Retention and Mobilisation of Trinitrotoluene, Aniline, Nitrobenzene and Toluene by Soil Organic Matter

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Abstract

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For decades, the fate of trinitrotoluene (TNT) in soil environments has interested researchers around the world. Due to its toxicity and locally high concentrations in soils and sediments, a proper risk assessment requires detailed knowledge of the type of bonds formed between TNT (and its degradation products) and major soil components. In the work described in this thesis the effects of different soil constituents were investigated using a fractional factorial experimental design, and kinetic and equilibrium experiments. A major concern was the distribution of TNT between dissolved (DOM) and particulate soil organic matter (POM). Free TNT in solution, TNT bound to POM and to different size fractions of DOM were determined using ^{14}C-labelled compounds, reversed-phase chromatography (RPC) and size-exclusion chromatography (SEC). The distributions of aniline, nitrobenzene and toluene between POM and DOM were also determined. These compounds have similar properties to TNT and its degradation products.

The results conclusively showed that soil organic matter (SOM) is the most important variable for the binding of TNT and its degradation derivatives in acid, organic rich soils. In experiments involving γ-radiation, dialysis and varying concentrations of DOM and POM, it was shown that biologically mediated reductive degradation of TNT is crucial for the binding of TNT derivatives to DOM and POM. The reactive TNT derivatives mainly bind to DOM functional groups via a pH-dependent reaction. Smaller DOM molecules were found to be more reactive than larger ones. Non-degraded TNT was found to bind mainly to POM by a pH-independent hydrophobic partitioning process. Even though a relatively small fraction of TNT was bound to DOM in our experiments, compared to POM, continuous degradation of TNT over time will result in a large potential transport of TNT derivatives with DOM to ground and surface waters. The question remaining to be answered is: how bioavailable and toxic are TNT derivatives that are bound to DOM?

Keywords: TNT, humus, sorption, desorption, HPLC, SEC, size-exclusion chromatography, fractional factorial experimental design

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Contents

Appendix

Papers I-IV

This thesis is based on the following papers, which will be referred to by their respective Roman numerals:

I. Eriksson J. and Skyllberg U. (2003) Determination of variables affecting sorption of aniline and trinitrotoluene (TNT) in soil using a multivariate experimental design. (Manuscript)

II. Eriksson J. and Skyllberg U. (2001) Binding of 2,4,6-trinitrotoluene and its degradation products in a soil organic matter two-phase system. *Journal of Environmental Quality 30*, 2053-2061.

III. Eriksson J., Frankki S. and Skyllberg U. (2003) Distribution of 2,4,6-trinitrotoluene, nitrobenzene, aniline and toluene between dissolved and particulate soil organic matter. (Manuscript)

IV. Eriksson J. and Skyllberg U. (2003) Binding of aniline and 2,4,6-trinitrotoluene to natural dissolved organic matter - a size-exclusion chromatography study. (Manuscript)

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Abbreviations

ADNT	Aminodinitrotoluene
CEC	Cation exchange capacity
DANT	Diaminonitrotoluene
DOM	Dissolved organic matter
DOC	Dissolved organic carbon
EDA	Electron donor-acceptor complex
HA	Humic acid
HADNT	Hydroxylaminodinitrotoluene
HPLC	High-performance liquid chromatography
IS	Ionic strength
NAC	Nitro-aromatic compound
NOM	Natural soil organic matter
OM	Organic matter
PLS	Partial least squares
POM	Particulate organic matter
POC	Particulate organic carbon
RPC	Reversed-phase chromatography
SD	Standard deviation
SEC	Size-exclusion chromatography
SOM	Soil organic matter
SOC	Soil organic carbon
TAT	Triaminotoluene
TNT	Trinitrotoluene

Introduction

Background

Disposed ammunition, an environmental issue?

During the 1990's the Swedish armed forces were legally required to investigate, map and classify old dumping sites of disposed ammunition. These sites were mainly located in lakes, the Baltic Sea and sealed mineshafts. As part of this assignment, the Swedish Defence Research Agency (FOI) was commissioned to perform several environmental risk assessments regarding the pollutant content of dumped ammunition. Besides this task, FOI was commissioned by the defence industry to investigate old open sites at which ammunition and explosives were burnt. In these studies a lack of knowledge was noted concerning the binding of compounds derived from munitions to natural soil organic matter (NOM). Therefore, research studies including those outlined in this thesis were proposed by FOI and funded by the Swedish armed forces.

Historically, the most important and widely used explosive is trinitrotoluene (TNT). It was synthesized for the first time in 1863 by J. Wilbrand (Yinon, 1990), and from the beginning of the 20th century it was used as a military explosive, mainly because of the relative safety of handling it during the manufacture and moulding of munitions. During World Wars I and II TNT was produced in millions of tons.

Since TNT and its degradation products were found to be toxic, contamination by these materials has been the subject of rather extensive research in recent decades. The toxicity was noticed first in manufacturing plants where the munitions workers often became ill, typically showing symptoms of toxic jaundice, liver damage and, ultimately, death (O'Donovan, 1921; Panton & Bates, 1921; Yinon, 1990). Studies later confirmed the toxic effects on different organisms (Smock, Stoneburner & Clark, 1976; Won, DiSalvo & Ng, 1976; Kaplan & Kaplan, 1982; Spiker, Crawford & Crawford, 1992; Drzyzga *et al.*, 1995). The extensive use of TNT during World War II led to severe contamination problems, often adjacent to manufacturing sites where large volumes of process water containing explosives were released without much treatment. It was often called 'pink water' or 'red water' because of its reddish colour. One munitions plant alone could daily generate up to 1900 m^3 of process water (Yinon, 1990). When located close to aquifers the risk of contamination was severe and threatened drinking water resources (Lenke *et al.*, 1993; Hildenbrand & Neumann, 1995). It is not only the manufactured explosive itself that causes contamination problem, but also the various degradation products formed and transformed in the environment by both biological and abiotic processes. Purification by removal of asymmetrical TNT-isomers with sulphite washing also produced highly polar nitrosulfonic acids, which are mobile because of their high water solubility and have been found in leachate water (Schmidt *et al.*, 1998).

In Sweden, old ammunition was commonly dumped between the end of the 1940's and 1970. After 1968, disposal in lakes was banned by law and in 1970 disposal in seas was also prohibited. In total, 104 locations of documented dumping sites have been found so far. However, in Sweden the scale of these problems is much smaller than in some countries, for example Germany and the USA, due to the comparably low volumes manufactured here.

Theoretical considerations

Previous related work

Binding affinity experiments

Most previous studies on the fate of TNT in soil have been either site-specific, with soils containing only small concentrations of SOM (often less than one percent of dry mass) or laboratory experiments using purified systems with clays or humic acids (HA). Few studies (*e.g.* Achtnich *et al.*, 1999a; Sheremata *et al.*, 1999) have focused on the interaction between TNT and SOM. However, no study, to our knowledge, has considered simultaneously the binding of TNT to dissolved (DOM) and particulate (POM) organic matter.

Several studies of purified clay systems have revealed that a specific type of bonding occurs between nitro aromatic compounds (NAC), including TNT, and clay surfaces (Haderlein & Schwarzenbach, 1993, 1994; Haderlein, Weissmahr & Schwarzenbach, 1996; Weissmahr *et al.*, 1997, 1999; Weissmahr, Haderlein & Schwarzenbach, 1998). This type of bonding is described as an electron donor-acceptor (EDA) complex between the electron-deficient NAC and the electron-rich clays. The clays' ability to form bonds is due to substitution of Si^{4+} with Al^{3+} in the clay structure, giving them a permanent, structural negative charge. Highly negatively charged clays, such as smectites, have the greatest capacity to bind NAC. Addition of NOM was not found to inhibit EDA complex formation between clays and TNT at low NOM concentration (4‰ weight of the total adsorptive material), (Weissmahr *et al.*, 1999).

Several studies have used natural site-soils, with varying concentrations of organic matter, to determine the sorptive properties of soil towards TNT and other related compounds (Pennington & Patrick, 1990; Brannon *et al.*, 1992; Grant *et al.*, 1995; Comfort *et al.*, 1995; Selim, Xue & Iskandar, 1995; Xue, Iskandar & Selim, 1995; Hundal *et al.*, 1997; Sheremata *et al.*, 1999). These studies have shown the importance of an anaerobic environment to increase the binding of TNT (and its derivatives) to soil. Positive correlations between binding capacity and cation exchange capacity (CEC) have also been found (Pennington & Patrick, 1990). In addition, adsorption and degradation have been found to be concentration dependent and non-linear models like the Langmuir isotherm have often been used to describe TNT adsorption to soil. In one study, indications that TNT binds more strongly to fulvic acids, as compared to humic acids and humin, have been found (Hundal *et al.*, 1997). Furthermore, approximately one third of TNT bound to the solid phase of soil was irreversibly bound. Sorption-desorption hysteresis was mainly associated with SOM-rich soils. In general, the binding has been found to be stronger for TNT degradation products than for TNT itself.

Degradation of TNT

Biotic and/or abiotic degradation of TNT in soil usually follows a reductive pathway through reduction of the nitro groups ($-NO_2$) of the molecule. This pathway also predominates under aerobic conditions in soils. Fig. 1 shows a schematic diagram of the reductive pathway. The main degradation path for TNT is a step-wise reduction of $-NO_2$ to amino ($-NH_2$), via the reactive intermediate nitroso (-NO) and hydroxylamino (-NHOH) compounds. The order of the main degradation derivatives is TNT → Aminodinitrotoluene (ADNT) → Diaminonitrotoluene (DANT) → Triaminotoluene (TAT). The different steps have different reaction rates, with the first step being faster than the following step and so forth. In an anaerobic environment, the reactions are more rapid than in an aerobic surrounding. However, the last step, in which TAT is formed, demands a highly anaerobic environment with a redox potential below approximately –250 mV (Held *et al.*, 1997). Reactions between nitroso and hydroxylamine intermediates lead to the formation of two-ring structures like azoxy- and azo-complexes. In anaerobic environments, the reduction is mediated by nitroreductase enzymes produced by fungi or bacteria, and oxidation of reduced ions (*e.g.* Fe^{2+} → Fe^{3+}) may occur as a consequence of the TNT reduction (Rugge *et al.*, 1998). For more detailed information on degradation pathways of TNT and other NACs see Rieger & Knackmuss (1995) and Hawari *et al.*, (2000).

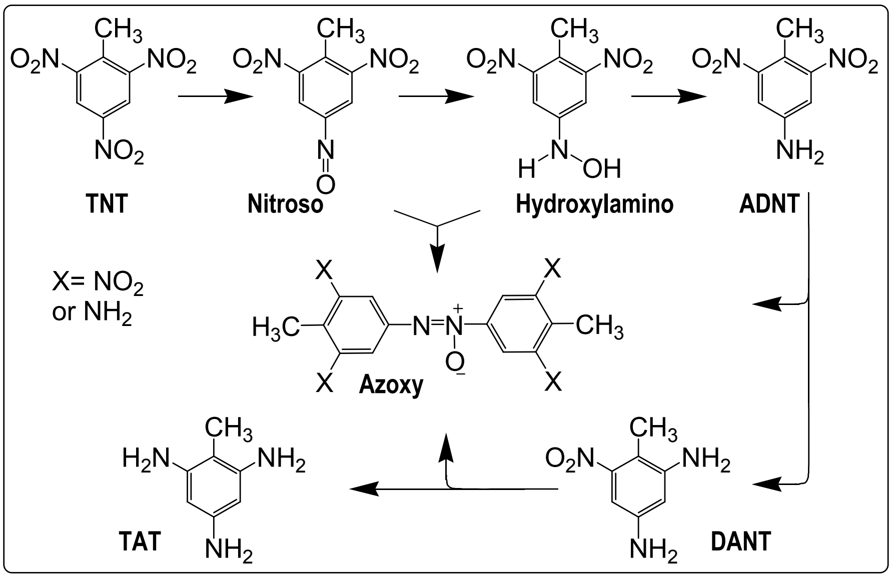


Figure 1. Reductive degradation pathway and main degradation products of TNT. The steps from nitro functional groups to the amino group are repeated for every nitro group of TNT to form multi-amino degradation products. These reactions are favoured in an anaerobic environment.

Reaction mechanisms between organic contaminants and SOM

Different types of binding mechanisms are responsible for the binding of TNT and its degradation products to SOM. Some of these mechanisms have been identified by use of spectroscopic techniques, whereas others are more speculative. Three different types of chemical/physical interactions can be identified: hydrophobic partitioning, electrostatic interactions and covalent bond formation.

The hydrophobic partitioning reaction occurs mainly between non-polar organic contaminants (or non-polar parts of a larger molecule) and similarly non-polar moieties of SOM (see, for example., Chiou, 1989). In common parlance, the non-polar organic contaminant can be said to 'dissolve' in hydrophobic parts of the SOM backbone. This process is driven by changes in entropy. An adsorption isotherm describing a pure hydrophobic partitioning process in SOM will be linear, thus there will be no limitation in the amount of sites in SOM available for sorption.

Electrostatic interactions and covalent bond formation reactions occur between functional groups in the organic contaminant and SOM. Because individual functional groups are involved in the binding, both of these types of reaction are said to be *specific*, as opposed to hydrophobic partitioning reactions that are *non-specific*. An adsorption isotherm describing a specific reaction with SOM will show an adsorption maximum, indicating that there is a limitation of adsorbing SOM sites. Since SOM is composed of very complex chemical structures, there are plenty of different types of reactive functional groups available for specific interactions with molecules of organic contaminants. The electrostatic interactions can be further divided into attractions between ionised groups, hydrogen bonding, van der Waals attractions and formation of EDA-complexes. As opposed to electrostatic attraction, covalent bond formation involves overlapping of orbitals ('electron densities'). Covalent bonds are often stronger than electrostatic associations, and in short-term experiments the former may show a stronger tendency towards irreversibility.

