Soils contaminated with explosives: Environmental fate and evaluation of state-of-the-art remediation processes (IUPAC Technical Report)*

Dimitrios Kalderis[1,‡], Albert L. Juhasz[2], Raj Boopathy[3], and Steve Comfort[4]

[1]*Department of Natural Resources and the Environment, Technological and Educational Institute of Crete, Chania 73100, Crete, Greece;* [2]*Centre for Environmental Risk Assessment and Remediation, University of South Australia, Mawson Lakes Campus, Adelaide, 5095, Australia;* [3]*Department of Biological Sciences, Nicholls State University, Thibodaux, LA 70310, USA;* [4]*School of Natural Resources, University of Nebraska, 205 Kiesselbach, Lincoln, NE, USA*

Abstract: An explosion occurs when a large amount of energy is suddenly released. This energy may come from an over-pressurized steam boiler, from the products of a chemical reaction involving explosive materials, or from a nuclear reaction that is uncontrolled. In order for an explosion to occur, there must be a local accumulation of energy at the site of the explosion, which is suddenly released. This release of energy can be dissipated as blast waves, propulsion of debris, or by the emission of thermal and ionizing radiation.

Modern explosives or energetic materials are nitrogen-containing organic compounds with the potential for self-oxidation to small gaseous molecules (N_2, H_2O, and CO_2). Explosives are classified as primary or secondary based on their susceptibility of initiation. Primary explosives are highly susceptible to initiation and are often used to ignite secondary explosives, such as TNT (2,4,6-trinitrotoluene), RDX (1,3,5-trinitroperhydro-1,3,5-triazine), HMX (1,3,5,7-tetranitro-1,3,5,7-tetrazocane), and tetryl (*N*-methyl-*N*-2,4,6-tetranitro-aniline).

Keywords: explosives; high melting explosive (HMX); IUPAC Chemistry and the Environment Division; rapid detention explosive (RDX); soil contamination; 2,4,6-trinitro-toluene (TNT);

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1. INTRODUCTION

1.1 General

An explosion occurs when a large amount of energy is suddenly released. This energy may come from an over-pressurized steam boiler, from the products of a chemical reaction involving explosive materials, or from a nuclear reaction that is uncontrolled. In order for an explosion to occur, there must be a local accumulation of energy at the site of the explosion, which is suddenly released [1]. This release of energy can be dissipated as blast waves, propulsion of debris, or by the emission of thermal and ionizing radiation.

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It has been estimated that there are hundreds of explosives-contaminated sites within the United States, and even a greater number in Europe and Asia. TNT, rapid detention explosive (RDX), and high melting explosive (HMX) are the most commonly found explosive substances in soil. Figure 1 shows the structures of common explosive contaminants found in soil. All of them are popular materials used by military forces, and due to improper handling and disposal techniques these substances and their derivatives have polluted environments to levels that threaten the health of humans, livestock, wildlife, and entire ecosystems. Additional sources of contamination include explosives machining, casting and curing, laboratory testing, and open burning/open detonation (OB/OD) of outdated munitions. Unexploded ordnance (UXO) from military operations worldwide also poses a serious environmental threat due to the release of toxic substances from the corroding ordnance, in addition to the risks associated with the potential for an accidental detonation. Several European countries are now dealing with the issue of uncontrollable sea dumping or land burying of old, outdated munitions. Soil contamination can also result from the disposal of wastewaters, which are divided into red and pink water. Red water is generated during the manufacturing of TNT, and pink water is the washwater associated with the load, assembly, and packing of materials that have been in contact with TNT. All the above activities cause different levels of contamination that can be toxic to ecological receptors that inhabit the impacted sites and adjacent areas exposed to offsite migration of contaminants.
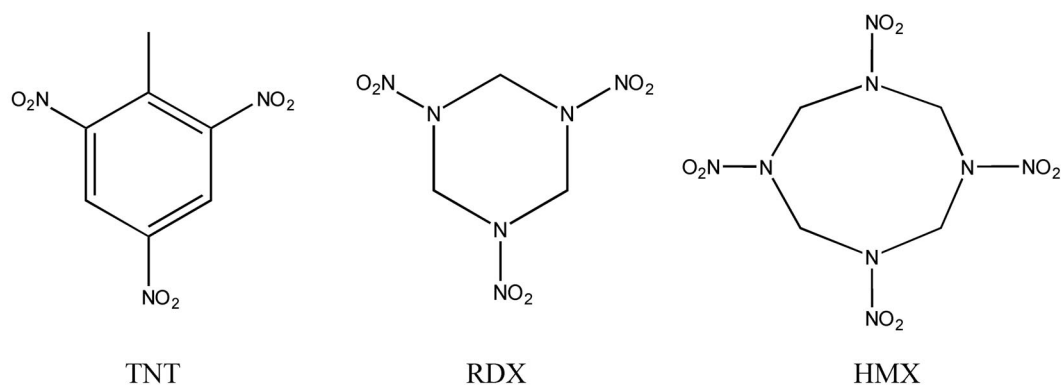

Fig. 1 Common explosives found on soil: TNT (2,4,6-trinitrotoluene), RDX (1,3,5-trinitro-1,3,5-triazinane), and HMX (1,3,5,7-tetranitro-1,3,5,7-tetrazocane).

Managing sites contaminated with energetic materials has become an international problem shared by many countries, but few have the technical expertise of the methodology for the effective characterization of environmental impacts from the explosives substances and their degradation products. Some countries, including Canada and the United States, have developed and tested novel "green" energetic materials that are designed to reduce environmental damage during military activities [2,3]. Since the protection of the environment is nowadays one of the highest priorities in most countries, the development of these materials would put enormous constraint not just on the advancements in the explosives-manufacturing industry in any country, but also on the continuation of the existing, outdated technologies.

Many explosives-contaminated sites contain substances in the soil, sediment, and surface- and/or groundwater at a wide range of concentrations. Live-fire military training can deposit millimeter-sized particles of high explosives (HE) on surface soils when rounds do not explode as intended. It was estimated by Taylor et al. [4] that as much as 2 % (by mass) of a TNT-filled 155-mm round remained as residuals on the soil surface after high-order (i.e., "complete") detonations, which translates to 140 g of explosive residues per round. If the round undergoes a low-order detonation, up to 3 kg of TNT could be deposited on the surrounding area.

Rainfall-driven dissolution of the particles then begins a process whereby aqueous solutions can enter the soil and groundwater as contaminants. Taylor et al. [5] simulated the rainfall-driven dissolution and outdoor weathering effects on TNT, Tritonal, Comp B, and Octol. Mass balance data revealed that TNT in the water samples accounts for only about one-third of the TNT lost from the chunks. The creation of phototransformation products on the solid chunks, and their subsequent dissolution or sublimation, probably accounts for the other two-thirds. Although these products cannot, as yet, be quantified, they are intrinsic to the outdoor weathering and fate of TNT-based explosives. The large, low-solubility HMX crystals appeared to impede but not control TNT dissolution. That is, neither limiting model case (independent or HMX-controlled dissolution) fits the observed TNT data. The reduced influence of HMX on TNT dissolution, compared with the controlling role of RDX in Comp B, is consistent with less constraint of TNT diffusion into the surrounding water layer. Probably the largest source of uncertainty to estimating HE aqueous influx into range soils results from the poorly quantified population distribution of the HE particles on a range of interest. The number and size distribution of these particles depends on many factors, including the munitions used, how many were fired, their detonation probabilities (high-order, low-order, or dud), and weathering and mechanical dissagregation [4]. Dontsova et al. [6] evaluated the dissolution of 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT) from the propellant formulation, M1 (87.6 % nitrocellulose, 7.3 % 2,4-DNT, 0.57 % 2,6-DNT, 1.06 % diphenylamine, 3.48 % dibutyl phthalate) and their subsequent transport in soil. M1 dissolution was limited by DNT diffusion from the interior of the pellet, resulting in an exponential decrease in dissolution rate with time. The HYDRUS-1D model accurately described release and transport of 2,4- and 2,6-DNT from M1 propellant. Dissolution rates for M1 in the stirred reactor and column studies were similar, indicating that batch dissolution rates are potentially useful to represent field conditions. At higher levels and due to their persistence and mobility, many explosives can leach to the groundwater and have a direct or indirect adverse effect on human health and the environment. Furthermore, photolysis can yield more toxic by-products.

1.2 Case studies

Still to date, there are many cases of soil contaminated with explosives, either nearby manufacturing sites, or in areas where military activities have occurred in the last century. However, due to the lack of detailed information regarding these activities, reports on actual explosives-contamination cases are rather scarce.

A typical example of explosives-contaminated soil is that of a former World War I ammunition destruction facility in Belgium. The soil samples obtained were analyzed for total arsenic, copper, lead,

organoarsenicals, nitroaromatic explosives, and sulfur mustard derivatives. Even after more than 80 years, considerable amount of contamination is still present, mainly by lead, copper, arsenic, and nitroaromatic metabolites such as 1,3-dinitrobenzene [7]. This is indicative of the persistence of nitroaromatic explosives and their metabolites in the environment.

During an evaluation of bioremediation methods, Clark and Boopathy [8] used soil from the Louisiana Army Ammunition Plant (LAAP) contaminated with 10 000, 1900, and 900 mg/kg of TNT, RDX, and HMX, respectively. These high levels of contamination resulted from incineration of explosives-contaminated soils and sludges. In another U.S. Army facility, that of Aberdeen Proving Ground, testing of armor-piercing ammunitions has resulted in the deposition of more than 70 000 kg of depleted uranium (DU) to local soils and sediments [9].

More than 100 000 tons of TNT were produced at the former ammunition site Werk Tanne in Clausthal-Zellerfeld, Germany. The production of explosives and consequent detonation in 1944 by the Allies caused great pollution in this area. One main problem of the 2,4,6-TNT production was the resulting wastewater. Altogether, 5 400 000 m^3 of toxic wastewater were produced during the operation time of this site. The environmental damage was additionally increased by the destruction of the facility. A study performed by Eisentraeger et al. [10] showed that today, this site remains highly polluted with explosives and their metabolites as well as with polycyclic aromatic hydrocarbons (PAHs) and heavy metals. Consequently, a risk for contamination of drinking-water resources exists around Clausthal-Zellerfeld, because pollutant leaching to the soil groundwater path from this site is possible.

A number of different types of live-fire and demolition ranges have been studied at U.S. and Canadian military bases (Fig. 2). These include hand grenade, rifle grenade, antitank rocket, demolition, tank firing, mortar, artillery, C-130 gunship, and bombing ranges. Training at these ranges is conducted with different types of munitions that contain a variety of energetic formulations. At many ranges, the area where the weapon is fired is separate from the impact area where detonations occur.

Generally, energetic residues at the firing points are composed of compounds used in propellant formulations, whereas residues at the impact areas are compounds used as HE in the munition warheads, or white phosphorus (WP) from smoke rounds.

The Pantex Plant is another case of contamination from HE. The site was used during World War II (1942–1945) by the U.S. Army for loading conventional ammunition shells and bombs. The current primary mission of the Pantex Plant is the disassembly of nuclear weapons. Pantex is also responsible for assembly, surveillance, storage, maintenance, modification, repair, and non-explosive testing of nuclear weapons components and the manufacture of chemical HE components. Current operations involve short-term handling (but not processing) of encapsulated uranium, plutonium, and tritium, as well as a variety of industrial chemicals [11]. The plant is surrounded by agricultural and range lands. Pantex Plant's solid waste management unit is a 3-acre area contaminated with the HE RDX and some TNT and HMX from the past disposal of explosives machining waste. The contamination is most concentrated at 10 m just below the soil surface, but continues downward in places to 85 m. HE contamination from this unit has been found offsite and has the potential to leach into the Ogallala aquifer, the region's primary source of drinking water. Since 1998, contamination in a small area of the unit has been biodegraded through a small-scale project that involved injecting nitrogen gas into the contaminated soil to create an anaerobic (oxygen-free) environment, which stimulated naturally occurring anaerobic microorganisms that destroyed the HE contaminants.

UXO is another life-threatening aspect of soil contamination. UXO can pose two kinds of risks to civilians: (1) explosion risks, with the potential to cause immediate physical harm, and (2) toxicity-related risks, due to the leakage of explosives and other munitions constituents into the surrounding soil and/or water [12]. Depending on soil properties, climatic conditions and UXO characteristics, the time to perforation (i.e., corrosion breakthrough of the metallic casing) can vary from roughly 10 years to several thousand years. Understanding the relative rate of corrosion significantly improves the assessment of risk to humans and the environment posed by the toxic energetic and constituent materials encased in UXO. Many UXO are comprised of HE, the metallic container, and lesser quantities of fuse
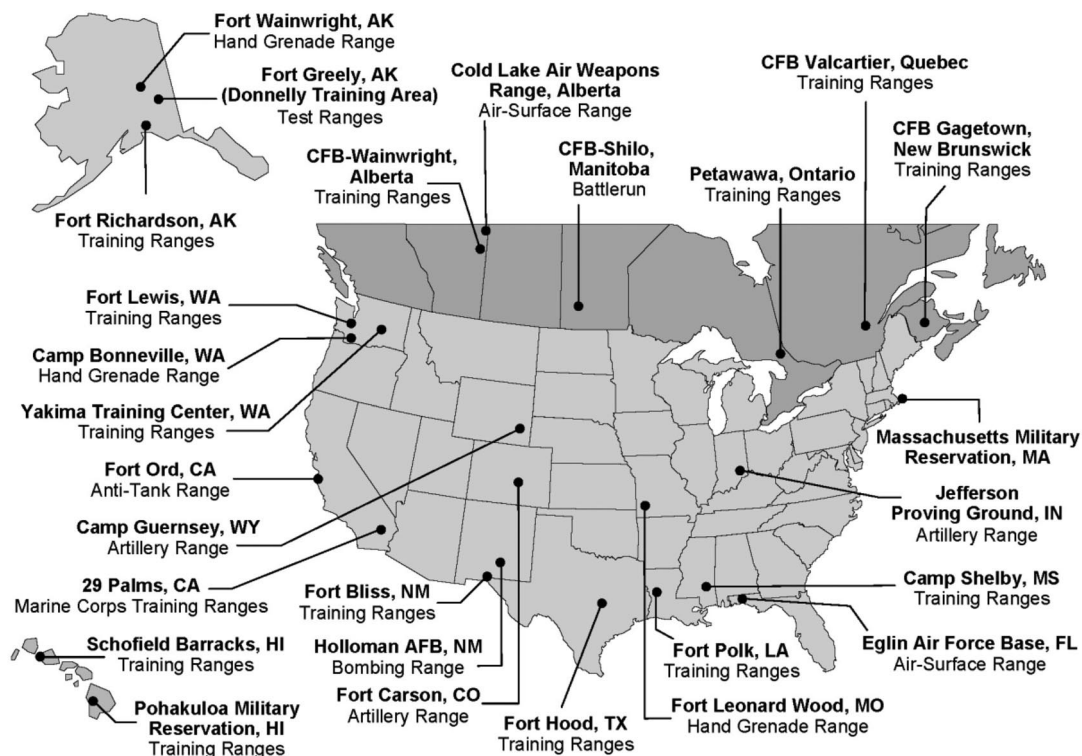
Fig. 2 U.S. and Canadian test and training ranges where explosive residues were detected in the soil (from U.S. Army Corps of Engineers [13]).

materials. Worldwide, the annual toll of casualties from landmines and UXO is estimated at 15000–20000 [14]. Some 1976 sites at closed military bases in the United States are contaminated with UXO and are slated for clean-up, at an estimated cost of 15 to 140 billion USD. Because no available technology can guarantee full removal of UXO, information about explosion probability is needed to assess the residual risks of civilian reuse of closed military bases and to make decisions about how much to invest in clean-up. Current expert beliefs vary greatly on the probability of UXO explosion in contact with humans [12,15,16].

