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Final Report on Residual Dinitrotoluenes in Settling Ponds and Spoils Disposal Area Soils at Badger Army Ammunition Plant: Microcosm and Soil Column Studies

Prepared for

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Executive Summary

The purpose of the research described was to address issues arising from recent investigations on the residual dinitrotoluenes (DNTs) in the Settling Ponds and Spoils Disposal Areas at the Badger Army Ammunition Plant. Badger Army Ammunition Plant was constructed in 1942 to produce propellants for use in cannon, rocket and small arms ammunition for World War II. Production of explosives was terminated in 1975, and remedial investigation and activities associated with contaminated soil and groundwater started in 1988. Recent investigations by Olin indicate that concentrations of both 2,4-DNT and 2,6-DNT are very low and inconsistent with fate and transport models that predict DNT in the groundwater at or close to the water solubility concentration. The Wisconsin Department of Natural Resources Administrative Code discourages the use of models for determination of biodegradation rates and pathways in deference to actual field data. The models overestimate the DNT concentration in the groundwater, mostly likely due to the omission of certain fate processes in the model which include:

1) aerobic biodegradation of DNT in the surface soils and the vadose zone, and
2) root-zone plant assisted biodegradation (phytoremediation).

Based on our previous experience and research involving contaminated media obtained from locations at Badger, it seemed likely that one or both of these mechanisms is active without intervention and that natural attenuation may be an effective strategy for managing the contamination that exists at the Settling Ponds and Spoils Disposal Areas. The goal of the present work was to determine the contribution of biodegradation to the fate and transport of DNT at the Settling Ponds and Spoils Disposal Areas.

The first objective of this study was designed to provide a yes or no answer as to whether there are microorganisms capable of growth on 2,4-DNT in the soil and subsurface material from the Settling Ponds and Spoils Disposal Areas. Microcosms and most probable number (MPN) plates constructed with soil and water from the sites showed that biological activity is responsible for the destruction of DNT in materials from the two sites. Concentrations of DNT in the samples from the site were negligible, so DNT was added to the microcosms where it was degraded rapidly and repeatedly. Finally, isolation and identification of the DNT-degrading bacteria provided conclusive evidence of the biodegradation potential at the BAAP site.

The second objective was to evaluate the rate and extent of natural attenuation of DNTs in the vadose zone soils under simulated field conditions including rainwater infiltration, transport and degradation. In batch sorption experiments 2,4- and 2,6-DNT behaved similarly for each soil, giving similar isotherms (shape and magnitude) and derived K_d values, but varied greatly depending on the soil. Samples from Settling Ponds OP1 and OP4 demonstrate relatively fast desorption and after a few steps show undetectable levels of DNT being desorbed indicating that soils from the Settling Ponds loosely bind DNT when compared to soils from the Spoils Disposal Area.

2,4-DNT was never detected after 123 days of operation in the effluent from columns designed to simulate leaching of DNT into the vadose zone, nitrite was found but at less than stoichiometric amounts. Breakthrough occurred in the column fed 2,6-DNT at 22

days and reached equilibrium at around 40 days. Nitrite was never observed in 2,6-DNT column effluent. Because it is less readily degradable, 2,6-DNT can be considered a control for sorption and transport. 2,6-DNT has not been detected at levels of concern at the site. The results indicate clearly that 2,4-DNT, but not 2,6-DNT was biodegraded in the soil columns under conditions that simulate rainwater.

The microcosm and MPN studies both demonstrated the presence of bacteria at the Settling Pond and Spoils Disposal areas that are capable of degradation of 2,4-DNT. Isolation of individual strains capable of growth on 2,4-DNT is conclusive evidence of the presence of the appropriate bacteria at all three sites for natural attenuation to occur. *Furthermore, the presence of substantial populations of DNT-degrading bacteria is strong* evidence that they are degrading DNT at the site. We have never detected DNT-degrading bacteria in uncontaminated sites or in sites where DNT degradation is not taking place.

There are differences among the sites with regard to the number of organisms present and the rate at which they are capable of 2,4-DNT degradation. The number and variety of isolates from each site was consistent with the rate and extent of 2,4-DNT degradation in the various microcosms. The differences between microcosms constructed with minimal medium and microcosms constructed with ground water suggest that some essential nutrients might be limiting or that the buffering capacity of the ground water is limited. Our results further indicate that the sorbed 2,4-DNT can be biodegraded until a residual concentration is reached that varies depending upon the soil type. The residual material is not likely to be mobile and should be biodegraded if it is released from the soil.

Introduction

The purpose of the research described in this report was to address issues arising from recent investigations on the residual dinitrotoluenes (DNTs) in the Settling Ponds and Spoils Disposal Areas at the Badger Army Ammunition Plant. The studies described herein briefly document the history and problems of DNT contamination in the Settling Ponds and Spoils Disposal Areas, followed by a summary of key concerns based on the prior investigations raised by the Development of Site-Specific Soil Residual Contaminant Levels proposal submitted in May of 2002. It is anticipated that this report will provide information critical to decision makers and on-site remedial personnel for a better prediction of DNT's behavior and a better design of site-specific remedial strategy to protect human health, ecosystem health, and groundwater quality at the Badger site.

Badger Army Ammunition Plant was constructed in 1942 to produce propellants for use in cannon, rocket and small arms ammunition for World War II. The Settling Ponds and Spoils Disposal Areas are located along the southern boundary of Badger Army Ammunition Plant. There are four Settling Ponds and five Disposal Areas along with Final Creek and the Main Ditch from the rocket production areas. The Settling Ponds (~55 acres) were used for aeration and settling basins for the treated effluent from production wastewater. Sediments in the ponds were removed via dredging between 1973

to the late 1970's, and were placed in the Spoils Disposal Areas (~13 acres). Production of explosives was terminated in 1975, and remedial investigation and activities associated with contaminated soil and groundwater started in 1988. Currently the Settling Ponds Areas are covered with vegetation with only a few acres filled with water in Settling Pond 1 (ECCI 2002).

Recent investigations by Olin have determined the extent of DNT contamination in various locations of the Settling Ponds and Spoils Disposal Areas. Results from these extensive sampling efforts indicate that concentrations of both 2,4-DNT and 2,6-DNT are very low – generally several orders of magnitude lower than the contaminated soils from the Propellant Burning Ground (up to 28% by weight). For instance, the concentrations of 2,4-DNT were 1.9–470, 0.64–110, and 660 mg/kg in the Spoils Disposal Areas, Settling Ponds, and Final Creek, respectively. For 2,6-DNT, the maximum concentrations were 0.11– 32, ND–6.80, and 41 mg/kg in the Spoils Disposal Areas, Settling Ponds, and Final Creek, respectively. All 2,6-DNT concentrations are below the direct contact Residual Contact Level (RCL) of 78 mg/kg. In contrast, several areas have 2,4-DNT contamination above the direct contact RCL of 160 mg/kg (ECCI 2002).

The development of Site-Specific Soil Residual Contaminant Levels proposal predicted the DNT concentration in underlying groundwater in the Settling Ponds and Spoils Disposal Areas using existing fate and transport models (SESOIL and AT123D). Modeling predicts that DNT will leach into groundwater at or close to the water solubility concentration. This model prediction, however, is not consistent with site observations. The Wisconsin Department of Natural Resources Administrative Code, Chapter NR 720.19 discourages the use of models for determination of biodegradation rates and pathways in deference to actual field data. The models overestimate the DNT concentration in the groundwater, mostly likely due to the omission of certain fate processes in the model. Specifically, these processes include:

3) aerobic biodegradation of DNT in the surface soils and the vadose zone, and
4) root-zone plant assisted biodegradation (phytoremediation).

As summarized below and supported by our previous studies, we believe that these two processes may contribute significantly to the disappearance of DNTs and explain why predicted DNT concentrations are substantially greater than measured concentrations.

Aerobic biodegradation of DNT: Naturally occurring aerobic bacteria that are able to metabolize DNT as their sole source of energy have been identified at Badger. Our previous work on soils from Badger's Propellant Burning Ground has shown that rapid degradation of DNTs can occur in the presence of indigenous bacteria as long as sufficient nutrients are present (Zhang, Nishino et al. 2000; Nishino and Spain 2001; Zhang, Daprato et al. 2001; Fortner, Zhang et al. 2003). In some cases, 2,4-DNT (if the concentration is too high) inhibited the biodegradation of 2,6-DNT. The lower concentration of DNT in the Settling Ponds and Spoils Disposal Areas (when compared to soils in Propellant Burning Ground) should allow for bacterial biodegradation without concerns of toxicity. Furthermore, low rates of degradation within the vadose zone may be sufficient to attenuate DNT before reaching the water table. We measured DNT degradation in vadose zone samples taken from the Propellant Burning Ground over a 7-

day test period without nutrient addition, suggesting that attenuation processes were taking place at that location without intervention (Fortner, Zhang et al. 2003).

Root-zone plant assisted biodegradation: The role of plants could be significant in the attenuation of DNT at the locations of interest in this study. This is especially true where the levels of DNT contamination are low and dense vegetation is present, including the Settling Ponds and Spoils Disposal Areas. The potential of phytoremediation has been demonstrated in our previous studies on explosives compounds including TNT, RDX and HMX (Hughes, Shanks et al. 1997; Bhadra, Spanggord et al. 1999; Bhadra, Wayment et al. 1999). In an analogous fashion, it is likely that plants can interact with DNT through various mechanisms, including uptake of DNT into plant tissue (phytoextraction), metabolism into bound residues via enzymes within the plant (phytodegradation), or plant-enhanced root-zone bacterial degradation (rhizodegradation). Additionally, plants transport significant amount of water by evapotranspiration and therefore have the potential to reduce the flux of DNT from leaching into the underlying aquifer.

Based on our previous experience and research involving contaminated media obtained from locations at Badger, we believe that it is quite likely that one or both of these mechanisms is active without intervention and that natural attenuation may be an effective strategy for managing the contamination that exists at the Settling Ponds and Spoils Disposal Areas. Natural attenuation, that includes or combines bacterial degradation and phytoremediation, is an environmentally friendly and potentially cost-effective alternative to other intrusive options such as ex-situ slurry reactors, composting, and incineration. Also, natural attenuation offers ecological improvement, simplicity in concept, and aesthetic advantages with strong public acceptance (Burken, Shanks et al. 2000).

Objectives

The overarching objective of the studies was to provide the information needed to assess the role of naturally occurring biological fate processes in the attenuation of DNT contamination in soils and the vadose zone at Settling Ponds and Spoils Disposal Areas. This work had two components that were implemented in a staged approach to allow for an expansion of scope if needed. In the first stage, studies addressed the role of aerobic bacterial breakdown of DNT. The studies for Stage 2, if necessary, are to be focused on the role of indigenous plant and associated microbes in pot studies. Because indigenous plants are suited for growth under conditions at Badger, these experiments (if needed) are to be conducted on site or at a facility with a controlled environment that simulates site conditions.