Fig. 2a illustrates the electrostatic attraction between a protonated aniline molecule and a negatively charged carboxylic group of SOM or some other negatively charged site (*e.g.* a permanently charged 2:1-clay). The TNT derivatives ADNT and DANT form similar electrostatic attractions with SOM. Aniline may also form covalent bonds with SOM carbonyl groups through nucleophilic addition reactions (Fig. 2b). TNT derivatives with a nitroso-group are believed to bind covalently with nucleophilic groups (Fig. 2c). It has been proposed that other TNT degradation products like the azoxy-complex are initially electrostatically attracted, but with time (hours-days) form covalent bonds with SOM (Fig. 2d). Fig. 2e presents a nucleophilic addition reaction for 4-ADNT, resulting in covalent bond formation with SOM carbonyl groups. All amino derivatives are believed to be able to bind to SOM in a similar way.

Aniline, nitrobenzene and toluene

Besides TNT, the binding of aniline, nitrobenzene and toluene to SOM were studied for several reasons. First, it is of great interest to gain more information about the binding of these relatively simple compounds in the same experimental DOM - POM systems used for the TNT studies. Secondly, many of the TNT derivatives believed to be active in binding to SOM are chemically/biologically unstable, so results of binding experiments are very difficult to interpret. Macroscopic affinity studies of TNT, ADNT and DANT binding to humic acids have given essentially similar results, indicating that all three compounds are rapidly degraded to a number of reactive compounds with quite similar reactivity (*e.g.*, Li *et al.*, 1997). We therefore used aniline, nitrobenzene and toluene as models describing the reactivity of the three different types of functional groups of TNT, ADNT, DANT and TAT; *i.e.* amino, nitro and methyl groups, respectively. As shown in Fig. 3, the only difference in structure between these three compounds is the functional group attached to the benzene ring. Thus, any difference in binding could be attributed to these functional groups.

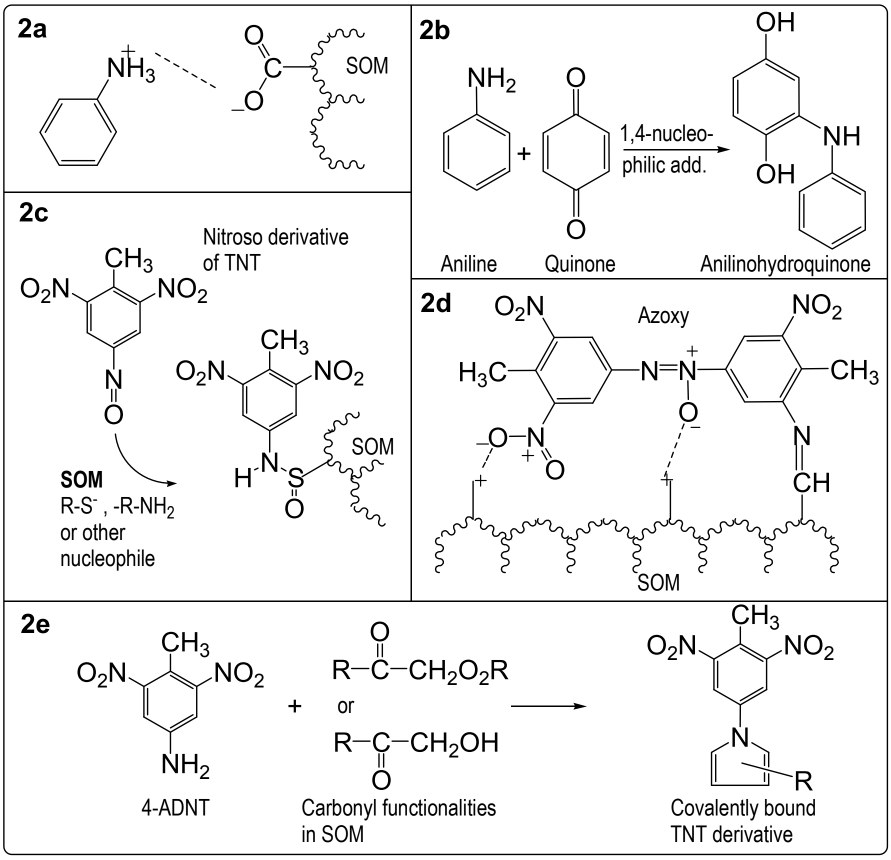


Figure 2a. Electrostatic attraction between protonated aniline and a carboxylic group in SOM. *2b*. Covalent bond formation through a 1,4 nucleophilic addition reaction of aniline to quinone. *2c*. Covalent bonding between a nitroso derivative of TNT and a nucleophilic $R\text{-}S^-$-group in SOM (Modified from Ahmad & Hughes, 2002). *2d*. Proposed electrostatic interactions (dashed lines) in combination with covalent bonding between an azoxy derivative of TNT and SOM (Modified from Achtnich *et al.* 1999a). *2e*. A nucleophilic addition reaction of 4-ADNT to carbonyl groups in SOM (Modified from Thorn, Pennington & Hayes, 2002).

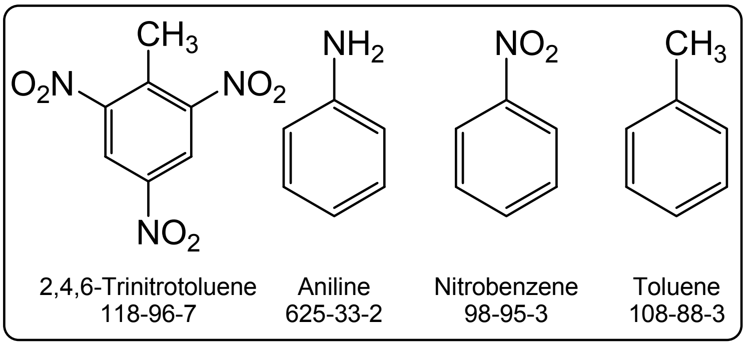
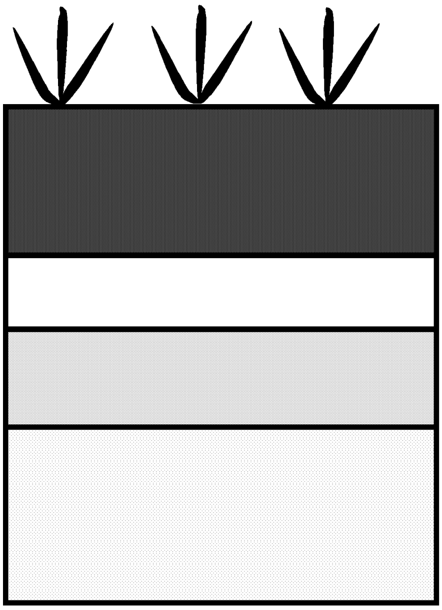


Figure 3. Structural formulas and common names of the investigated compounds, including their Chemical Abstracts Service (CAS) registry numbers.

Spodosol – the typical forest soil of Scandinavia

The most common type of soil profile in Scandinavia is the Spodosol (or Podzol in the old Russian/Swedish classification system), Fig. 4. A main characteristic of the Spodosol is the accumulation of rather acid SOM in the surface horizons. Organic matter from plants and animals accumulates and forms a distinct organic horizon (O-horizon) on top of the mineral soil. With time, plant debris decomposes and is transformed by the microbial community (mainly fungi) into humified organic matter. Soil organic matter is a term covering all forms of organic matter (dissolved, particulate, humified and non-humified) in soils. The excess of water in a humid climate (as in Scandinavia) results in leaching of DOM from the O-horizon into the mineral soil. In the mineral soil DOM is arrested by sorption reactions. Little DOM is arrested in the elluvial (E) horizon, as compared to the Bs (illuvial or Spodic) horizon where DOM is adsorbed to reactive mineral surfaces. The downward DOM and soil water movement in the soil profile leads to surface etching (weathering) of soil minerals, leaving a white/grey horizon of less soluble quartz and feldspar minerals in the E-horizon. Further down in the profile the dissolved metal ions (including Al-ions) precipitate together with DOM when pH rises to form the Bs-horizon. At greater depth in the soil (the C-horizon), the parent mineral material is more or less unaltered by pedogenic (soil forming) processes. Very little DOM reaches the C-horizon (Fig. 4). Under oxygen limited conditions in soils with a high ground water table, *e.g.* in discharge areas, the microbial activity is low and the O-horizon may be partly transformed into a peaty O-horizon or even into a peat soil (>30 cm deep organic layer). Mineral soils that would develop into Spodosols under well-drained conditions often develop into Gleysols under poorly drained conditions.

In recharge areas the water movement is downward all year round. Because DOM strongly adsorbs to mineral surfaces in the B-horizon, very little DOM originating in soils from recharge areas reaches draining streams and lakes. In discharge areas, however, the water movement is not necessarily downward, rather more or less lateral in direction. Lateral flow not only changes the soil formation (no E-horizon is formed) but also results in transport of DOM, and other aqueous phase constituents, from the soil into draining streams and eventually into lakes. Thus, soil processes occurring in discharge areas have a strong influence on the chemistry in streams and lakes. Since discharge areas are often problematic for the cultivation of crops and other important human activities, low-lying areas in the terrain not seldom have been used for secondary human activities such as dumping pollutants. Environmental problems arising from this practice are obvious. For these reasons, peaty O-horizons from discharge areas were used in the experiments in this study.



Horizon	pH	OM (%)
O	4.0	80-95
E	4.0	1-3
Bs	5.0	2-5
C	6.5	<1

Figure 4. A Spodosol soil profile showing its main horizontal zones. The pH and percentage organic matter are approximate values for an ordinary Spodosol from a temperate region.

Soil remediation of TNT contaminants

Incineration of contaminated soils is costly, so other remediation techniques have been extensively investigated. Bioremediation in slurry, or composting with the addition of bacterial strains that degrade NAC should be more cost effective. Many studies have examined these alternatives with varying degrees of success (McCormick, Feeherry & Levinson, 1976; Walker & Kaplan, 1992; Williams, Ziegenfuss & Sisk, 1992; Preuss, Fimpel & Diekert, 1993; Boopathy & Kulpa, 1994; Michels & Gottschalk, 1994; Boopathy *et al.*, 1994; Gorontzy *et al.*, 1994; Bradley & Chapelle, 1995; Spain, 1995; Heijman *et al.*, 1995; Pennington *et al.*, 1995; Roberts, Ahmad & Pendharkar, 1996; Breitung *et al.*, 1996; Holliger *et al.*, 1997; Krumholz *et al.*, 1997; Boopathy, Widrig & Manning, 1997; Dawel *et al.*, 1997; Widrig, Boopathy & Manning, 1997; Held *et al.*, 1997; Daun *et al.*, 1998; Vorbeck *et al.*, 1998; Bruns-Nagel *et al.*, 1998, 2000; Lenke *et al.*, 1998; Drzyzga *et al.*, 1998; Achtnich *et al.*, 1999a-b; Knicker *et al.*, 1999; Thorn, Pennington & Hayes, 2002). Phyto-remediation has also been investigated. In this approach, crops growing on contaminated soils may extract the contaminants from the soil and the contaminants can then be harvested together with the crop (Peterson *et al.*, 1996; Qaisi, Thibodeaux & Adrian, 1996; Thompson, Ramer & Schnoor, 1998).

Production of CO_2 originating from the contaminants can provide evidence of successful remediation, especially if the rate of degradation is high. Transformation of TNT occurs, but mineralization of TNT to CO_2 and other fundamental products is often negligible or marginal (Hawari *et al.*, 2000). Another remediation approach is to incorporate the substance irreversibly into the soil matrix, thereby inhibiting the possibility of desorption. Typically, covalent bond formation is taken as an indication that the substance is irreversibly incorporated into the structure of SOM and that it may eventually decompose during SOM degradation and turnover. In the studies of Achtnich *et al.* (1999a), Bruns-Nagel *et al.* (2000) and Thorn, Pennington & Hayes (2002), ^{15}N-labeled TNT and nuclear magnetic resonance (NMR) spectroscopy were used to determine the type of bonds formed between TNT and SOM. There are strong indications that the di- and tri- amino degradation products preferentially undergo 1,2-nucleophilic addition reactions with carbonyl groups or quinones, resulting in a covalent bonding to SOM (Thorn & Kennedy, 2002). Aniline and TNT monoamino degradation products also form covalent bonds to carbonyl groups and quinones, but via 1,4-nucleophilic addition reactions. These reactions are favoured by aerobic conditions, which explains the success in previous studies of using a two-step composting process with an anaerobic first step enhancing the degradation of TNT, followed by an aerobic step favouring covalent binding of the degradation products. In a recent study, humic acid showed no reactivity towards hydroxylamino derivatives, whereas substantial binding between nitroso groups and humic acids was detected (Ahmad & Hughes, 2002). Other findings of this study included the importance of the proteinaceous fraction of HA for degradation of TNT via a reaction between thiol groups and nitroso compounds.

The evidence presented for covalent bonding of TNT derivatives to SOM, implying that incorporation of TNT into SOM can be an acceptable method for bioremediation of contaminated soils, has to be further supported by long-term studies to evaluate the possible release of bound secondary contaminants (derivatives). Of special importance is to make sure that the formation of dissolved organic matter during SOM decomposition does not result in the leaching of potentially toxic and bioavailable TNT derivatives.

Objectives

- To determine the major factors underlying the retention and mobilisation of TNT in organic rich soils using a multivariate experimental design (**Paper I**).
- To determine the importance of particulate and dissolved organic matter as sorbents of TNT and its derivatives in soil, using kinetic and equilibrium experiments (**Papers II** and **III**).

