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Fate of 2,4,6-Trinitrotoluene and Its Metabolites in Natural and Model Soil Systems

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The sorption−desorption characteristics of 2,4,6-trinitrotoluene (TNT), 4-amino-2,6-dinitrotoluene (4-ADNT), and 2,4-diamino-6-nitrotoluene (2,4-DANT) within a natural topsoil, an illite shale, and a sandy aquifer material (Borden sand) were studied. The sorption capacity constant (K_d^s) of the three nitroaromatic compounds (NACs) increased with the number of amino groups (i.e., 2,4-DANT > 4-ADNT > TNT) for topsoil, and there was significant sorption−desorption hysteresis. Traces of 4-*N*-acetylamino-2-amino-6-nitrotoluene (4-*N*-AcANT) formed during sorption of 2,4-DANT by nonsterile topsoil (22 h), but this did not account for the hysteresis. For longer contact times (66 h), 4-*N*-AcANT accounted for 26% of the biotic disappearance of 2,4-DANT, and traces of 2-*N*-acetylamino-2-amino-6-nitrotoluene (2-*N*-AcANT) were detected. For illite, the K_d^s increased with the number of nitro groups (i.e., TNT > 4-ADNT > 2,4-DANT), and there was also sorption−desorption hysteresis. Most of the 2,4-DANT was neither desorbed nor extractable by acetonitrile from illite or topsoil. Sorption of the NACs by Borden sand was slight or nonexistent. This study illustrates that soil and NAC type will have a significant effect on the K_d^s as well as the formation of acetylated metabolites.

Introduction

Soils contaminated with 2,4,6-trinitrotoluene (TNT) from activities in the munitions and defense industries is a worldwide environmental problem. In natural and engineered systems it has been demonstrated that, although TNT may undergo biotransformations, it is not completely mineralized (*1, 2*). In particular, the nitro groups of TNT undergo reduction reactions to form amino derivatives that include 4-amino-2,6-dinitrotoluene (4-ADNT), 2-amino-4,6-dinitrotoluene (2-ADNT), 2,4-diamino-6-nitrotoluene (2,4-DANT), and 2,6-diamino-4-nitrotoluene (2,6-DANT) (*3, 4*). TNT and its amino derivatives are energetic nitroaromatic compounds (NACs). Such NACs are toxic, mutagenic, and persistent in the environment (*5*−*7*). The absence of TNT mineralization in natural and engineered systems has been attributed to the possibility that 2,4,6-triaminotoluene (an amino derivative formed anaerobically) is a dead-end product that misroutes TNT from mineralization (*8*) and also to the irreversible sorption of TNT and its metabolites by soil (*3*). The kinetics, extent, and reversibility of the sorption of TNT and its metabolites will have an effect on their availability for subsequent biodegradation as well as their transport properties. Many studies have examined the sorption−desorption behavior of TNT and its metabolites in soil (*3, 7, 9*−*15*). In these cases, sorption by nonsterile soil was studied in batch reactors, and sorption was estimated by difference. However, under nonsterile conditions, many microorganisms are able to catalyze the nonspecific reduction of the nitro groups of TNT to form amino derivatives (*16*), as such, sorption may be overestimated. To ensure that contaminant losses from the aqueous phase in a soil slurry are due to abiotic processes such as sorption, soil can be sterilized to eliminate microbial activity (*17*−*19*).

Sorption reversibility can be estimated by conducting desorption studies. Desorption requires a longer period of time, and this will have a detrimental effect on the efficiency of various remediation options (*20*). To accurately predict sorption reversibility, Huang et al. (*20*) proposed a 'minimal artifact' experimental protocol to study equilibrium sorption−desorption processes whereby all of the sorption points are implemented as the initial condition for desorption. Sorption−desorption hysteresis is then evaluated by comparing the sorption capacity constants for sorption, K_d^s, and desorption, K_d^d.

The objective of the present study was to examine the sorption−desorption behavior of TNT, 4-ADNT, and 2,4-DANT using natural and model sterile and nonsterile soils using the methods of Huang et al. (*20*). The NAC desorption behavior and the efficiencies of acetonitrile extraction were used to assess the mechanisms by which the NACs may bind with various soil constituents. In addition, highly sophisticated analytical tools (high-performance liquid chromatography coupled with mass spectroscopy) were used to identify transformation products that may form in nonsterile soil.

Experimental Section

Chemicals. TNT (>99% purity) was obtained from Defence Research Establishment Valcartier (Valcartier, PQ, Canada), 4-ADNT (>99% purity) was supplied by Omega Inc. (Lévis, PQ, Canada), and 2,4-DANT (>99% purity) was supplied by AccuStandard Inc. (New Haven, CT).

Soils. An agricultural topsoil was obtained from Varennes, PQ, Canada. A sandy aquifer material was obtained from Canadian Forces Base Borden, Ontario (*21*). The illite, a green shale from Rochester, NY, was purchased from Ward's Earth Science (St-Catharines, ON, Canada). Properties of the three geological materials are summarized in Table 1. Illite was saturated with K^+ by washing with 0.2 N KCl at pH 2 four times, followed by four washings with 0.2 N KCl at pH 7 (*13*). The pH of the clay suspension was adjusted to 5.5 with 1 N KOH, and excess electrolyte was removed by one wash with distilled water.