Between 2000 and 2002, Afghanistan had one of the highest numbers of casualties due to landmines and UXO in the world [17]. Landmines pose a considerable public health threat and economic burden in post-conflict situations. Most victims are civilians, and many are children and women. UXO currently causes more injuries than landmines and, as opposed to landmines, predominantly injures children. Because UXO are usually more visible than landmines, these injuries may be more amenable to prevention through targeted educational messages.

In another study, Bilukha et al. [14] reported that Chechnya, a small Republic in the North Caucasus region of the Russian Federation, has a land mass about half the size of Belgium (15300 km^2) and a population of roughly 1 million people. For more than a decade, the population of this tiny Republic has been among those most heavily affected by landmines and UXO worldwide. Their survey shows that for several years during the past decade, the rate of injury in Chechnya from these devices was higher than in any other country in the world for which data were available. In 2000 and 2001, injury rates in Chechnya were 10 times that of the world's most affected countries—Afghanistan, Cambodia, and Angola.

The situation is similar in Cambodia, a country located in Southeast Asia. Mortar bombs, rocket-propelled and rifle grenades, artillery shells, cluster bomb submunitions, and aircraft bombs, as well as a variety of antipersonnel and antitank mines are scattered by the millions in two vast areas of Cambodia. The country signed peace agreements in 1991 and has reached a complete ceasefire since 1994. National and international demining organizations, such as the Mines Advisory Group (MAG) have been active for more than 10 years. The demining process is extremely slow, due to the absence of reliable minefield maps, and only a small percentage of land has been "cleaned" and is therefore relatively safe [18].

To maximize safety and reduce hazards associated with procedures for disposing of UXO, the Explosive Ordnance Disposal (EOD) community in the United States has developed methods to neutralize UXO without releasing maximum energy, that is, to produce low-order detonations. This is desirable when UXO are located near buildings, harbors, or other structures or populated areas, or on roads where troops must move. In previous studies, the Naval EOD Technical Division developed the main charge disrupter (MCD) to produce low-order detonations. The results indicate that the MCD not only provides a safer disposal option for UXO, but also the explosive substances found in the soil after the explosion have lower concentrations and are located at a closer distance to the explosion center, compared to OB/OD methods [19].

1.3 Purpose of the study

The purpose of this report is to

- provide an overview of the environmental fate and degradation pathways of the explosive substances that commonly contaminated soil,
- estimate the human and environmental risk associated with explosives-contaminated soil, and
- examine the current treatment methods of remediation.

This Technical Report focuses on TNT, HMX, and RDX, which are mostly responsible for the explosives contamination found in soils. Compared to these, other explosives such as nitroglycerine (1,3-dinitrooxypropan-2-yl nitrate, NG) and PETN ([3-nitrooxy-2,2-bis(nitrooxymethyl)propyl]nitrate, common name pentaerythritol tetranitrate) are seldom found in soils; subsequently, the literature addressing PETN- and NG-contaminated soils is scarce. From a practical point of view, this study aims to provide a reference tool for the decision-maker environmental engineer, the scientist working in relevant fields, but also to any party interested in being introduced to the issue of explosives-contaminated soil.

There are numerous publications on this topic, however, this study contains a list of references extensive enough to guide the reader toward more comprehensive databases and the work of all the major research teams dealing with this issue. Additionally, the evaluation of the current treatment methods available will point out the advantages and disadvantages of each process and lead scientists to work toward improving these processes and developing new ones.

2. ENVIRONMENTAL FATE

Explosive compounds may enter the environment during their production (e.g., wastewater lagoons), disposal (e.g., burn pits), storage, or usage (dispersed or unexploded ordnance) resulting in contamination of groundwater, surface water, marine, and terrestrial environments. Following entry into the environment, both abiotic and biotic processes will influence the fate of explosive compounds [20,21]. The rate and extent of transport and transformation will be governed by the physico-chemical properties of the explosive compounds (solubility, K_{ow}, vapor pressure, Henry's law constant), environmental factors (weather conditions, soil properties, pH, redox), and biological factors including the presence and/or

absence of explosives-degrading microorganisms. Processes that influence the environmental fate of explosive compounds may be broadly divided into two categories:

a. Processes that influence transport

- dissolution
- volatilization
- adsorption

b. Processes that influence transformation

- photolysis
- hydrolysis
- reduction
- biodegradation

In the following sections, a description of the aforementioned processes will be provided for TNT, RDX, HMX, and PETN. Figure 3 provides a schematic representation of the major degradation pathways for these explosives.
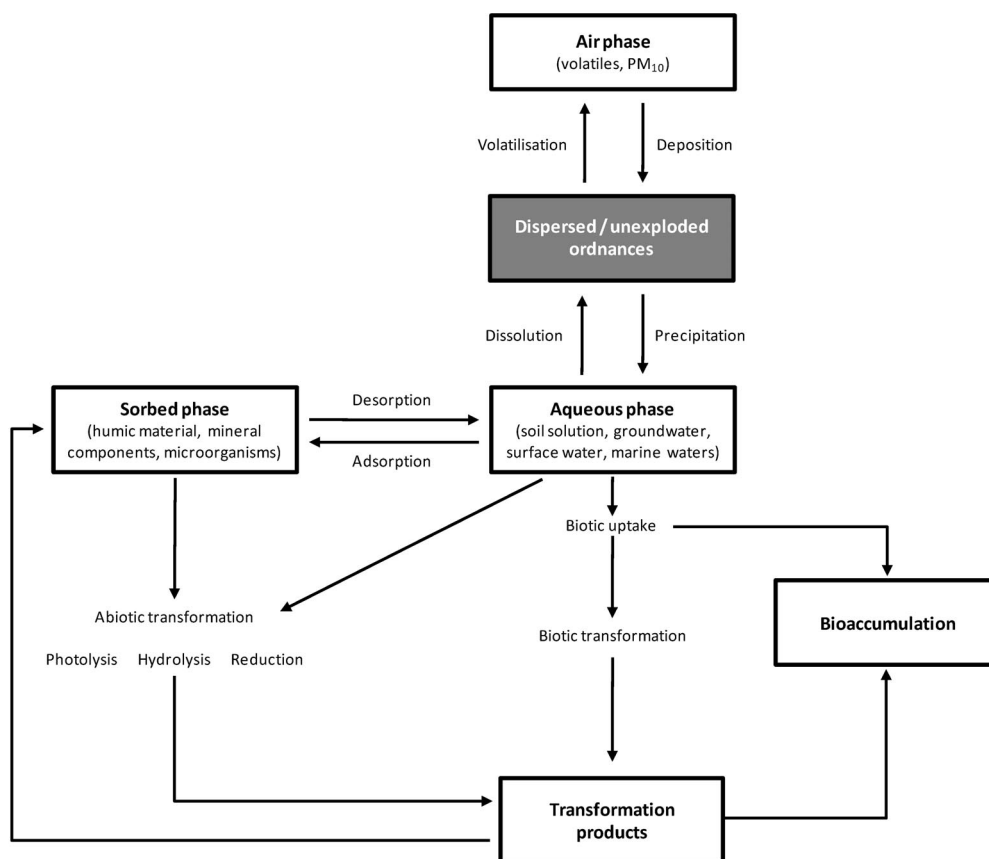

Fig. 3 Environmental fate of explosive compounds (adapted from Brannon and Myers [22]).

2.1 Dissolution

The primary mechanism for the transport and dissemination of explosive compounds throughout the environment is via dissolution into water [23]. Due to the aqueous solubility of TNT (130 mg/L), RDX (42 mg/L), HMX (5 mg/L), and PETN (43 mg/L) (Table 1), discrete particles resulting from low-order detonation may dissolve slowly over time, resulting in a constant release of explosive compounds to groundwater, surface water, marine environments, or subsurface soil over extended time periods [24]. TNT transformation products have a greater potential for transport due to their higher aqueous solubilities compared to the parent compound. In a study to compare transport processes of explosives in saline and fresh water systems, Brannon et al. [25] determined that dissolution rates for TNT, RDX, and HMX showed a close agreement between conditions tested. However, environmental parameters, such as temperature, will influence the rate of dissolution. For example, Lynch et al. [28] and Lynch [23] deter-

Table 1 Physical and chemical properties of TNT, RDX, HMX, and PETN (data sourced from [26,27]; Material Safety Data Sheets).

Name	Explosive compounds			
	TNT	RDX	HMX	PETN
CAS Number	38082-89-2	00121-82-4	026914-41-0	78-11-5
Synonyms and commercial names	2,4,6-Trinitrotoluene, trinitrotol, trilite, tolite, trinol, trotyl, tritolol, tritone, trotol, triton	Cyclotrimethylene-trinitramide, cyclonite, hexogen, composition A-6	Cyclotetromethylene-tetranitramine, octogen, octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (four distinct polymorphic forms, α, β, γ, and δ)	Corpent, pentrite, nitropenta
Chemical formula	$C_7H_5N_3O_6$	$C_3H_6N_6O_6$	$C_4H_8N_8O_8$	$C_5H_8N_4O_{12}$
Melting point (°C)	80–82	204	276–280	141
Solubility in water	130 mg/L[a]	42 mg/L[a]	5 mg/L[b]	43 mg/L[b]
Mass density ρ (g/cm^3)	1.5–1.6	1.82	1.96	1.77
Vapor pressure p (1 bar, 20°C)	7.2×10^{-9}	5.3×10^{-12}	4.3×10^{-17}	1.36×10^{-13}
Henry's Law constant: k_H (bar m^3 mol^{-1})	4.57×10^{-7} to 1.1×10^{-8}	6.3×10^{-8} to 1.96×10^{-11}	2.6×10^{-15}	1.2×10^{-11}
Octanol/water partitioning coefficient (K_{ow})	1.86	0.86	0.061	1.61
Appearance and odor	Yellow flakes with bitter almond odor	White or gray powder, odorless	White or gray powder, odorless	White tetragonal crystals

[a]Solubility at 20 °C.
[b]Solubility at 25 °C.

mined that explosive dissolution rates double with every 10 °C increase in temperature from 3 to 33 °C. In addition, solubility maxima may be influenced by soil physico-chemical properties which may influence explosive adsorption onto soil particles [29]). Furey et al. [30] determined that geosorbents (e.g., iron, clay, humic acid) may influence the elution rate of TNT and RDX from soil. Both iron and iron-bearing clays reduced the effective elution rates of TNT and RDX, however, humic acids had a greater influence on TNT elution than RDX. This is probably due to the significant difference between the K_{ow} values of TNT and RDX.

2.2 Volatilization

Due to low vapor pressures and Henry's law constants (Tables 1 and 2), volatilization of TNT, TNT transformation products, RDX, HMX, and PETN from solid or aqueous phases is insignificant [31]. As a result, volatilization's contribution to explosive compounds environmental fate is negligible.

Table 2 Physical and chemical properties of selected TNT transformation products (data sourced from [26,27]; Material Safety Data Sheets).

| Name | TNT transformation products | | | |
	2,4-DNT	2,6-DNT	2-M-3,5-DNA	4-M-2,6-DNA
CAS Number	121-14-2	606-20-2	35572-78-2	19406-51-0
Synonyms and commercial names	2,4-Dinitrotoluene	2,6-Dinitrotoluene	2-Methyl-3,5-dinitroaniline	4-Methyl-3,5-dinitroaniline
Chemical formula	$C_7H_6N_2O_4$	$C_7H_6N_2O_4$	$C_7H_7N_3O_4$	$C_7H_7N_3O_4$
Melting point (°C)	70	64-66	176	171
Solubility in water	270 mg/L[a]	206 mg/L[b]	17 mg/L	36 mg/L
Mass density ρ (g/cm^3)	1.32	1.28	No data	No data
Vapor pressure p (1 bar, 20 °C)	2.9×10^{-7}[c]	7.5×10^{-7}	5.3×10^{-8}	2.6×10^{-8}
Henry's Law constant: k_H (bar m^3 mol^{-1})	$(1.3–1.86) \times 10^{-7}$	4.86×10^{-7} to 9.26×10^{-8}	1.19×10^{-7}	1.74×10^{-7}
Octanol/water partitioning coefficient (K_{ow})	1.98	2.02	2.8	2.62
Appearance and odor	Yellow needles or monoclinic prisms	Yellow to red rhombic needles	–	–

[a]Solubility at 22 °C.
[b]Solubility at 25 °C.
[c]Vapor pressure at 25 °C.

2.3 Adsorption

Adsorption of explosive compounds onto a variety of surfaces (e.g., colloidal and humic material, mineral components, microorganisms) may occur following dissolution of the explosive compound and interaction with the sorbent surface. Both the physico-chemical properties of the solute and sorbent and environmental factors will influence sorption reactions (e.g., hydrophobic partitioning, hydrogen bond-

ing, ion exchange, chemisorption) and therefore the extent of adsorption [32,33]. The number of amino groups on nitroaromatic compounds influences the sorption capacity of these compounds. Sheremata et al. [34] determined that sorption capacity constants increased with an increase in the number of amino groups (2,4-DANT[a] higher than 4-ADNT[b] higher than TNT). Clay minerals have a significant impact on the adsorption of nitroaromatics in soils, however, sorption is greatly influenced by the clay's exchangeable cations [35–37]. Haderlein et al. [35] determined that TNT adsorption was greatest when K^+ or NH_4^+ were the dominant cations, whereas adsorption was negligible for homoionic Na^+, Ca^{2+}, Mg^{2+}, and Al^{3+} clays. Furthermore, the adsorption of TNT onto clays increased in the order of montmorillonite, kaolinite [35]. The organic carbon fraction in soil also plays a major role in the adsorption of explosive compounds. Yamamoto et al. [38] determined that the surface partition coefficient (K_d) values for TNT, 2,4-DNT, and RDX were dependent on the organic carbon fraction. In addition, TNT and 2,4-DNT were more strongly adsorbed compared to RDX. However, soil organic carbon content does not significantly affect HMX sorption [39].

2.4 Photolysis

Transformation of explosive compounds may occur as a result of a number of abiotic processes that are dependent on environmental conditions. McGrath [26] identified photolysis as a major transformation process of explosive compounds in surface waters, however, in soils transformation may only occur near the soil surface. Transformation may occur via direct absorption of light energy (direct photolysis) or as a result of energy transfer from peroxide, ozone (O_3), or humic compounds (photosensitized compounds, i.e., indirect photolysis). Consequently, light intensity and wavelength will influence the rate and extent of photolysis.

The photolysis of TNT in production water is readily recognized by the change in color to red or pink following exposure to sunlight. Phototransformation of TNT results in the formation of nitrobenzenes (NBs), benzaldehydes, azoxydicarboxylic acids, and nitrophenols as a result of the oxidation of methyl groups, reduction of nitro groups and dimer formation [26]. RDX and HMX may also undergo photolysis resulting in the formation of azoxy compounds, ammonia, formaldehyde, nitrate, nitrite, nitrous oxide, and *n*-nitroso-methylenediamine [40]. Direct photolysis of energetic compounds may result in half-lives ranging from 0.5 to 22 h (TNT), 9 h to 14 d (RDX) and 1.4 to 70 d (HMX); however, these rates may be enhanced as a result of indirect photolysis due to the presence of sensitizing humic substances or nitrates. In addition, photo-Fenton reactions may result in the rapid oxidation of energetic compounds. Section 3.2.6.2 provides further details on Fenton and photo-Fenton reactions for energetic wastewaters and contaminated soils.