- To compare the binding of TNT and its derivatives to particulate and dissolved organic matter with the binding of aniline, nitrobenzene and toluene (**Papers III** and **IV**).
- To determine the association of TNT and aniline to different size fractions of dissolved organic matter, using size-exclusion chromatography (**Paper IV**).

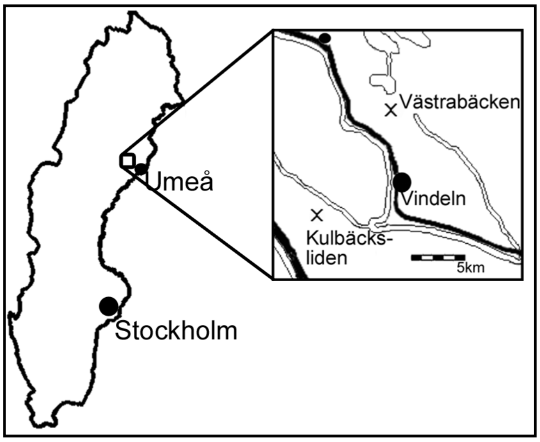
Materials and methods

Soil organic matter

Description

The SOM material used in the laboratory experiments presented in **Papers I, III** and **IV** was taken from a 30 cm thick organic horizon of a Gleysol (Soil Survey Staff, 1992) near the stream Västrabäcken within the grounds of Svartberget Research Station, Vindeln, Sweden (Fig. 5). This SOM sample was highly decomposed, consisting of black-brown humic material without any visible structure from original litter and debris. In the study presented in **Paper II** the SOM material used was a less decomposed fermentation layer of a 10 cm thick organic horizon from a Spodosol soil located in the Kulbäcklidens experimental area (Fig. 5). All collected SOM material was passed through a 4-mm cutting sieve to homogenize it and to separate it from plant roots and large, undecomposed pieces of debris, then stored in the dark at 4 ˚C until used (within four months) in laboratory experiments.

Figure 5. Sampling locations, near Vindeln in Sweden, of the two SOM samples used in this study (marked with "X" in the enlarged section). Material from Kulbäckliden was used in the studies described in Paper II and Västrabäcken humus in the other three papers.



The SOM samples were further divided into particulate soil organic matter (POM) and dissolved soil organic matter (DOM) fractions. Since DOM consists of organic matter with a continuum of molecular sizes, a definition is required. In this thesis, the DOM fraction is defined as SOM remaining in soil solution after either filtration through a 0.45 μM filter or following centrifugation at 14,600g for 10 minutes. Comparisons showed no difference in DOC concentration (absorbance at 254 nm) between filtered and centrifuged DOM. DOM alone is referred to as single-phase SOM systems and experiments with both DOM and POM are referred to as two-phase SOM systems.

Preparation of DOM - POM experimental systems

DOM stock solution

For laboratory experiments, a DOM stock solution was prepared from the SOM sample. This was obtained by adding a Na^+ saturated metal-cation chelating resin to a sample of homogenised SOM (**Paper II**). The major polyvalent cations like Al^{3+} and Ca^{2+} that bind and flocculate organic substances in the SOM sample were complexed by the resin and DOM was released into solution. After centrifugation and filtration, the stock solution of DOM was mixed with original SOM, or H^+-saturated SOM (see below), to create manipulated DOM-POM systems with varying concentrations of adsorbed major cations (Na^+, Ca^{2+}, Al^{3+}), pH and ionic strength.

Hydrogen ion washing of SOM

To obtain a homo-ionic material for the DOM-POM systems used in the experiments, the SOM sample from Västrabäcken was sequentially acid washed with diluted HCl solutions. After the complete protonation of the SOM sample, it was rinsed with distilled water until no chloride ions could be detected by adding a saturated $Ag(NO_3)_2$ solution. All washing was done carefully to minimise the loss of organic matter. Despite this, some losses of DOM were inevitable.

DOM-POM kinetic batch experiments

Non-protonated SOM samples were used for the time-dependent adsorption experiments reported in **Paper II** (non-sterile conditions) and **Paper III** (sterile and non-sterile conditions). After at least a week of pre-equilibration of DOM with POM in a larger batch, the aqueous phase with DOM was separated from the solid phase (POM) by centrifugation at 14,600 g. Duplicates of aqueous phase with DOM and POM were mixed corresponding to a 50:1 mass:dry mass ratio in 4 or 7 mL glass vials. After 24 h of mixing by turning the vials end-over-end, TNT, nitrobenzene, aniline or toluene was added to them. Except for TNT, non-labelled compounds were used in the kinetic experiments. For radiated series all lab-ware were autoclaved, heated at 160 °C for 2 h or washed in methanol. The DOM-solutions were filtered with a sterile 0.22 µm filter to remove micro-organisms. The SOM sample was treated with γ-radiation. The microbial activity in each vial was tested by a plate-count method described in Trevors (1996). The overall biological activity in the radiated and untreated soils was determined by monitoring respiration according to a method described in Nordgren (1992). The radiated systems are henceforth referred to as sterile and non-radiated as non-sterile systems, respectively. Blanks without SOM or DOM were prepared and sampled to monitor possible side reactions that might occur, for instance with the glass walls of the vessels.

DOM-POM equilibrium experiments

Manipulated DOM-POM systems were created with a varied composition of adsorbed H, Na, Ca and Al ions at constant ionic strength and at different pH-values, set by adding different concentrations of NaOH + NaCl (Na-system), $Ca(OH)_2$ + $CaCl_2$ (Ca-system) and 3NaOH + $AlCl_3$ (Al-system), to original SOM (**Paper II**), and to H^+-saturated POM (**Paper III**). The DOM concentration was kept as constant as possible by addition of a DOM stock solution until the DOM concentration was stable over time. After at least a week of pre-equilibration of DOM with POM in a larger batch, the aqueous phase with DOM and the solid phase (POM) were separated by high-speed centrifugation. Duplicates of aqueous phase with DOM and POM were mixed corresponding to a 50:1 mass:dry mass ratio in 4 or 7 mL glass vials. Different concentrations of ^{14}C-labelled TNT, nitrobenzene, aniline or toluene were added to duplicate vials. The DOM-POM systems were equilibrated in darkness at 22 °C by end-over-end shaking. After 22 h (TNT, nitrobenzene and toluene) or 72 h (aniline), the DOM-solution and POM were separated by centrifugation and concentrations of H^+, DOM, analytes in solution and analytes associated with DOM were determined. For all series, blanks without DOM + POM, but otherwise treated in the same way as the other samples, were monitored for possible side reactions (*e.g.* with glass walls).

Chemicals

Selected properties of the investigated chemicals are reported in Table 1. Aniline, nitrobenzene and toluene all have a benzene ring with different substituents. Toluene is often referred to as volatile, nitrobenzene and aniline as semi-volatile and TNT as non-volatile. The low vapour pressure of TNT makes its air-borne fraction negligible in mass balance calculations.

Table 1. *Chemical properties of studied organic substances*

Name	**Formula**	**Mw**	**pK_a**	**Boiling point (°C)**	**log K_{ow}**	**Solubility in water ($g \times L^{-1}$)**
TNT	$C_7H_5N_3O_6$	227.13	--	*250*[†]	1.86[§]	0.13[††]
Nitrobenzene	$C_6H_5NO_2$	123.11	--	210[‡]	1.84[#]	2.00[‡]
Aniline	C_6H_7N	93.13	4.6[‡]	185[‡]	0.90[#]	34.97[‡]
Toluene	C_7H_8	92.13	--	111[‡]	2.59[#]	0.67[‡]

[†] Beginning of decomposition (Yinon, 1990)

[‡] Solubility was determined at 25 °C (Anonymous, 2001)

[§] Haderlein, Weissmahr & Schwarzenbach (1996)

[#] Average of tabulated values in Hansch & Leo (1979)

[††] Determined at 20 °C (Yinon, 1990)

-- not applicable

Analyses

Reversed Phase Chromatography (RPC)

To separate the DOM fraction from the free analytes, an end-capped reversed phase C_{18}-column was used in a Waters HPLC system. This system was also used to quantify the free concentrations of the different analytes and possible degradation products. A schematic diagram of the apparatus is presented in Fig. 6. The major reactions responsible for separation in this system are the hydrophobic interactions between the analytes, the stationary phase (column) and the mobile phase (eluent) with varying water:organic solvent ratios. The mobile phase was adjusted to ensure adequate separation of the DOM and the compound of interest. The DOM fraction includes constituents with a continuum of properties from highly hydrophilic to more hydrophobic, and may cause separation problems with very hydrophilic compounds of interest. In this study, however, we encountered no separation problems.

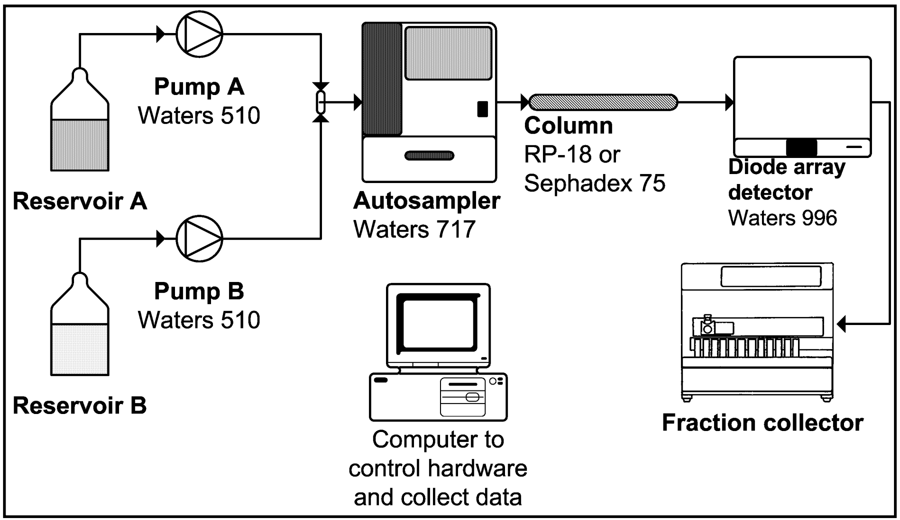


Figure 6. Schematic diagram of the High Performance Liquid Chromatography apparatus used in this study. Waters Millennium software was used to process the data and to monitor the hardware.

Size-Exclusion Chromatography (SEC)

As the name suggests, SEC is supposed to mainly separate molecules with different sizes. In reality, it is not strictly the size of the molecule, but rather differences in the volume of the hydrated molecules that are responsible for the separation in the column. In addition to the volume of the molecule, electrostatic effects and hydrophobic interactions may also play a significant role in the separation process. In order to minimize electrostatic forces, a high ionic strength in the mobile phase solution is maintained, and the water:organic solvent ratio is adjusted to minimize undesired hydrophobic interactions.

Molecular size is expressed in units of Daltons (Da) and is equivalent to the molecular weight. For small substances such as TNT, the molecular size is a well-defined property but for DOM, which represents an undefined continuum of molecular sizes that is very sensitive to changes in factors such as pH and ionic strength, size is not clearly defined. Two statistical descriptions of the DOM molecular size continuum are the weight- and number-averaged molecular masses (Yau, Kirkland & Bly, 1979). The weight-averaged mass is equal to or larger than the number-averaged mass, and the ratio between them indicates the breadth of the molecular mass distribution. A ratio close to one indicates a monodisperse system and high values indicate cross-linked polymers.

Carbon-14 determination

^{14}C-labelling was used in laboratory experiments to detect the DOM-associated fraction of the added organic substances. The HPLC eluates were fractionated and the fraction containing the DOM was collected in scintillation vials for analysis on a liquid scintillation system. Any quenching and background activities were corrected for before calculating the ^{14}C labelled substance concentration. For solid samples, a carbon combustion apparatus was used to detect sorbed substances after desorption in the studies reported in **Paper I**. The CO_2 collected in the carbon-trap was analysed by liquid scintillation. It is assumed that any molecular weight difference in carbon isotopic abundance had no influence on the studied processes. All substances were labelled in the benzene/toluene ring.

For degradable chemicals like TNT it is not easy to distinguish between sorbed TNT and sorbed degradation products. Therefore, the sum of all degradation products and TNT, determined by measuring ^{14}C activity in DOM or POM, will henceforth be referred to as TNT*.

DOM and SOM characterisation

The DOM concentration was determined by light absorption at 254 nm, either with a stand-alone photometer or online with the HPLC equipment. These measurements were correlated to total organic carbon measurements by a Schimadzu 5000 TOC analyser. SOM organic carbon was determined by dry combustion in a CHN elemental analyser. Metal cations adsorbed to original SOM samples were determined by sequential $CuCl_2$ extraction using a method of Skyllberg & Borggaard (1998) and the total acidity was determined at pH 8.2 (Thomas, 1982). Total cation-exchange capacity at pH 8.2 was determined for original SOM samples as the sum of total acidity and charges of adsorbed Na, K, Mg and Ca.

Data evaluation

Isotherms

Isotherms are commonly used to describe sorption data mathematically. To ensure a good foundation for data evaluation a minimum of five concentration levels should be used and preferably more. Isotherms describe the relationship between sorbed concentration and free substance concentration in solution. There are a number of different types of isotherms but the most fundamental distinction is between linear (equation [1]) and non-linear isotherms. The non-linear isotherms can be fitted with Freundlich [2] and Langmuir [3] equations. The linear isotherm is the simplest form, and if it can be fitted to data capturing a large range of concentrations of free substance it may be indicative of hydrophobic partitioning. The linear isotherm suggests there is not a limiting binding capacity for the substance being considered. The Langmuir equation may be used to determine a limited number of specific bonding sites, which is characteristic of specific chemical interactions. The Freundlich model is a purely empirical model, used strictly to parameterise and compare data. It is important not to use a more complex model than necessary to describe the sorption data. If linear models are sufficient, the non-linear models will not add significant information for interpretation of the governing binding processes.