Qualitative mineralogical analysis of topsoil and Borden sand was determined by X-ray diffraction (Geochemical Laboratories, McGill University, Montreal, PQ, Canada) and was provided by the supplier for the illite (Table 1).

Soil Sterilization. The topsoil and K^+-illite were sterilized by γ-irradiation from a cobalt-60 source at the Canadian Irradiation Centre (Laval, PQ, Canada) with minimum and maximum doses of 35.4 and 40.4 kGy, respectively. γ-Irradiation was examined for sterilization since it has minimal

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TABLE 1. Properties of Three Geological Materials Used in Sorption−Desorption Experiments

	particle size distribution					
soil/clay	% clay (<2 μm)	% silt (2−53 μm)	% sand (>53 μm)	% organic matter	pH	CEC[a] (mequiv/100 g)
topsoil	4	12	83	8.4	5.6	14.6
Borden sand	2	2	96	0.02	8.4	0
illite	100	0	0	0	6.0	9.0

soil/clay	major elements	minor elements	trace elements (<0.05%)
topsoil	quartz (SiO_2)	albite-ordered ($Na{\cdot}Al{\cdot}Si_3O_8$)	crossite ($Na_2(Fe,Mg)_5(Si,Al)_8O_{22}(OH)_2$)
Borden sand	quartz (SiO_2) albite-calcian, disordered $(Na,Ca)(Si,Al)_4O_8$		crossite ($Na_2(Fe,Mg)_5(Si,Al)_8O_{22}(OH)_2$)
illite	illite $(K,H_3O)(Al,Mg,Fe)_2(Si,Al)_4O_{10}[(OH)_2,H_2O]$		

[a] CEC, cation exchange capacity.

impact on the sorption of other contaminants by soil (*17*). Irradiated soil was combined with 0.1% sodium pyrophosphate, and three dilutions were spread plated onto tryptone-yeast extract agar (*22*). The absence of colony forming units (CFUs) after 20 days incubation at 35 °C is indicative that γ-irradiation was effective.

Sorption−Desorption Kinetics. Sorption−desorption kinetics (0.5−44 h) were studied using the methods described subsequently for sorption−desorption.

Sorption Experiments (Nonsterile Soil). Sorption was conducted in batch reactors at 25 °C. Aqueous NAC solutions were prepared from stock solutions in acetonitrile. The initial TNT concentrations were 5, 10, 15, 30, and 50 mg/L. Lower initial 4-ADNT and 2,4-DANT concentrations were employed to reflect levels found in contaminated soil (*10*, *12*) (0.5, 1.0, 1.5, 3.0, and 5.0 mg/L). In 16-mL borosilicate centrifuge tubes, with Teflon-coated screw caps, 15 mL of the aqueous NAC solutions were combined with 2 g of topsoil, 2 g of Borden sand, and 1 g of illite. A lower mass of illite was used due to extensive sorption. For topsoil and Borden sand, the background solution was distilled water, and for illite it was 0.1 N KCl.

Centrifuge tubes were wrapped in aluminum foil and agitated on a Wrist Action shaker (Burrell Corp., Pittsburgh, PA) for 22 h. The tubes were centrifuged for 30 min at 3500 rpm, and the supernatant was filtered using a Millex-HV 0.45 μm filter unit (Millipore Corp., Bedford, MA). Sorption of the NACs by the filter unit was negligible, as reported by Jenkins et al. (*23*) for TNT and this particular filter unit. The mass of NAC sorbed by soil was calculated by difference. All experiments were conducted in triplicate.

Sorption (Sterile and Nonsterile Soils). To verify that the results for 22 h sorption were not coupled with NAC biotic losses, sorption was studied with nonsterile and sterile soils. Soil-to-solution ratios of 1:7.5 and 1:15 were implemented for topsoil and illite, respectively. The initial NACs were 7 mg/L TNT, 2 mg/L 4-ADNT, and 2 mg/L 2,4-DANT.

To study the formation of acetylated products, sorption of 2,4-DANT and 4-ADNT was conducted using sterile and nonsterile topsoil (initially at 5 and 10 mg/L). The residence time was increased from 22 to 66 h since some results indicated that these products accumulated with time.

Sorption by Dissolved Organic Matter. Sorption of TNT, 4-ADNT, and 2,4-DANT by dissolved organic matter (DOM) was investigated using the methods of Chiou et al. (*24*). Specifically, a quantity of each NAC in excess of its aqueous solubility was combined with distilled water or a DOM solution (obtained by equilibrating distilled water with topsoil, as described for sorption). After 22 h, the solutions were filtered with Millex-HV 0.45 μm filter units prior to analysis.

Desorption Experiments. Desorption was conducted by adding 15 mL of distilled water (for topsoil and Borden sand) or 0.1 N KCl (for illite) to the soil pellets for all five initial concentrations following sorption. The interstitial solution volume was estimated gravimetrically. A second desorption step that lasted 88 h was also employed.