The leachate, ground- and surface waters of former ammunition sites in Germany were analyzed by Godejohann et al. [41]. They identified the presence of several nitrobenzoic acids in mass fractions up to 160 µg/kg for 2,4-dinitro-benzoic acid (2,4-DNBA) and 86 µg/kg for 2-amino-4,6-dinitro-benzoic acid (2-A-4,6-DNBA). 2-amino-4,6-dinitrobenzoic acid, known as a phototransformation product of TNT, is one of the major acidic contaminants in almost all samples investigated in this study. The compound has been overlooked during routine investigations of water samples of former ammunition sites due to the fact that 2-A-4,6-DNBA is not commercially available and only extractable by solid-phase extraction (SPE) under acidic conditions. 4-Amino-nitrobenzoic acid, 2-amino-nitrobenzoic acid, and 2-amino-4-nitrobenzoic acid were also detected in the drain water samples from a former ammunition

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[a]2,4-DANT derived from the name 2,4-diamino-6-nitrotoluene, deprecated by IUPAC nomenclature, stands for 6-methyl-5-nitrobenzene-1,3-diamine.
[b]4-ADNT derived from the name 4-diamino-2,6-dinitrotoluene, deprecated by IUPAC nomenclature, stands for 4-methyl-3,5-dinitroaniline.

samples by Schmidt et al. [42]. Later, Preiss et al. [43] confirmed the presence of several methyl-, amino-, and hydroxy-nitrobenzoic acids in samples obtained from former German ammunition sites. The leachates and groundwater samples were analyzed by liquid chromatography (LC)-mass spectrometry (MS) and LC-NMR hyphenated techniques to characterize the range of highly polar nitroaromatic compounds. Hennecke et al. [44] investigated the phototransformation processes of explosives in natural water/sediment systems. They concluded that for TNT, pH and the presence of natural photosensitizers have strong influence on the photolysis rate. The major metabolites identified were 2-A-4,6-DNBA, 4-A-2,6-DNBA, 2,4,6-trinitrobenzoic acid, 1,3,5-trinitrobenzene, and 3,3,5,5-tetranitroazoxybenzene-2,2-dicarboxylic acid.

2.5 Hydrolysis

Explosive compounds may be transformed as a result of hydrolysis, however, elevated pH conditions are required for amine, amide, nitrile, and carboxylic acid functional groups to be susceptible [45]. While it is unlikely that pH conditions in the environment will be sufficiently high for hydrolytic reactions to take place, alkaline hydrolysis has been investigated as in situ and ex situ treatment technologies for explosives-contaminated soils. TNT hydrolysis is dependent on the reaction pH and initial TNT concentration [46]. At a pH of 12, Bajpai et al. [47] observed more than 95 % reduction in TNT concentration compared to 20 by 25 % reduction at pH 11. In addition, a higher treatment pH is required for the destruction of 2A- and 4A-DNT compared to TNT [48]. Thorn et al. [49] proposed that the initial step in the alkaline hydrolysis of TNT was nucleophilic substitution of the nitro and methyl groups of TNT by hydroxide ions. RDX and HMX may undergo alkaline hydrolysis at pH value higher than 10 leading to the formation of NO_2, HCHO, HCOOH, NH_3, and N_2O [46,50]. 4,6-Dinitro-2,4,6-triazahexanal and 5-hydroxy-4-nitro-2,4-diaza-pentanal were identified as by-products leading to the formation of the ring cleavage product 4-nitro-2,4-diazabutanal. The efficacy of alkaline hydrolysis may be enhanced with an increase in reaction temperature [51–54], however, thermal runaway may occur at temperatures greater then 120 °C [53].

2.6 Reduction

Reduction of explosive compounds may occur as a result of abiotic reactions where nitro groups are reduced to amino groups [26]. These processes are dependent on pH and redox potential and require activation by catalysts such as iron, clay minerals, or organic macromolecules. TNT, RDX, and HMX reduction by iron (magnetite, ferrous iron, zero valent iron) has been reported by a number of researchers [33,55–60]. The resulting transformation products (e.g., aromatic polyamines, MNX, DNX, TNX) may be further metabolized via biotic processes or may adsorb to soil constituents.

2.7 TNT degradation

A wide variety of organisms have been shown to have the capacity to degrade TNT (Table 3). For bacteria, TNT degradation may occur under aerobic or anaerobic conditions where the explosive compound serves as a carbon and/or nitrogen source [61]. Alternatively, degradation may proceed as a result of cometabolism where an additional substrate serves as the carbon and energy source. Under both aerobic (Fig. 4) and anaerobic conditions (Fig. 5), TNT is transformed to amino derivatives via nonspecific NAD(P)H-dependent nitroreductase. 2-ADNT, 4-ADNT, 2,4-DANT, and 2,6-DANT are the predominant metabolites of TNT although triaminotoluene may be formed under anaerobic conditions. These compounds may undergo further transformation via biotic or abiotic processes. Transformation products may covalently bind to surfaces, thereby reducing availability for further transformation and transport. Under aerobic conditions, Meisenheimer complexes may be formed [62] as a result of the nucleo-

philic attack by a hydride ion on the aromatic ring. While mineralization of TNT has been reported in mixed or undefined culture systems, reports of its mineralization (under aerobic or anaerobic conditions) in pure bacterial systems are lacking.

Due to the actions of nonspecific extracellular enzyme systems (lignin peroxidase, manganese peroxidase, laccase), fungi have the capacity to mineralize TNT. In order to stimulate the production of these enzymes, supply of a growth substrate is required for TNT degradation. Numerous fungi have been shown to transform TNT to varying degrees (Table 4) depending on species, culture conditions, and supplied substrate; however, *Phanerochaete chrysosporium* is the most widely studied fungal isolate. The TNT degradation pathway for *P. chrysosporium* is shown in Fig. 6. The initial reaction in the transformation of TNT involves reduction of nitro groups to nitroso-DNT which may be further transformed to 2-hydroxylamino-4,6-dinitrotoluene and 4-hydroxylamino-2,6-dinitrotoluene (HADNT) and mono- and diaminotoluene (ADNT, DANT) intermediates. As shown in Fig. 6, *P. chrysosporium* and other fungal species may further transform ADNT and DANT to azo, azoxy, phenolic, and acetylated derivatives leading to the mineralization of the parent compound. In TNT mineralization experiments, *P. chrysosporium* species were shown to completely transform TNT, however, mineralization ranged from 10 to 40 % [63]. While litter-decaying basidiomycetes (e.g., *Agaricus eastivalia*, *Agrocybe praecox*, *Clitocybe odora*) were also able to transform TNT, the extent of mineralization was small, ranging from 5 to 15 %. Similar to bacteria, micromycetous fungi (e.g., *Alternaria* sp., *Aspergillus terrus*, *Fusarium* sp., *Mucor mucedo*, *Penicillum* sp., *Rhizoctonia* sp.) were able to transform but not mineralize TNT.
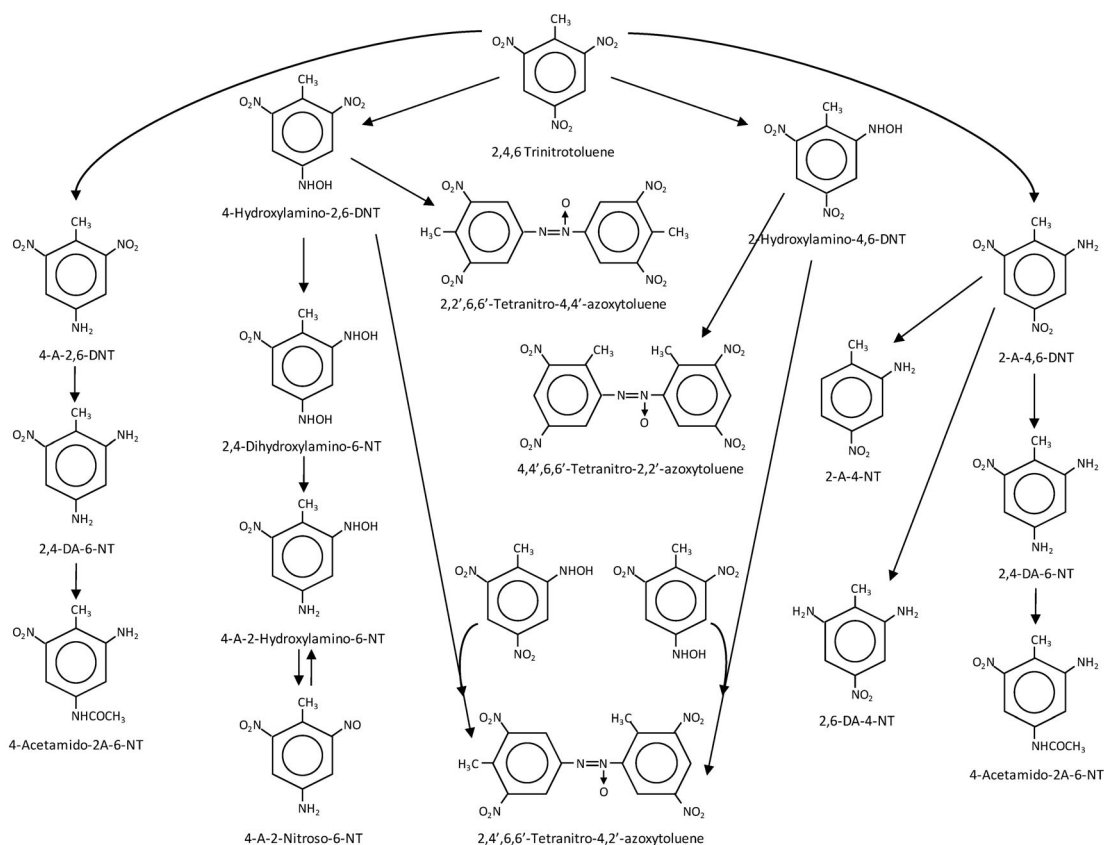

Fig. 4 Metabolic pathway for the bacterial degradation of 2,4,6-trinitrotoluene (TNT) under aerobic conditions.

Table 3 Aerobic and anaerobic bacterial species that degrade TNT, RDX, HMX, and PETN (adapted from Juhasz and Naidu [21]).

Compound	Bacterial species		References
	Degradation under aerobic conditions	Degradation under anaerobic conditions	
TNT	*Acinetobacter johnsoni, Acinetobacter junii, Agrobacterium* sp. 2PC, *Alcaligenes eutrophus, Anabaena* sp., *Arthrobacter globiformis, Arthrobacter* sp. RP17, *Bacillus cereus, B. subtilis, Bacillus* sp., *Corynebacterium glutamicum, Corynebacterium* sp. Nap2, *Cytophaga pectinovora, Flavobacterium odoratum, Klebsiella* sp. 1PC, *Klebsiella pnueomoniae, Micrococcus luteus, Mycobacterium* sp. HL4-NT-1, *M. vaccae* strain JOB5, *Myxococcus xanthus, Pseudomonas aeruginosa, P. aeruginosa* MA01, *P. cepacia, P. fluorescens, P. pseudoalcaligenes* JS52, *P. putida, P. putida* strain KP-T202, *Pseudomonas* sp. clone A, *Pseudomonas* sp. Tol1A, *Pseudomonas* sp. JS150, *Pseudomonas* sp. JLR11, *Pseudomonas* DFC49, *Pseudomonas* sp., *Pseudomonas putida* JLR11, *Rahnella aquitilis* BFB, *R. erthropolis, R. globerulus, R. rhodocrouss, Raoultella terrigena, Rhizobium* sp. T10, *Rhizobium* sp. B5, *Rhizobium* sp. M8, *Rhodococcus* sp. TF2, *Sphingomonas capsulata, Staphylococcus* sp., *Streptomyces albus, S. chromofuscus* A11, *S. griseus*	*Clostridium acetobutylicum, C. bifermentans, C. nitrophenolicum, C. pasteurianum, Enterobacter cloacae* PB2, *Escherichia coli, Serratia marcescens, Veillonella alkalscens, Desulfobacterium indolicum, Desulfovibrio* sp., *D. gigas, D. desulfuricans, D. vulgaris, Desulfovibrio* sp., *Methanococcus* strain B, *M. deltae, M. thermolithotrophicus, Methanosarcina barkeri*	[62,65–103]

(*continues on next page*)

Table 3 (*Continued*).

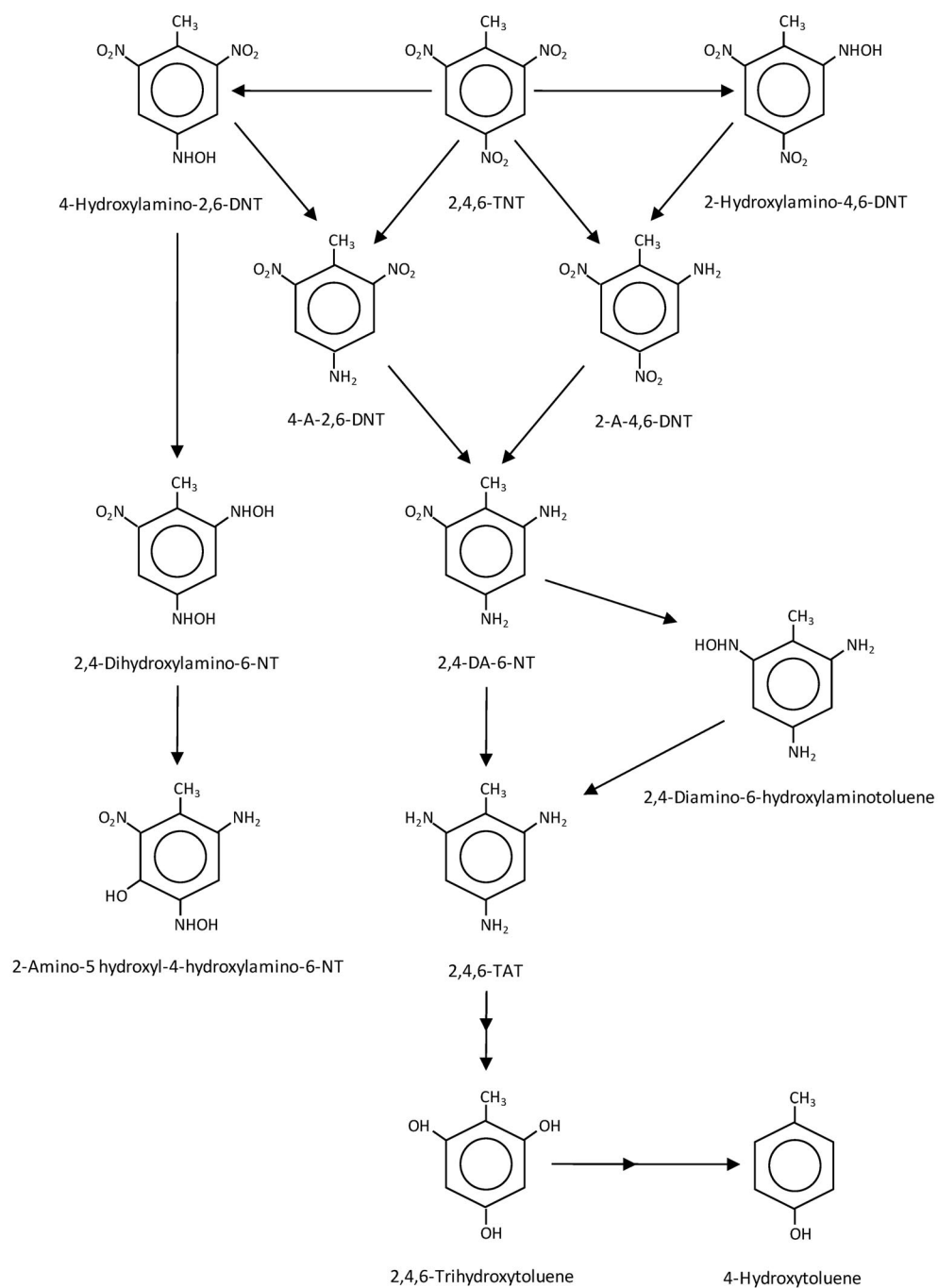| Compound	Bacterial species		References
	Degradation under aerobic conditions	Degradation under anaerobic conditions	
RDX	*Burkholderia* sp., *Methylobacterium* sp. strain BJ001, *Pseudomonas fluorescens, Pseudomonas putida, Rhizobium rhizogenes, Rhodococcus rhodochrous, Rhodococcus* sp. strain A, *Rhodococcus* sp. strain D22, *Rhodococcus* sp. strain DN22, *Rhodococcus* sp. strain YH11, *Shewanella Halifaxensis, Shewanella sediminis, Stenotrophomonas maltophilia*	*Acetobacterium malicum* strain HAAP-1, *Acetobacterium paludosum, Bacillus* sp., *Bacullus* HAW-OC6, *Citrobacter freundii, Clostridium acetobutylicum* ATCC 824, *Clostridium bifermentans, Clostridium butyricum, Clostridium celerecreseens, Clostridium saccharolyticum, Clostridium* sp. strain EDB2, *Desulfovibria* spp., *Desulfovibrio desulfuricans, Enterobacter cloacae* ATCC 43560, *Geobacter metallireducens, Gordonia* sp. KTR9, *Halomonas* HAW-OC4, *Klebsiella pneumoniae* strain SCZ-1, *Marinobacter* HAW-OC1, *Morgenella morganii, Providencia rettgeri, Pseudoalteromonas* HAW-OC2, *Pseudoalteromonas* HAW-OC5, *Pseudomonas* sp., *Williansia* sp. KTR4	[73,82,104–139]
HMX	*Methylobacterium* sp.	*Clostridium* sp. strain EDB2, *Clostridium bifermentans* sp. strain HAW-1	[67,110,111,140–145]
PETN	*Enterobacter cloacae*		[146,147]