Specific Objectives and Performers

1. Confirm the presence and activity of aerobic DNT degrading bacteria in samples from contaminated soils and underlying vadose zone materials (*Phase 1: Spain*).
2. Evaluate rate and extent of natural attenuation of DNTs in the vadose zone soils under simulated field conditions including rainwater infiltration, transport and degradation (*Phase 1: Hughes*).

3. Test the potential of phytoremediation of DNT-contaminated soils using indigenous plants from the Badger site (*Phase 2: Hughes*).

Here we report the results of work on the first two objectives, which were accomplished separately in the labs of Spain and Hughes. There is some overlap in that microcosm experiments were done in both labs to provide assurance of consistency in the results. A portion of the work was done at Tyndall AFB, FL prior to the move of the Spain lab to the Georgia Institute of Technology.

Deliverables

The primary deliverable for Phase 1 of this project is this final report prepared and submitted to John P. Hansen, P.E., Chief Environmental Engineer, Olin Corporation - Badger Army Ammunition Plant, Baraboo, WI that details the methods, results, implications of findings, and a recommendation regarding the need for Phase 2 studies. Specifically, this report addresses the findings of microcosm and soil column studies, including findings on the presence/absence of organisms capable of DNT mineralization, the impact of degradation processes on leaching from site materials, and estimates of in situ degradation rates as a function of site conditions. After submission of the report, Drs. Spain and Hughes will travel to Badger and present the findings to site personnel as appropriate and discuss implications regarding the efficacy of natural attenuation processes to treat DNT in these locations.

Objective 1. Confirm the presence and activity of aerobic DNT degrading bacteria in samples from contaminated soils and underlying vadose zone materials (*Phase 1: Spain*).

The studies described in this section were designed to extend current understanding of the fate of dinitrotoluene (DNT) in soils and groundwater to the Settling Ponds and Spoils Disposal Areas at the Badger Army Ammunition Plant (BAAP). The primary issue addressed in this study was whether natural attenuation is a major mechanism for the disappearance of DNT from the Settling Ponds and Spoils Disposal Areas. Generally, three questions need to be addressed to determine whether biological activity will effect natural attenuation. The first is whether biodegradation of the contaminant has ever been reported. The second question is whether suitable bacteria active against the contaminants of concern are present at the site. The third question is whether the conditions at the site are appropriate and sufficient to sustain biodegradative activity through completion of monitored natural attenuation (Spain 1997).

Both 2,4- and 2,6-DNT are biodegradable (Fig. 1.1), although 2,4-DNT is much more readily degraded than is 2,6-DNT (Nishino, Paoli et al. 2000; Nishino, Spain et al. 2000). Because bacteria use 2,4- and 2,6-DNT as growth substrates, the compounds are mineralized, and the final end products are cell mass, H_2O, CO_2, and NO_2^-. Roughly a quarter of the available NO_2^- from DNT is used by the DNT-degrading bacteria to satisfy their own nitrogen requirements and the balance is released into the growth medium. Our previous work with materials from the Badger site yielded bacterial isolates that grow on

both DNT isomers. The finding of rapid and complete DNT mineralization provided the basis for a highly successful in situ bioremediation system at the Propellant Burning Ground (Cuffin, Lafferty et al. 2001). A similar effort was designed to provide a yes or no answer as to whether there are microorganisms capable of growth on 2,4-DNT in the site materials from the Settling Ponds and Spoils Disposal Areas. First, microcosms and most probable number (MPN) plates constructed with soil and water from the two sites were used to determine that biological activity can cause the destruction of DNT in materials from the two sites. Second, sustained degradative activity was demonstrated by respiking and transferring microcosms and MPN plates that demonstrated DNT degradation. And, finally, isolation and identification of the DNT-degrading bacteria provided conclusive evidence of the biodegradation potential at the BAAP site.

Materials and Methods

Soil samples. Soil cores were collected from Settling Pond 1, OP1-48; Settling Pond 4, OP4-07; and from Spoils Site 1, SSB-03-17 on 28 June, 2004 and shipped on ice by overnight courier to Tyndall AFB, FL. Sample locations and core depths were based on historic sampling data for the Settling Ponds and Spoils Site areas, and were chosen because of previous findings of DNT contamination in the soil. Analyses performed in 1997 (Settling Ponds) and 2003 (Spoil Site 1) indicated 2,4-DNT soil concentrations between 0.57 and 52 mg/kg in the upper soil layers (John Hansen, personal communication). Groundwater from an uncontaminated upgradient monitoring well was shipped a day later.

The surface of the sleeves containing the soil cores were wiped down with a 10% bleach solution, then aseptically extruded from the core sleeves. Cores were approximately 5 cm diameter x 90 cm long. Five cm of soil from the top and bottom of the core was discarded and the remaining core was divided into top, middle, and bottom sections. Each core section was separately sampled and analyzed for DNT, pH, moisture, nitrite, nitrate, total organic carbon, and DNT-degrading bacteria by a most probable number (MPN) technique (below).

DNT extraction and analysis. DNT was extracted by the method of Jenkins (Jenkins and Walsh 1987) with modifications. A sample of soil from each core section was weighed and added to a 10 x volume (wt/vol) of acetonitrile (ACN). The soil was dispersed in the ACN with a vortex mixer, then allowed to stand 15 minutes, after which it was again mixed with a vortex mixer. A portion of the slurry was clarified by centrifugation, the pellet discarded, and the supernatant analyzed for 2,4- and 2,6-DNT by reverse phase high performance liquid chromatography (HPLC) on an Agilent 1100 system equipped with a diode array detector. Separation was performed with a Chromolith Speedrod (50 mm, Merck, FRG) with a mobile phase consisting of Part A: water with trifluoroacetic acid (1 ml L^{-1}), and Part B: ACN with trifluoroacetic acid (0.5 ml L^{-1}). Initial conditions were 98:2 A:B, with a flow rate of 1 ml min^{-1}. Between 1 and 7 minutes a linear gradient changed the proportions to 50:50 A:B, and increased the flow rate to 1.5 ml min^{-1}, The final conditions were held for 1 min. The column temperature was 35°C, and the autosampler was maintained at 5°C. Compounds were detected by A_{254}.

pH was measured directly in the soil with an ISFET (ion sensitive field effect transistor) pH electrode. Soil from each core section was also used to make 20% (wet wt/vol) soil slurries in nitrogen-free minimal medium (BLK) (Bruhn, Lenke et al. 1987). The slurries were used for nitrite and nitrate analysis (Smibert and Krieg 1994), and for MPN analysis. MPN plates were constructed in 96-well microtiter plates. The slurries were serially diluted into BLK containing 2,4-DNT (100 μM). The wells were inoculated in an array of 8 replicates, serially diluted 12 times at 1:10. Two MPN plates were constructed per core section. The MPN plates were incubated at room temperature, sealed in a plexiglass box at 100% humidity. After 6 weeks the plates were subsampled for nitrite analysis. Nitrite release from 2,4-DNT is a good indicator of mineralization (Nishino and Spain 2002). The MPN was calculated from an 8-replicate table (Garthwright 2001).

Samples from each core section were weighed and dried at room temperature to determine soil moisture. The dried soils were ashed at 600°C to determine the organic content.

Microcosm construction. The top, middle, and bottom subsections were composited and microcosms were constructed in triplicate from each core. Soil (20% wet wt) was added to either BLKN or filter sterilized ground water from BAAP. Killed controls (60 mg/L $HgCl_2$ plus 0.8% glutaraldehyde) were constructed in duplicate in BLKN only. 2,4-DNT (100 μM) was added to all microcosms. Microcosms were incubated in the dark, at 14°C, with shaking at 250 rpm.

Microcosms were sampled periodically and analyzed for DNT concentration, nitrite, and ammonia release. Because the DNT sorbed to the soil, preparation of samples for DNT analysis included an organic extraction step. The microcosm was shaken to suspend the soil immediately before sampling with a wide-bore pipette tip. Slurry (50 μl) was added to a microcentrifuge tube with a filter insert (Millipore Ultrafree-MC, 0.22 μm pore) and centrifuged at 14,000 rpm for 4 min. ACN (100 μl) was added to the pellet retained on the filter, then centrifuged for an additional 8 min. The combined culture fluid and ACN rinse was analyzed by HPLC for quantification of 2,4-DNT. Starting with the 11th sampling period the analysis protocol was changed and the culture fluid and ACN rinse were separately analyzed by HPLC. The concentration of 2,4-DNT measured in the ACN extract was multiplied by 2 to account for the dilution factor, then averaged with the 2,4-DNT measured in the culture fluid and the results reported as a single number. Nitrite and ammonia were measured in a separate sample.

Isolation of 2,4-DNT-degrading bacteria. MPN samples that were positive for nitrite release were enriched for 2,4-DNT-degrading bacteria. The highest dilution that was positive for nitrite release was used to inoculate the initial well of a fresh 96-well microtiter plate containing 2,4-DNT (100 μM) in BLKN. The wells were serially diluted 12 times at 1:10. The plates were incubated at room temperature for 3 weeks, then subsamples were taken for nitrite analysis. The highest dilutions that showed nitrite release were transferred to fresh microtiter plates containing 2,4-DNT in BLKN and incubated 3 more weeks. Wells that were positive for nitrite release at the end of the incubation period were used to inoculate 2,4-DNT agar plates (Set A) (Nishino, Paoli et

al. 2000). The plates were incubated at 30°C for 2 weeks until individual colonies were visible. Colonies were streaked onto fresh 2,4-DNT agar plates (Set B) and ¼-strength tryptic soy agar (1/4 TSA) plates and incubated at 30°C. Cultures that grew on the Set B 2,4-DNT agar plates and appeared pure on the 1/4 TSA plates were transferred onto 2,4-DNT agar plates (Set C), BLKN agar plates (no carbon source), and 1/4 TSA plates from the Set B 2,4-DNT plates. Cultures that grew on the Set C 2,4-DNT agar plates, but not on the BLKN control plates, and were pure on the 1/4 TSA plates were sent to MIDI Labs (Newark, DE) for 16S rDNA analysis.

Results and Discussion

No 2,4- or 2,6-DNT was detected in any soil sample. The minimum detection limit for the initial soil extracts was 2 mg/L. The sensitivity was low because of the high dilution with the organic solvent required to ensure extraction in the presence of water (Jenkins and Walsh 1987). The initial organic extract was not concentrated to analyze for µg levels of DNT because the objective was to determine how much 2,4-DNT to add to the microcosms to obtain an initial concentration of 100 µM in all microcosms. The concentration was chosen because it is a reasonable amount to find in field contaminated soil and groundwater, it is enough to support growth of bacteria and induction of the DNT-degradation pathway, and it is in the linear range for DNT and nitrite analysis. The detection limit for routine samples from the microcosms was 1 µM (0.2 mg/L).

pH, soil moisture and organic content are presented in Table 1.1. The cores from the two settling ponds were fairly uniform silty clay from top to bottom. The core from the spoils site was highly organic in the upper two-thirds, but the composition changed markedly and the lowest third was almost pure sand. The change is reflected in the % moisture and organic content.