Material Balances. For sorption and desorption, each NAC was extracted from the soil using acetonitrile, as described in EPA SW-846 Method 8330 (*25*). Briefly, the soil was combined with 10.0 mL of acetonitrile, vortexed, and placed in a sonicator bath (60 Hz) cooled to 22 °C (Blackstone Ultrasonics, Jamestown, NY) for 18 h. After sedimentation, 5.0 mL of the supernatant was combined with 5.0 mL of a 5 g/L $CaCl_2$ solution. The solutions were agitated and settled for 15 min prior to sample preparation for HPLC/LC/MS analysis. Based on a material balance, the percent recovery of an NAC was calculated as follows:

$$\% \text{ recovery} = ((NAC_{solid} + NAC_{aqueous})/NAC_{total}) \times 100\% \quad (1)$$

where NAC_{solid} is the NAC recovered by acetonitrile extraction, $NAC_{aqueous}$ is the NAC present in the aqueous phase, and NAC_{total} is the total amount of NAC present. All terms in eq 1 are expressed in units of moles.

The efficiency of acetonitrile extraction for a particular NAC was calculated as follows:

$$\% \text{ efficiency of extraction} = (NAC_{solid}/(NAC_{total} - NAC_{aqueous})) \times 100\% \quad (2)$$

Analytical Methods. The NAC concentrations were determined by reversed-phase high-pressure liquid chromatography (HPLC) with a photodiode array (PDA) detector. The Waters (Waters Associates, Milford, MA) HPLC system consisted of a model 600 pump, 717 Plus autosampler, and a 996 PDA detector ($\lambda = 254$ nm). The system was outfitted with Millennium data acquisition software. Separations were performed on a C-8 column.

Liquid chromatography/mass spectrometry (LC/MS) was used to identify and verify TNT metabolites. This consisted of a Micromass Platform II benchtop single quadrupole mass detector fronted by a Hewlett-Packard 1100 series HPLC system. Analyte ionization was conducted under the negative electronspray (ES) mode at 30 V and 90 °C. Confirmation of the identity of targeted metabolites was accomplished by

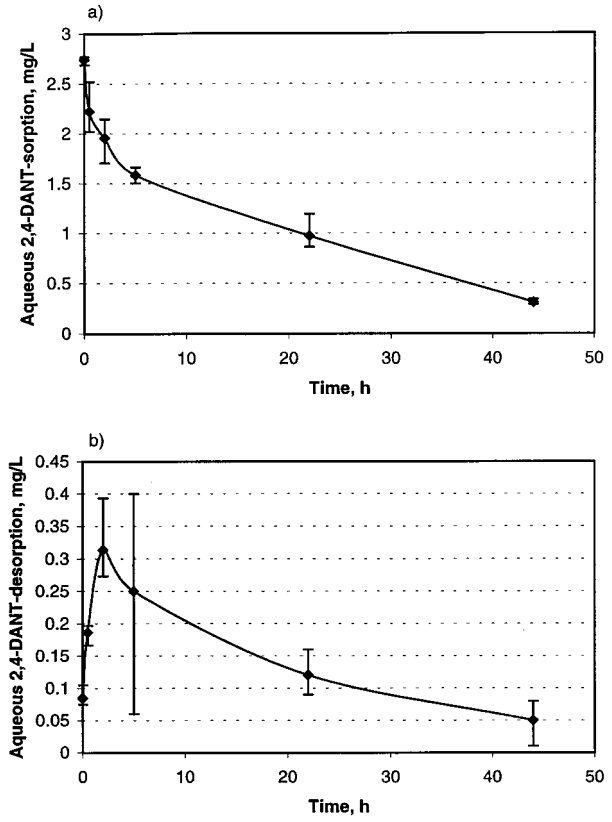

FIGURE 1. Kinetics of 2,4-DANT (a) sorption and (b) desorption by and from nonsterile topsoil (vertical bars denote minima and maxima).

comparison with commercially available reference compounds. Standards for the acetylated metabolites were synthesized from the corresponding amine using the acetic anhydride/bicarbonate method (*26*).

Results and Discussion

Sorption Kinetics (Nonsterile Soil). In most cases, the NAC disappearance from the aqueous phase during sorption was stable between 22 and 44 h. However, the 2,4-DANT continued to disappear from the aqueous phase from 0.5 to 44 h (Figure 1 a). Traces of 4-*N*-acetylamino-2-amino-6-nitrotoluene (4-*N*-AcANT) were detected at 22 h in the aqueous phase, and when 4-*N*-AcANT was included in a material balance for topsoil, the percent recovery (eq 1) increased from 43 to 48%.