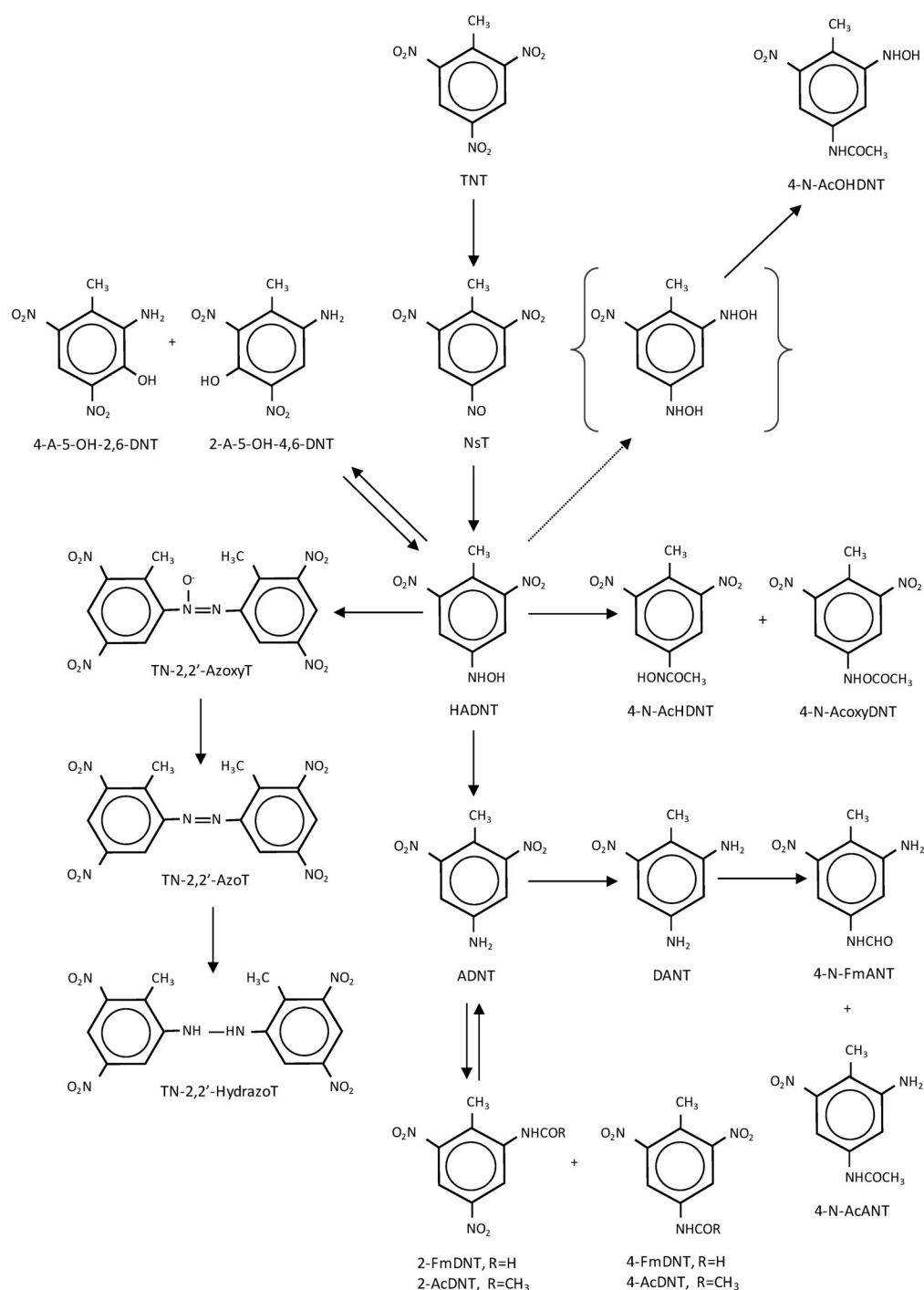**Fig. 5** Metabolic pathway for the bacterial degradation of 2,4,6-TNT under anaerobic conditions.

Fig. 6 TNT degradation pathway for *Phanerochaete chrysosporium* (reproduced from Hawari et al. [64] with permission from Springer). Compounds in brackets represent unidentified products.

2.8 RDX degradation

Figure 7 shows the degradation pathways for RDX under aerobic and anaerobic conditions. Bacterial mineralization of RDX has been shown to occur under aerobic conditions following utilization of the compound as a nitrogen source [110,112] (Table 3). While a proposed degradation pathway is outlined in Fig. 7, only 4-nitro-2,4-diazabutanal has been identified as an aerobic transformation product of RDX. However, the majority of research into RDX biodegradability has been undertaken using anaerobic conditions. Under anaerobic conditions, degradation of RDX may proceed via reduction and ring cleavage or via direct ring cleavage as outlined in Fig. 7. Sequential reduction of the nitro groups results in the formation of 1-nitroso-3,5-dinitro-1,3,5-triazinane (MNX), 1,3-dinitroso-nitro-1,3,5-triazinane (DNX), and 1,3,5-trinitroso-1,3,5-triazinane (TNX). It is proposed that further transformation of MNX, DNX, and TNX results in the formation of hydroxylamine derivatives, although these products are yet to be isolated. Ring cleavage of the hydroxylamine derivatives results in the formation of a number of low-molecular-weight products including dimethylhydrazine, hydrazine, formaldehyde, and methanol.
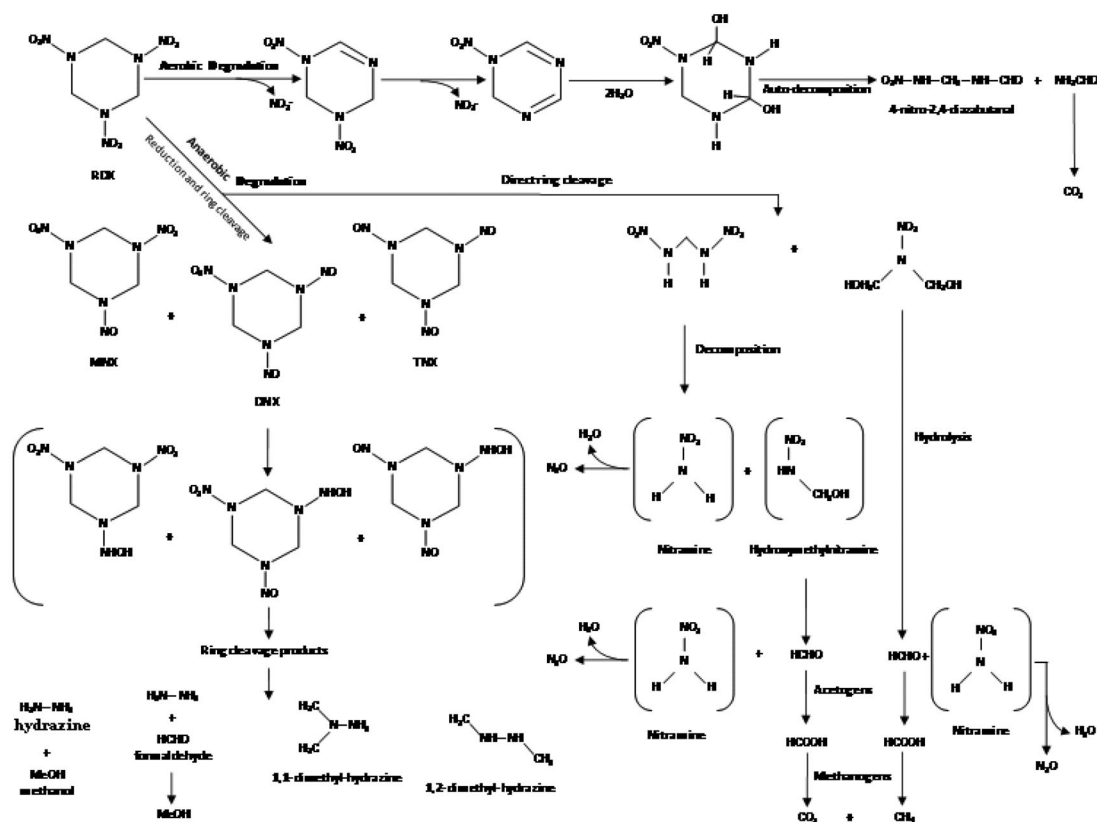
Fig. 7 Bacterial degradation of 1,3,5-trinitro-1,3,5-triazinane (RDX) under aerobic and anaerobic conditions.

Hawari et al. [148] and Fournier et al. [149] proposed that RDX may undergo transformation via direct ring cleavage, resulting in the formation of methylenedinitramine and bis(hydroxymethyl)nitramine (Fig. 7). These products may undergo further transformation, resulting in the production of nitramine and formaldehyde. Nitramine may be abiotically transformed via hydrolysis to nitrous oxide, whereas the actions of acetogenic and methanogenic bacteria convert formaldehyde to carbon dioxide.

As with TNT, the majority of investigations on the fungal degradation of RDX have focused on *P. chrysosporium* (Table 4). The white rot fungus has been shown to utilize the nitramine as a sole nitrogen source [150,151]. In the study of Sheremata and Hawari [151], *P. chrysosporium* completely removed RDX (62 mg/L) from liquid medium containing glycerol as the predominant carbon source. Extensive mineralization of the compound occurred (about 53 %) while 11 % of the ^{14}C was incorporated into fungal biomass with a further 28 % remaining in the aqueous phase as unidentified metabolites. The major by-product of the fungal degradation of RDX was N_2O with 62 % of RDX's nitrogen being converted to this end product.

Table 4 Fungal degradation of TNT, RDX, HMX, and PETN under aerobic conditions (adapted from Juhasz and Naidu [21]).

Compound	Fungal species	References
TNT	*Absidia* sp., *Acremonium* sp., *Agaricus aestivalis* TMAest1, *Agaricus bisporus* MWA80-7, *Agrocybe praecox* TM70.84, *Agrocybe praecox* YM70.3.1, *Alternaria* sp. TMRZ/WN2, *Aspergillus terrus* MWi458, *Bjerkandera adjusta* DSM 3375, *Ceratocystis coerulescens*, *Clitocybe odora* TM3, *Clitocybula dusenii* DSM 11238, *Clitocybula dusenii* TMB12, *Coprinus comatus* TM6, *Cunninghamella elegans* DSM1980, *Cyathus stercoreus* 36910, *Cylindrocarpon* sp., *Fomes fomentarius* MWF01-4, *Fusarium* sp. TMS21, *Gliocladium* sp., *Heterobasidion annosum* TM5P2, *Hypoloma fasciculare* TM5.2, *Irpex lacteus*, *Kuehneromyces mutabilis* TME, *Lentinus lepideus, Lepista nebularis* TM2, *Mucor mucedo* DSM810, *Nematoloma forwardii* DSM 11239, *Neurospora crassa* TM, *Paxillus involutus* TM2, *Penicillium frequentans* ATCC 96048, *Penicillium* sp. DSM 11168, *Phanerochaete chrysosporium* ATCC 1767, *P. chrysosporium* ATCC 24725, *P. chrysosporium* BKM-F-1767, *P. chrysosporium* DSM 1556, *P. chrysosporium*, *P. sordida* HHB-8922, *Phlebia brevispora* HHB-7030, *Phlebia radiata* ATCC 64658, *Pleurotus ostreatus* TMPost, *Rhizoctonia solani* Mwi5, *Stropharia rugoso-annulata* DSM11373*, Stropharia rugosoannulata*, *Trametes modesta*, *Trametes sueveolens* MWT03-2, *Trametes versicolor* DSM 11269, *Trametes versicolor* TM5, *Trichoderma harzianum*, *Trichoderma* sp.	[152–177]
RDX	*Acremonium* sp., *Aspergillus niger*, *Bullera* sp., *Cladosporium cladosporioides*, *Penicillium* sp., *Phanerochaete chrysospotium*, *Rhodotorula* sp.	[119,150,178–181]
HMX	*Phanerochaete chrysosporium*, *Pleurotus ostreatus*	[182,183]

2.9 HMX degradation

Limited information is available regarding the bacterial and fungal degradation of HMX (Tables 3 and 4). While Figure 8 illustrates the degradation pathway for HMX, the majority of transformation products are proposed (in brackets) and are yet to be isolated and identified. As with RDX, bacterial degradation may occur via reduction of the nitro groups to form nitroso intermediates (1-nitroso-3,5,7-trinitro-1,3,5,7-tetrazocane, 1,3-dinitroso-5,7-dinitro-1,3,5,7-tetrazocane, 1,5-dinitroso-3,7-dinitro-1,3,5,7-tetrazocane). It has been proposed that nitroso intermediates may be formed as a result of both aerobic and anaerobic HMX degradation. Hawari et al. [64] proposed an alternative pathway for HMX degradation involving ring oxidation, which results in the transient production of methylenedinitramine and bis(hydroxymethyl)nitramine. These products may be further transformed to nitrous oxide and formaldehyde, which then may be converted to nitrogen, carbon dioxide, and methane via denitrification or methanogenesis.
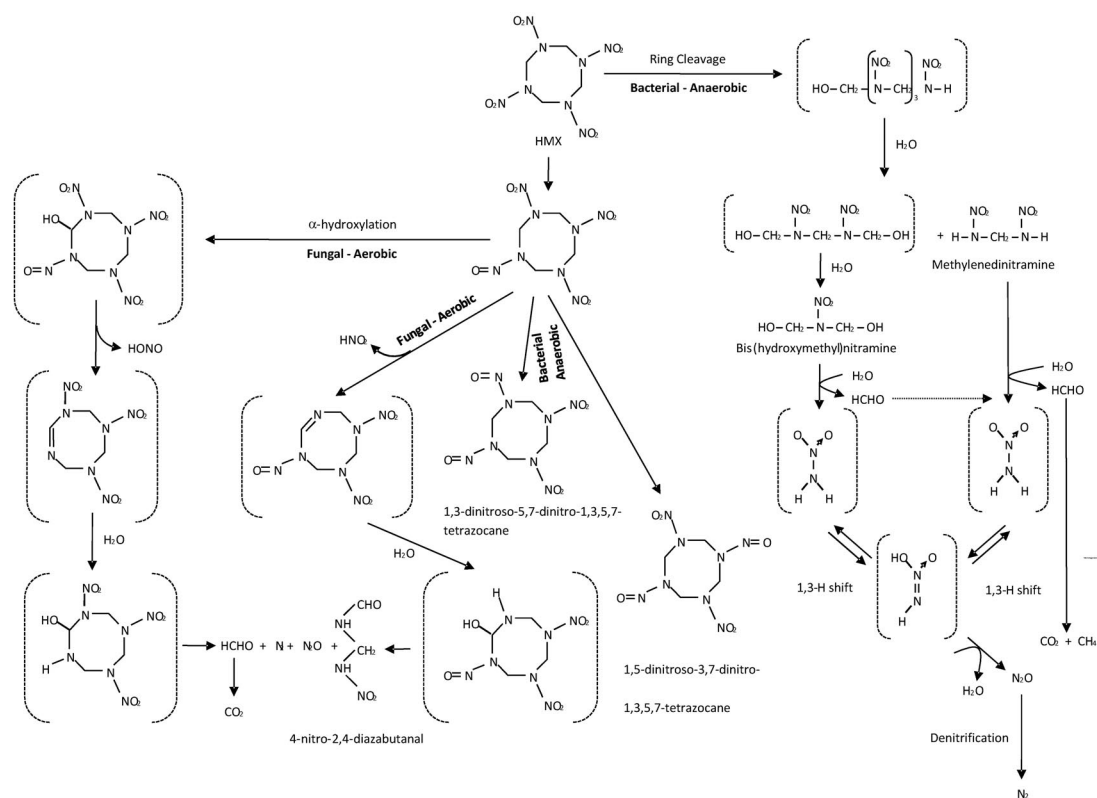
Fig. 8 Metabolic pathways for the bacterial and fungal degradation of 1,3,5,7-tetranitro-1,3,5,7-tetrazocane (HMX).