Microcosms. After 3 weeks incubation, all microcosms constructed with soil from SSB-0317 degraded the initial 2,4-DNT, and degraded the second addition of 2,4-DNT in one week (Fig. 1.2). DNT degradation was accompanied by stoichiometric release of nitrite. The considerable organic content of the composited soil from core SSB-0317 rapidly sorbed 2,4-DNT from the aqueous phase, making it difficult to determine whether biological activity was responsible for the DNT disappearance, but killed controls showed no nitrite release as 2,4-DNT disappeared from the aqueous phase. The tendency of the 2,4-DNT to partition to the soil, becoming less available for biodegradation is illustrated in Table 1.2 for 3 individual microcosms.

All three microcosms constructed with minimal medium and soil from OP4-07, and one of the microcosms constructed with ground water and soil from OP4-07 degraded the initial 2,4-DNT within six weeks (Fig. 1.3). Nitrite release was stoichiometric in only one of the microcosms, and increases in nitrate were not detected. Small amounts of 2-amino-4-nitrotoluene were detected in the OP4-07 microcosms, indicating some reductive activity. No DNT degradation was detected in killed controls. The resumption of 2,4-DNT degradation after the second addition of 2,4-DNT was highly variable among the replicate microcosms.

Microcosms constructed with soil from OP1-48 showed an initial rapid disappearance of 2,4-DNT that ceased after 3 weeks. The initial disappearance was not accompanied by nitrite release. The lack of 2,4-DNT-degrading bacteria, lack of nitrite release, and a comparable decrease in the 2,4-DNT in the killed control microcosms for OP1-48 suggest that the decrease in 2,4-DNT was due to sorption.

Nitrate was analysed but not detected in all microcosms in which nitrite release was not stoichiometric to 2,4-DNT disappearance. Nitrite oxidizing bacteria are abundant in environmental samples and have in some cases been the mechanism for nitrite loss from 2,4-DNT degradation systems (Lendenmann, Spain et al. 1998). Lack of nitrate rules out the possibility of nitrite loss through oxidation.

Ammonia release in all non-sterile microcosms was rapid and immediate and occurred prior to any DNT disappearance, and in concentrations much greater than the amount of DNT added, and so cannot be used to monitor or explain DNT disappearance. No ammonia was detected in killed controls, indicating that biological activity was responsible for the ammonia release. But the timing and amount of ammonia release suggests that there is a substantial amount of biological activity in the microcosms that is unrelated to DNT biodegradation. The most likely source of the ammonia is the substantial quantity of organic matter in the Settling Ponds and Spoils Disposal Areas soils.

The microcosm studies were terminated 2 weeks earlier than planned on 18 September due to Hurricane Ivan induced power failure of the incubator containing the microcosms. The microcosms clearly showed biological degradation of DNT in soil from SSB-0317 and OP4-07, but not in soil from OP1-48. Degradation of 2,4-DNT in microcosms constructed with nitrogen-free minimal medium was somewhat better than in microcosms constructed with filter-sterilized groundwater from BAAP. The result suggests that some nutrients might be limiting for degradation of 100 μM concentrations of 2,4-DNT at the Settling Pond and Spoils Disposal sites. We found earlier that phosphorus is limiting for degradation of very high concentrations of 2,4-DNT at the Propellant Burning Grounds (Nishino and Spain 2001; Fortner, Zhang et al. 2003). That the rate of disappearance of DNT was reasonably rapid even at 14°C, the soil temperature for BAAP, suggests that DNT degradation by the indigenous organisms is not inhibited by the cool soil temperatures.

MPN analysis. A most probable number technique was used to detect 2,4-DNT-degrading bacteria in each of the original subsections of the soil cores (Table 1.3, Fig. 1.). The MPNs are reported as number of bacteria per gram dry weight of soil, corrected for the % moisture of the soil cores. The MPNs confirm the results of the microcosm study. There are abundant 2,4-DNT-degrading bacteria in the SSB-0317 soil, fewer 2,4-DNT-degrading bacteria present in the OP4-07 soil, and still fewer in the OP1-48 soil.

Isolation of 2,4-DNT-degrading bacteria. The wells that were positive for nitrite release in the MPN plates served as the inocula for enrichment cultures. 68 enrichments

eventually yielded 12 isolates that could be grown in pure culture when 2,4-DNT was supplied as the only growth substrate. Partial 16S rDNA analysis was used to identify the isolates against the MicroSeq Database (Table 1.4). Soil from OP1-48, which showed the least degradation of 2,4-DNT, also yielded the smallest variety of 2,4-DNT-degrading bacteria. All three isolates had identical partial 16S rDNA sequences. More variety was seen in the isolates from the OP4-07 soil, although the sequences of the two rhodococci were identical. Finally, the soil from SSB 03-17 yielded the greatest variety of 2,4-DNT-degrading isolates. Isolate 192 is distinct from the *Pseudomonas* sp. from the OP1-48 samples. Thus a greater variety of DNT-degrading strains was isolated from soils that supported more rapid DNT-degradation. Whether that was because the different bacteria somehow complemented each other to provide more rapid DNT-degradation, or if it was simply a function of higher numbers of DNT-degrading bacteria cannot be stated. The limited number of soil samples and bacterial isolates chosen for identification precludes any more meaningful analysis of the distribution of DNT-degrading strains within or among the sample sites. It should be emphasized that specific strains, not species of bacteria, possess the pathways for biodegradation of 2,4- and/or 2,6-DNT. Bacteria that are able to degrade the two isomers have only been isolated from sites that have had the specific contaminants.

Objective 2. Evaluate rate and extent of natural attenuation of DNTs in the vadose zone soils under simulated field conditions including rainwater infiltration, transport and degradation (*Phase 1: Hughes*).

Materials and Methods

Chemicals

2,4-DNT (97%) and 2,6-DNT (98%) (Table 2.1) were obtained from Sigma-Aldrich. The following reagent grade chemicals were used for mineral media constituents: $CaCl_2.2H_20$, $CaSO_4.5H_2O$, $FeSO_4.7H_2O$, H_3BO_3, K_2HPO_4, KH_2PO_4, $MgSO_4.7H_2O$, NaCl, $Na_2MO{\cdot}6H_2O$, and $ZnSO_4{\cdot}7H_2O$ (Spanggord, Spain et al. 1991). ACN (HPLC grade, 99.9%, Fisher Scientific), reagent grade trifluoroacetic anhydride (Fisher Scientific) and acetic acid (Acros, glacial reagent) were used for HPLC analysis. $NaNO_2$ (Fisher Scientific) was used for preparing calibration curve for NO_2^- analysis. NaOH was used to adjust pH when needed.

Soil Samples

Soil cores analyzed in this study were collected as described above at BAAP from Settling Pond 1 at depth 0-3 feet, labeled OP1; Settling Pond 4 at a depth of 0-3 feet, labeled OP4 and from Spoils Site 1 at depth of 3-6 feet, labeled SSB. Upon arrival the soils were homogenized and debris removed (pebbles, plant matter, etc.). They were transferred into stainless steel containers and stored at 4°C. The soils were air-dried, ground, and sieved (2 mm) for column and sorption studies. Soil samples were analyzed by the Department of Crop and Soil Science, University of Georgia, at their commercial

laboratory for environmental analysis. Methods of analysis and results of soil properties are summarized in Table 2.2.

Soil Extraction Procedure

For each homogenized soil (OP1, OP4 and SSB), five sub samples (approximately 0.05 g each) were extracted and averaged to determine the soil associated DNT concentrations. Using a Ultrafree-MC 0.22 μm two chamber centrifugal filter device (Millipore), 50 mg of soil and 200 μL of ACN was added to the top filter unit and allowed to equilibrate (10 minutes). The unit was centrifuged (5 minutes at 7000 RPM) allowing for separation of the ACN through the top filter unit into the bottom collection unit. This process was repeated twice more for a total of three ACN extractions per soil sub sample (Zhang, Nishino et al. 2000; Fortner, Zhang et al. 2003). The 600 μL of ACN in the bottom of the collection unit was then analyzed for DNT via HPLC analysis.

Batch Sorption

Modified from Haderlein and Schwarzenbach, batch sorption experiments were carried out in 50 ml Teflon© (PTFE) centrifuge tubes with 3 g (constant mass) of soil and 30 ml (constant volume) of D.I. water (pH 7) at various DNT concentrations (2.5, 5, 10, 25 and 50 mg/L) (Haderlein and Schwarzenbach 1993). The samples were prepared in triplicate for each initial condition. Samples were continuously mixed end over end at approximately 1 rpm at room temperature (22°C). Sodium azide (1g/L) was added to all experiments to inhibit biological activity. For each soil, controls were prepared with both 2,4- and 2,6-DNT with no soil and an initial phase concentration of approximately 50 mg/L to assess other losses from the aqueous phase. Soil free controls demonstrated that other reactions and or losses within the bottles were insignificant. After 24 hrs, to ensure equilibrium (ASTM method D4646-87el, 1985 and Pennington et al 1990), each sample tube was centrifuged (1500g, 22°C for 20 minutes) and the supernatant was collected and analyzed for aqueous phase DNT concentration with HPLC. Sorbed phase concentrations were calculated by the difference between the initial and equilibrium concentrations.

Batch Desorption

Modified from Fu *et al.*, experimental analysis of soil associated DNT desorbing back into the aqueous phase was as follows (Fu, Kan et al. 1994). Because of very low or non-detectable soil-associated DNT in the soil samples taken from the field, an initial adsorption step was necessary for each soil (OP1, OP4 and SSB) which was completed with 50 mg/L DNT using the same solids to volume ratio (1:10) as described above in the sorption protocol. The desorption process was then initiated by separating the solids from the system (via centrifugation 1500g, 22°C for 20 minutes) and replacing the supernatant with DNT free D.I. water (sodium azide 1 g/L). Done in triplicate, the samples were continuously mixed end over end at approximately 1 rpm at room temperature (22°C) for 24 hours. Once equilibrium was reached, the aqueous phase was analyzed for DNT that desorbed from the solid. This process was designated as one

desorption step. Depending on the soil characteristics (% organic matter, cation exchange capacity, etc), solution parameters (pH, ionic strength, etc.) and the properties of the chemical of interest (partition coefficients, etc.) the number of desorption steps necessary can vary widely and in some cases adsorption becomes irreversible.

As soil SSB was the only soil from the site with detectable levels of associated DNT (30 mg 2,4-DNT/kg soil, determined through the solvent extraction procedure described above), it was of interest to determine the desorption rates of the DNT present at the site. As described above, in 50 mL PTFE centrifuge tubes in duplicate, 15g of SSB soil and 10 ml of DNT free D.I. water (sodium azide) were mixed end over end at approximately 1 rpm at room temperature (22°C) for 5 days (140 hours). The equilibrated system was then centrifuged (5000 rpm, 4°C for 5 minutes), the supernatant was filtered (0.22 μm PTFE) and analyzed via HPLC for desorbed aqueous DNT concentrations.

