research may be required to fully explain the observed results, including determination of partition coefficient for the explosives in vegetable oils.

The levels of breakdown products in the acetonitrile extracts as determined by HPLC analysis were near or below the detection limit of the method for these compounds because the initial concentrations of TNT, RDX, and HMX in this study were reasonably low (4.9, 1.7, and 0.3 mg/kg, respectively). The only two clear observations from the analysis of these extracts were 2-amino-4,6-DNT was detected as the primary TNT metabolite when any breakdown products were present and the occurrence and concentrations of RDX breakdown products were in the order MNX $>$ DNX $\gg$ TNX, which is essentially opposite of what was observed in the slurry microcosms. The reason for this difference is unclear, but may reflect differences in microbial populations carrying out the reactions in these two environments (aqueous slurry vs soil), or the different times of the two assays (i.e., the soil assay was performed for a shorter period). Mixing of the parent compounds in the soil or chemical bioavailability also may have played a role in the occurrence of degradation products in the samples.

The chloroform fumigation–extraction procedure indicated that the amount of the added dpm associated with microbial biomass was very low ($<$5%) in the $[^{14}C]$TNT treatments (Table 3). Greater amounts and a wider range of microbial biomass–associated dpm were observed for $[^{14}C]$RDX (0–15%) and $[^{14}C]$HMX (3–13%). Higher levels of incorporated dpm appeared to be associated with samples receiving molasses addition for RDX and in those receiving soybean oil for HMX. This relationship seems reasonable, because these two substrates also stimulated the greatest mineralization of the explosives, which likely resulted in more ^{14}C dpm being available for assimilation into microbial biomass. These results are in the range observed in other studies. For example, Manning et al. [47] determined that up to 30% of $[^{14}C]$TNT was in the cell protein microbial biomass of soil bioslurry reactors fed molasses [47]. In contrast, Waisner et al. [35] found that a negligible amount ($<$3%) of $[^{14}C]$RDX was incorporated into microbial cell membranes [35] in soil–water slurry microcosms.

Conclusion

These experiments and those in the companion paper examining sorption of explosive compounds [21] clearly indicate that sorption and biodegradation of explosives residues can be enhanced at impact ranges by the application of readily available and inexpensive amendments. Sphagnum peat moss clearly proved to be the best sorbent based on isotherm data, and crude soybean oil resulted in the highest degree of mineralization of RDX and HMX, and nearly the same mineralization of TNT as molasses. The combination of peat moss and soybean oil in unsaturated soil stimulated significant RDX mineralization, but little HMX mineralization compared to soybean oil in slurried soil. Based on these results, laboratory-scale soil column experiments currently are being conducted to examine the effectiveness of peat moss, alone and in combination with crude soybean oil, for reducing the downward migration and promoting the biodegradation of explosives residues deposited at the soil surface. The initial results from

these studies suggest that soil treatment with these amendments may help to protect groundwater resources at live fire ranges.

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