Sorption (Sterile and Nonsterile Soils). There was little difference between the masses of the NACs sorbed in sterile and nonsterile soils at 22 h (data not shown). Therefore, the NAC disappearance with nonsterile soil was predominantly due to abiotic processes such as sorption. Although 4-*N*-AcANT formed from 2,4-DANT with nonsterile topsoil, the quantities were not sufficient to affect the net disappearance of 2,4-DANT from the aqueous phase as compared to sterile topsoil. For 2,4-DANT sorption by sterile topsoil, there was no difference between the aqueous phase concentrations measured at 22 and 44 h (data not shown). Hence, the observed disappearance of 2,4-DANT from the aqueous phase between 22 and 44 h with nonsterile topsoil (Figure 1a) was attributed to biotic processes. Therefore, 22 h was sufficient to achieve apparent equilibrium with respect to the abiotic process of sorption. Hence, nonsterile soils were used to study sorption for 22 h.

Desorption Kinetics. For all NACs examined, except for 2,4-DANT and topsoil, apparent equilibrium for desorption was achieved by 22 h (data not shown). For 2,4-DANT, following an initial phase of desorption at 0.5 h, there was a continued disappearance of 2,4-DANT in the aqueous phase (Figure 1 b), and 4-*N*-AcANT was detected in the aqueous phase (about 0.05 mg/L from 5 to 44 h).

Acetylation Products from 2,4-DANT and 4-ADNT. The 4-*N*-AcANT was only detected in experiments involving 2,4-DANT and topsoil. This is the first instance (to our knowledge) that 4-*N*-AcANT has been detected in natural uninoculated soil. To further investigate the formation of 4-*N*-AcANT in topsoil, conditions that could favor its formation (i.e., greater 2,4-DANT concentrations and a longer residence time) were implemented for sterile and nonsterile topsoil. For nonsterile topsoil, 4-*N*-AcANT was quantified by HPLC, and traces of 2-*N*-AcANT were detected (presence of both confirmed by LC/MS). From peak areas of LC/MS chromatograms, the ratio of 2-*N*-AcANT to 4-*N*-AcANT that formed (i.e., 10 mg/L 2,4-DANT initially and 66 h residence time) was 1:4.5. That 4-*N*-AcANT was produced in greater quantities than 2-*N*-AcANT is consistent with the fact that the amino group in the para position in 2,4-DANT is more reactive than that in the ortho position (*27*). Neither *N*-AcANT isomer was detected in experiments involving sterile topsoil, hence their formation was microbially mediated. The microbially mediated acetylation of the amino group in aniline in soil is thought to occur for the purpose of detoxification (*28*). It is possible that a similar detoxification reaction occurs for 2,4-DANT in soil.

Gicrease and Murphy (*27*) and Alvarez et al. (*29*) were the first to report on the occurrence of 4-*N*-AcANT from the acetylation of 2,4-DANT. In the former, 2,4-DANT was acetylated to 4-*N*-AcANT by a *Pseudomonas fluorescens* culture isolated from TNT-contaminated soils. In the latter study, pure 2,4-DANT was acetylated by *Pseudomonas aeruginosa* MA01 to 4-*N*-AcANT under anaerobic conditions. Bruns-Nagel et al. (*30*) observed the formation of 4-*N*-AcANT in an anaerobic/aerobic composting system designed to treat TNT-contaminated soil. We recently demonstrated that the two *N*-AcANT isomers disappear with time during TNT transformation by *Phanerochaete chrysosporium* (*31*). The results of the present study differ from those cited above since the formation of the acetylated product occurred in soil that was simply spiked with 2,4-DANT. Furthermore, the present system contained only indigenous microorganisms that had not been previously exposed to TNT or its metabolites.

The fraction of 2,4-DANT that biotically disappeared and that was subsequently recovered as 4-*N*-AcANT can be expressed as follows:

$$x_{2,4-\mathrm{DANT}} = N_{4-N-\mathrm{AcANT}}/((N_{2,4-\mathrm{DANT}})_{\mathrm{abiotic}} - (N_{2,4-\mathrm{DANT}})_{\mathrm{biotic}}) \quad (3)$$

where $(N_{2,4-\mathrm{DANT}})_{\mathrm{abiotic}}$ is the total amount of 2,4-DANT recovered (i.e., aqueous and sorbed) from sterile topsoil, $(N_{2,4-\mathrm{DANT}})_{\mathrm{biotic}}$ is the total amount of 2,4-DANT recovered from nonsterile topsoil, and $N_{4-N-\mathrm{AcANT}}$ is the total amount of 4-*N*-AcANT recovered from nonsterile topsoil. All terms in eq 3 are expressed in units of moles (66 h contact).

Values of $x_{2,4-\mathrm{DANT}}$ were calculated as 26 and 21% for 2,4-DANT initially at 5 and 10 mg/L, respectively. Furthermore, the "biotic disappearance" (i.e., $((N_{2,4-\mathrm{DANT}})_{\mathrm{abiotic}} - (N_{2,4-\mathrm{DANT}})_{\mathrm{biotic}})$) was calculated as 32 and 28% of the total quantities of 2,4-DANT applied (i.e., 5 and 10 mg/L, respectively). Therefore, transformation of 2,4-DANT to 4-*N*-AcANT is a significant process governing the fate of 2,4-DANT in some natural soils. Consistent with the results of Hundal et al. (*10*), traces of 2-ADNT and 4-ADNT were detected in the sorption−desorption experiments of TNT with nonsterile topsoil (results discussed subsequently), but 2,4-DANT and 2,6-DANT were never detected. For 2,4-DANT,