Since several different types of sorption processes may occur at the same time, it is possible to combine linear isotherms with non-linear ones, or to describe simultaneous binding to both a weak and a strong binding site by combining two non-linear isotherms (Xia & Pignatello, 2001). Equation [4] describes a situation where some of the molecules of a substance occupy a non-limited binding capacity (hydrophobic partitioning) and some of the molecules bind to a limited number of functional groups (Langmuir isotherm). This situation is very realistic for most organic compounds with hydrophobic moieties (*e.g.* benzene rings) and more or less reactive functional groups (*e.g.* amino groups).

Linear: $$C_s = K_{OC} \times C_w \qquad [1]$$

Freundlich: $$C_s = K_f \times C_w^N \qquad [2]$$

Langmuir: $$C_s = \frac{q_{max} \times K_L \times C_w}{1 + K_L \times C_w} \qquad [3]$$

Langmuir + linear: $$C_s = \frac{q_{max} \times K_L \times C_w}{1 + K_L \times C_w} + K_{OC} \times C_w \qquad [4]$$

In this thesis, C_S represents the concentration of sorbed substance expressed in relation to the mass of organic carbon in DOM or POM (mol×kg^{-1} C). C_W is the equilibrium concentration of uncomplexed compound (mol×L^{-1}) in solution. K_{OC} and K_f are partitioning coefficients for the linear and Freundlich equations, respectively (L×kg^{-1} C), and N is the power of C_W in the Freundlich equation. In the Langmuir equation, q_{max} is the maximum sorption capacity, assuming the sorbate is arranged in a monolayer (µmol×g^{-1} C) at the adsorbing surface, and K_L is the Langmuir binding constant.

Mobility isotherms

From an environmental point of view, the mobile fraction of a toxic substance may be of most interest with respect to the potential hazard it poses to natural ecosystems. The toxicity and bioavailability of organic contaminants sorbed to DOM and POM remains uncertain, but it is a potential pool of contamination. Since DOM bound contaminant is most prone to transportation, it may be of interest to describe the relationship between the total concentration of potentially mobile compound (free compound + DOM associated compound) and the soil-bound concentration of the compound. So called ‘mobility isotherms’ (describing the level of soil bound compound as a function of potentially mobile compound) were calculated for TNT*, aniline, nitrobenzene and toluene in the study presented in **Paper III**.

Experimental design

In **Paper I** a fractional factorial design (FrF) was used to evaluate how seven different factors affected sorption and desorption of aniline and TNT in the soil. Data were evaluated by partial least squares projection to latent structures (PLS) analysis, after appropriate transformation of the data distribution. This is an efficient method to determine the most important factors affecting the investigated response. One drawback of the fractional design is the inherent confounding of variables that must be considered in the evaluation. Only three levels were investigated in the FrF and this makes it impossible to determine curvature in the functions describing the processes. To obtain more detailed information, response surface modelling (RSM) experimental designs could be used.

Results and discussion

Kinetic experiments

There are several reasons for conducting a kinetic study. One is to determine the reaction time required for a chemical equilibrium to be attained. Kinetic experiments may also be used to gain knowledge about reaction mechanisms. In **Paper II** the kinetics governing the adsorption of TNT* to POM and DOM were investigated, and in **Paper III** the kinetics describing the adsorption of TNT*, aniline, nitrobenzene and toluene to SOM were examined, under both sterile and non-sterile conditions.

Aniline, nitrobenzene and toluene

All three compounds, aniline, nitrobenzene and toluene, showed a rapid initial decline in free concentrations when added to SOM, indicative of almost instant binding. Free nitrobenzene and toluene in solution reached equilibrium with their respective SOM-associated fractions within approximately 45 minutes. In contrast, aniline sorption showed a biphasic pattern with a slower decay after an initially rapid sorption (Fig. 7). This pattern has been taken to indicate that bonding rearrangements occur, from an initially reversible electrostatic attraction, or hydrophobic partitioning, to an irreversible covalent bonding (Weber, Spidle & Thorn, 1996; Fabrege-Duque *et al.*, 2000). The proposed irreversible covalent bonding has been suggested to involve SOM functional groups, such as quinones.

Effects of γ-radiation

There were no major differences in aniline and nitrobenzene concentrations following treatment with γ-radiation in experiments designed to determine the effects (if any) of sterilisation (**Paper III**). Thus, biodegradation of aniline and nitrobenzene had little effect on the binding of these compounds to SOM. In the toluene non-sterile series, on the other hand, the free concentration of toluene continued to decrease almost linearly with time and after nine days virtually no toluene remained in solution. This suggests that biodegradation played a significant role in the removal of toluene from solution and its incorporation into SOM.

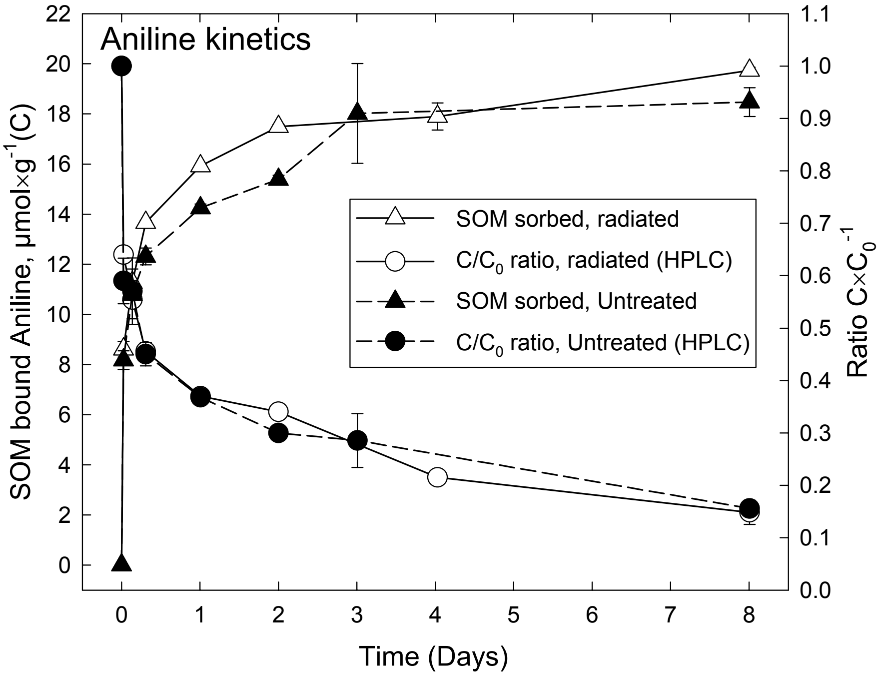


Figure 7. Aniline sorption and degradation kinetics in both radiated and non-irradiated SOM systems. Aniline has a typical biphasic degradation pattern, indicating that combinations of different types of binding are involved. C_0 refers to initial aniline concentration in solution. Error bars represent one standard deviation of the mean from duplicates.

TNT

TNT concentration kinetics in a SOM environment can be divided into two phases: an initially rapid sorption of TNT to POM and DOM followed by a slower decrease in free TNT concentration due to a combination of degradation and sorption processes. In the studies described in **Papers II** and **III**, the concentration of TNT was followed for at least a week in order to monitor processes other than initial binding. A decrease in TNT concentration involves sorption of TNT, as well as both biotic and abiotic transformations in combination with binding to soil constituents, *i.e.* SOM.

Effects of γ-radiation

Biotic transformations of TNT were shown to be closely related to microbial activity, which in turn is related to the SOM concentration in the soil system. For TNT, both the biodegradation and the extent of the association to SOM increased with increasing SOM (POM+DOM) concentrations in the studied system (**Paper II**). Interestingly, a dialysis experiment showed that it was the concentration of DOM, rather than the absolute amount, that was most critical for the biological degradation. Since the total SOM concentration is important for DOM sorption of degradable compounds like TNT, it is impossible to obtain relevant results for TNT* sorption to DOM (or humic substances) using laboratory samples that do not also contain solid phase organic matter (*i.e.* POM).

In **Paper III,** γ-radiation of SOM was shown to have a substantial effect on the binding of TNT* to DOM, whereas the binding to POM (expressed as $\mu mol \times g^{-1}$ [C]) was affected less (Fig. 8). The most likely explanation for the much stronger binding of TNT* to DOM in non-sterile systems is that TNT degradation products with reactive functional groups (ADNT, DANT, HADNT, nitroso- and azoxy-compounds) have much higher reactivity towards certain functional groups in DOM than TNT does. Based on findings in recent studies, the functional groups in SOM involved in these reactions may be carbonyls and quinones, as in the reactions with aniline (Thorn & Kennedy, 2002). On an organic C basis, the concentrations of these types of functional groups are lower in POM, as suggested by the equilibrium studies for aniline (see below). The minor effect radiation had on the binding of TNT* to POM suggests that a large part of the TNT* associated with POM is non-degraded TNT. Some biological activity also remained after radiation. Interestingly, this eventually led to the degradation of TNT even in the sterile system resulting, after 28 days, in a very similar distribution of TNT* bound to DOM and POM to that seen in the non-sterile system (Fig. 8). Thus, this observation gives additional support for the conclusion that the binding of TNT, especially to DOM, is highly dependent on the formation of reactive TNT derivatives.

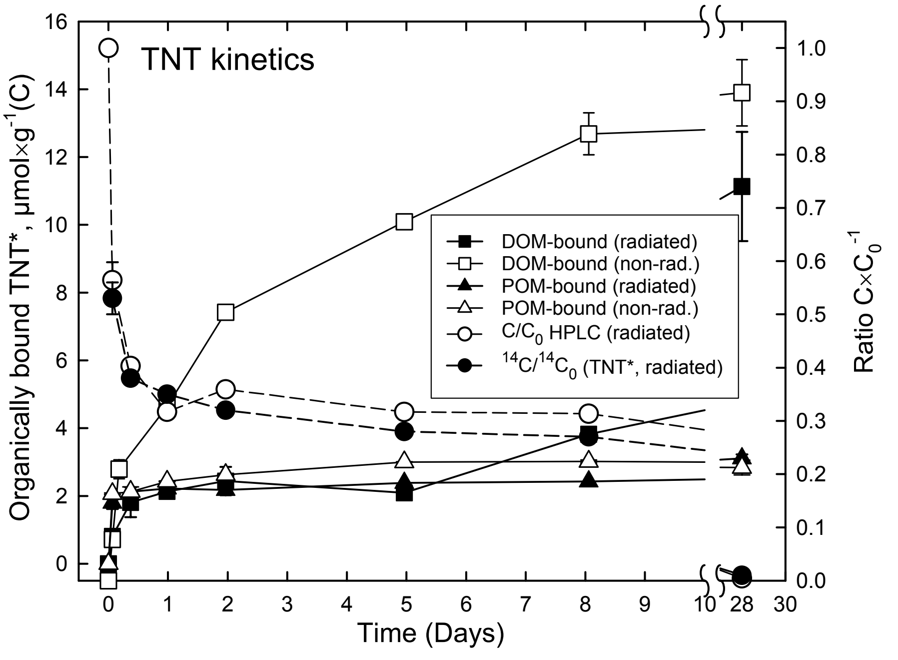


Figure 8. TNT sorption kinetics in a irradiated system. Note that DOM associated TNT* from a non-irradiated is illustrated in the same figure. C_0 refers to initial concentration of TNT in solution. Error bars represent one standard deviation of the mean from duplicates.

Indirectly, DOM sorption provides a measure of both sorption and degradation of TNT in a SOM environment. The formation of amino groups (or possibly other reactive groups) makes sorption processes of TNT similar to those of aniline. The slow binding of TNT* to DOM indicates that several types of binding are involved. Transformation from mainly electrostatic and hydrophobic interactions at first, to some degree of covalent bonding, as for aniline, is a plausible explanation for the kinetic patterns.

Ionic and pH effects on TNT binding kinetics to DOM

Ionic strength showed only small effects on the binding kinetics, whereas a pH increase from 3.7 to 6.0 greatly increased the binding of TNT* to DOM (**Paper II**). It is uncertain if the change in pH affected the microbial activity in the systems, thereby changing the degradation and binding of TNT* to DOM. In the one-phase DOM system, a 50-fold increase in ionic strength only decreased the TNT* binding to DOM to a minor extent. These observations are consistent with results from the sterile and non-sterile kinetic experiments, collectively indicating that the binding of TNT* to DOM involves mainly the binding of TNT degradation products. In addition, the pH effect indicates that pH-dependent functional groups of DOM may be involved in the binding.

Equilibrium experiments

Important variables – fractional factorial experimental design

In order to identify the most important factors affecting sorption of TNT and aniline in soil, and to quantify possible interactions between factors, a fractional factorial design was used to optimise the number and types of laboratory experiments. The effects of the following seven factors: pH, Ca/Na-ratio, adsorbent concentration, temperature, and levels of montmorillonite, kaolinite and SOM, on sorption and desorption were investigated, at three levels for each factor (**Paper I**). The results identified SOM as the most important factor for the binding of TNT and aniline, accounting for most of the variance for both sorption and desorption coefficients. For sorption coefficients, montmorillonite was the second most important factor, whereas the adsorbent concentration was the second most important factor for desorption. Some interaction factors were also of significance.