Soil Column Tests

Two glass columns (5 cm ID x 20 cm) with Teflon® endplates (Spectrum Chromatography, Houston, TX) were packed with soil OP1, one designated with a 2,4-DNT feed and other with 2,6-DNT influent feed. The columns were packed with 2 cm of 3 mm glass beads to prevent wash out of sand to prevent clogging of the effluent line, followed by 10 cm of OP1 soil sample (approximately 300 g) and 5 cm of 6 mm glass beads to uniformly distribute the influent (Figure 2.1). To each column, 10 ml of D.I. water (equilibrated to the atmosphere) with a DNT concentration of 10 mg/L was fed daily to simulate a low concentration (leaching) influent of DNT into a vadose zone. The hydraulic retention time of was approximately 8 days. Column effluent was collected daily and analyzed for DNT and nitrite.

Screening DNT Degrading Activity:

All soils were screened for the ability of indigenous microbial populations to degrade DNT. Batch tests were carried out in shake flasks with 5 g from each soil sample suspended in 50 ml mineral medium (Spanggord, Spain et al. 1991) spiked with either 2,4-DNT (200 mg/L) or 2,6-DNT (200 mg/L). Abiotic controls were maintained in parallel with 1 g/L sodium azide. Periodic samples from the flask were filtered (0.22 μm, PTFE) and analyzed for DNT, nitrite and pH. To maintain actively degrading DNT cultures, 1 ml of the culture from these shake flasks was transferred to the fresh media with DNT (200 mg/L) after 7 days. Based on widely cited reports by this lab and others, microbial DNT degradation can be demonstrated by the loss of DNT with a corresponding increase in nitrite, which then decreases the pH. (Spanggord, Spain et al. 1991; Lendenmann, Spain et al. 1998; Nishino, Spain et al. 1999; Smets, Riefler et al. 1999; Zhang, Nishino et al. 2000; Zhang, Daprato et al. 2001; Fortner, Zhang et al. 2003).

Sample Analysis

All aqueous samples taken in the study were filtered through a 0.22 μm filter unit (PTFE,

Millipore) prior to analysis. Separation and quantification of DNT was by HPLC (Agilent) equipped with a diode array detector. Two HPLC methods were used throughout the project. DNT was separated by either using a Zorbax® SB-C18 with a mobile phase of 68:32 (water (with 0.1% acetic acid):ACN) at a flow rate of 1 ml/min or a Hybercarb® porous graphite column (100 x 3 mm, 5µm, Thermo Hypersil, UK) with mobile phase of 96:4 (ACN : water (0.55% trifluoroacetic anhydride)) at a flow rate of 0.7 ml/min (Fortner, Zhang et al. 2003). For both methods, 2,4-DNT was quantified at 246 nm and 2,6-DNT at 230 nm. For each batch of samples analyzed a new calibration curve was performed using known 2,4-DNT and 2,6-DNT standards to assure the data quality and consistency. The detection limits for for DNT were approximately 0.2 mg/L using the first method discussed (Zorbax® SB-18 column) and 0.02 mg/L for the second (Hypercarb® graphite column). Nitrite was measured colorimetrically following Standard Methods for Wastewater Treatment method 4500-NO_2 (Greenberg, Clesceri et al. 1992). Nitrate was measured using ion chromatography (Dionex IC) following Standard Method for Wastewater Treatment method 4500-NO_3 (Greenberg, Clesceri et al. 1992). pH was measured with a Denver Instrument pH 220.

Results and Analysis

Batch Sorption Studies

All experimentally derived DNT sorption isotherms with BAAP soils appear to be Freundlich (Figure 2.3), where $C_s = K_d \cdot C_w^n$ and $n < 1$. For all K_d values calculated and used, only the linear range of the isotherms was examined. 2,4- and 2,6-DNT behaved similarly for each soil, giving comparable isotherms (shape and magnitude) and derived K_d values (Figure 2.3). For example 2,4- and 2,6-DNT K_d values for OP1 where 2.0 and 1.2 L/kg respectively (Table 2.5). The isotherm data of DNT adsorption to BAAP soils (Figure 2.3, Table 2.5) varies depending on the soil (Table 2.2). For example soil OP1 with a high sand, low clay and low OC content had relatively low K_d values (K_d =2.0 L/kg 2,4-DNT; 1.2 L/kg 2,6-DNT) when compared to SSB a low sand, high clay and moderate amount of OC content (K_d =263 L/kg 2,4-DNT; 337 L/kg 2,6-DNT). Soil OP4 which has a clay content and OC content between OP1 and SSB displayed DNT isotherms and K_d values between those observed for OP1 and SSB. The partitioning value K_d, is a relatively simple experimental value assigned to a single compound that sums all partitioning processes involved in a solid – water system; however, it is a complex issue in that there are many variables involved making it difficult to say exactly what percentage each variable plays in partitioning. Major variables to consider include mineral properties such as surface area and ion exchange capacity; organic carbon present; and the partitioning compound's properties such as K_{oc}, ionization constants, etc.

Batch Desorption Studies

2,4- and 2,6-DNT desorbed similarly within each given soil (Figure 2.3). For example, with BAAP soil SSB over the course of 15 desorption steps, 2,4- and 2,6-DNT desorbed quite similarly. Similar to adsorption, the desorption analysis of DNT from BAAP soils depended on what type of soil was being tested. Soil SSB sorbed much more DNT than

soil OP1 or OP4 (as discussed above) yet, the first few desorption analyses showed aqueous phase concentrations similar to those in OP1 and OP4. SSB however, continued to desorb DNT for many more steps than OP1 and OP4, which went to non detectable levels after a few steps. For reasons that probably control the K_d discussed above, desorption appears to be a factor of soil type and properties. SSB with a higher clay and OC content demonstrates a desorption process that slowly decreases DNT concentration in the aqueous phase over many steps (which can be thought of as pore volumes in a soil). OP1 and OP4 demonstrate relatively fast desorption and after a few steps show undetectable levels of DNT being desorbed indicating that OP1 and OP4 loosely bind DNT when compared to SSB.

Soil SSB was the only soil received with a detectable level of DNT; 30 mg/kg of 2,4-DNT was observed (ACN extraction discussed above). However, when SSB was analyzed for desorption of the 30 mg/kg DNT present (as received from the site), no DNT was observed to desorb into the aqueous phase after 5 days, indicating a tightly bound association with the soil. This observation, a lack of 2,4-DNT leaching from SSB, was expected based on experimentally derived K_d and desorption values.

Soil Column Experiments

Both columns were operated for over 3 months; 123 days (116 days of effluent collection) for the 2,4-DNT feed and 116 days (106 days of effluent collection) for the 2,6-feed (Figure 2.2). Each column had an ~8 day hydraulic retention time.

2,4-DNT Column

With an influent of 10 mL/day and an average concentration of 9.2 mg/L 2,4-DNT, the total volume of column influent in study was 1.23 liters of solution with a total mass applied of 10.7 mg 2,4-DNT. Over the course of the study, 2,4-DNT was never observed in the effluent solution (Figure 2.2). Nitrite was observed at elevated levels, indicative of biological DNT degradation, however at lower levels than expected. Nitrate was not observed in the effluent. Based on these observations, a simplified, steady state first order rate constant describing apparent 2,4-DNT loss was calculated to be $k_{apparent} \geq 0.57$ d^{-1}. Because the effluent concentration is below our detection limit, we assume an effluent value of 0.1 (half of the detection of 0.2 mg/L), thus the rate must be greater than or equal to the calculated value, but not less than.

2,6-DNT Column

With an influent of 10 mL/day and an average concentration of 9.5 mg/L 2,6-DNT, the total volume of column influent in study was 1.06 liters of solution with a total applied mass of 11.0 mg 2,6-DNT. On day 22, 2,6-DNT was observed in the effluent and reached an equilibrium around day 40 (Figure 2.2). From day 40 to day 106 the average 2,6-DNT concentration was approximately 3.6 ± 0.4 mg/L. During the same period, an unknown metabolite appeared and reached steady state in the effluent. The metabolite was later identified as 2-amino-6-nitrotoluene. Over the course of the study, neither

nitrite nor nitrate was ever observed in effluent analysis. Based on observations at steady state, a simplified first order rate constant describing apparent 2,6-DNT loss in the column was calculated to be $k_{apparent} \approx 0.12\ d^{-1}$.

Microbial DNT Degrading Screening

For all BAAP soils (OP1, OP4 and SSB) indigenous microbes were able to metabolize 2,4-DNT as demonstrated by loss of 2,4-DNT and the corresponding increase in nitrite. For all soils, this activity was transferable. Figure 2.4 shows 2,4-DNT degrading activity of indigenous BAAP cultures after being transferred to fresh media and 200 mg/L 2,4-DNT. In contrast, we did not find any BAAP soils (OP1, OP4 or SSB) to be source of indigenous microbes capable to metabolizing 2,6-DNT. These findings, while not as extensive, are consistent with the results from section 1.

Conclusions

The microcosm and MPN studies both demonstrated the presence of bacteria at the Settling Pond and Spoils Disposal areas that are capable of degradation of 2,4-DNT. Isolation of individual strains capable of growth on 2,4-DNT is conclusive evidence of the presence of the appropriate biology at all three sites for natural attenuation to occur. Furthermore, the presence of substantial populations of DNT-degrading bacteria is strong evidence that they are degrading DNT at the site. We have never detected DNT-degrading bacteria in uncontaminated sites or in sites where DNT degradation is not taking place. There are differences among the sites with regard to the number of organisms present and the rate at which they are capable of 2,4-DNT degradation. The number and variety of isolates from each site was consistent with the rate and extent of 2,4-DNT degradation in the various microcosms. The differences between microcosms constructed with minimal medium and microcosms constructed with ground water suggest that some essential nutrients might be limiting. The tendency of the DNT to sorb to the soil and be unavailable to the bacteria present should be tested, but our results indicate that the sorbed DNT can be biodegraded until a residual concentration is reached that varies depending upon the soil type. The residual material is not likely to be mobile and should be biodegraded if it is released from the soil. It should be repeated here that the results presented here are based on the degradation of 2,4-DNT added to the soil and biodegraded by the indigenous bacteria.

All Settling Pond and Spoils Disposal soils examined (OP1, OP4 and SSB) had the propensity to sorb DNT, however, depending on the soil type and properties, the soils did so at different capacities. Soil SSB had the greatest capacity which was evident in the relatively large K_d values that where experimentally determined (K_d = 2,4-DNT 263 L/kg; 2,6-DNT 337 L/kg). In contrast, soils OP1 and OP4 were observed to have lower K_d values, thus less soil associated DNT at equilibrium. The differences in soil K_d values is reflective of site heterogeneity with regard to soil types and profiles. Desorption data shows similar trends with higher clay and organic content soils, SSB in particular, having desorbing more DNT over time than OP1 and OP4. These observations were strengthened by additional desorption studies examining the background 2,4-DNT levels

associated with SSB upon arrival from BAAP. Despite having an appreciable 2,4-DNT concentration associated with the soil SSB (30 mg/kg 2,4-DNT), after five days of suspended conditions in water, aqueous phase concentrations remained below detection limits.