Under nitrogen-limiting conditions, *P. chrysosporium* has been shown to mineralize HMX [183]. After 25 days incubation, 97 % of HMX was removed via reduction, which resulted in the accumulation of 1-NO-HMX in the culture medium. The concentration of 1-NO-HMX peaked following 12 days incubation after which the decrease in concentration corresponded to the liberation of $^{14}CO_2$ and the accumulation of 4-nitro-2,4-diazabutanal. Fournier et al. [183] proposed that 1-NO-HMX undergoes N-denitration resulting in the formation of an α-hydroxy-alkylnitramine intermediate. Alternatively, α-hydroxylation of 1-NO-HMX may result in unstable intermediates which decompose to the ring cleavage product 4-nitro-2,4-diazabutanal. Further incubation (44 d) resulted in approximately 69 % of ^{14}C recovered as $^{14}CO_2$ while nitrite and nitrous oxide were also detected.

2.10 PETN degradation

Limited information is available regarding the bacterial and fungal degradation of PETN (Table 3). Binks et al. [146] isolated *Enterobacter cloacae* strain PB2 from munitions-contaminated soil with the ability to utilize PETN as a sole source of nitrogen. Utilization of the compound resulted in the formation of 3-hydroxy-2,2-bis-[(nitroxy)methyl]-propanal and 2,2-bis-[(nitroxy)methyl]-propanedial (Fig. 9). Transformation of PETN occurred as a result of PETN reductase activity [184], which catalyzed the reduction of two nitro groups of PETN to alcohol groups.

$$O_2N-O-CH_2 \quad CH_2-O-NO_2$$
$$\diagdown C \diagup$$
$$O_2N-O-CH_2 \quad CH_2-O-NO_2 \qquad \text{PETN}$$

↓ NADPH

NO$_2^-$ ← → NADP

↓

$$O_2N-O-CH_2 \quad CH_2-OH$$
$$\diagdown C \diagup$$
$$O_2N-O-CH_2 \quad CH_2-O-NO_2$$

↓ NADPH

NO$_2^-$ ← → NADP

↓

$$O_2N-O-CH_2 \quad CH_2-OH$$
$$\diagdown C \diagup$$
$$HO-CH_2 \quad CH_2-O-NO_2$$
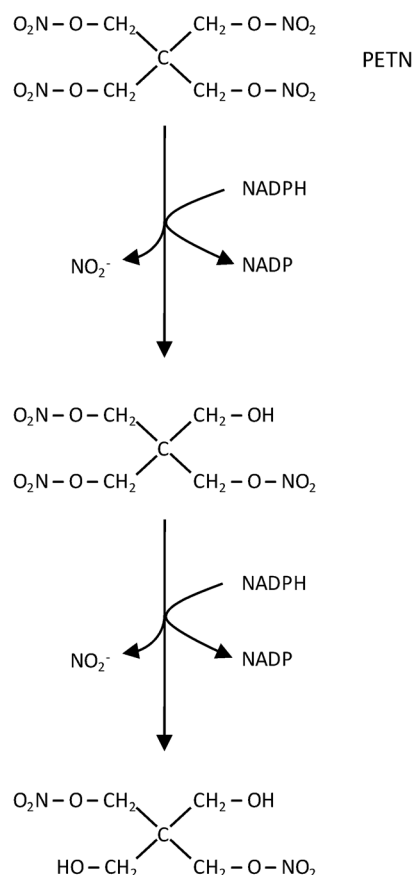
Fig. 9 Metabolic pathway for the bacterial degradation of PETN under aerobic conditions.

2.11 Toxicity of explosives

Following entry into the soil and aquatic and marine environment as a result of manufacture and usage, explosive compounds have the potential to exert toxic effects on a variety of receptors. For humans and some vertebrates, exposure to explosive compounds may occur via a number of exposure routes including inhalation, ingestion, and dermal absorption. While data on the adverse health effects of explosive exposure to humans is predominantly based on information collected from munitions plant workers (epidemiological studies), a considerable amount of information is available from animal studies for the aforementioned exposure routes. While toxicological data is available for a number of ecological receptors (e.g., microorganisms, algae, invertebrates, plants), the major focus of these studies has been on the effects of TNT with limited data available for other explosives such as the nitramines. Although explosive compounds have the potential to exert toxic effects, long-term exposure may result in genotoxic effects. Grummt et al. [185] studied the genotoxicity of nitrosulfonic acids, nitrobenzoic acids, and nitrobenzylalcohols, pollutants commonly found in groundwater near ammunition facilities. The genotoxicity of these compounds was evaluated in Salmonella/microsome assays (in strains TA100 and TA98, with and without S9 and in TA98NR without S9), in chromosomal aberration (CA) tests with Chinese hamster fibroblasts (V79), and in micronucleus (MN) assays with human hepatoma (HepG2) cells. All compounds except the sulfonic acids were positive in the bacterial mutagenicity tests, with 2,4,6-trinitrobenzoic acid (2,4,6-TNBA) producing the strongest response. Studies on mice, rats, and

dogs have demonstrated that TNT exposure may result in a variety of adverse health effects including genotoxic and carcinogenetic effects [186].

2.11.1 Bacteria and algae

A readily available method for the assessment of explosive toxicity in water and soil extract samples is the Microtox assay. As outlined in Table 5, the *Vibrio fischeri*-based assay has been utilized for the assessment of explosive compounds and their transformation products by a number of researchers. In the study by Dodard et al. [187], TNT was shown to be more toxic than its transformation products, with DNT toxicity varying depending on the position of the nitro groups. 2,6-DNT was more toxic than 2,4-DNT while toxicity decreased with further transformation to mono- and diamino products (Table 5). RDX was shown to be less toxic than TNT [188] while HMX was not toxic up to its limit of water solubility.

TNT IC_{50} values for algal growth inhibition, germination, germling length, and cell number using *Selenastrum capricornitum* and *Ulva fasciata* are similar to those obtained using the Microtox assay, however, 2,4- and 2,6-DNT exerted greater toxicological responses by algal species compared to *V. fischeri* (Table 5). In addition, IC_{50} values for RDX (8.1–12 mg/L) are more than 4 times lower than those obtained using the Microtox assay, however, a dearth of information is available for HMX and other explosive transformation products.

2.11.2 Invertebrates

The acute toxicity of explosive compounds to invertebrates has been assessed for marine, freshwater, and terrestrial organisms, although only limited studies have been conducted to assess the effect of explosive transformation production on these organisms. The Naval Facilities Engineering Service Center (NFESC) developed marine porewater and sediment toxicity tests to assess the toxicity of ordnance compounds toward *Arbacia punctulata*, *Dinophilus gyrociliatus*, and *Mysidopsis bahia* [193,194]. The end points for toxicity assessment were fertilization, embryo development, and juvenile survival. The embryological development test was more sensitive than the fertilization test for *A. punctulata* with IC_{50} values for TNT of 12 and more than 103 mg/L, respectively (Table 5). TNT transformation products (2,4- and 2,6-DNT) were of low toxicity when fertilization was the toxicological end point, however, 2,6-DNT was highly toxic to *A. punctulata* embryo development with IC_{50} values ranging from about 0.029 to 36.9 mg/L [193]. For *D. gyrociliatus* and *M. bahia*, TNT IC_{50} values ranged from 0.98 to 7.7, while the toxicity of dinitrotoluenes was 3 to 5 times lower (Table 5). RDX exhibited low toxicity toward *A. punctulata*, *D. gyrociliatus*, and *M. bahia*; an IC_{50} value of 26 mg/L was determined for *D. gyrociliatus* eggs laid per female. In survival studies conducted with *Daphnia magna* and spiked water, TNT exhibited the greatest toxicity (IC_{50} values from 0.8 to 11.9 mg/L) compared to 2,4-DNT and 2,6-DNT. In addition, IC_{50} values for 2,6-DNT were 2-fold greater than 2,4-DNT (Table 5), following a similar toxicity trend for the dinitrotoluenes to that observed for Microtox and algal tests.

A number of studies have utilized earthworms for assessing the toxicity of explosive compounds in spiked soils. TNT IC_{50} values ranging from 132 to 570 mg/kg have been determined for *Eisenia andrei*, *Eisenia fetida*, and the potworm *Enchytraeus crypticus* using survival as the toxicological end point [198–200,204]. Similar IC_{50} values have been determined for 2-ADNT and 4-ADNA (from 201 to 228 and 99 to 111 mg/kg, respectively) although limited studies have been conducted using these transformation products. Recently, the effect of TNT and its metabolites (2,4-DNT, 2A-DNT, 4A-DNT) on cricket (*Acheta domesticus*) reproduction was studied [196]. The relative toxicity of TNT and its metabolites in soil generally showed the following trend, from the lowest to the highest toxicity: TNT, 2A-DNT, 4A-DNT, 2,4-DNT. In addition, toxicity appeared to be higher in sand than in sandy loam soil or in the topical exposure test. After 45 days of exposure in sandy loam soil, the EC_{20}, EC_{50}, and EC_{95} were 14, 116, and 10837 mg/kg for TNT, 1.7, 32, and 16711 mg/kg for 2A-DNT, and 1.9, 9, 296 mg/kg for 4A-DNT and 0.4, 5.7, and 1437 mg/kg for 2,4-DNT. The term half-maximal effective concentration

Table 5 Ecotoxicity of energetic compounds to aquatic, marine, and terrestrial receptors (adapted from Juhasz and Naidu [21]).

Organism	Test	Matrix	$IC_{50}{}^a$ (mg/kg)									References
			TNT	2,4-DNT	2,6-DNT	2-ADNT	4-ADNT	2,4-DANT	2,6-DANT	RDX	HMX	
Microorganisms												
Vibrio fischeri	Microtox (15 min)	Spiked water, Extracts from spiked soils	0.3–1.5	36–73	2.5–3.6	>22	>26	53–81	>51; >101	>42	>6.5	[187–189]
V. fischeri	Microtox (30 min)	Spiked water, Extracts from spiked soils	2.0–3.6			21–230	10–122	48	>100			[190–192]
Algae												
Selenastrum capricornutum	Growth inhibition (96 h)	Extracts from spiked soils	0.5–1.0			1.9–3.4	10–13	23–96	29–43			[188,189]
Ulva fasciata	Germination	Spiked seawater, spiked porewater, porewater from spiked sediment	2.5	2.5	0.092–17					12		[193–195]
U. fasciata	Germling length	Spiked seawater, spiked porewater, porewater from spiked sediment	0.76	1.7	<0.087–8					8.1		[193–195]

Table 5 (*Continued*).

Organism	Test	Matrix	IC$_{50}$^{a} (mg/kg)									References
			TNT	2,4-DNT	2,6-DNT	2-ADNT	4-ADNT	2,4-DANT	2,6-DANT	RDX	HMX	
U. fasciata	Germling cell number	Spiked seawater, spiked porewater, porewater from spiked sediment	1.4	2.1	<0.087–9					9.8		[193–195]
Invertebrates												
Acheta domesticus	Survival	Spiked soil	116	5.7		32	9					[196]
Arbacia punctulata	Fertilization	Spiked seawater	>103	68	>84					>75		[193]
A. punctulata	Embryo development	Spiked seawater, spiked porewater	12	51	<0.029–36.9					>75		[193,194]
Copepod	Survival	Porewater from spiked sediment				49–62						[195]
	Embryo and nauplii survival	Porewater from spiked sediment			60–69							[195]
Daphnia magna	Survival (48 h)	Spiked water	0.8–11.9	47.5	21.8							[197]
Dinophilus gyrociliatus	Survival	Spiked seawater, spiked porewater	7.7	21	0.046–21.1					>49		[193,194]
D. gyrociliatus	Laid eggs/female	Spiked seawater	1.8	5.7	2.1					26		[193,194]

Table 5 (*Continued*).

| Organism | Test | Matrix | IC$_{50}$^{a} (mg/kg) | | | | | | | | | References |
			TNT	2,4-DNT	2,6-DNT	2-ADNT	4-ADNT	2,4-DANT	2,6-DANT	RDX	HMX	
Eisenia andrei	Survival (7 d)	Spiked OECD artificial soil; Spiked forest soil	204–400									[198]
E. andrei	Survival (14 d)	Spiked OECD artificial soil; Spiked forest soil	132–222			201–228	99–111					[199–201]
Eisenia fetida	Survival (14 d)	Spiked forest soil	325									[202]
Enchytraeus albidus	Survival (21 d)	Spiked artificial soil	422 ± 63									[203]
	Fecundity (42 d)	Spiked artificial soil	111 ± 34									[203]
Enchytraeus crypticus	Survival (7 d)	Spiked Lufa 2.2 soil	570									[204]
Eohaustorius estuarius	Survival (10 d)	Spiked sediment	28–36									[205]
Folsomia candida	Survival (7 d)	Spiked Lufa 2.2 soil	185									[204]

Table 5 (*Continued*).

Organism	Test	Matrix	IC$_{50}$[a] (mg/kg)									References
			TNT	2,4-DNT	2,6-DNT	2-ADNT	4-ADNT	2,4-DANT	2,6-DANT	RDX	HMX	
Hyalella azteca	Survival (10 d)	Sediments	30					3460				[197]
Lumbriculus variegatus	Survival (48 h)	Spiked water	6.5									[197]
	Survival (48 h)	Spiked water	5.2									[197]
Mysidopsis bahia	Juvenile survival	Spiked seawater	0.98	5.4	5.6					>47		[206,207]
Vertebrates												
Cyprinodon variegatus	Survival	Spiked seawater	2.3			8.6		>51		9.8–9.9	> aqueous solubility	[197,208]
Danio rerio	Survival (96 h)	Spiked water								23.0–23.6		[197]
Ictarus punctatus	Survival (96 h)	Spiked water	2.4									[197]
Lepomis macrochirus	Survival (96 h)	Spiked water	2.6									[206]
Menidia beryllina	Survival	Spiked seawater								7.1		[197]
Oncorhyncus mykiss	Survival (96 h)	Spiked water	0.8	32.8	18.5							[197,209,210]
Pimephales promelas	Survival (96 h)	Spiked water	0.5–12.7									[193]
Sciaenops ocellatus	Larvae survival	Spiked seawater	8.2	48	34					>68		[211]
Xenopus laevis	FETAX assay (96 h)	Spiked water	3.8			33	23					
Plants												
Echinochloa crusgalli L.	Seedling emergence	Freshly amended soil	129–858	5	4							[212,213]
		Aged soil	398–948	4	3							

Table 5 (*Continued*).