In systems with SOM alone the binding aniline increased when pH increased from 3.7 to 5.0. It is well known that organic cations show an adsorption maximum around at its pKa-value. When montmorillonite was mixed with SOM the pH had no significant effect on sorption. This may be explained by competing binding processes involving SOM and montmorillonite. If clay binding is electrostatic, it should be favoured when the aniline molecule is positively charged, thus when pH is below the pKa value of 4.6. Covalent bond formation with SOM is favoured by neutral species of aniline and the predominance of quinone over hydroquinone. This situation is favoured by a higher pH.

The results of the statistical model give good directions for further experiments and identify factors that need to be investigated in more detail to derive quantitative and mechanistic descriptions of sorption and desorption processes in soil. To gain detailed information and to obtain higher order model terms, a response surface method (RSM) could be used, and could prove to be more useful than the isotherm models used in many studies.

Aniline, nitrobenzene and toluene

Besides the new, specific information gained by studying the sorption of aniline and nitrobenzene to a mixed DOM-POM system, these compounds could be seen as model compounds for TNT and its degradation products. The reductive amino-degradation products of TNT (ADNT and DANT) have recently been shown in ^{15}N-NMR spectroscopy studies to bind to humic acids in a similar way to the amino group of aniline (Thorn & Kennedy, 2002). The benzene ring of nitrobenzene, which has an unreactive nitro group, may be assumed to show a hydrophobic partitioning similar to TNT (which has three nitro groups).

Based on the kinetic experiments, an equilibration time of 72 h was used for aniline, and 22 h for nitrobenzene and toluene equilibrium experiments (**Paper III**). The adsorption isotherms were, as expected, very different for aniline and nitrobenzene. Aniline binding to both DOM and POM was best fitted by a Langmuir isotherm (Fig. 9), whereas NB data were best fitted by linear isotherms, for both DOM and POM (Fig. 10). The pH was similar in both studies (4.9-5.1), and changing between Na and Al as the predominant cation adsorbed to POM made no significant difference to the results. The non-linear isotherm for aniline is in agreement with recent studies showing a specific interaction between aniline amino groups and functional groups in organic substances of soil. The binding capacity (q_{max}, equation [3]), as determined by the Langmuir isotherm, was 124 μmol×g^{-1} [C] for POM and 160 μmol×g^{-1} [C] for DOM, indicating that DOM has a slightly higher density of functional groups that are reactive towards aniline, on an organic C basis. In contrast to aniline, nitrobenzene showed linear isotherms indicative of a hydrophobic partitioning type of binding to both DOM and POM over a large concentration range. The partitioning coefficient for the linear model (K_{OC}) was on an average 70 L×kg^{-1} [C] for POM and 36 L×kg^{-1} [C] for DOM. Thus, the volume of hydrophobic moieties for molecules of the size of nitrobenzene appears to be twice as high in POM, as compared to DOM.

In Fig. 11 average data and fitted adsorption isotherms are illustrated on a logarithmic scale. For all substances the combined isotherm of equation 4 was used, representing a mixed linear + Langmuir model. With this model aniline also showed some hydrophobic partitioning (K_{OC} = 31 L×kg^{-1} [C] for POM and 29 L×kg^{-1} [C] for DOM), while q_{max} values were 104 μmol×g^{-1} [C] for POM and 143 μmol×g^{-1} [C] for DOM. Thus, the hydrophobic partitioning of aniline was quite similar to the hydrophobic partitioning of nitrobenzene to DOM, and the specific binding capacity was approximately 40% higher for DOM.

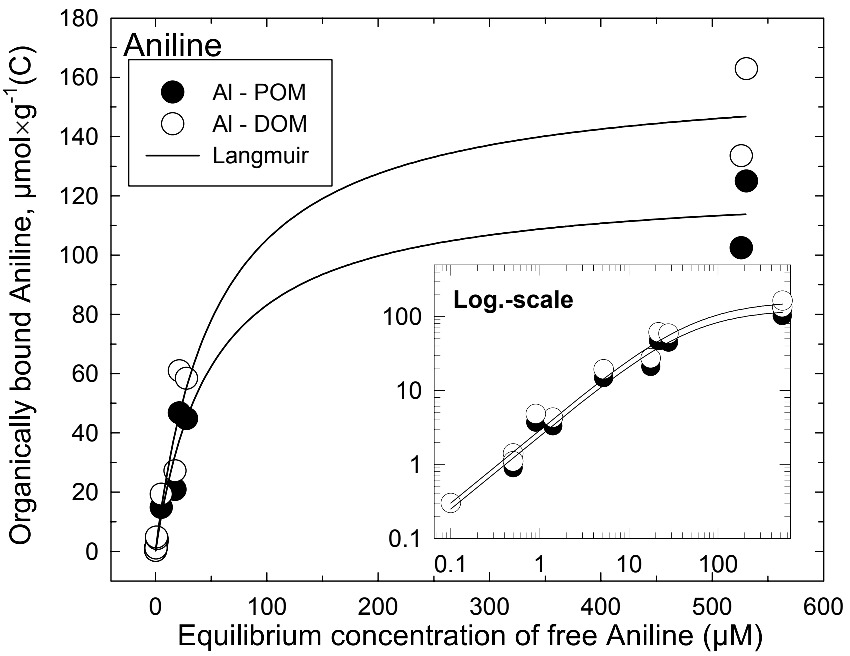


Figure 9. Adsorption isotherms describing the association of aniline to POM and DOM. Aluminium-dominated system characteristics: [DOM] = 570 mg (C)×L^{-1}, [POM] = 9.5 g (C)×L^{-1}, Na_{ads} = 340 $mmol_C$×kg^{-1} (POM), Al_{ads} = 210 $mmol_C$×kg^{-1} (POM), and pH = 4.9. Solid lines represent fit of eq [3].

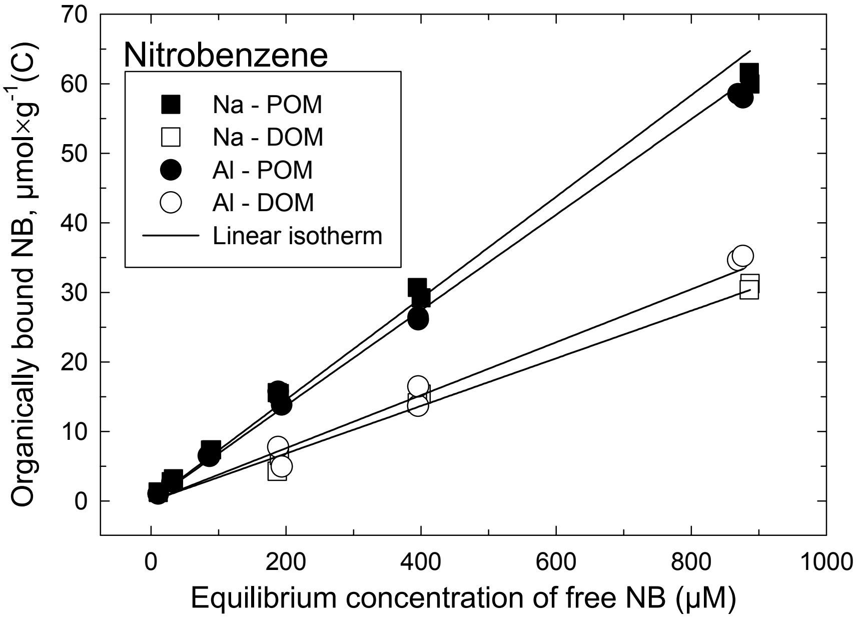


Figure 10. Adsorption isotherms describing the association of nitrobenzene to DOM and POM. Sodium-dominated system characteristics: [DOM] = 500 mg (C)×L^{-1}, [POM] = 8.5 g (C)×L^{-1}, Na_{ads}= 600 $mmol_C$×kg^{-1} (POM), Al_{ads}< 10 mmolckg-1 (POM) and pH = 5.1. Aluminium-dominated system characteristics: [DOM] = 590 mg (C)×L^{-1}, [POM] = 9.6 g (C)×L^{-1}, Na_{ads}= 80 $mmol_C$×kg^{-1} (POM), Al_{ads}= 240 $mmol_C$×kg^{-1} (POM), and pH = 5.1.

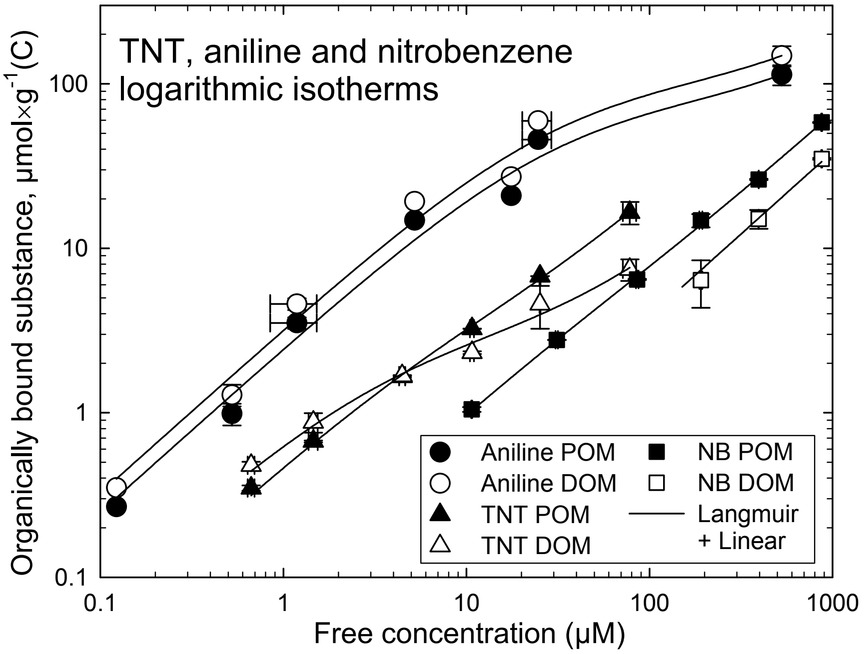


Figure 11. Adsorption isotherms for the binding of TNT*, aniline and NB to DOM and POM with Al as the predominant adsorbed metal cation at pH 5.0. Solid lines show the fit of equation 4 to data on a logarithmic scale.

In **Paper IV** the DOM-associated aniline was fractionated into different size classes using size-exclusion chromatography (SEC). Results are given in Fig. 12. Even if aniline was bound to all DOM size fractions there was an increase in associated aniline (expressed as µmol×g^{-1} [C]) with decreasing size of DOM. For aniline, the association with the smallest of three size fractions (<3.5 kDa) was 3.5 times higher than for the largest size fraction (>40 kDa), on an organic C basis, at pH 3.7. At pH 5.1 up to 8.5 times more aniline was associated with the smallest fraction, as compared to the largest. The effect of pH on aniline binding to DOM is consistent with expectations, given that the pKa value of the amino group is 4.6 and that the neutral form of the amino groups is most reactive in nucleophilic addition reactions towards carbonyl and quinone groups in DOM (Weber, Spidle & Thorn, 1996).

An artefactual effect in the SEC study that probably exaggerated the adsorptive capacity of small molecules is that the ratio between UV absorption and organic C is substantially lower for smaller DOM molecules and humic substances than for their larger counterparts (Specht, Kumke & Frimmel, 2000). This effect was not corrected for in our studies since organic C equivalents were calculated from UV absorption data using a linear equation. However, this effect is far from large enough to explain the increase in reactivity associated with reductions in DOM size. Thus, it seems that the density of functional groups in DOM that are reactive towards aniline (likely carbonyl and quinone groups), increased with decreasing size of DOM molecules.

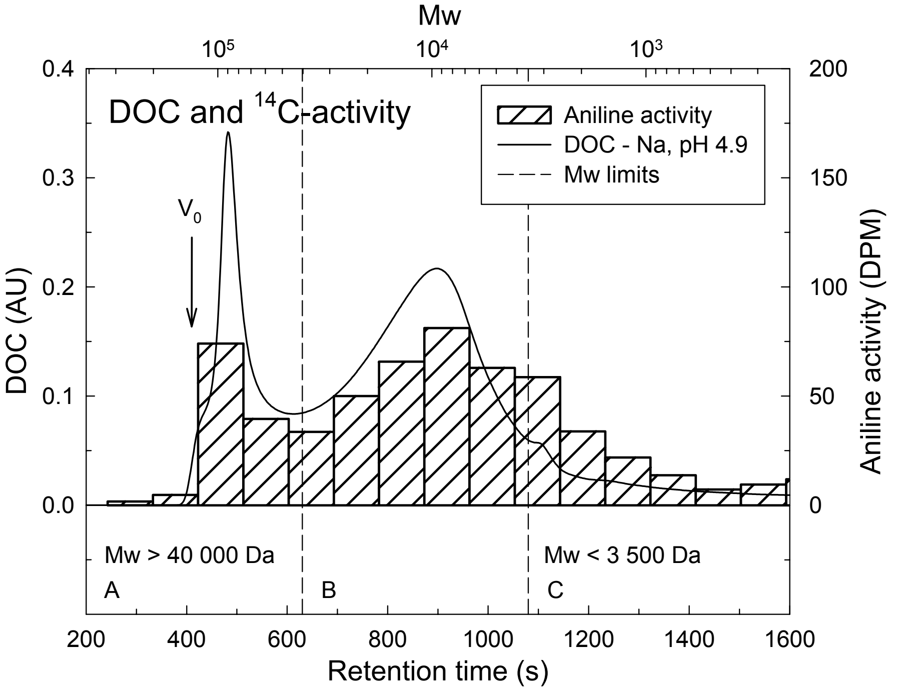


Figure 12. A SEC chromatogram for Västrabäcken DOM and associated ^{14}C-activity of collected fractions. There is a preference for aniline to bind to small-sized DOM fractions, suggesting they have higher densities of functional groups that are reactive towards aniline, as compared to DOM fractions of higher sizes.