Effluent data from the OP1 soil column study suggests that 2,4-DNT was being lost to processes within the column as compared 2,6-DNT which reached an equilibrium effluent concentration of 3.6 mg/L around 40 days of operation. Throughout 123 days of column operation, 2,4-DNT was never observed in the effluent. However, elevated levels of nitrite were observed as compared to 2,6-DNT column which showed none. Positive identification of multiple 2,4-DNT degrading cultures in all BAAP soils as we discuss briefly (objective 2) and Nishino and Spain discuss extensively (objective 1) indicate that 2,4-DNT degrading bacteria are ubiquitous to the site. These findings, coupled with the low K_d value of OP1 strongly suggest that biological degradation is responsible for some if not all 2,4-DNT loss in the 2,4-DNT soil column study.

Because 2,6-DNT is not a contaminant of concern in the Settling Ponds and Spoils Disposal Areas, no effort was made to isolate or identify 2,6-DNT-degrading bacteria in soils from the sites. In our experience and to our knowledge, no 2,6-DNT-degrading bacteria have ever been isolated from soils that did not have significant (mg kg^{-1}) 2,6-DNT contamination, and none were anticipated to be found in this study based upon the low reported levels of 2,6-DNT contamination. The 2,6-DNT column study confirmed our conjecture; 2,6-DNT was not mineralized (no nitrite release) but was transformed to 2-amino-6-nitrotoluene. The reduction of a single nitro group is commonly observed when bacteria are present that cannot grow on 2,6-DNT (Nishino, unpublished results). Given the low reported 2,6-DNT concentrations in the soil at the Settling Ponds and Spoils Disposal Areas, we would not anticipate the development of a microbial community at the sites that can degrade (grow on) 2,6-DNT. A compound can only be a growth substrate if there is enough present for bacteria to realize a net benefit after expending the energy necessary to process the compound. Clearly, however, microorganisms are present at the sites with the capacity to incidentally transform or cometabolize 2,6-DNT. With time, complete disappearance of 2,6-DNT may come about via transformation into 2-amino-6-nitrotoluene and/or through washout of the parent or daughter compounds.

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Table 1.1. Initial soil parameters.

Core (Top, Middle, Bottom)	OP1-48 T	OP1-48 M	OP1-48 B	OP4-07 T	OP4-07 M	OP4-07 B	SSB-0317 T	SSB-0317 M	SSB-0317 B
pH	4.41	4.69	5.11	7.59	7.72	7.34	8.29	7.63	6.78
% soil moisture	15.92	12.73	8.99	18.75	19.31	22.25	24.66	24.56	8.06
% organic	3.10	2.49	1.11	3.39	3.42	4.77	13.80	10.60	1.81

Table 1.2. Partitioning of 2,4-DNT between aqueous and solid phases in representative microcosm slurries. Water and soil columns indicate the proportion of 2,4-DNT measured in each phase, μM columns indicate the total concentration of 2,4-DNT in the microcosm.

Date	OP1-48 BLKN #1			SSB 03-17 BLKN #1			SSB 03-17 KC #1		
	water	soil	μM	water	soil	μM	water	soil	μM
08/18[a]	-	-	nd*	0	1.00	14.9	0	1.00	88.2
08/18[b]	.14	.86	105.2	.28	.72	105.8	.35	.65	91.5
08/19	.02	.98	88.6	.21	.79	98.1	.32	.38	87.9
08/22	.11	.89	80.2	.28	.72	108.9	.26	.74	97.2
08/28	.10	.90	61.1	.28	.72	103.2	.31	.69	99.8
09/04	0	1.00	50.3	.27	.73	87.0	.25	.75	94.8
09/11	.03	.97	27.1	.26	.74	38.5	.27	.73	82.1

a. Day 46 immediately before spike with DNT (OP1-48) or subculture (SSB 03-17 microcosms) to fresh microcosms

b. Day 46 immediately after spike/subculture

* nd = not detected

Table 1.3. Number of 2,4-DNT-degrading bacteria per g-soil, results are for replicate plates.

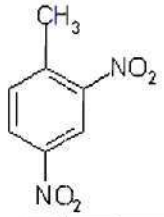
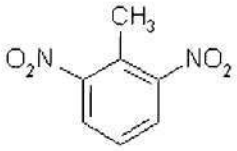
OP1-48 Top	OP1-48 Middle	OP1-48 Bottom	OP4-07 Top	OP4-07 Middle	OP4-07 Bottom	SSB-0317 Top	SSB-0317 Middle	SSB-0317 Bottom
< dl	< dl	1.68E+5	3.51E+5	3.54E+5	7.72E+4	7.31E+9	7.30E+8	8.56E+6
< dl	7.64E+4	1.15E+5	3.51E+5	1.32E+5	4.70E+5	7.31E+9	4.87E+8	2.76E+6

< dl = below detection limit of 3.5E+04

Table 1.4. Identification of 2,4-DNT-degrading bacteria based on partial 16S rDNA analysis.

Source	Isolate	Closest Match	% Difference	Confidence Level
OP1-48 middle	181	*Pseudomonas* sp.	0.57	Species
OP1-48 middle	182	*Pseudomonas* sp.	0.57	Species
OP1-48 bottom	183	*Pseudomonas* sp.	0.57	Species
OP4-07 top	184	*Variovorax paradoxus*	0.57	Species
OP4-07 middle	185	*Rhodococcus/Tsukamurella* sp.	0.10	Species
OP4-07 bottom	186	*Rhodococcus/Tsukamurella* sp.	0.10	Species
OP4-07 bottom	187	*Achromobacter piechaudii*	0.10	Species
SSB 03-17 top	188	*Sphingobacterium faecium*	0.85	Species
SSB 03-17 top	189	*Acidovorax temperans*	1.54	Genus
SSB 03-17 top	190	*Xanthomonas* sp.	0.19	Species
SSB 03-17 top	192	*Pseudomonas* sp.	0.57	Species
SSB 03-17 bottom	194	*Agrobacterium rubi*	2.13	Genus

Table 2.1: Chemical Properties of 2,4-DNT and 2,6-DNT

Properties	2,4-DNT	2,6-DNT
Chemical Formula	$C_7H_6N_2O_4$	$C_7H_6N_2O_4$
Chemical Structure		
Molecular Weight (g/mole)	182.1	182.1
Aqueous Solubility (mg/L)	280 (25°C) [a] 270 (22°C) [b]	208 (25°C) [a]
Log K_{ow}	1.98 [a, b]	2.02 [a] 1.89 [a, b]
Log K_{oc}	1.98 [a, b]	1.89 [a, b]

[a] Rosenblatt, et al. (1991), [b] Verschueren, K. (2001), Handbook of Environmental Data on organic Chemicals, John Wiley and Sons, Inc., New York.

Table 2.2: Soil Physical and Chemical Properties

Physical parameters	OP1	OP4	SSB
pH[a]	5.7	6.2	6.3
Moisture, %	1.35	3.45	5.25
EC (meq/100 g)[b]			
Na	0.02	0.02	0.04
Mg	1.12	1.71	2.65
K	0.12	0.19	0.23
Ca	3.6	14.99	36.35
CEC (meq/100 g)[c]	7.28	19.46	26.09
Texture Distribution, %[d]			
Clay	8.36	17.16	13.52
Silt	10.43	74.64	49.96
Sand	81.21	8.2	36.52
Soil Type	Loamy Sand	Silt Loam	Loam
Plant Available Nutrients (ppm)			
TOC %[e]	0.179	1.982	3.346
Ammonia N[f]	5	14	15
Nitrate N[g]	24.68	2.26	12.44
Nitrite N[h]	0.12	0.49	1.11
Phosphate[i]	140	191	197
Mn[j]	502	286.3	696.8
Fe[j]	9980.7	13405.9	6621.5
Cu[j]	21.3	15.7	59.7
Zn[j]	119.9	65.8	86.3
Mo[j]	0.18	0.19	0.62

Methods used for the analysis of soil samples:

[a] pH using combined calomel glass electrode. [b]EC was determined using ammonium acetate method, Methods of Soil Analysis Part II- Chemical and Microbiological Properties, Second edition, Agronomy #9, ASA. Page 160. [c] CEC was done by displacing the cations on the soil with 1 M K acetate solution. The K is then displaced with 1 M ammonium acetate. K ions were determined by ICP/MS. [d] Texture was determined using hydrometer procedure, Black, C.A. (eds.), Methods of Soil Analysis. Part 1. Am.Soc.Agron., Madison, WI, 1965. [e] TOC was determined by ignition in a LECO CNS 2000 analyzer (LECO Corporation, St. Joseph, MI 49085-2396. Prior to ignition, the samples were treated with 10% HCl to decompose inorganic carbon and dried at 105°C. [f] Ammonia N colorimetrically. [g] Nitrate was done by ion chromatography. [h] Nitrite N was done by flow injection analysis using OI Analytical analyzer. [i]Phosphate was done colorimetrically with the molybdate blue reaction. [j] Elements analysis: Soils were digested using EPA Method 3051: "Microwave assisted acid digestion of sediments, sludges, soils, and oils" The digestate was analyzed for the required elements using EPA Method 200.8". Determination of trace elements in waters and wastes by inductively coupled plasma - mass spectrometry".

Table 2.3: Extractable DNT associated with BAAP soils

Soil Sample	2,4-DNT (mg/kg)	2,6-DNT (mg/kg)
OP1 (0-3 ft)	0	0
OP4 (0-3 ft)	0	0
SSB (3-6 ft)	30	0

Table 2.4: Experimental K_d values of 2,4-DNT and 2,6-DNT for all three BAAP soils

Soil Sample	K_d (L/kg)	
	2,4-DNT	2,6-DNT
OP1 (0-3 ft)	2.002	1.214
OP4 (0-3 ft)	10.23	3.85
SSB (3-6 ft)	263.56	337.25