Organism	Test	Matrix	IC$_{50}$^{a} (mg/kg)									References
			TNT	2,4-DNT	2,6-DNT	2-ADNT	4-ADNT	2,4-DANT	2,6-DANT	RDX	HMX	
Hordeum vulgare	Seedling emergence	Spiked artificial soil	8133									[214]
		Spiked forest soil	2500								>1866	
Lactuca sativa L.	Seedling emergence	Spiked artificial soil	>3120									[214]
Lollum perenne	Seedling emergence	Freshly amended soil	45–518	4	89							[212,213,215]
		Aged soil	69–143	8	20					>1540		
Medicago sativa L.	Seedling emergence	Freshly amended soil	64–911	5	4							[212,213]
		Aged soil	50–188	10	5							
Smooth bromegrass	Seedling emergence	Spiked nutrient solution	45									[216]

^{a}The half maximal inhibitory concentration (IC$_{50}$) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function.

(EC_{50}) refers to the concentration of a drug, antibody, or toxicant that induces a response halfway between the baseline and maximum after some specified exposure time. These results suggest that parent TNT and metabolites are toxic to cricket eggs at relatively high concentrations and these toxic effects are manifested as a decrease in hatching success.

2.11.3 Vertebrates

The salamander (*Ambystoma tigrinum*) has been suggested as a bioindicator of effects in the evaluation of toxicity of explosives at contaminated sites [217–219]. Dermal exposure was determined to be the most important exposure pathway for uptake of TNT from contaminated soil by the salamander. Trace amounts of TNT were detected only in the skin and liver of exposed salamanders, while 2,6-DANT was found only in liver and kidney tissues [218]. Skin was concluded to be important in the primary reduction of TNT. When salamanders were exposed to TNT in soil (1 mg/kg) and fed earthworms exposed to TNT in the same soil, no difference between control and treated animals were observed for weight gain, organ-to-body-weight ratios, function of spleenic phagocytic cells, nor peripheral hematological parameters [219]. However, the liver exhibited heavily pigmented iron-rich phagocytes (melanomacrophages) and growth rate was slower during treatment.

A variety of fish species have been utilized to assess the toxicity of explosive compounds (Table 5). In studies conducted by Lotufo and Lydy [207], juvenile sheepshead minnows (*Cyprinodon variegatus*) were exposed to the explosive compounds TNT, RDX, HMX, and to the TNT transformation products 2-ADNT and 2,4-DANT in five separate water-only experiments. The results showed that because of the fast elimination rate of TNT and its transformation products and the exceedingly low bioaccumulative potential of RDX and HMX, exposure conditions likely associated with the presence of explosives in aquatic systems are unlikely to pose unacceptable risks to fish. However, the acute 96-h IC_{50} of RDX for juvenile fathead minnow was estimated at 12.7 mg/L and for bluegill, at 6 mg/L [210]. Mukhi et al. [208] investigated the toxicity of RDX to larval zebrafish (*Danio rerio*). The 96-h IC_{50} was estimated at 22.98 and 25.64 mg/L in two different tests. The estimated no observed effective concentration (NOEC) values of RDX on lethality were 13.27 ± 0.05 and 15.32 ± 0.30 mg/L, and the lowest observed effective concentration (LOEC) values were 16.52 ± 0.05 and 19.09 ± 0.23 mg/L in these two tests, respectively. The 96-h IC_{50} for vertebral deformities on survivors from one of the acute lethality tests was estimated at 20.84 mg/L with NOEC and LOEC of 9.75 ± 0.34 and 12.84 ± 0.34 mg/L, respectively. The authors concluded that the chronic effects of RDX in aquatic vertebrates need to be determined for an adequate assessment of the ecological risk of RDX. The acute toxicity of TNT is greater than RDX with IC_{50} values ranging from 0.5 to 8.2 mg/L for a number of fish species (see able 5).

The toxicological effects of explosive compounds on mammals have been studied extensively with information being detailed in the Agency for Toxic Substances and Disease Registry [186,220,221]. Studies on mice, rats, and dogs have demonstrated that TNT exposure may result in a variety of adverse health effects including cardiovascular, gastrointestinal, hematological, hepatic, renal, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenetic effects [186]. Recent mammalian studies conducted on mice and rats [222,223] administered up to 601 mg TNT per kg body weight to whitefooted mice over a 14-day period. While fatalities were not observed [222], several indicators of nonspecific immunity, including increased spleen weight, were related to dose. Results of dosing studies with hispid cotton rats suggest that hepatic enzymes and hemolytic anemia may be useful biomarkers of terrestrial contamination by explosives [223]. Increased spleen weight, hemolytic anemia and elevated methemoglobin, and increased weight and histological changes of the liver were among the effects reported. In humans, TNT is considered a mutagen and exposure may result in liver damage. Other symptoms of TNT exposure include dermatitis, vomiting, anemia, toxic hepatitis, and urine discoloration [186].

Epidemiological studies have identified that RDX exposure results in neurological effects such as seizures and convulsions. Other symptoms of RDX exposure in humans include nausea and vomiting, while in animals, minor hepatic and renal effects have been reported [220]. Cyclonitramines are gener-

ally considered to be toxic to living organisms [224], and thus the presence of RDX in the natural environment could be detrimental to the health of the ecosystem. Toxic effects of RDX have been well documented in various laboratory animals including mammals and birds [208 and refs. therein, 225]. Limited data is available regarding the health effects of HMX exposure to humans. In animals, high doses may adversely affect the gastrointestinal tract and kidneys and result in hepatotoxicity. Mild hematological effects may occur following large doses of HMX (larger than 4000 mg/kg per day) including a decrease in hemoglobin and packed cell volume and an increase in methemoglobin [221].

2.11.4 Plants

Limited information is available regarding the phytotoxicity of energetic compounds to plants. Of the studies conducted, root elongation, seedling emergence, biomass production, and photosynthesis parameters have been used as phytotoxicity indicators. The majority of studies have been undertaken using agricultural species (Table 6) with more recent studies investigating phytotoxic effects on rye grass [212,226,227] and Arabidopsis [228]. While impacts on the aforementioned indicator parameters have been reported, few studies have determined NOEC, LOEC, EC_{20}, or EC_{50} values for explosive compounds (Table 5). NOEC values for TNT range from approximately 50 mg/kg (*Brassica rapa*, *Lepidium sativum*, *Hordeum vugare*) to 150 mg/kg (*Avena sativa*) [214,229,230]. For *Medicago sativa*, *Echinochloa crusgall* L., and *Lolium perenne* L., EC_{20} values for TNT range from 43 to 62 mg/kg while DNT was found to be more phytotoxic (EC_{20} values ranging from 3 to 24 mg/kg) than TNT [212]. Similarly, Best et al. [227] determined that the EC_{20} value for TNT metabolites was 3.75 mg/kg for *Lolium perenne*. Robidoux et al. [214] determined that *Hordeum vugare* was not affected by HMX fractions (up to 1866 ± 438 mg/kg) while Best et al. [215] determined that *Lolium perenne* was not impacted by RDX up to 1540 mg/kg. The variability in NOEC, LOEC, EC_{20}, and EC_{50} values for individual compounds may be expected due to species specific differences in explosives uptake, in addition to differences in soil physico-chemical parameters and soil-explosive residence time, which will both influence adsorption and therefore phytoavailability.

Table 6 Plants used for the assessment of explosive compound phytotoxicity.

Plant	Test	Matrix	References
Allium cepa, Avena sativa, Arabidopsis thaliana, *Brassica rapa* Metzg, *Bromus inermis* Leyss, *Cucumis sativus Echinochloa crusgalli* L., *Festuca arundinacea, Hordeum vulgare, Lactuca sativa* L., *Lens culinaris* Med., *Lolium perenne* L., *Lepidium sativum* L., *Medicago sativa* L., *Oriza sativa, Panicum virgatum* L., *Raphamus sativus, Rumex crispus, Sinapis alba* L., *Triticum aestirum* L.	Root elongation, seedling emergence, fresh shoot biomass, photosynthesis	Water, sand/soil mixture, antitank firing range soil	[212,214,216,226–240]

3. STATE-OF-THE-ART TREATMENT PROCESSES FOR REMEDIATION OF EXPLOSIVES-CONTAMINATED SOIL

3.1 Biodegradation and bioremediation methods

Many xenobiotic chemicals introduced into the environment for agricultural and industrial use are nitro-substituted aromatics. Nitro groups in the aromatic ring are often implicated as the cause of the persistence and toxicity of such compounds. Nitroaromatic compounds enter soil, water, and food by several routes such as use of pesticides, plastics, pharmaceuticals, landfill dumping of industrial wastes, and the military use of explosives. The nitroaromatic compound, TNT, is introduced into soil and water ecosystems mainly by military activities like the manufacture, loading, and disposal of explosives and propellants. This contamination problem may increase in the future because of the demilitarization and disposal of unwanted weapons systems.

Biotransformation of TNT and other nitroaromatics by aerobic bacteria in the laboratory has been reported frequently [91,120,241–248]. Biodegradation of 2,4-DNT by a *Pseudomonas* sp. has been reported to occur via 4-methyl-5-nitrocatechol in a dioxygenase-mediated reaction [249]. Duque et al. [244] successfully constructed a *Pseudomonas* hybrid strain that mineralized TNT. White rot fungus has been shown to mineralize radiolabeled TNT [157]. The work of Spiker et al. [168] showed that *Phanerochaete chrysosporium* is not a good candidate for bioremediation of TNT-contaminated sites containing a high concentration of explosives because of its high sensitivity to contaminants. Michels and Gottschalk [164] showed that the lignin peroxidase activity of *P. chrysosporium* is inhibited by the TNT intermediate hydroxylamino-dinitrotoluene. Valli et al. [250] found that 2,4-DNT is degraded completely by the white rot fungus.

Ecological observations suggest that sulfate-reducing and methanogenic bacteria might metabolize nitroaromatic compounds under anaerobic conditions if appropriate electron donors and acceptors are present in the environment, but this ability had not been demonstrated until recently. Under anaerobic conditions, the sulfate-reducing bacterium, *Desulfovibrio* sp. (B strain) transformed TNT to toluene [251,252] by reduction. Gorontzy et al. [253] reported that under anaerobic conditions, methanogenic bacteria reduced nitrophenols and nitrobenzoic acids. Preuss et al. [99] demonstrated conversion of TNT to methylbenzenetriamine by a *Desulfovibrio* sp.

3.1.1 Anaerobic biotransformation of nitroaromatic compounds

The anaerobic bacterial metabolism of nitroaromatics has not been studied as extensively as of aerobic pathways, perhaps because of the difficulty in working with anaerobic cultures and the slow growth of anaerobes. Earlier studies on anaerobic metabolism of nitroaromatic compounds by McCormick et al. [91] laid the foundations for such study and established the usefulness of anaerobic organisms. Successful demonstration of degradation of 1,2,3-trinitro-1,3,5-triazinane (RDX) by sewage sludge [120,254] under anaerobic conditions further demonstrated the usefulness of anaerobes in waste treatment. RDX was reduced sequentially by the anaerobes to the nitroso derivatives, which were further converted to formaldehyde and methanol. Hallas and Alexander [255] showed successful transformation of NB, nitrobenzoic acid, nitrotoluene, and nitroaniline by sewage sludge under anaerobic conditions.

Methanogens are obligate anaerobes that grow in an environment with an oxidation-reduction potential of less than –300 mV. They transform various substrates to C_1 products such as CH_4 and HCOOH. The role of some novel compounds and the mechanism of single carbon flow in these bacteria remain to be formally proved, along with the arrangement of the electron transport chain. Because of the limited substrate capabilities, the metabolism of more complex molecules to methane depends on the activity of non-methanogens in association with the methanogens. Under pure culture conditions, methanogens have not been reported to degrade aromatic compounds. The studies of Gorontzy et al. [253] on microbial transformation of nitroaromatic compounds by methanogenic bacteria revealed that methanogens can transform nitroaromatic compounds to corresponding amino compounds. Boopathy

and Kulpa [256] isolated a methanogen, *Methanococcus* sp., from a lake sediment which transformed TNT to 6-methyl-5-nitro-1,3-diamine. This organism also transformed NB and nitrophenol. The intermediates observed were amino derivatives of the parent compounds. According to some reports, the reductive transformation of nitroaromatic compounds leads to detoxification of the substance [257,258]. The specific enzymes responsible for the reduction process in methanogens have not been characterized yet. Angermeier and Simon [259] suggested that the reduction of aromatic compounds may be catalyzed by hydrogenase and ferredoxin. The observation of sulfate reducers and methanogenic bacteria by many workers [68,99,252,253,256,260] suggests that these organisms could be exploited for bioremediation under anaerobic conditions by supplying proper electron donors and acceptors.

Boopathy et al. [260] showed that TNT can be transformed under anaerobic conditions by using different electron acceptors. A soil sample collected from the Joliet Army Ammunition Plant, Joliet, IL was incubated under sulfate-reducing, nitrate reducing and methanogenic conditions. The results showed that TNT was transformed under all three conditions. However, when no electron acceptor was supplied no TNT was transformed. The intermediates observed during the study were 2,6-dinitrotoluene-4-amine and 4,6-dinitrotoluene-2-amine. This study showed that if the appropriate electron acceptor is present in the system, anaerobic bacteria will reduce TNT to amino compounds.

3.1.2 Sulfate-reducing bacteria

Although oxygen is the most widely used electron acceptor in energy metabolism, a number of different kinds of bacteria are able to reduce other compounds and hence use them as electron acceptors. This process of anaerobic respiration is less energy-efficient, but it allows these bacteria to live in environments where oxygen is absent.

Sulfate-reducing bacteria are obligate anaerobes that are conveniently considered together because of their shared ability to perform dissimilatory sulfate reduction, a process analogous to aerobic respiration in that the sulfate ion acts as an electron acceptor, like oxygen in the aerobic process. The genera of sulfate reducers are defined on the basis of morphology rather than physiology. All sulfate reducers are gram negative, except *Desulfotomaculum*. The most frequently encountered genus is *Desulfovibrio*.

The use of various non-fermentable aromatic compounds in the absence of oxygen or nitrate is apparently of the natural roles of sulfate-reducing bacteria. Aromatic compounds with more than two hydroxyl groups are readily degraded by fermenting bacteria [261]. Several new types of sulfate-reducing bacteria have been isolated directly with aromatic compounds [262–264]. Most of these isolates are extremely versatile sulfate reducers that use many aliphatic compounds. Aromatic compounds oxidized by sulfate-reducing bacteria include benzoate, phenol, p-cresol, aniline, and the *N*-heterocyclic compounds like nicotinate, indole, and quinoline. All the known degraders of aromatic compounds are complete oxidizers. The sulfate-reducers employ reactions like those detected in denitrifying bacteria, phototrophic bacteria, and methanogenic co-cultures using aromatic compounds [265–268]. The sulfate-reducing bacteria are capable of carrying out the following reactions: activation of benzoate to benzoyl CoA [269,270], caroboxylation of phenol to 4-hydroxybenzoate [271,272], or the reductive removal of hydroxyl groups [273].

3.1.3 Metabolism of TNT and other nitroaromatic compounds by sulfate-reducing bacteria

Boopathy and co-workers [252] showed that a sulfate-reducing bacterium, *Desulfovibrio* sp. (B strain) can convert TNT to toluene. This organism, isolated from an anaerobic digester treating furfural-containing wastewater [274], used nitrate as electron acceptor apart from using sulfate as electron acceptor. It also used nitrate as a nitrogen source. Further experiments showed that this bacterium could use the nitro group present in TNT molecules either as an electron acceptor or as a nitrogen source.

Some sulfate-reducing bacteria can use nitrate in addition to sulfate as their terminal electron acceptor [275]. The reaction is coupled to electron transfer phosphorylation [276,277] and is catalyzed by a respiratory nitrite reductase that has a molecular mass of 65 KDa and contains six c-type hemes.