TNT

Based on the kinetic experiments, equilibration times of 20-22 h were chosen in order to approach equilibrium between free and bound TNT and to avoid extensive biological degradation of TNT.

DOM isotherms

One apparent feature of isotherms describing the binding of TNT* to DOM was their clear non-linearity, indicating a specific type of binding occurred. This was seen for DOM extracted from the less decomposed O horizon sample used in the study reported in **Paper II**, as well as for DOM extracted from the more humified O-horizon sample used in the study reported in **Paper III**. In both studies, non-linear Langmuir and Freundlich isotherms, in contrast to a pure linear model, could be used to provide satisfactory descriptions of the data. In general, the isotherms fitted to DOM data were more non-linear (*i.e.* given by a much lower N-value of the Freundlich isotherm, **Paper II**) than isotherms fitted to POM data (Fig. 13).

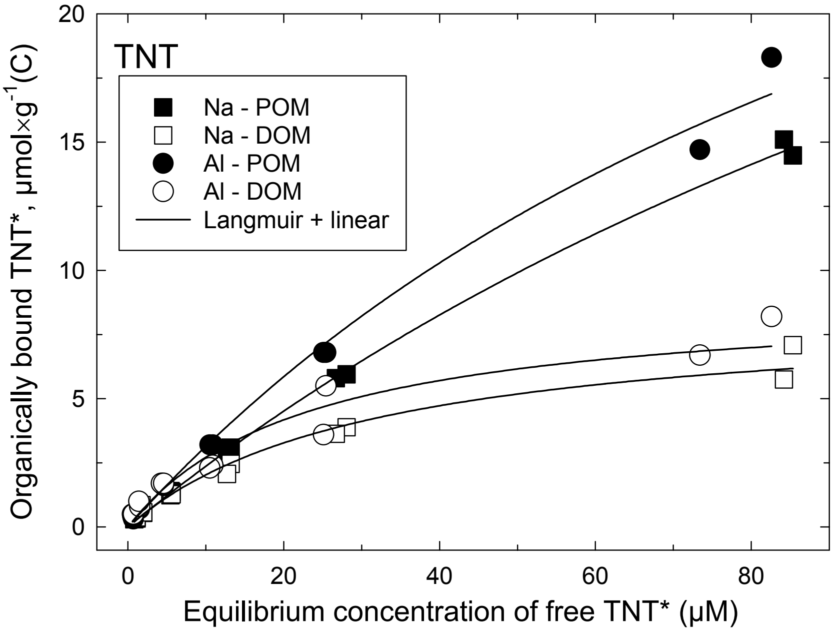


Figure 13. Adsorption data for the binding of TNT* to POM and DOM at pH 5.0 with Na and Al as the predominante adsorbed metal cations. The solid line shows the fit of a combined linear + Langmuir isotherm equation [4].

The concentration of SOM (POM+DOM) had a great influence on the binding of TNT* to DOM (**Paper II**). This is presumably because the degradation of TNT was stimulated by the concentration of organic matter in the system, and the degradation products showed a high reactivity towards DOM (see kinetic study above). The correlation between increases in the degradation of TNT and SOM concentration may be due to microbes associated with SOM, and it is possible that some SOM fractions were used as energy substrates for the reduction of TNT.

The pH-value had a strong influence on the binding of TNT* to DOM, and in line with findings in the kinetic studies, reductions in pH generally resulted in less TNT* binding (**Paper II)**. When comparing the Langmuir constants for DOM sorption, both the binding constant and the sorption capacity increased with rises in pH (4.4-6.2) in the two-phase system, although the capacity seems to have more or less two discrete levels (**Paper II**). The strong pH-effect is consistent with the conclusion that functional groups are involved in the binding of TNT* to DOM. In contrast, the binding of TNT* to POM was essentially insensitive to pH (see below). There are several possible explanations for the way the change in H^+-concentration affected DOM and TNT* binding. When negatively charged functional groups like carboxyls become protonated and uncharged, the change leads, in turn, to a flocculation and contraction of DOM structure. It is not only the structure of DOM that changes with pH and ionic composition, but also its chemical properties and possibly its binding parameters (Myneni *et al.*, 1999).

The composition of the major cations adsorbed to POM did not greatly affect the sorption of TNT* to DOM. Neither did the origin of the DOM seem to matter significantly. The DOM used in **Paper II** had approximately the same sorption capacity and a slightly higher Langmuir constant compared to the Västrabäcken DOM used in **Paper III** at the same pH and ionic strength.

In **Paper III** the binding of TNT* to DOM was modelled by a mixed linear + Langmuir isotherm (equation [4]). The outcome of the model is illustrated together with collected adsorption data in Fig. 13. The partitioning coefficient for DOM (K_{DOC}; 41-68 L×kg^{-1} [C]) was quite similar to that of nitrobenzene (34-38 L×kg^{-1} [C]), indicating that the hydrophobic partitioning to DOM was similar for the two compounds. The specific bonding properties, as described by the Langmuir parameters K_L and q_{max}, was much lower than for aniline. This is not surprising given that only a small proportion of the TNT* in solution is represented by degradation products with reactivity comparable to aniline.

A SEC study was conducted in order to determine whether TNT, like aniline, showed preference for any of the DOM size fractions. Just as for aniline, TNT* (determined as ^{14}C activity) showed increasing association with decreasing size of DOM fractions. Approximately ten times more TNT* was associated with the smallest DOM fraction (<3.5 kDa), as compared to the largest fraction (>40 kDa). Thus, even if UV absorbance per organic C atom decreased as the DOM size fractions increased (see above), it is apparent that the smaller DOM fractions show a higher reactivity towards TNT* than larger DOM fractions. In accordance with the binding kinetics and equilibrium experiments, we can conclude that specific binding between TNT derivatives and functional groups is of utmost importance for the binding of TNT to DOM, and that the density of reactive functional groups increases with decreasing DOM size. The only other SEC study of TNT we know of did not find any preferential binding of TNT towards smaller HA-molecules (Achtnich *et al.*, 1999b). The most likely reason for this difference in results, was the long equilibrium time (80 days) used in the cited slurry experiments, leaving most TNT* bound tightly to the organic matter (due to biodegradation and subsequent strong, covalent bonding).

A method comparison of SEC and RPC for the determination of TNT*associated with DOM is presented in **Paper IV**. If the binding of TNT* to all DOM size fractions are summarized, the SEC method gives a lower estimate than RPC of TNT* binding to DOM. There may be several reasons for this difference. Even small errors in background subtraction for just one of the many SEC fractions may lead to quite a large underestimation of ^{14}C activity when figures for all fractions are summed. In the RPC method the sorption of DOM (and associated TNT*) within the column is corrected for, but there may still be an error. However, despite the discrepancy, the sorption coefficients derived using the two independent methods were of the same order of magnitude.

POM isotherms

In sharp contrast to DOM, a linear adsorption isotherm was almost as successful as the non-linear Freundlich and Langmuir isotherms for describing the binding of TNT* to POM. This was reflected by the absolute TNT* binding capacity being five times higher for POM (42-49 µmol×g^{-1} [C]) than for DOM (8.5-9.0 µmol×g^{-1} [C]), using the Langmuir isotherm for data collected at pH 5.0 for SOM from the highly decomposed O horizon (**Paper III**). In the pH-dependent study (**Paper II**), using SOM from the less decomposed O horizon, the binding capacity of POM was between 7 (pH 6.2) and 22 (pH 5.2) times greater than for DOM.

Another important observation was that the binding of TNT* to POM was much less pH-dependent than the binding of TNT* to DOM (**Paper II**). In addition, kinetic studies showed that the binding of TNT* to POM was rapid, regardless of whether the material had been irradiated, and only weakly dependent on the formation of TNT degradation products (**Paper III**). Collectively, these observations strongly suggest that weak, hydrophobic partitioning was the predominant binding mechanism between TNT* and POM within 22–24 h. Since a specific type of binding of TNT* to functional groups in POM (similar to that suggested for its interaction with DOM) appears to occur, a dual-mode sorption model can be applied to the POM data, combining specific and hydrophobic partitioning at low concentrations and mainly partitioning with little specific binding at high TNT concentrations. This dual-mode sorption model is similar to models used for the binding of other substances to SOM (Xing & Pignatello, 1997; Zhu, Chen & Shen, 2000).

In **Paper II** a dual-mode model was used to describe the binding of TNT* to POM. This model was based on the assumption that the specific bonding of TNT* to functional groups was identical (on an organic C basis) in both DOM and POM, and that specific interactions were the only binding mechanisms involved in interactions with DOM. Based on this very simple model, a K_{OC} of 205 (± 22) $L \times kg^{-1}$ [C] was determined for the hydrophobic partitioning of TNT* in POM (using data within the pH-interval 4.4–6.2). If the same model was used for SOM from the more decomposed O-horizon a K_{OC} of 90 $L \times kg^{-1}$ [C] was obtained (at pH 5.0). Thus, the more decomposed SOM showed weaker hydrophobic partitioning than SOM from the less decomposed O horizon. The specific bonding parameters of TNT* in DOM were very similar for the two SOM materials: (**Paper II**; pH 5.18, $K_L = 43$ nM^{-1}, $q_{max}= 8.9$ $\mu mol \times g^{-1}$ [C]): **Paper III**; pH 5.0, $K_L = 32\text{-}43$ nM^{-1}, $q_{max}= 8.5\text{-}9.0$ $\mu mol \times g^{-1}$ [C]).

The difference in K_{OC} values obtained for the two soil organic materials may be caused by differences in SOM decomposition (although we do not have any good chemical data on this visual character). The fact that SOM from Västrabäcken was hydrogen ion saturated and had a controlled composition of adsorbed cations, whereas the Kulbäckliden SOM sample did not, should also be considered. However, the composition of the adsorbed metals did not have any significant effect on the TNT* binding (**Paper III**).

In **Paper III** the mixed linear + Langmuir model (equation [4]) was used to describe the binding of TNT* to POM (and DOM). The independent fitting of linear and non-linear contributions resulted in specific bonding parameters that were slightly greater for DOM ($K_L = 99\text{-}330$ nM^{-1}, $q_{max}= 2.4\text{-}3.3$ $\mu mol \times g^{-1}$ [C]), as compared to POM ($K_L = 52\text{-}150$ nM^{-1}, $q_{max}= 2.1\text{-}2.8$ $\mu mol \times g^{-1}$ [C]). On average, for preparations differing in the amounts of Na and Al adsorbed to the POM, the specific binding capacity (q_{max}) of DOM was in average 25% greater than for POM. This can be compared with a 38% difference in specific bonding for aniline to DOM and POM. Thus, the density of functional groups with a reactivity towards TNT* degradation products was greater for DOM (on an organic C basis), as compared to POM. In contrast, the density of hydrophobic moieties was substantially greater for POM (K_{OC} = 146-187 L×kg^{-1} [C]), as compared to DOM (K_{OC} = 41-68 L×kg^{-1} [C]). This is consistent with the observation that POM had double the density, compared to DOM, of moieties that could participate in the hydrophobic partitioning of nitrobenzene. The fit of the mixed model to POM data is illustrated in Fig. 13.

*Modelling specific and non-specific binding of TNT**

In contrast to nitrobenzene and aniline, which did not undergo any significant transformation during the experiments, the binding of TNT to DOM and POM is highly dependent on the composition and concentration of TNT degradation products. Although the mixed linear + Langmuir model (Eq. 4) may be used to separate specific and non-specific (hydrophobic) binding of nitrobenzene and aniline, the binding of TNT* (y-axis) will not be properly reflected by the independent variable (free TNT*, x-axis), since only some of the TNT* derivatives are involved in the specific binding. Thus, the TNT isotherms could be considered to reflect a mixture of hydrophobic partitioning, properly described by the independent variable, and specific bonding, which is not properly described by the independent variable. One way to try to model the two mechanisms of TNT binding in a mixed model that is not subject to these errors would be to use nitrobenzene as a model compound for the hydrophobic partitioning of TNT* and aniline as a model compound for the binding of TNT derivatives. Based on these assumptions, the concentration of TNT* derivatives could then be calculated.

The first assumption is quite plausible since TNT*, aniline and nitrobenzene all have K_{OC} values of a similar order of magnitude for their associations to DOM and POM according to calculations based on the mixed model (equation [4]). Therefore, the K_{OC} value of nitrobenzene was used in equation [4] to calculate theoretical K_L and q_{max} values for the association of TNT and aniline to POM and DOM. These theoretical Langmuir parameters were in turn used to calculate the percentage degradation of TNT, assuming that the theoretical Langmuir parameters represented the binding solely of TNT derivatives to POM and DOM with a reactivity identical to aniline. The modelling calculation showed that approximately 5-8% of the TNT* was represented by TNT derivatives after 22 h of equilibration, assuming that all specifically bound TNT and its products had a reactivity similar to aniline. The fact that the percentage degradation of TNT was quite constant over the entire range of TNT concentrations (especially for the isotherms describing the binding to POM, Table 2) and was similar for POM and DOM, supports the relevance of the model and the assumptions made. Of course, this is a highly simplified calculation, since we know that there are a number of TNT derivatives, some with less reactivity than aniline and possibly some with greater reactivity. Overall, however, it may provide a reasonable estimate for the percentage of TNT* involved in specific bonding with DOM and POM.