TABLE 2. Summary of Regression Parameters for TNT, 4-ADNT, and 2,4-DANT Sorption and Desorption by and from Topsoil and Illite

	sorption by topsoil			sorption by illite		
NAC	K_d^s (L/kg)	n	r^2	K_d^s (L/kg)	n	r^2
TNT	6.38	0.816	0.980	223.63	0.469	0.969
4-ADNT	7.91	0.695	0.956	58.52	0.677	0.835
2,4-DANT	11.96	0.711	0.915	19.73	0.809	0.959

	desorption from topsoil			desorption from illite		
NAC	K_d^d (L/kg)	n	r^2	K_d^d (L/kg)	n	r^2
TNT	12.01	0.820	0.968	265.98	0.401	0.990
4-ADNT	14.95	1.000	0.777	87.56	1.000	0.723
2,4-DANT	36.58	0.635	0.841	81.85	1.000	0.932

this may be due to its unstable nature in natural soil, as demonstrated here. Hundal et al. (*10*) reported that 4-ADNT and 2-ADNT (present as transformation products of TNT) removed from soil (with 3 M $CaCl_2$ or extracted with acetonitrile) decreased with the initial contact time, and this was partly attributed to transformation processes.

Comfort et al. (*12*) performed a material balance for the case of TNT that was fed to soil columns containing uncontaminated natural soil. For pulse feeds of 6.3 and 70 mg/L of TNT, the percent recoveries were 35 and 81% (eq 1), respectively. The NACs included in the material balance were TNT, 4-ADNT, and 2-ADNT; however, several other unidentified degradation peaks were detected by HPLC. Since 2,4-DANT can be reduced from 4-ADNT by facultative microorganisms (*32*), it is possible that this metabolite formed during the course of the column experiments. The formation of 2,4-DANT with subsequent acetylation may explain some of the observed discrepancies that were reported by Comfort et al. (*12*) in the material balances noted above.

In the present study, acetylation of 4-ADNT did not occur. Gilcrease and Murphy (*27*) reported that although 2,4-DANT was acetylated by *P. fluorescens*, no acetylation of 4-ADNT occurred. It was speculated that the lower basicity of 4-ADNT, as compared to 2,4-DANT, prevented its acetylation since it is less nucleophilic.

Sorption and Desorption Isotherms. The sorption−desorption data were fitted to the following Freundlich sorption isotherm using the method of least squares:

$$x/m = K_d^s C^n \quad (4)$$

where x/m is the solid-phase solute concentration at apparent equilibrium (μg/g), C is the aqueous-phase solute concentration at apparent equilibrium (mg/L), K_d^s is the sorption capacity constant (L/kg), and n is a dimensionless constant that relates to the intensity of sorption and heterogeneity of sorption sites. This model has been applied to sorption data of TNT and its metabolites (*7*), and its linearized form (i.e., $n = 1$) can sometimes be applied over limited concentration ranges (*34*). For desorption, K_d^s is replaced by K_d^d.

In Table 2, the regression parameters for TNT, 4-ADNT, and 2,4-DANT sorption and desorption by topsoil and illite are presented for the isotherm that provided the best fit for the data. The K_d^s and K_d^d are reported in terms of three−five significant figures. However, for purposes other than data analysis, these values should be rounded off to two significant figures. For topsoil, it was concluded that the three NACs were not sorbed by the DOM during sorption since there were no increases in their apparent solubilities (in DOM) as compared with their true solubilities (in pure water).