This nitrite reductase known as the hexaheme cytochrome C_3 is widely distributed in strict and facultative aerobes [278,279]. This nitrite reductase is unrelated to the regulated nitrite reductase (nonheme iron siroheme containing) found in many plants and bacteria [280], where its function is nitrogen assimilation. According to Steenkamp and Peck [277], nitrite reductase is closely associated with a hydrogenase and is probably a transmembrane protein. This conclusion is based on the presence of proton-releasing and nitrite-binding sites on the periplasmic aspect of the cytoplasmic membrane and a benzyl viologen-binding site on the cytoplasmic side of the membrane.

TNT (100 mg/L) was metabolized by *Desulfovibrio* sp. (B strain) within 10 days [252], with pyruvate as the main substrate, sulfate as the electron acceptor and TNT as the sole nitrogen source. Boopathy et al. [252] showed that under different growth conditions that this bacterium used TNT as its sole source of nitrogen. This result indicates that the isolate has the necessary enzymes to use the nitro groups present in TNT molecules as a nitrogen source.

Apart from pyruvate, lactate served as the best substrate for TNT metabolism, followed by H_2 + CO_2, ethanol, and formate. Comparison of the rate of TNT biotransformation by *Desulfovibrio* sp. with that of other sulfate-reducing bacteria showed that this new isolate has a unique metabolic ability to degrade TNT. *Desulfovibrio* sp. transformed 100 % of TNT present in a relatively short period of time (7 d). Other *Desulfovibrio* spp. (ATCC cultures) converted 59–72 % TNT within 21 to 23 days, whereas *Desulfobacterium indolicum* transformed 82 % of TNT in 36 days of incubation [252].

Mass spectral analyses showed that various intermediates were produced depending upon the culture conditions of the isolate. When ammonium was the main nitrogen source, 2,4-diamino-6-nitrotoluene was the major intermediate. When TNT was the sole source of carbon and energy, it was first reduced to 4-amino-2,6-dinitrotoluene and then to 2,4-diamino-6-nitrotoluene. When TNT was the sole source of nitrogen, all the TNT in the medium was converted to 2,4-diamino-6-nitrotoluene within 10 days of incubation and traces of 2- and 4-amino compounds were identified. Later these intermediates were converted to toluene. The quantitative analysis of the aqueous and gas phases of the culture bottle by gas chromatograph showed a good mass balance of TNT to toluene [252].

Nitroaromatic compounds are considered resistant to microbial attack [281,282], partly because the reduction of electron density in the aromatic ring by the nitro groups can hinder electrophilic attack by oxygenases and thus prevent aerobic degradation of nitroaromatic compounds [283]. Under anaerobic conditions, the sulfate-reducing bacteria metabolized TNT. Of all the metabolites produced, the formation of toluene from TNT seems to be very novel and significant.

TNT was reduced to nitrotoluenediamines by the isolate through dinitrotoluene-2-amines and dinitrotoluene-4-amines when pyruvate served as the main substrate in the presence of sulfate and ammonia, in a simple reduction process carried out by the enzyme nitrite reductase. The cell free extract showed high activity of nitrite reductase. The nitroreductase activity was monitored photometrically at 325 nm by the consumption of diaminonitrotoluene. Most *Desulfovibrio* spp. have nitrite reductase enzymes that reduce nitrate to ammonia [263]. This isolate reduced the nitro groups present in TNT to amino groups. When TNT served as the sole source of nitrogen, toluene was formed from the TNT. McCormick et al. [91] showed that TNT was reduced by H_2 in the presence of enzyme preparations of *Veillonella alkalescens* to toluenetriamine: 3 mol H_2 is required to reduce each nitro group to the amino group. Preuss et al. [99] observed the formation of methylbenzenetriamine from TNT by a sulfate-reducing bacterium isolated from sewage sludge.

Boopathy et al. [252] showed the formation of toluene from triaminotoluene and in the process, the isolate used the ammonium released from the original TNT molecule as a nitrogen source for growth. This is achieved by reduction of nitro groups followed by reductive deamination. A significant quantity of toluene concentration was observed in the culture sample [252], and virtually no nitrite ions were detected during TNT metabolism. The aromatic ring structure was not cleaved, and no metabolites other than toluene appeared even after six months of incubation. Reductive deamination is catalyzed by a deaminase enzyme in *Pseudomonas* sp. [284]. Reductive deamination reactions were postulated first for 2-aminobenzoate degradation by methanogenic enrichment cultures [285]. Reductive

dehydroxylation of gentizate to benzoate and acetate was demonstrated in the fermenting bacterium HQGO1 [286].

Beller et al. [287] and Edwards et al. [288] demonstrated the complete mineralization of toluene under sulfate-reducing conditions. These toluene-degrading sulfate reducers could be used in combination with the *Desulfovibrio* sp. described by Boopathy et al. [252] to degrade TNT completely to CO_2.

The *Desulfovibrio* sp. (B strain) [252] also metabolized other nitroaromatics such as 2,4-dinitrophenol (2,4-DNP), 2,4-DNT, 2,6-DNT, and aniline. As shown by Boopathy and Kulpa [260], the *Desulfovibrio* sp. used all the nitroaromatics studied as a sole source of nitrogen. It also used 2,4-DNT, 2,6-DNT, and 2,4-DNP as electron acceptors in the absence of sulfate. The GC/MS analyses of the culture samples showed the presence of phenol from 2,4-DNP and benzene from aniline as intermediates. Gorontzy et al. [253] showed transformation of nitrophenols and nitrobenzoic acids by the sulfate reducers *Desulfovibrio desulfuricans*, *D. gigas*, *Desulfococcus multivorans*, and *Desulfotomaculum orientis*. All of the nitroaromatics were transformed to corresponding amino compounds.

Schnell et al. [264] isolated a new sulfate-reducing bacterium, *Desulfobacter anilini*, which degraded aniline completely to carbon dioxide and ammonia with stoichiometric reduction of sulfate to sulfide. This is the first obligate anaerobic bacterium observed to grow in pure culture with aniline as its sole electron donor and carbon source. The organism oxidizes aniline completely to carbon dioxide and releases the amino nitrogen quantitatively as ammonia. Two metabolic pathways were suggested. First, aniline could be carboxylated to 2-aminobenzoate or 4-aminobenzoate, with the aminobenzoate then reductively deaminated to benzoate and metabolized further [289]. Alternatively, aniline could be deaminated hydrolytically to phenol, which is subsequently degraded either by carboxylation to 4-hydroxybenzoate or by reductive transformation to cyclohexanol or cyclohexanone. Both pathways appear possible, because the bacterial strain used each of these intermediates as a sole source of carbon.

Schnell and Schink [290] reported that *D. anilini* degraded aniline via reductive deamination of 4-aminobenzoyl CoA. The first step, the carboxylation of aniline to 4-aminobenzoate, is followed by activation of 4-aminobenzoate to 4-aminobenzoyl CoA, which is reductively deaminated to benzoyl CoA. This product enters the normal benzoate pathway leading to three acetyl CoA. Carbon monoxide dehydrogenase and formate dehydrogenase are present in *D. anilini* indicating that acetyl residues are oxidized via the carbon monoxide dehydrogenase pathway [264].

Schnell and Schink [291] isolated a sulfate-reducing bacterium that oxidized 3-aminobenzoate to carbon dioxide with concomitant reduction of sulfate to sulfide and release of ammonium. High activity of carbon monoxide dehydrogenase indicated that acetyl CoA is oxidized via the carbon monoxide dehydrogenase pathway, although 2-oxoglutarate synthase activity was found as well. Similar activity was found with pyruvate as substrate. Perhaps both synthase activities can be attributed to an enzyme needed in assimilatory metabolism. Carbon monoxide dehydrogenase and pyruvate synthase are probably also key enzymes during autotrophic growth with hydrogen and sulfate. The complete oxidation of 3-aminobenzoate yields –186 kJ/mol according to the following equation:

$$2C_7H_6NO_2^- + 7SO_4^{2-} + 11H^+ = 14CO_2 + 2NH_4^+ + 7HS^- + 4H_2O, \Delta_r G^{\circ'} = -180 \text{ kJ/mol}$$
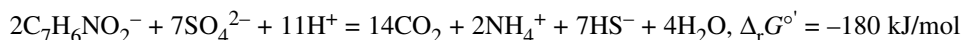
The first step in degradation of 3-aminobenzoate by this new sulfate-reducing bacterium was found to be activation to 3-aminobenzoyl CoA [291]. Further reduction of 3-aminobenzoyl CoA did not yield benzoyl CoA, but rather a product tentatively described as a reduced CoA-ester. The activation of benzoyl CoA depends on the presence of the cofactors, ATP and Mg^{2+}. Acyl-CoA synthetase reactions were identified as the initial step in the degradation of benzoate by anaerobic bacteria.

3.1.4 Bioremediation of TNT under sulfate-reducing conditions

Soil and water in most U.S. military facilities are contaminated with explosive chemicals, mainly because of the manufacture, loading, and disposal of explosives and propellants. This contamination problem may increase in future because of demilitarization and disposal of unwanted weapon systems. Disposal of obsolete explosives is a problem for the military and the associated industries because of

the polluting effect of explosives in the environment [292]. TNT is the major contaminant in many U.S. Army ammunition facilities. TNT represents an environmental hazard because it has toxicological effects on number of organisms [157,293] and it is mutagenic [294]. The disposal of large quantities of TNT in an environmentally acceptable manner poses serious difficulties. The present approach to the remediation of TNT contamination is incineration of soil, a very costly and destructive process. Bioremediation would be a safe and cost-effective method for treating TNT contamination. Biological removal of explosives from soil has been demonstrated using aerobic/anoxic soil slurry rectors [295–298]. A well-defined sulfate-reducing consortium, consisting of *Desulfovibrio* spp., namely, *D. desulfuricans* strain A, *D. desulfuricans*, strain B, *D. gigas*, and *D. vulgaris*, was isolated from a creek sediment [69]. The ability of this consortium to degrade and remediate TNT was explored.

The consortium was grown in anaerobic serum bottles under various growth conditions including TNT as the sole carbon source, cometabolic condition with pyruvate (30 mM) as co-substrate, and heat inactivated control as shown in Boopathy and Manning [69]. Figure 10 shows the results of bacterial growth. Growth was observed in all conditions except in the killed control. The maximum growth was observed under cometabolic conditions and bacteria also grew under the conditions where TNT served as the sole carbon source. Figure 11 shows the removal of TNT under various culture conditions. In all the cultures, the initial TNT concentration was 100 mg/L. In the killed control, the TNT mass concentration remained constant throughout the experiment, indicating that no physical or chemical removal of TNT occurred. TNT removal was fastest in the cometabolic condition, where 100 % of TNT was removed within 10 days of incubation. TNT removal in the culture condition where TNT served as the sole carbon source was very slow, but 100 % of the TNT was still removed within 25 days. The results show that the consortium can remove TNT faster in the presence of an additional carbon source like pyruvate. This could be due to an increase in the bacterial cell numbers in the pyruvate-containing cultures.
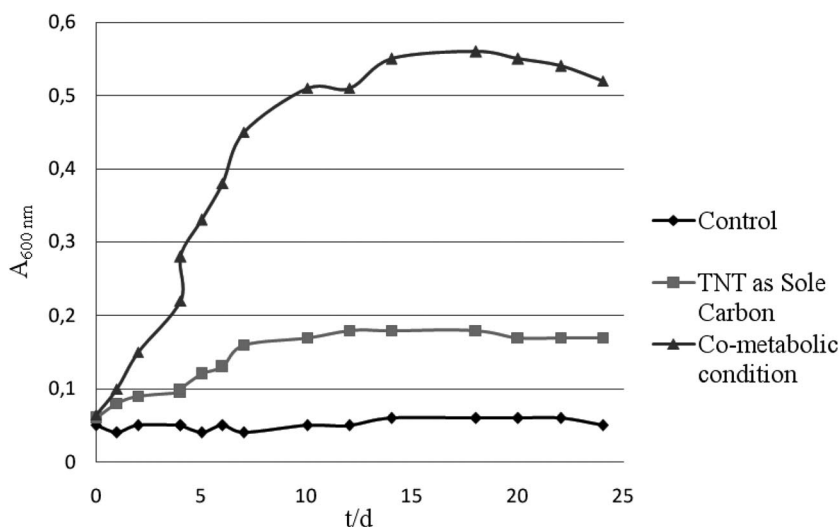

Fig. 10 Growth of sulfate-reducing bacteria under various growth conditions.
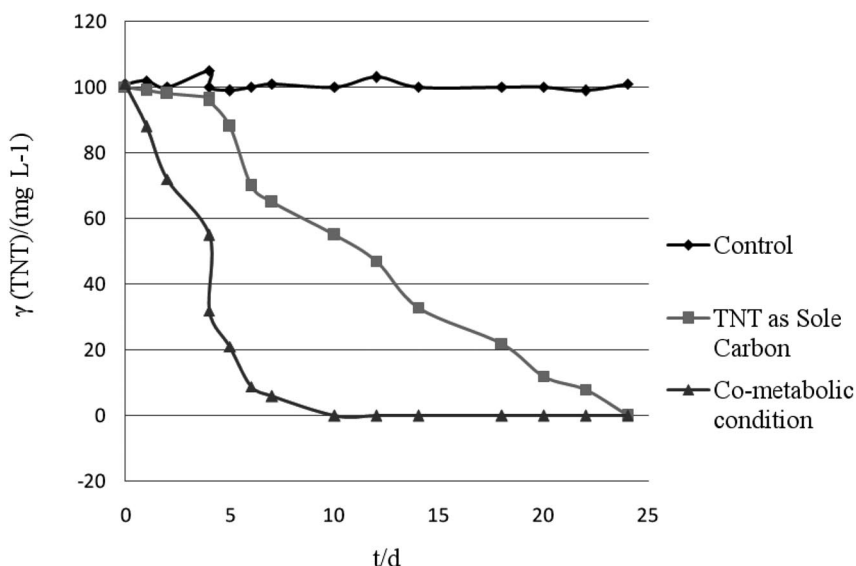
Fig. 11 Removal of TNT by sulfate-reducing bacteria under various growth conditions over a period of 25 days.

The GC-MS analysis of culture samples with and without pyruvate revealed the presence of various intermediates, which were identified by comparison of their GC retention times and their mass spectra with authentic standards. The first intermediates observed were 4-ADNT and 2-ADNT. The ratio of 4-ADNT and 2-ADNT formed from the TNT metabolism was approximately 80:20. These products were further reduced to 2,4-DANT. Other compounds appearing in the culture medium in order were nitrobenzoic acid, cyclohexanone, 2-methylpentanoic acid, butyric acid, and acetic acid. All of these compounds were identified in cultures with both TNT and pyruvate as carbon sources as well as in the cultures that received TNT alone as a carbon source. These intermediates were not present in the control.

A radiolabeled study was conducted with TNT uniformly ring-labeled ^{14}C TNT (hereafter denoted [14C]-TNT) in the culture condition, where TNT served as the sole carbon source. The experimental procedure used by Boopathy and Manning [69] was used in this study. The results of the radiolabeled study are presented in Table 7. The data showed the production of various metabolites and biomass at the end of the experiment on day 30. TNT was not mineralized, as there was no production of CO_2. Most of the TNT was converted to acetic acid (49 %) and 27 % of TNT was assimilated into cell biomass. Apart from acetate, the other major intermediates present in the culture medium were nitrobenzoic acid (6 %) and butyric acid (9.5 %). In the killed control, TNT was reduced to a smaller extent to 4-ADNT (3 %), yet nearly 95 % of the original TNT was recovered unaltered. Traces of cyclohexanone were observed, which accounted for 0.01 % of the original [14C] TNT. This radiolabeled study showed a reasonable mass balance with a recovery of 95 % of [14C] TNT. Since the ring carbons of TNT were uniformly labeled, conversion of TNT to acetic acid and butyric acid clearly denotes ring cleavage.

Table 7 Results of radiolabeled TNT study: Relative mass balance for TNT metabolism by *Desulfovibrio* consortium.