Table 2. *Calculation of percentages of TNT derivatives in solution in equilibrium with TNT* adsorbed to POM and DOM after 22 h. It was assumed that the TNT degradation products have a similar reactivity to aniline towards POM and DOM*

TNT in solution µM	**POM**[†] % degradation	**DOM**[‡] % degradation
1	8.3	11.2
2	8.2	10.2
5	8.0	8.0
10	7.6	5.9
20	6.9	3.9
40	5.9	2.3
80	4.6	1.3

[†] Langmuir parameters used in specific binding calculations were: Aniline K_L=32, q_{max}=81 µmol×g^{-1}; TNT K_L=13, q_{max}=21 µmol×g^{-1}

[‡] Langmuir parameters used in specific binding calculations were: Aniline K_L=23, q_{max}=137 µmol×g^{-1}; TNT K_L=113, q_{max}=4.5 µmol×g^{-1}

Environmental implications

To pose a threat to surface and ground waters a pollutant must be mobile. The pollutant may be transported as a free compound in the aqueous phase, or in association with particles, colloids or dissolved inorganic or organic compounds. Of course, the toxicity of pollutants may also vary, according to whether they are free or associated with colloids. Since soil solution + DOM was separated from POM by high-speed centrifugation, colloids and smaller particles were included in our DOM samples.

The mass ratio between POM and DOM we used in our studies generally applies to both organic and mineral soils. In pure organic soils (100% POM), DOM concentrations of 300-800 mg C×L^{-1} are on the high side, but in A, AB, E, B and BC horizons of different types of northern forest soils, POM concentrations in the range 1-10% and DOM concentrations in the range 3-30 mg C×L^{-1} are quite normal (Kalbitz *et al.*, 2000). Thus, our chosen POM:DOM mass ratios can be seen as a relevant compromise for most soil horizons.

For predictive purposes we determined linear isotherm parameters describing the relationship between stationary (POM-associated) and mobile (DOM-associated + free compounds) fractions of TNT*, nitrobenzene, aniline and toluene in **Paper III**. Within the concentration range 0-100 µM, linear isotherms could reasonably well describe the relationship between fractions retained in the soil and mobile fractions for all four compounds. We call these relationships ‘mobility isotherms’. We conclude that in an organic soil with a pH around 5 and a ratio of 1% POM: 5-6 mg C×L^{-1} DOM, the aqueous concentrations (DOM-associated + free compound) of aniline:TNT:toluene:nitrobenzene would be found in ratios of 1 : 3.5 : 3.5 : 8.2 under the same load of pollutants in the soil (Fig. 14). Note that the composition of major cations (Na, Al) adsorbed to POM had little effect on the isotherm parameters for all four of the compounds.

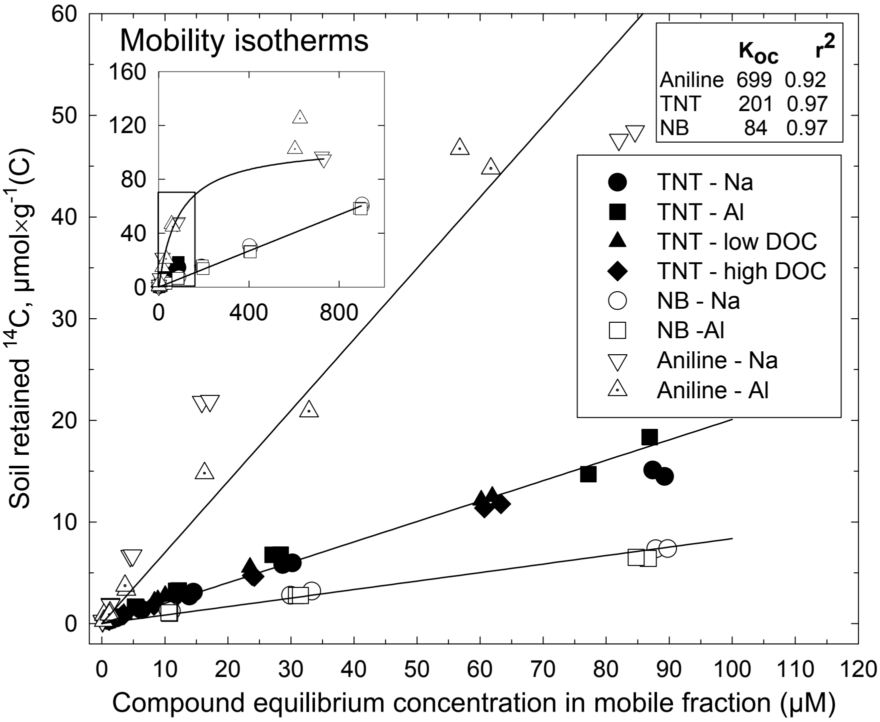


Figure 14. Average isotherms for Na and Al systems describing the relationship between soil-bound (POM-associated) compound and potentially mobile compound (sum of DOM-associated and free compound). The small inset graph shows the complete concentration range for aniline and NB.

The importance of DOM for the transportation of contaminants depends on various factors. The transportation of DOM itself is heavily dependent on solution chemistry as well as water movements. As previously discussed, higher DOM size fractions are most easily flocculated in soil. The most environmentally important fractions of DOM should therefore be those that are not prone to flocculation, but still have a high reactivity towards the contaminants. This fraction of DOM is likely small-sized and hydrophilic in structure. Both aniline and TNT showed a great preference to associate with smaller DOM molecules. In addition, the amount of the substance associated with the DOM pool was low in comparison to the amount associated with POM. Therefore, a change in DOM concentration did not influence the 'mobility isotherms' substantially for TNT, nor for the other compounds (**Paper III**).

However, it should be noted that the TNT derivatives showed a preference for DOM, as compared to POM. Thus, even if a relatively small fraction of total TNT was bound to DOM in our experiments (after 22 h) continuous degradation of TNT over time may result in transport of TNT derivatives with DOM to ground and surface waters. Leaching of freshly produced DOM from the topsoil may, over a longer time-span, deplete TNT derivatives bound to POM in deeper soil, and transport it to ground and surface waters.

If no transport of DOM occurs in a soil, the only contaminants transported will be free forms in the aqueous phase in the soil. In **Paper I,** the desorption of aniline and TNT was studied in 17 different mixed systems in which the effects of seven factors was examined. The only factors found to significantly influence desorption were concentrations of SOM and the substance itself. SOM had a negative effect on desorption, whereas the concentration of the substance had a positive influence. For TNT, the temperature had a negative influence, supporting the observation that degradation of TNT and subsequent incorporation of these species into SOM and DOM is of fundamental importance for the fate of TNT in organic soils.

Major conclusions

- The concentration of SOM was the most important variable for sorption and desorption of aniline and TNT in organic rich, acidic soil.
- TNT and its degradation products (TNT*) bind to dissolved and particulate soil organic matter via both hydrophobic (non-specific) and specific interactions. The binding to DOM is mainly specific, likely involving amino and/or nitroso and azoxy groups of TNT derivatives and pH-dependent, oxygen containing functional groups of DOM. The binding to POM is mainly hydrophobic in character and insensitive to pH and composition of adsorbed major cations. Consequently, the binding of TNT to DOM was much more dependent on biological degradation than the binding of TNT to POM.
- Adsorption of nitrobenzene indicated that the mass of hydrophobic moieties in POM (on an organic C basis) was approximately twice as high as the mass of hydrophobic moieties in DOM. Adsorption of aniline indicated that the density of functional groups showing a strong reactivity towards aniline in DOM was 38% higher than the density in POM.
- Based on the assumption that nitrobenzene can be used as a model for hydrophobic partitioning, it was calculated that 5-8% of TNT* was represented by degradation products with a specific binding reactivity similar to aniline, after 22 h of equilibration with SOM.
- Small-sized fractions of DOM showed a greater reactivity towards both TNT and aniline, as compared to larger sized DOM fractions.
- Even though a relatively small fraction of TNT* was bound to DOM in our experiments (after 22 h), as compared to POM, continuous degradation of TNT over time will result in substantial potential transport of TNT derivatives with DOM to ground and surface waters. The question remaining to be answered is: how bioavailable and toxic are TNT derivatives bound to DOM?

References

Anonymous. 2001. *The MERCK INDEX, thirteenth edition*. Merck Research Laboratories. Whitehouse Station, NJ.

Achtnich, C., Fernandes, E., Bollag, J.M., Knackmuss, H.J. & Lenke, H. 1999a. Covalent binding of reduced metabolites of [$^{15}N_3$]TNT to soil organic matter during a bioremediation process analyzed by ^{15}N NMR spectroscopy. *Environmental Science and Technology 33*, 4448-4456.

Achtnich, C., Sieglen, U., Knackmuss, H.J. & Lenke, H. 1999b. Irreversible binding of biologically reduced 2,4,6-trinitrotoluene to soil. *Environmental Toxicology and Chemistry 18*, 2416-2423.

Ahmad, F. & Hughes, J.B. 2002. Reactivity of Partially Reduced Arylhydroxylamine and Nitrosoarene Metabolites of 2,4,6-Trinitrotoluene (TNT) toward Biomass and Humic Acids. *Environmental Science and Technology 36*, 4370-4381.

Boopathy, R. & Kulpa, C.F. 1994. Biotransformation of 2,4,6-trinitrotoluene (TNT) by a *Methanococcus* sp. (strain B) isolated from a lake sediment. *Canadian Journal of Microbiology 40*, 273-278.

Boopathy, R., Widrig, D.L. & Manning, J.F. 1997. *In situ* bioremediation of explosives-contaminated soil: A soil column study. *Bioresource Technology 59*, 169-176.

Boopathy, R., Wilson, M., Montemagno, C.D., Manning Jnr, J.F. & Kulpa, C.F. 1994. Biotransformation of 2,4,6-trinitrotoluene (TNT) by soil bacteria isolated from TNT-contaminated Soil. *Bioresource Technology 47*, 19-24.

Bradley, P.M. & Chapelle, F.H. 1995. Factors affecting microbial 2,4,6-trinitrotoluene mineralization in contaminated soil. *Environmental Science and Technology 29*, 802-806.

Brannon, J.M., Adrian, D.D., Pennington, J.C. & Meyers, T.E. 1992. Slow release of PCB, TNT, and RDX from soils and sediments. *Technical Report EL-92-38*. NTIS. Springfield, VA.

Breitung, J., Bruns-Nagel, D., Steinbach, K., Kaminski, L., Gemsa, D. & von Löw, E. 1996. Bioremediation of 2,4,6-trinitrotoluene-contaminated soils by two different aerated compost systems. *Applied Microbiology and Biotechnology 44*, 795-800.

Bruns-Nagel, D., Drzyzga, O., Steinbach, K., Schmidt, T.C., von Löw, E., Gorontzy, T., Blotevogel, K.H. & Gemsa, D. 1998. Anaerobic/aerobic composting of 2,4,6-trinitrotoluene-contaminated soil in a reactor system. *Environmental Science and Technology 32*, 1676-1679.

Bruns-Nagel, D., Knicker, H., Drzyzga, O., Butehorn, U., Steinbach, K., Gemsa, D. & von Löw, E. 2000. Characterization of ^{15}N-TNT residues after an anaerobic/aerobic treatment of soil/molasses mixtures by solid state ^{15}N-NMR spectroscopy. 2. Systematic investigation of whole soil and different humic fractions. *Environmental Science and Technology 34*, 1549-1556.

Chiou, C.T. 1989. Theoretical considerations of partition uptake of nonionic organic compounds by soil organic matter. *In* Sawhney, B.L. and K. Brown (ed.) *Reactions and Movement of Organic Chemicals in Soils*. Soil Socience Society of America. Madison, Wisconsin. 1-29.

Comfort, S.D., Shea, P.J., Hundal, L.S., Li, Z., Woodbury, B.L., Martin, J.L. & Powers, W.L. 1995. TNT transport and fate in contaminated soil. *Journal of Environmental Quality 24*, 1174-1182.

Daun, G., Lenke, H., Reuss, M. & Knackmuss, H.J. 1998. Biological treatment of TNT-contaminated soil. 1. Anaerobic cometabolic reduction and interaction of TNT and metabolites with soil components. *Environmental Science and Technology 32*, 1956-1963.

Dawel, G., Kastner, M., Michels, J., Poppitz, W., Gunther, W. & Fritsche, W. 1997. Structure of a laccase-mediated product of coupling of 2,4-diamino-6-nitrotoluene to guaiacol, a model for coupling of 2,4,6-trinitrotoluene metabolites to a humic organic soil matrix. *Applied and Environmental Microbiology 63*, 2560-2565.

Drzyzga, O., Bruns-Nagel, D., Gorontzy, T., Blotevogel, K.H., Gemsa, D. & von Löw, E. 1998. Incorporation of ^{14}C-labeled 2,4,6-trinitrotoluene metabolites into different soil fractions after anaerobic and anaerobic-aerobic treatment of soil/molasses mixtures. *Environmental Science and Technology 32*, 3529-3535.

Drzyzga, O., Gorontzy, T., Schmidt, A. & Blotevogel, K.H. 1995. Toxicity of explosives and related-compounds to the luminescent bacterium *Vibrio-Fischeri* nrrl-b-11177. *Archives of Environmental Contamination and Toxicology 28*, 229-235.

Fabrege-Duque, J.R., Jafvert, C.T., Li, H. & Lee, L.S. 2000. Modeling abiotic processes of aniline in water-saturated soils. *Environmental Science and Technology 34*, 1687-1693.

Gorontzy, T., Drzyzga, O., Kahl, M.W., Bruns-Nagel, D., Breitung, J., von Löw, E. & Blotevogel, K.H. 1994. Microbial-degradation of explosives and related-compounds. *Critical Reviews in Microbiology 20*, 265-284.

Grant, C.L., Jenkins, T.F., Myers, K.F. & McCormick, E.F. 1995. Holding-time estimates for soils containing explosives residues: comparison of fortification vs field contamination. *Environmental Toxicology and Chemistry 14*, 1865-1874.