Test conditions: soil:water = 1:10 (w/v), temperature 22°C, 1g/L NaN_3

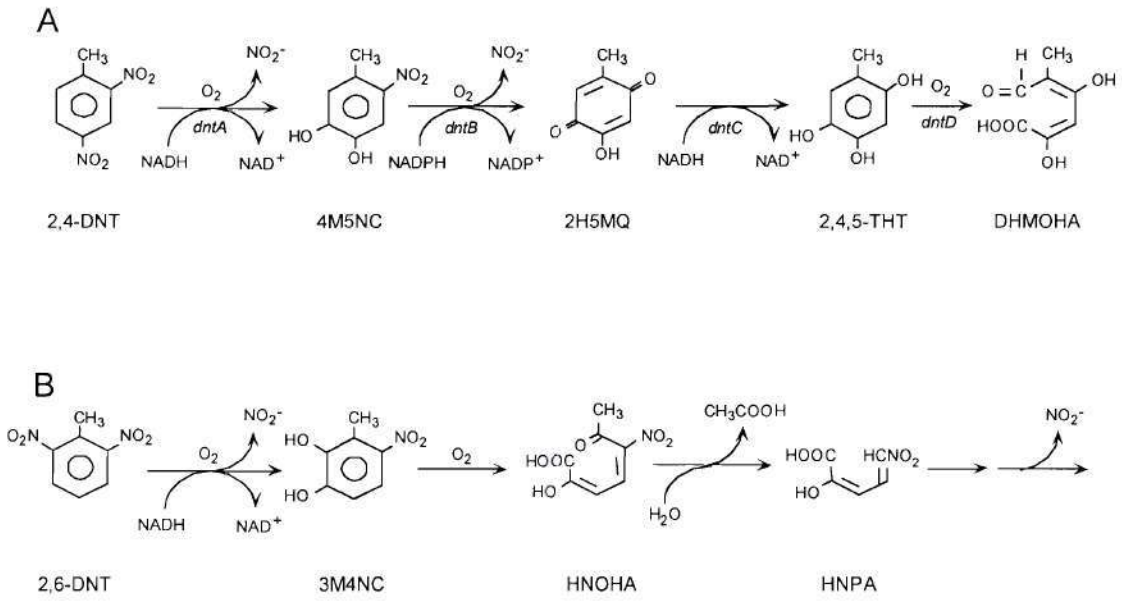


Fig 1.1. Initial steps in the pathways for biodegradation of A) 2,4-DNT, and B) 2,6-DNT (Nishino, Paoli et al. 2000). Both pathways feed into central metabolic pathways and result in mineralization of the compounds. During growth on DNT (either isomer), 40-50% of the carbon is released as CO_2, and 60-80% of the nitrogen is released as NO_2^-. If ammonia is provided as the nitrogen source, all the nitrogen is released from the DNT molecule as NO_2^-. Abbreviations are: 2,4-dinitrotoluene (2,4-DNT); 4-methyl-5-nitrocatechol (4M5NC); 2-hydroxy-5-methylquinone (2H5MQ); 2,4,5-trihydroxytoluene (2,4,5-THT); 2,4-dihydroxy-5-methyl-6-oxohexa-2,4-dienoic acid (DHMOHA); 2,6-dinitrotoluene (2,6-DNT); 3-methyl-4-nitrocatechol (3M4NC); 2-hydroxy-5-nitro-6-oxohepta-2,4-dienoic acid (HNOHA); and 2-hydroxy-5-nitropenta-2,4-dienoic acid (HNPA).

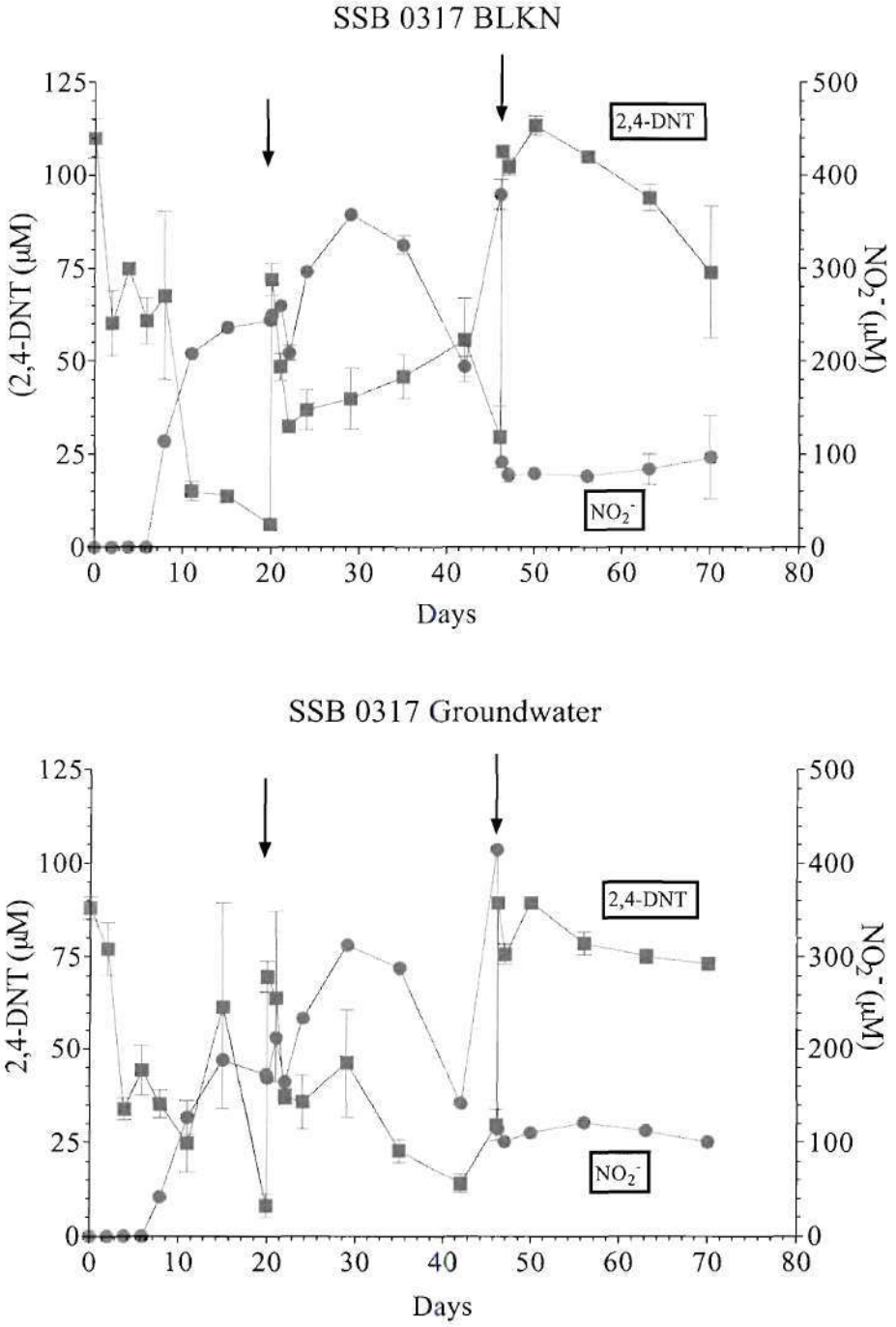


Figure 1.2. Disappearance of 2,4-DNT and release of nitrite in microcosms constructed with soil from SSB 03-17. 2,4-DNT concentration plotted is combined aqueous and sorbed concentrations. Arrows at 20 days indicate respike with 2,4-DNT, arrows at 46 days indicate 10% transfer to fresh media with 2,4-DNT.

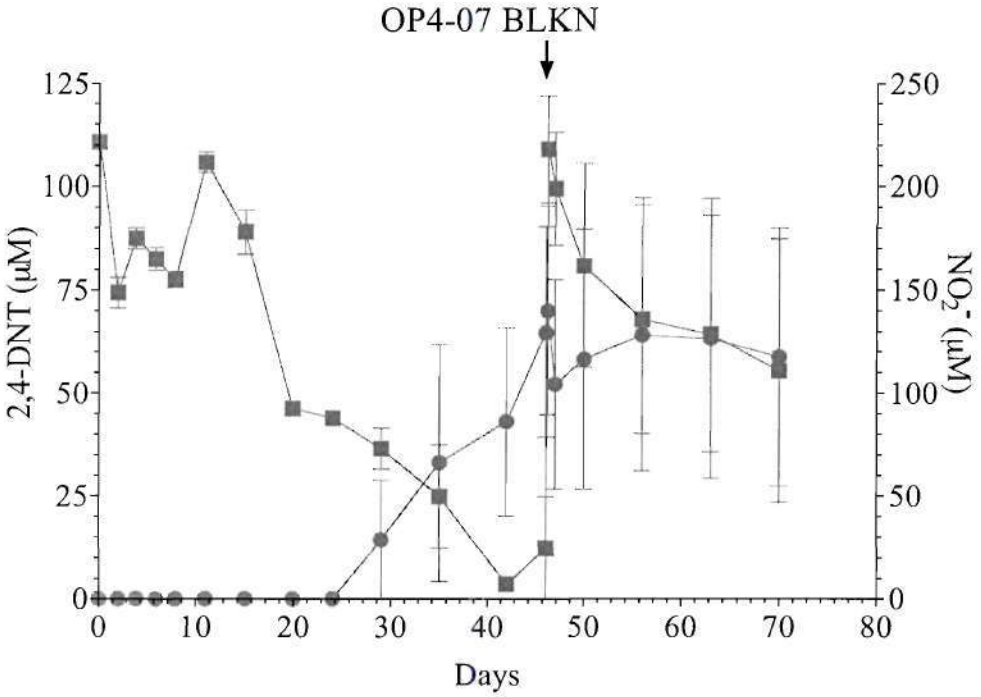


Figure 1.3. Disappearance of 2,4-DNT and release of nitrite in microcosms constructed with soil from OP4-07. 2,4-DNT concentration plotted is combined aqueous and sorbed concentrations. Arrow at 46 days indicate respike with 2,4-DNT.

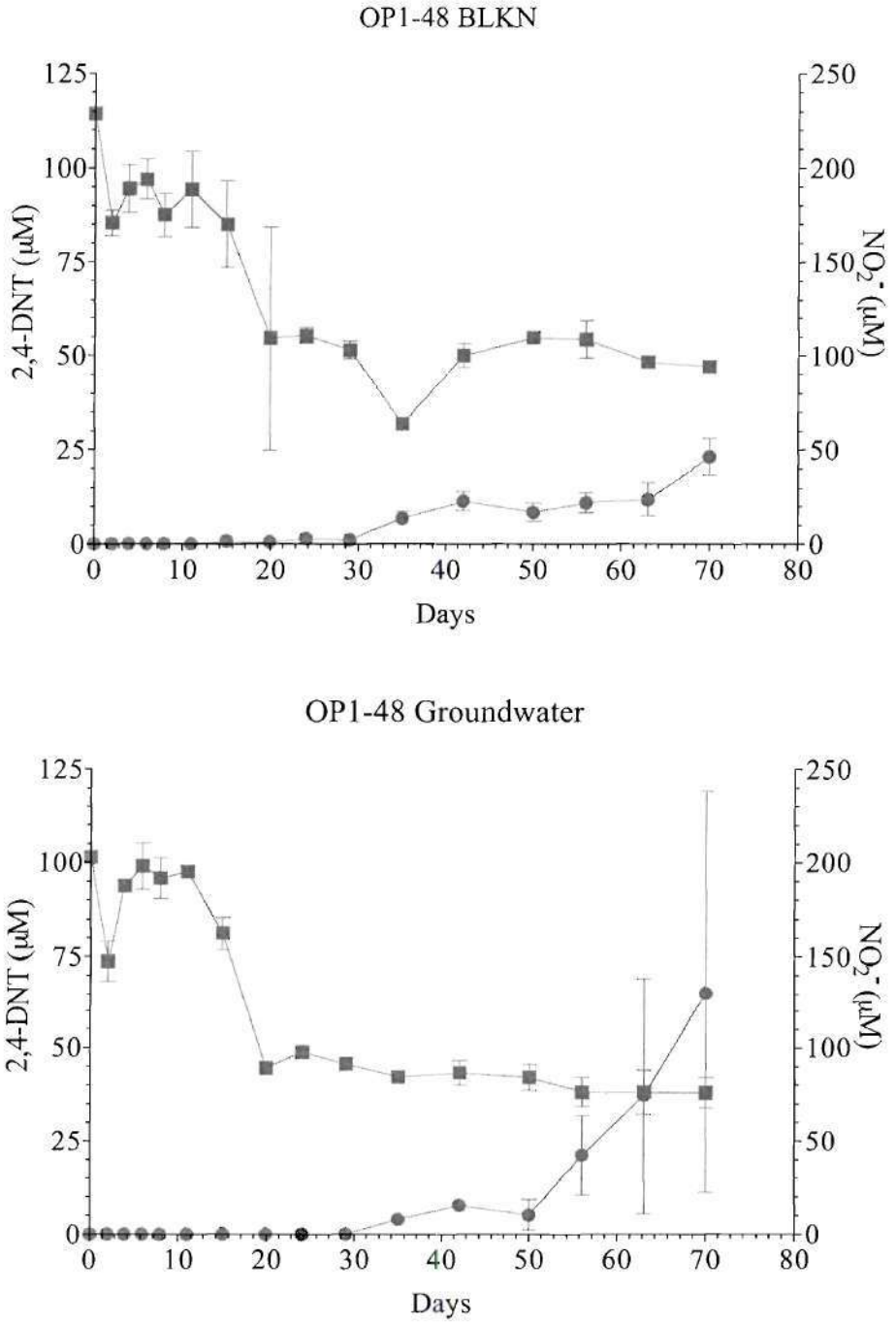


Figure 1.3. Disappearance of 2,4-DNT and release of nitrite in microcosms constructed with soil from OP1-48. 2,4-DNT concentration plotted is combined aqueous and sorbed concentrations.