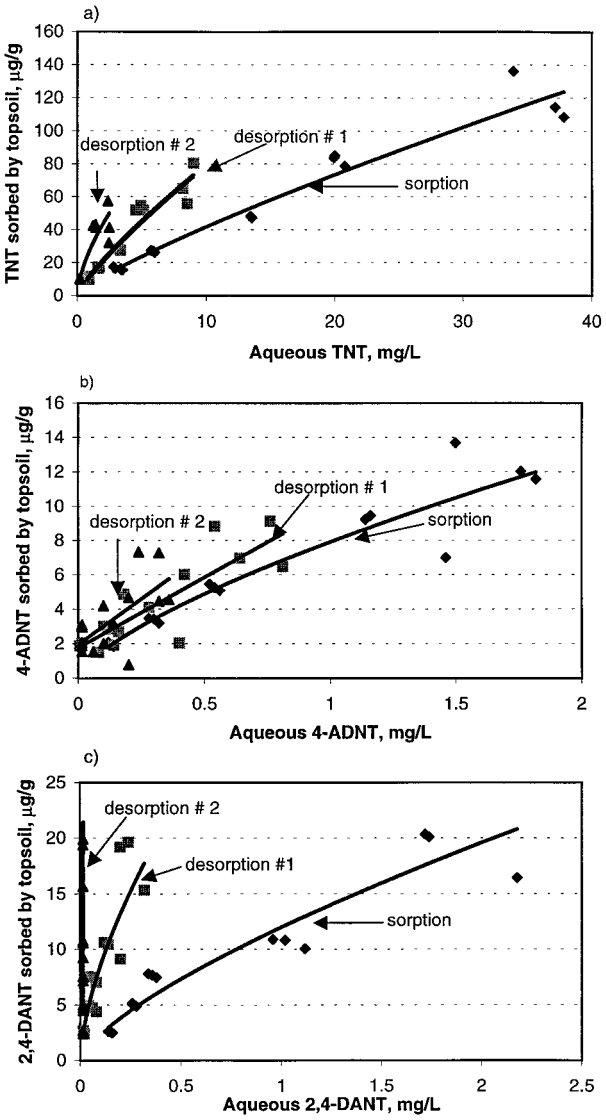

FIGURE 2. Sorption−desorption isotherms for topsoil and (a) TNT, (b) 4-ADNT, and (c) 2,4-DANT (lines denote fitted isotherms, regression parameters are reported in Table 2).

From Figure 2, there is sorption−desorption hysteresis for all NACs from topsoil. This is evidenced by the fact that the sorption−desorption isotherms do not coincide and since the K_d^d values are greater than the K_d^s values (Table 2). Hysteresis is most pronounced for 2,4-DANT (Figure 2c) and could be caused by 4-*N*-AcANT formation. However, some of this hysteresis is likely attributable to abiotic processes, such as chemisorption, since amines are capable of forming covalent bonds with organic carbon in humus (*35*).

As discussed previously, the K_d^s for 2,4-DANT with topsoil (i.e., 11.96 L/kg, Table 2) does not include biotic processes that may become significant for longer contact times. Particularly, an apparent K_d^s of 37.97 L/kg ($n = 0.44$, $r^2 = 0.68$) was calculated for nonsterile topsoil (2,4-DANT at 5 and 10 mg/L, 66 h). For sterile topsoil, a K_d^s of 13.75 L/kg ($n = 1$, $r^2 = 0.54$) was obtained, and this is comparable to the K_d^s of 11.96 L/kg (Table 2) obtained for shorter contact times with nonsterile topsoil (22 vs 66 h). Hence, caution should be exercised in applying reported K_d^s values to field situations.

Figure 3a depicts the masses of the three NACs sorbed by topsoil and the corresponding masses that were extracted with acetonitrile. The extraction efficiencies for TNT and

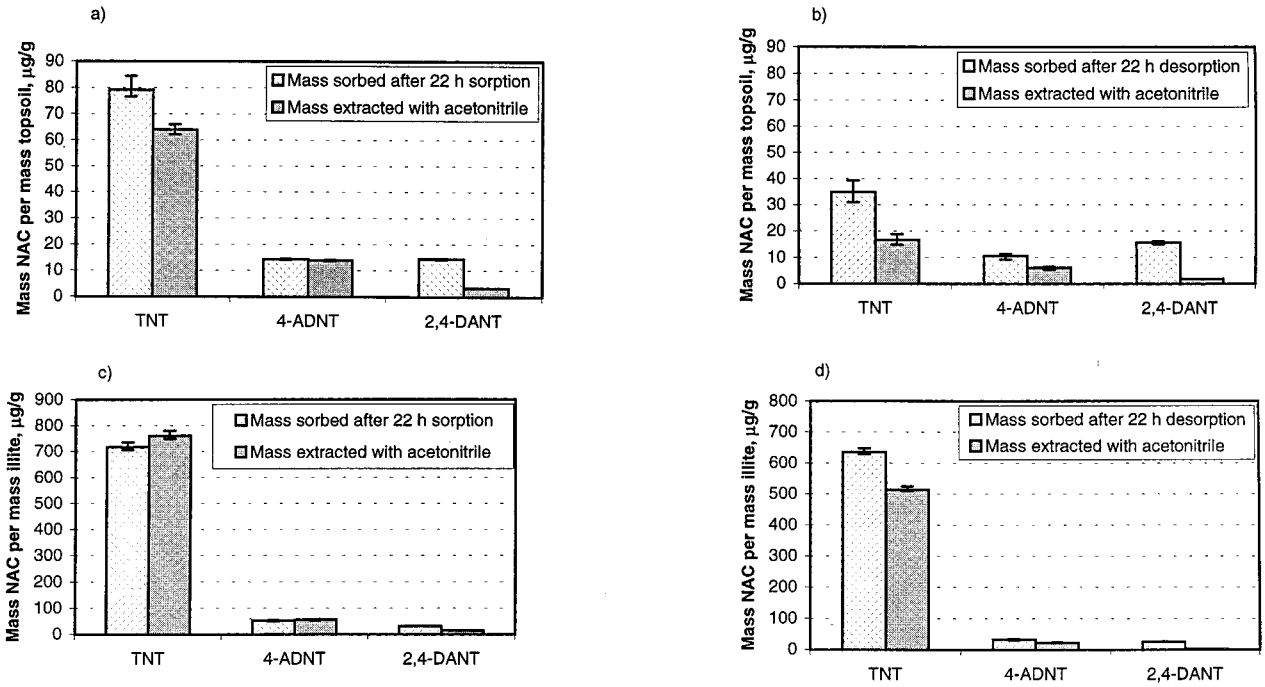

FIGURE 3. Masses of TNT, 4-ADNT, and 2,4-DANT (initial concentrations of 30, 3, and 3 mg/L, respectively) sorbed by topsoil and illite (soil-to-solution ratios of 1:7.5 and 1:15, respectively) and masses that were subsequently removed by acetonitrile extraction for the case of (a) sorption by topsoil, (b) desorption from topsoil, (c) sorption by illite, and (d) desorption from illite (vertical bars denote minima and maxima).