Haderlein, S.B. & Schwarzenbach, R.P. 1993. Adsorption of substituted nitrobenzenes and nitrophenols to mineral surfaces. *Environmental Science and Technology 27*, 316-326.

Haderlein, S.B. & Schwarzenbach, R.P. 1994. Sensing reactive mineral surfaces in natural sediments by nitroaromatic model compounds. *In* Dracos, and Stauffer (ed.) *Transport and Reactive Processes in Aquifers*. Balkema. Rotterdam. 67-72.

Haderlein, S.B., Weissmahr, K.W. & Schwarzenbach, R.P. 1996. Specific adsorption of nitroaromatic explosives and pesticides to clay minerals. *Environmental Science and Technology 30*, 612-622.

Hansch, C. & Leo, A. 1979. *Substituent constants for correlation analysis in chemistry and biology*. Wiley. New York.

Hawari, J., Beaudet, S., Halasz, A., Thiboutot, S. & Ampleman, G. 2000. Microbial degradation of explosives: biotransformation versus mineralization. *Applied Microbiology and Biotechnology 54*, 605-618.

Heijman, C.G., Grieder, E., Holliger, C. & Schwarzenbach, R.P. 1995. Reduction of nitroaromatic - compounds coupled to microbial iron reduction in laboratory aquifer columns. *Environmental Science and Technology 29*, 775-783.

Held, T., Draude, G., Schmidt, F.R.J., Brokamp, A. & Reis, K.H. 1997. Enhanced humification as an *in-situ* bioremediation technique for 2,4,6-trinitrotoluene (TNT) contaminated soils. *Environmental Technology 18*, 479-487.

Hildenbrand, M. & Neumann, V. 1995. REV-reactor and soil column studies of sorption and migration of explosives in Elsnig sandy aquifers. *Acta Universitatis Carolinae Geologica 39*, 131-135.

Holliger, C., Gaspard, S., Glod, G., Heijman, C., Schumacher, W., Schwarzenbach, R.P. & Vazquez, F. 1997. Contaminated environments in the subsurface and bioremediation: organic contaminants. *FEMS Microbiology Reviews 20*, 517-523.

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

Kalbitz, K., Solinger, S., Park, J.H., Michalzik, B. & Matzner, E. 2000. Controls on the dynamics of dissolved organic matter in soils: A review. *Soil Science 165*, 277-304.

Kaplan, D.L. & Kaplan, A.M. 1982. 2,4,6-Trinitrotoluene-surfactant complexes: Decomposition, mutagenicity, and soil leaching studies. *Environmental Science and Technology 16*, 566-571.

Knicker, H., Bruns-Nagel, D., Drzyzga, O., von Löw, E. & Steinbach, K. 1999. Characterization of ^{15}N-TNT residues after an anaerobic/aerobic treatment of soil/molasses mixtures by solid state ^{15}N-NMR spectroscopy. 1. Determination and optimization of relevant NMR spectroscopic parameters. *Environmental Science and Technology 33*, 343-349.

Krumholz, L.R., Li, J., Clarkson, W.W., Wilber, G.G. & Suflita, J.M. 1997. Transformations of TNT and related aminotoluenes in groundwater aquifer slurries under different electron-accepting conditions. *Journal of Industrial Microbiology and Biotechnology 18*, 161-169.

Lenke, H., Warrelmann, J., Daun, G., Hund, K., Sieglen, U., Walter, U. & Knackmuss, H.J. 1998. Biological treatment of TNT contaminated soil. 2. Biologically induced immobilization of the contaminants and full-scale application. *Environmental Science and Technology 32*, 1964-1971.

Lenke, V.H., Daun, G., Bryniok, D. & Knackmuss, H.-J. 1993. Biologiche sanierung von rüstungsaltlasten. *Spektrum der Wissenschaft Okt.*, 106-108.

Li, A.Z., Marx, K.A., Walker, J. & Kaplan, D.L. 1997. Trinitrotoluene and metabolites binding to humic acid. *Environmental Science and Technology 31*, 584-589.

McCormick, N.G., Feeherry, F.E. & Levinson, H.S. 1976. Microbial transformation of 2,4,6-trinitrotoluene and other nitroaromatic compounds. *Applied and Environmental Microbiology 31*, 949-958.

Michels, J. & Gottschalk, G. 1994. Inhibition of the lignin peroxidase of *Phanerochaete-chrysosporium*by hydroxylamino-dinitrotoluene, an early intermediate in the degradation of 2,4,6-trinitrotoluene. *Applied and Environmental Microbiology 60*, 187-194.

Myneni, S.C.B., Brown, J.T., Martinez, G.A. & Meyer-Ilse, W. 1999. Imaging of humic substance macromolecular structures in water and soils. *Science 286*, 1335-1337.

Nordgren, A. 1992. A method for determining microbially available N and available P in an organic soil. *Biology and Fertility of Soils 13*, 195-199.

O'Donovan, W.J. 1921. TNT sickness and toxic jaundice. *In* O'Donovan, W.J. (ed.) *T.N.T. poisoning and fate of T.N.T in the animal body. Special report series / Medical Research Council; 58*. His Majesty's Stationery Office. London. 7-31.

Panton, P.N. & Bates, H.E. 1921. Summary of an experimental investigation into TNT poisoning. *In* O'Donovan, W.J. (ed.) *T.N.T. poisoning and fate of T.N.T in the animal body. Special report series / Medical Research Council; 58*. His Majesty's Stationery Office. London. 62-71.

Pennington, J.C., Hayes, C.A., Myers, K.F., Ochman, M., Gunnison, D., Felt, D.R. & McCormick, E.F. 1995. Fate of 2,4,6-trinitrotoluene in a simulated compost system. *Chemosphere 30*, 429-438.

Pennington, J.C. & Patrick, W.H.J. 1990. Adsorption and desorption of 2,4,6-trinitrotoluene by soils. *Journal of Environmental Quality 19*, 559-567.

Peterson, M.M., Horst, G.L., Shea, P.J., Comfort, S.D. & Peterson, R.K.D. 1996. TNT and 4-amino-2,6-dinitrotoluene influence on germination and early seedling development of tall fescue. *Environmental Pollution 93*, 57-62.

Preuss, A., Fimpel, J. & Diekert, G. 1993. Anaerobic transformation of 2,4,6-trinitrotoluene (TNT). *Archives of Microbiology 159*, 345-353.

Qaisi, K.M., Thibodeaux, L.J. & Adrian, D. 1996. Aquatic plant augmented TNT degradation - analysis of reaction kinetics. *Journal of Environmental Science and Health Part A: Environmental Science and Engineering A31*, 1595-1603.

Rieger, P.-G. & Knackmuss, H.-J. 1995. Basic knowledge and perspectives on biodegradation of 2,4,6-trinitrotoluene and related nitroaromatic compounds in contaminated soil. *In* Spain, J.C. (ed.) *Biodegradation of nitroaromatic compounds*. Plenum press. New York. 1-18.

Roberts, D.J., Ahmad, F. & Pendharkar, S. 1996. Optimizing of an aerobic polishing stage to complete the anaerobic treatment of munitions-contaminated soils. *Environmental Science and Technology 30*, 2021-2026.

Rugge, K., Hofstetter, T.B., Haderlein, S.B., Bjerg, P.L., Knudsen, S., Zraunig, C., Mosbaek, H. & Christensen, T.H. 1998. Characterization of predominant reductants in an anaerobic leachate-contaminated aquifer by nitroaromatic probe compounds. *Environmental Science and Technology 32*, 23-31.

Schmidt, T.C., Steinbach, K., von Löw, E. & Stork, G. 1998. Highly polar metabolites of nitroaromatic compounds in ammunition wastewater. *Chemosphere 37*, 1079-1090.

Selim, H.M., Xue, S.K. & Iskandar, I.K. 1995. Transport of 2,4,6-trinitrotoluene and hexahydro-1,3,5-trinitro-1,3,5-triazine in soils. *Soil Science 160*, 328-339.

Sheremata, T.W., Thiboutot, S., Ampleman, G., Paquet, L., Halasz, A. & Hawari, J. 1999. Fate of 2,4,6-trinitrotoluene and its metabolites in natural and model soil systems. *Environmental Science and Technology 33*, 4002-4008.

Skyllberg, U. & Borggaard, O.K. 1998. Proton surface charge determination in Spodosol horizons with organically bound aluminum. *Geochimica et Cosmochimica Acta 62*, 1677-1689.

Smock, L.A., Stoneburner, D.L. & Clark, J.R. 1976. The toxic effects of trinitrotoluene (TNT) and its primary degradation products on two species of algae and the fathead minnow. *Water Research 10*, 537-543.

Soil Survey Staff. 1992. Keys to soil taxonomy, 5th edition. *SMSS technical monograph No.19*. Pocahontas Press, Inc. Blacksburg, VA.

Spain, J.C. 1995. Biodegradation of nitroaromatic compounds. *Annual Review of Microbiology 49*, 523-555.

Specht, C.H., Kumke, M.U. & Frimmel, F.H. 2000. Characterization of NOM adsorption to clay minerals by size exclusion chromatography. *Water Research 34*, 4063-4069.

Spiker, J.K., Crawford, D.L. & Crawford, R.L. 1992. Influence of 2,4,6-trinitrotoluene (TNT) concentration on the degradation of TNT in explosive-contaminated soils by the white rot fungus *Phanerochaete-chrysosporium*. *Applied and Environmental Microbiology 58*, 3199-3202.

Thomas, G.W. 1982. Exchangeable cations. *In* Page, A.L. et al. (ed.) *Methods of soil analysis, Part 2. Chemical and microbiological properties. Agronomy monograph No. 9, 2nd Edition*. ASA-SSSA. Madison, MI. 159-165.

Thompson, P.L., Ramer, L.A. & Schnoor, J.L. 1998. Uptake and transformation of TNT by hybrid poplar trees. *Environmental Science and Technology 32*, 975-980.

Thorn, K.A. & Kennedy, K.R. 2002. ^{15}N NMR investigation of the covalent binding of reduced TNT amines to soil humic acid, model compounds, and lignocellulose. *Environmental Science and Technology 36*, 3787-3796.

Thorn, K.A., Pennington, J.C. & Hayes, C.A. 2002. ^{15}N NMR investigation of the reduction and binding of TNT in an aerobic bench scale reactor simulating windrow composting. *Environmental Science and Technology 36*, 3797-3805.

Trevors, J.T. 1996. Sterilization and inhibition of microbial activity in soil. *Journal of Microbiological Methods 26*, 53-59.

Vorbeck, C., Lenke, H., Fischer, P., Spain, J.C. & Knackmuss, H.J. 1998. Initial reductive reactions in aerobic microbial metabolism of 2,4,6-trinitrotoluene. *Applied and Environmental Microbiology 64*, 246-252.

Walker, J.E. & Kaplan, D.L. 1992. Biological degradation of explosives and chemical agents. *Biodegradation 3*, 369-385.

Weber, E.J., Spidle, D.L. & Thorn, K.A. 1996. Covalent binding of aniline to humic substances. 1. kinetic studies. *Environmental Science and Technology 30*, 2755-2763.

Weissmahr, K.W., Haderlein, S.B. & Schwarzenbach, R.P. 1998. Complex formation of soil minerals with nitroaromatic explosives and other pi-acceptors. *Soil Science Society of America Journal 62*, 369-378.

Weissmahr, K.W., Haderlein, S.B., Schwarzenbach, R.P., Hany, R. & Nuesch, R. 1997. *In situ* spectroscopic investigations of adsorption mechanisms of nitroaromatic compounds at clay minerals. *Environmental Science and Technology 31*, 240-247.

Weissmahr, K.W., Hildenbrand, M., Schwarzenbach, R.P. & Haderlein, S.B. 1999. Laboratory and field scale evaluation of geochemical controls on groundwater transport of nitroaromatic amunition residues. *Environmental Science and Technology 33*, 2593-2600.

Widrig, D.L., Boopathy, R. & Manning, J.F. 1997. Bioremediation of TNT-contaminated soil: A laboratory study. *Environmental Toxicology and Chemistry 16*, 1141-1148.

Williams, R.T., Ziegenfuss, P.S. & Sisk, W.E. 1992. Composting of explosives and propellant contaminated soils under thermophilic and mesophilic conditions. *Journal of Industrial Microbiology 9*, 137-144.

Won, W.D., DiSalvo, L.H. & Ng, J. 1976. Toxicity and mutagenicity of 2,4,-6-trinitrotoluene and its microbial metabolites. *Applied and Environmental Microbiology 31*, 576-580.

Xia, G.S. & Pignatello, J.J. 2001. Detailed sorption isotherms of polar and apolar compounds in a high-organic soil. *Environmental Science and Technology 35*, 84-94.

Xing, B.S. & Pignatello, J.J. 1997. Dual-mode sorption of low-polarity compounds in glassy poly(vinyl chloride) and soil organic matter. *Environmental Science and Technology 31*, 792-799.

Xue, S.K., Iskandar, I.K. & Selim, H.M. 1995. Adsorption-desorption of 2,4,6-trinitrotoluene and hexahydro-1,3,5-trinitro-1,3,5-triazine in soils. *Soil Science 160*, 317-327.

Yau, W.W., Kirkland, J.J. & Bly, D.D. 1979. *Modern size-exclusion liquid chromatography*. Wiley. New York.

Yinon, J. 1990. *Toxicity and metabolism of explosives*. CRC Press. Boca Raton, FL.

Zhu, L.Z., Chen, B.L. & Shen, X.Y. 2000. Sorption of phenol, p-nitrophenol, and aniline to dual-cation organobentonites from water. *Environmental Science and Technology 34*, 468-475.

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The following quotation by John Tukey is for all scientists to bear in mind when seeking the truth: 'It is better to be approximately right than precisely wrong'.