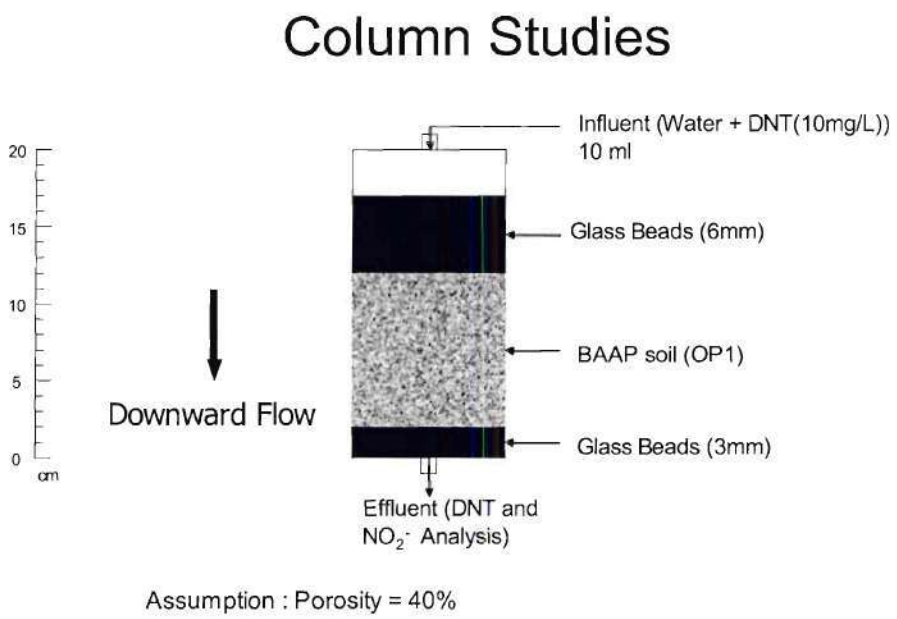


Figure 2.1. Soil Column Schematic

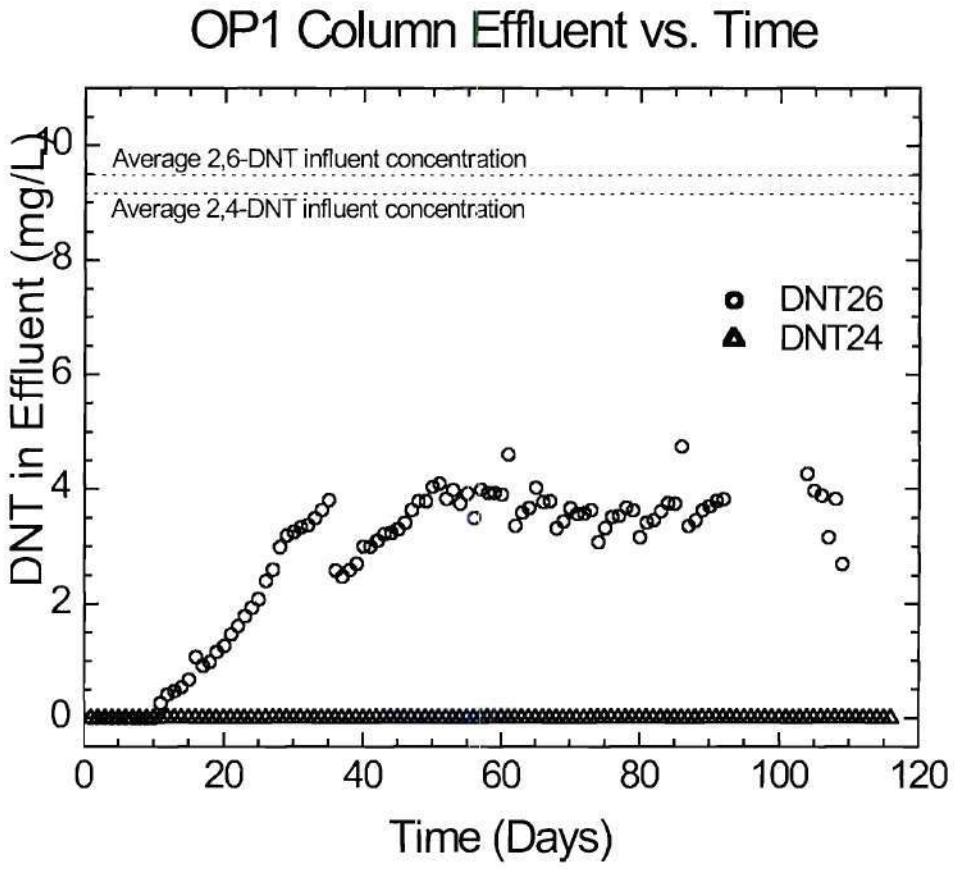


Figure 2.2: Soil OP1 Column effluent DNT concentration vs. time. Green circles represent 2,6-DNT concentrations; blue triangles represent 2,4-DNT concentrations.

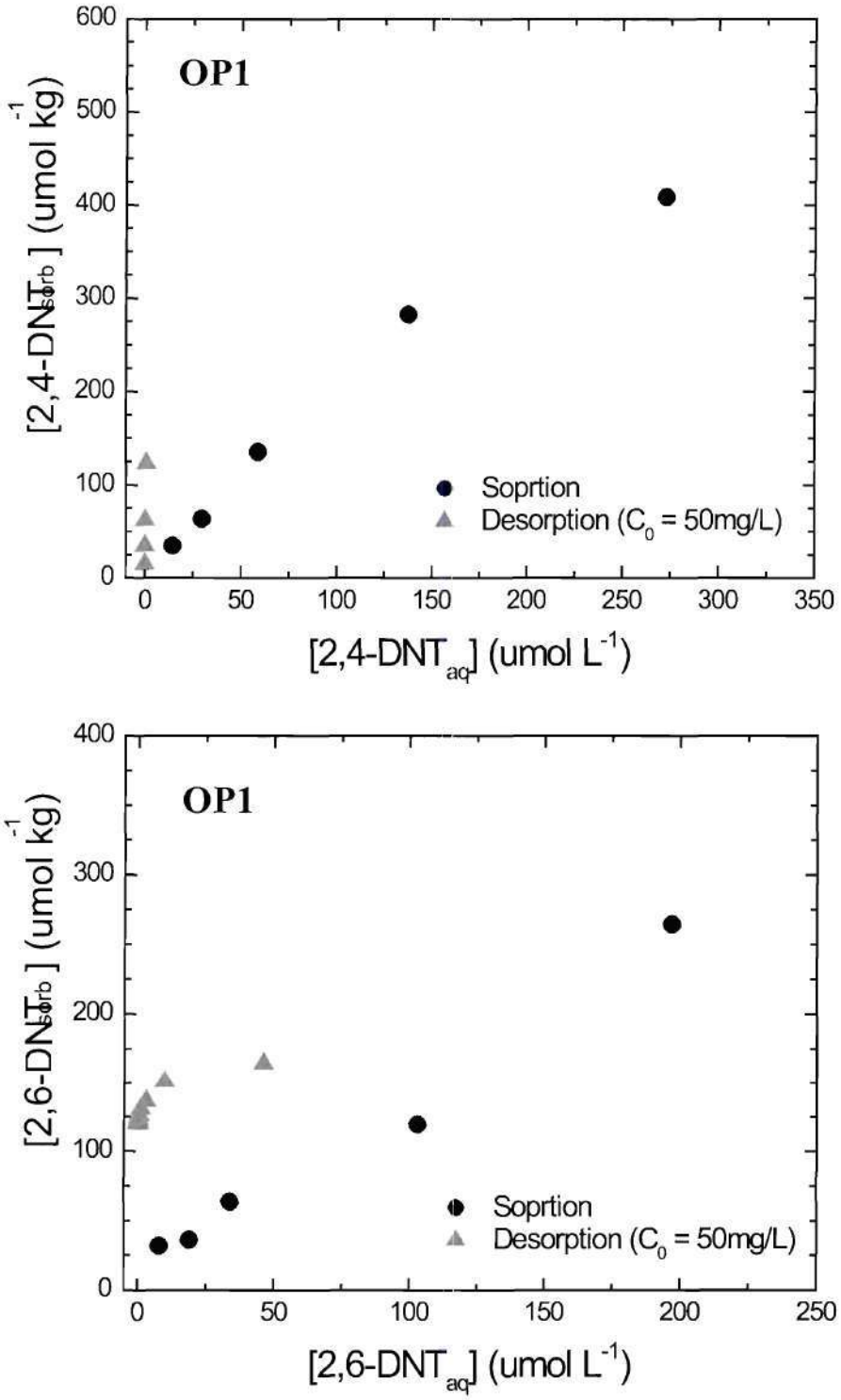


Figure 2.3.1: Sorption and desorption isotherms of BAAP soils OP1. Blue circles represent DNT adsorption data points for 2.5, 5, 10, 25, and 50 mg/L initial aqueous DNT concentration. Orange triangles represent desorption data with an initial aqueous concentration of 50 mg DNT/L.