4-ADNT were high (81 and 97% using eq 2, respectively) but declined for 2,4-DANT (22%). When 4-*N*-AcANT was included, the efficiency increased to 27%. In Figure 3b, the masses of the NACs that remained sorbed by the topsoil following desorption are depicted with the masses that were subsequently extracted with acetonitrile. The extractable fraction decreased following desorption (Figure 3b) as compared to sorption (Figure 3a). Hence, the fraction of extractable NAC decreased with time since 22 h desorption corresponds to 44 h contact (i.e., 22 h sorption and 22 h desorption). For all three NACs, there was greater hysteresis for the second desorption.

For the sorption isotherm of TNT and illite (Figure 4a), there is curvature at low surface coverage, thereby indicating the presence of specific binding sites of the sorbent (illite) to the sorbate (TNT) (*34*). Furthermore, the nitro groups of TNT have strong electron-withdrawing properties that will enhance its sorption by the siloxane surface of illite (*13, 14*). Desorption isotherms for TNT and 4-ADNT exhibited moderate hysteresis (Figure 4 a,b). For desorption of 4-ADNT, there was a reduced sorption capacity of the illite for 4-ADNT in the second phase of desorption. On the other hand, the desorption isotherm for 2,4-DANT exhibited a greater degree of hysteresis from the sorption isotherm (Figure 4c) than was observed for the other two NACs (Figure 4 a,b). These results are consistent with others where sorption−desorption nonsingularity for 2,4-DANT and a montmorillonite clay was reported (*13*). In the present study, hysteresis was more pronounced during the second phase of desorption (Figure 4c), despite the fact that 4-*N*-AcANT was never detected.

In Figure 3c, the masses of the three NACs sorbed by illite following sorption are depicted along with the extractable masses. The extraction efficiencies for TNT and 4-ADNT were high (106 and 108%, respectively) but decreased for 2,4-DANT (45%). Following desorption, the NAC masses extracted (Figure 3d) declined from those obtained following sorption. Therefore, as was the case for topsoil, the extractable fraction decreased with time. Since no significant quantities of known metabolites (other than 4-*N*-AcANT) were detected for topsoil and illite, the reported decreases in extractable fractions are likely due to irreversible sorption. The three NACs were not appreciably sorbed by Borden sand.

Mechanisms of NAC−Soil Interactions. The topsoil and the Borden sand did not contain illite, rather they contained traces of the clay mineral crossite (Table 2). Therefore, it is not likely that the difference in sorption−desorption characteristics of these two soils for the three NACs was due to differences in mineralogy since quartz and albite (only other minerals present) are considered as "nonreactive" minerals (*36*). Rather, the observed differences in the sorption−desorption characteristics of these two soils are likely due to differences in organic matter (8.4 vs 0.02% for topsoil and Borden sand, respectively). Amorphous iron oxides have been shown to be capable of reducing NACs such as those studied here (*37*). However, although the topsoil likely contained iron oxides, such abiotic reduction reactions probably did not occur during sorption−desorption since the present system was aerobic. This is evidenced by the absence of reduction products in the aqueous and acetonitrile extracts following sorption of the three NACs by sterile topsoil.

The pK_a values of 0.95 and 3.13 for 4-ADNT and 2,4-DANT have been reported (*13*), respectively. Since the pH of the three geological materials (Table 1) studied were greater than these pK_a values, the two NACs were not protonated. Hence, cation exchange is not the mechanism by which these NACs interacted with the three soils.

NAC sorption by clay or soil organic matter (SOM) was mediated by nitro or amino group interactions, respectively. The increase in K_d^s with NAC amino group substitution for topsoil observed here is consistent with results of others for NAC binding with humic acid (a component of SOM) (*9*). Particularly, the K_d^s increased with amino group substitution (2,6-DANT > 2-ADNT > TNT), and it was concluded that this was consistent with the increase in the partial positive charge of the amines. From this, it was proposed that the negatively charged carboxylic and phenolic acid sites in humic acid interacted with the partial positive charges of the

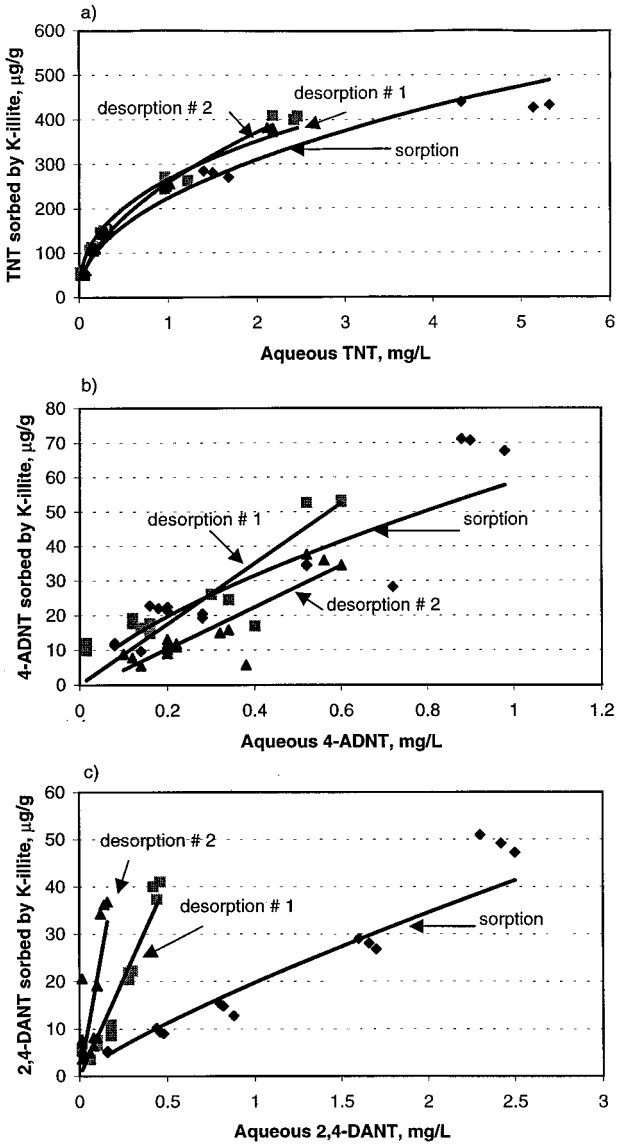

FIGURE 4. Sorption−desorption isotherms for illite and (a) TNT, (b) 4-ADNT, and (c) 2,4-DANT (lines denote fitted isotherms, regression parameters are reported in Table 2).

amino groups. However, the energy required to form these individual bonds is just a few kilocalories per mole; hence, they are not strong associations for small molecules (*38*). Furthermore, if such interactions were predominant, a greater recovery of 2,4-DANT from the topsoil (23%, eq 2) would be expected. More likely, the amino group in the para position of 2,4-DANT underwent irreversible reactions with the SOM in topsoil. Bollag et al. (*39*) showed that cross couplings, catalyzed by extracellular enzymes, can occur between amino aromatics (such as 2,6-diethylaniline) and aromatic acids that are ubiquitous to soil. As well, it is thought that the slow and not readily reversible addition of amino groups to quinoidal structures in humate followed by oxidation of the product to a nitrogen-substituted quinoid ring is possible (*40*). The amino group in the para position of 2,4-DANT may be more susceptible than 4-ADNT to cross couplings and addition to quinoidal structures in SOM because it is more basic (and hence more nucleophilic) and is less sterically hindered. Hence, the unique reactivity of the 2,4-DANT as compared to 4-ADNT may explain the fact that only 23% (eq 2) is extractable from topsoil (Figure 3a). The subsequent decrease in extractable 2,4-DANT following 22 h desorption (Figure 3b) may be due to the longer retention time (i.e., 44 h) since the extent of both reactions (i.e., cross coupling and addition to quinoidal structures) reportedly increase with time (*39*, *40*). Recently others have noted the irreversible binding of 2,4-DANT by sediment (*41*). Further decreases in extractable TNT and 4-ADNT from topsoil following desorption (Figure 3b) may be due to further reduction of nitro groups by soil microorganisms followed by irreversible reactions such as those noted above.

The 2,4-DANT was also not readily extractable with acetonitrile following sorption to illite (Figure 3c). Electron donor−acceptor (EDA) reactions between the amino group in the para position of the 2,4-DANT and the aluminum at the edge sites of the illite may explain this behavior, as has been reported for a variety of clays and amino aromatics (*42*). For example, transformation of benzidine by electron transfer from the amino groups to many clays gives rise to the blue monovalent radical cation in aqueous solution. The 'oxidizing sites' were identified as the aluminum in octahedral coordination at the crystal edges (*42*). In such an EDA system, free radical formation at the reactive para position of 2,4-DANT with subsequent dimer formation is also a possibility and would explain the apparent irreversible sorption behavior of this NAC by illite.

A variety of anaerobic and aerobic microorganisms are capable of reducing the first nitro group of TNT to produce either 2-ADNT or 4-ADNT (*16*). Subsequent reduction of either aminodinitrotoluene is mediated by several facultative or strictly anaerobic bacteria, and the exclusive product is 2,4-DANT. Our results indicate apparent irreversible sorption 2,4-DANT by clay (illite) and SOM (topsoil). Additionally, 2,4-DANT is converted to 4-*N*-AcANT by indigenous microorganisms not previously exposed to explosives. Therefore, future research will focus on 2,4-DANT in terms of identifying other transformation products as well as determining conditions conducive to its complete mineralization.

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