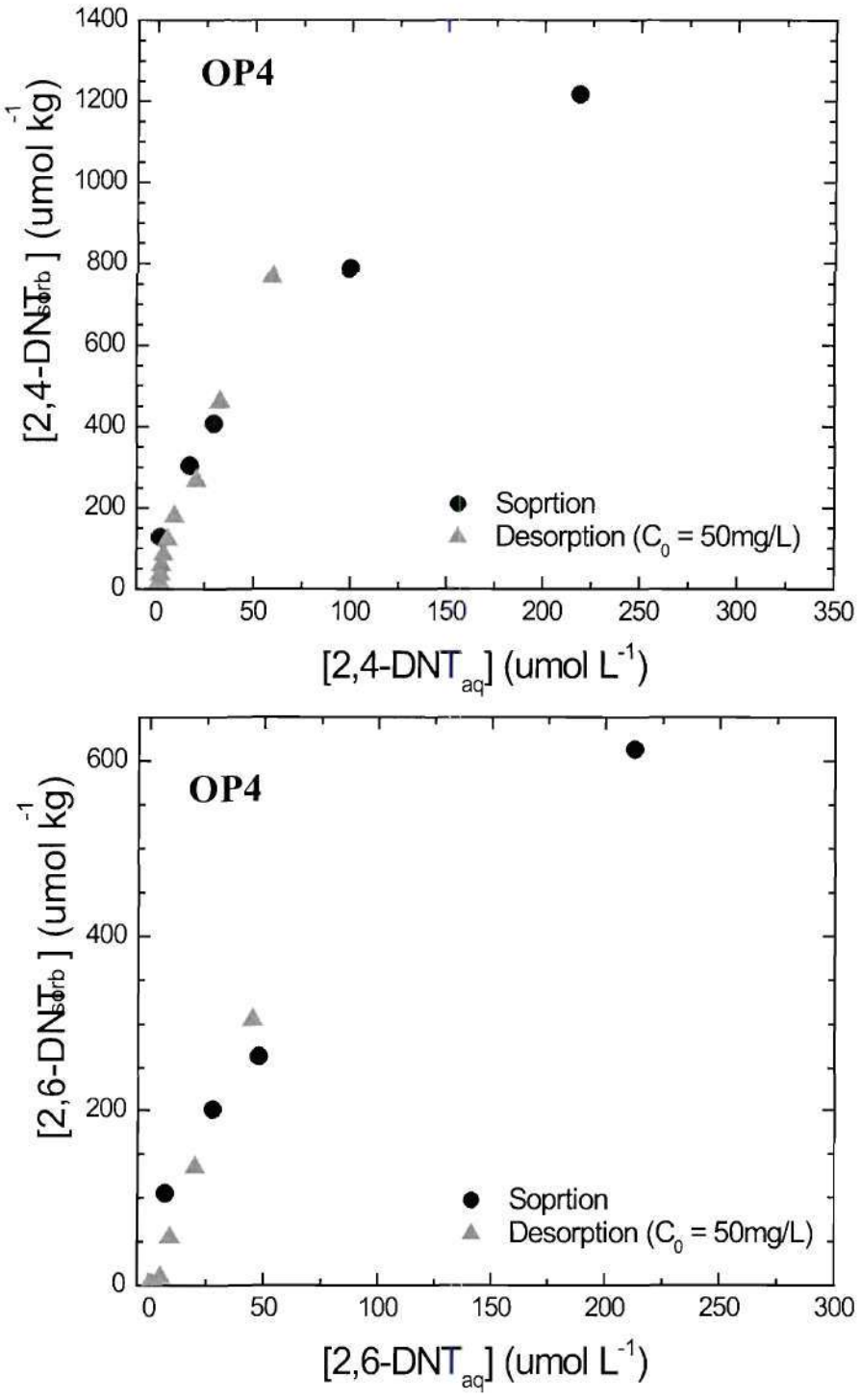


Figure 2.3.2: Sorption and Desorption Isotherms of BAAP soils OP4. Blue circles represent DNT adsorption data points for 2.5, 5, 10, 25, and 50 mg/L initial aqueous DNT concentration. Orange triangles represent desorption data with an initial aqueous concentration of 50 mg DNT/L.

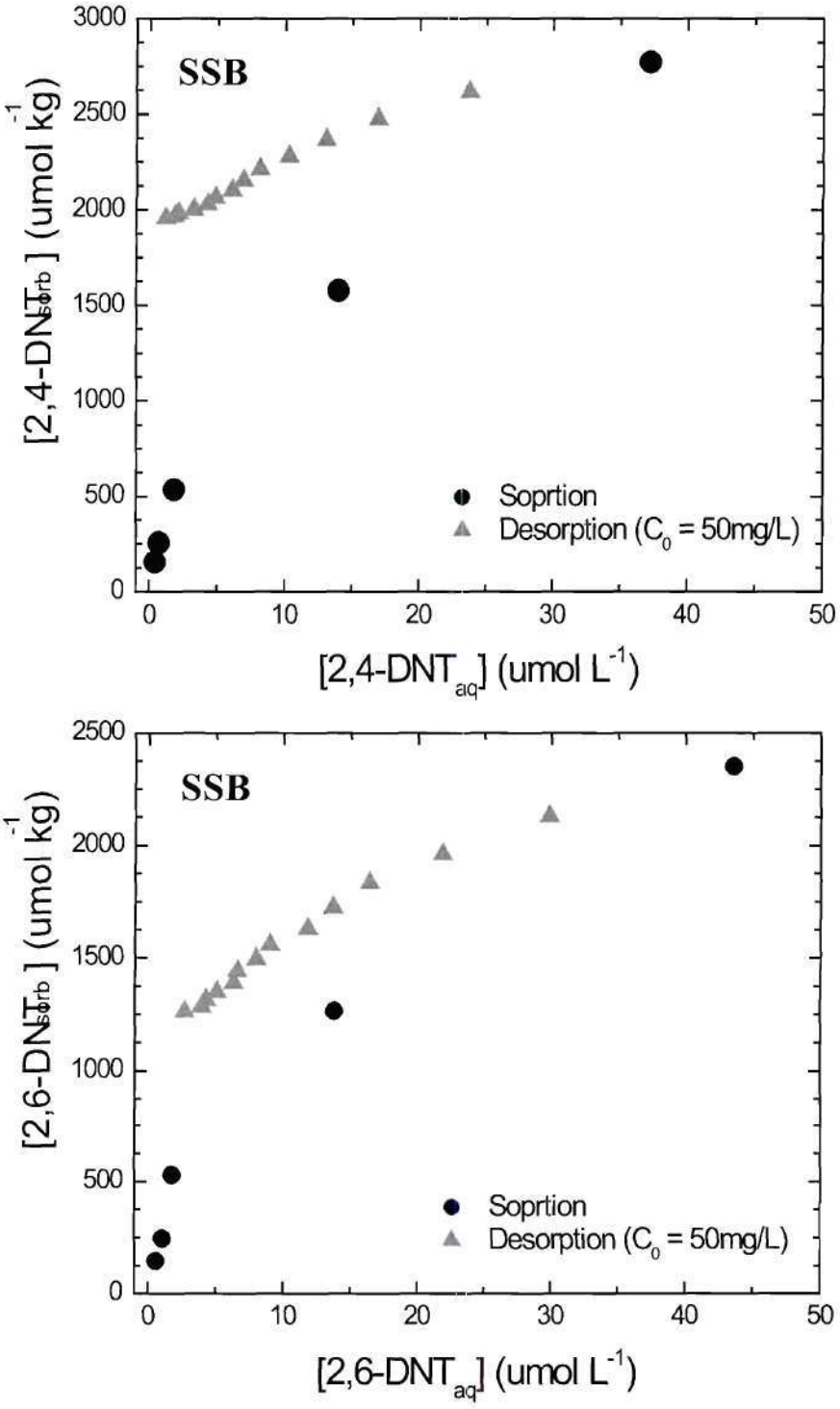


Figure 2.3.3: Sorption and desorption Isotherms of BAAP soils SSB. Blue circles represent DNT adsorption data points for 2.5, 5, 10, 25, and 50 mg/L initial aqueous DNT concentration. Orange triangles represent desorption data with an initial aqueous concentration of 50 mg DNT/L.

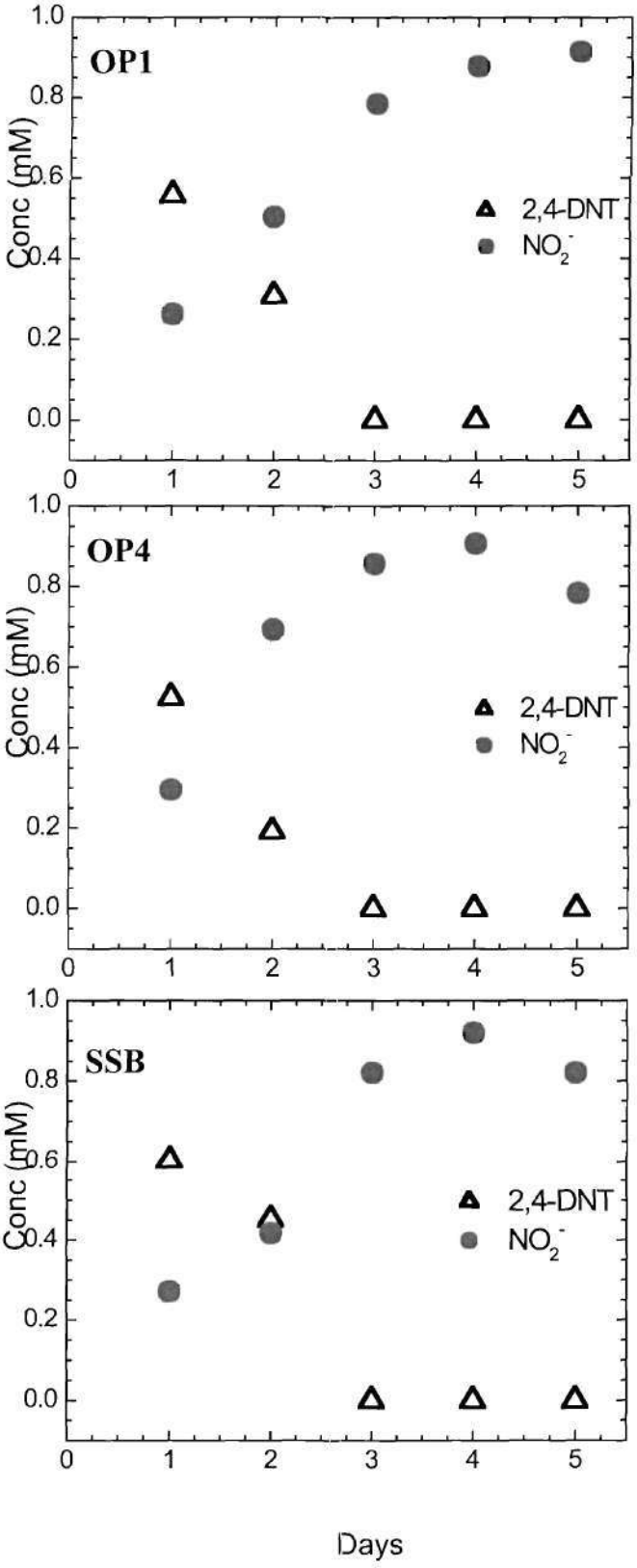


Figure 2.4. Microbial degradation of 2,4-DNT with corresponding production of NO_2^-