[^{14}C] TNT recovered	Active culture (%)	Killed control (%)
CO_2	0	0
Biomass	27.4	0
Acetic acid	49.5	0
Nitrobenzoic acid	6.2	0
Cyclohexanone	0.01	0
Butyric acid	9.5	0
2-Methyl pentanoic acid	0.2	0
4-ADNT	0.5	3.2
2-ADNT	0.9	0
2,4-DANT	0.7	0
TNT	0	94.5
Unrecovered	5.09	2.3

The production of various intermediates in both culture conditions (with TNT as the sole carbon source and cometabolic condition with pyruvate) suggested that the bacterial consortium has all the necessary enzymes to degrade TNT. The anaerobic metabolic pathway as shown in Fig. 12 was proposed for TNT metabolism by sulfate-reducing bacteria. TNT was reduced to 4-ADNT and 2-ADNT, which were further reduced to 2,4-DANT. These reductions may have been accomplished by the production of sulfide from sulfate by the *Desulfovibrio* spp., as demonstrated by Preuss et al. [99] and Gorontzy et al. [253]. The sulfide analysis showed 10.6 and 3.1 mM of sulfide on day 20 in the cultures with and without pyruvate, respectively. The large difference in sulfide production in the cultures with and without pyruvate may be due to the availability of higher electron donor in pyruvate-containing cultures compared to cultures with only TNT. The next metabolite identified was NB. There may be two or three intermediates between 2,4-DANT and NB, which were not identified. These compounds might be transient and thus not detected in the GC analysis. The NB was converted to cyclohexanone. This step was accomplished by ring cleavage, which under anaerobic conditions would generally be accomplished by a series of hydrogenation and dehydrogenation reactions [267], converting NB to cyclohexanone. Harwood and Gibson [267] reported that under anaerobic conditions, *Rhodopseudomonas palustris* produced pimelic acid from benzoic acidy by dehydrogenation and hydration reactions. The major intermediate observed in the study by Harwood and Gibson [267] was cyclohexanoic acid. Cyclohexanone was further converted to 2-methyl pentanoic acid. From 2-methyl pentanoic acid, butyric acid was formed, which was further converted to acetic acid. The radiolabeled study showed no production of CO_2 from TNT metabolism and the final end product is acetic acid. This acid can be easily removed under anaerobic conditions by various acetate utilizing sulfate-reducing and methanogenic bacteria.

The application of this consortium to the treatment of TNT-contaminated soil was evaluated using a TNT-contaminated soil collected from the Joliet Army Ammunition Plant (JAAP), Joliet, IL. The soil contained a very high fraction of TNT of 6000 mg/kg of soil. An anaerobic soil slurry reactor was designed based on the previous study by Boopathy et al. [299]. The anaerobic condition in the reactor was maintained by bubbling helium gas in the headspace of the reactor. The contaminated soil was sterilized using an autoclave. A 10 % soil slurry was made using sterile tap water containing 20 mM sodium sulfate as electron acceptor, 15 mM pyruvate as co-substrate, and 5 mM ammonium chloride as nitrogen source. A 5 % pre-grown inoculum of the sulfate-reducing consortium was added to the soil slurry reactor to start the bioremediation experiment. A control soil slurry reactor was maintained with similar conditions as described above except bacterial inoculum. The experiment was run for 125 days. The
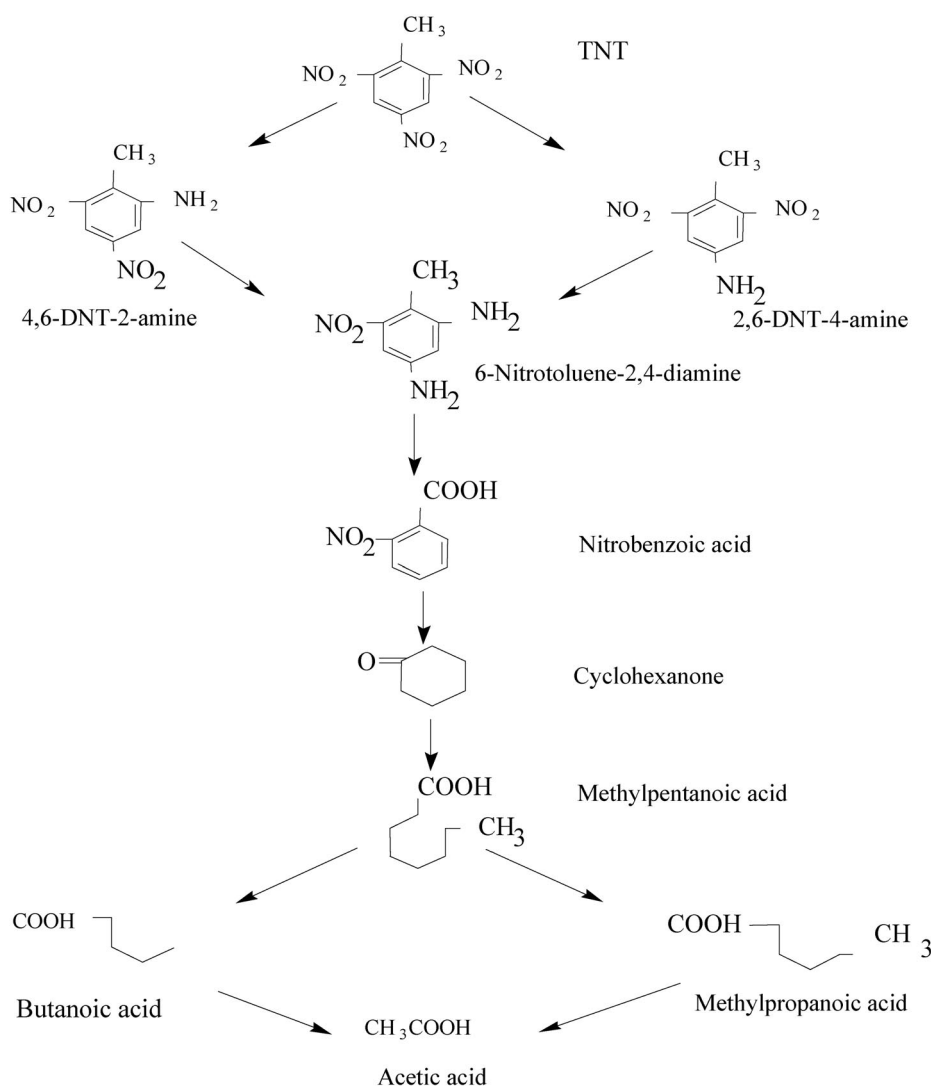
Fig. 12 TNT metabolic pathway by sulfate-reducing bacteria.

results shown in Fig. 13 indicated that the sulfate-reducing bacterial consortium effectively removed TNT compared to the control reactor. The TNT removal in the reactor with bacterial inoculum was almost 100 %, and in the control there was no TNT removal. This study showed that the sulfate-reducing bacteria can remove TNT under anaerobic conditions. This was the first report on a sulfate-reducing bacteria that can remove TNT in a soil slurry condition. This report on the removal of TNT in soil by the sulfate-reducing bacterial consortium in a soil slurry reactor may have significant implications for the decontamination of TNT-contaminated soil. Most munitions contamination is in the surface layer of soil, which can be excavated and treated in an anaerobic soil slurry reactor.
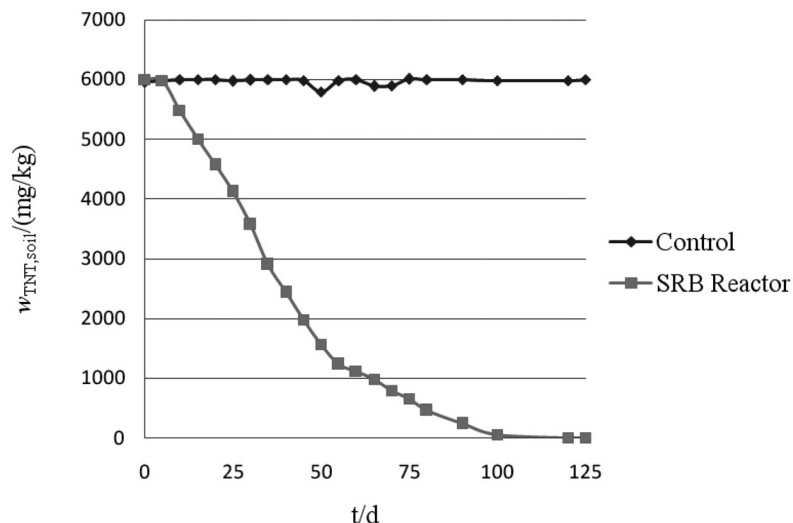
Fig. 13 TNT removal by sulfate-reducing bacteria in a soil slurry reactor over a 125-day period.

3.1.5 Composting

Composting is a biochemical process in which organic materials are biologically degraded, resulting in the production of organic or inorganic by-products and energy in the form of heat. Heat is trapped within the cornposting mass, leading to the phenomenon of self-heating that is characteristic of the composting process. Composting was the first biological treatment process to be tested, approved, and selected for use in remediating military sites [300]. Several types of composting systems exist, but static pile and windrow composting are the most commonly used in explosives remediation [301].

In any composting system, a readily degradable source of organic material such as manure or vegetable waste is mixed with contaminated soil, along with bulking agents to improve texture for optimal aeration. With highly contaminated soil, the addition of organic material also effectively dilutes the concentration of explosives and may prevent toxicity to the microbial population. The degradation of the organic material proceeds by aerobic and anaerobic processes, generating heat [302]. Systems vary in the means of aerating the pile, and some systems use no aeration. Static piles can be aerated by a ventilation manifold installed within the pile. Composting reactors have been designed which mechanically agitate the pile to provide aeration and distribute heat. In windrow composting, piles are aerated by periodic turning to expose previously buried surfaces. Windrow composting is the least expensive design since it requires only a simple liner or asphalt pad and no aeration manifold [301,303].

Water is added to prevent drying of the piles, and if contaminated water is used, treatment of both types of contaminated media can be combined. The end result according to laboratory tracer studies is the fixation of most of the TNT carbon to the humic material present at the end of the composting process [304] driven by the covalent bond formation between reduced amino metabolites and the organic fraction of the compost [305]. The main types of linkages identified in the study by Thorn and Kennedy [305] are aminohydroquinone, aminoquinone, heterocyclic and imine. RDX and HMX have also been reduced in compost systems to below treatment goals, although some methods have given poor results, particularly in removing HMX [300]. Leachability tests have shown that TNT and its metabolites are not released from composted material under simulated acid rain conditions or with solvent extraction [306]. Held et al. [307] studied enhanced humification as an in situ bioremediation method for TNT-contaminated soils. They suggested that if TNT or its metabolites are irreversibly incorporated into the humic matrix instead of being sorbed, then humification can be used in remediation.

Studies of ^{14}C-tracer to assess the potential hazards of inhaling dust from composted TNT-contaminated soil [308] have been conducted in rats. TNT, TNT-spiked soil, and composted TNT-spiked soil were compared with regard to elimination after introduction into the lungs. The results showed that unknown products of composting were slowly eliminated in urine, with some accumulation in the kidneys, thus determining these compounds to be bioavailable. Tests with bacterial tester strains, aquatic invertebrates, earthworms, and rats have shown near-complete reductions in the toxicity and mutagenicity of contaminated soil and leachates after composting at field scale [300].

Dalgren et al. [309] studied the anaerobic remediation of soil with mixed contaminants, including explosives. The scope of this study was to evaluate the decrease in explosives concentration in two industrially contaminated soils, one highly and one moderately contaminated, with different bioremediation methods applicable on excavated soil masses performed off-site. The commercially available method Daramend®, amended with Fe^0, was compared with a horse-manure-amended compost and a treatment with Fe^0 alone. The Daramend process utilizes native soil microorganisms for the degradation of persistent organic compounds by addition of a solid-phase fibrous organic amendment. In a moderately contaminated soil, Daramend and Fe^0 treatment gave significantly higher removal rates compared to compost and control treatments. The largest overall decrease in ecotoxicity, measured with bioluminescent bacteria (*V. fischeri*), was achieved with Fe^0 treatment. In a more contaminated soil, no degradation of contaminants and no decline in soil toxicity could be distinguished after the same time period. The toxicity and mutagenicity reduction after composting of explosives-contaminated soil was also assessed by Jarvis et al. [310]. Two bioassays were used (Mutatox and earthworm acute toxicity test) to evaluate the effectiveness of a pilot-scale composting demonstration in reducing environmental hazard. Explosives-contaminated soil was collected from a military installation and amended for composting in two adiabatic reactors. The unamended soil was lethal to all exposed earthworms, as were both amended replicates, prior to composting. Serial dilutions of the finished composts with artifcial soil had earthworm 14-day LC_{50} values of 35.7 and 100 % finished compost: artificial soil. Extracts of the initial materials were also toxic to bacteria in the Mutatox assay. Dilutions of those extracts to sublethal concentrations revealed a low level of mutagenicity. Extracts of the finished composts indicated reduced bacterial toxicity, but the mutagenicity was markedly increased by composting. The reduction in lethality reflected the attenuation of explosives caused by composting, as indicated by chemical analysis. However, the increased mutagenicity was a result that would not have been indicated by chemical analysis alone and is inferred to be the result of the formation of mutagenic metabolites of explosives during composting and their incomplete degradation.

In a slightly different set-up, Fuller et al. [311] evaluated peat moss plus soybean oil (PMSO) technology for reducing explosive residue transport to groundwater at military training ranges under field conditions. RDX and MNX fluxes were reduced by several hundredfold using a 10 cm layer of a mixture of 1:2 peat moss: crude soybean oil compared to the untreated control. While this research evaluated the effectiveness of a continuous surface-applied layer of PMSO, the actual field application of the technology as a buried, in-place treatment layer or as material tilled into the top 30 cm of the soil would be expected to mitigate downward explosive compound transport.

3.1.6 Phytoremediation methods

Phytoremediation offers an environmentally friendly, low-cost alternative to current remediation methods, however, this approach is hindered by the low inherent metabolic abilities of plants toward these xenobiotic compounds and the phytotoxicity of these compounds. As a result of recent advances in our knowledge of the biochemistry underlying endogenous plant detoxification systems and the use of genetic engineering to combine bacterial explosives-detoxifying genes with the phytoremediatory benefits of plants, this technology is now poised for testing in the field and in a wider range of plants, such as poplar trees and perennial grasses [312].

Phytoremediation encompasses several different technologies: phytoextraction involves bioconcentrating contaminants in the harvestable zones of the plant; phytostabilization reduces the bioavail-

ability of contaminants by binding them in plant tissues. In phytodegradation, the enzyme systems of the plants and plant-associated microorganisms degrade the toxic compounds, while phytovolatilization uses plants to voltatilize pollutants [313].

In a study performed by Riefler and Medina [314], the uptake of NG and 2,4-DNT from water by three common grasses, yellow nutsedge (*Cyperus escalantus*), yellow foxtail (*Setaria glauca*), and common rush (*Juncus effusus*), was investigated using hydroponic reactors. Rapid removal from solution by all grasses was observed, with yellow nutsedge removal rates being the highest. NG or 2,4-DNT accumulated in the tissues in all of the plants, except yellow foxtail did not accumulate NG. Overall, the three grasses tested appear to be good candidates for phytoremediation of propellant contamination.

Although plants can take up chemicals from the vapor, liquid, and solid phases, the movement of the organics within the plant usually occurs in solution. Chemicals most likely to be taken up are those where the decadic logarithm of the octanol-water partition coefficients $\log_{10}(K_{ow})$ is between 0.5 and 3.0. Nitroaromatic explosives are in this range: nitrotoluene has $\log_{10}(K_{ow}) = 2.37$, for 2,4-DNT $\log_{10}(K_{ow}) = 1.98$ [315]. Uptake efficiency depends on factors such as pH, pK_a, the soil's water and organic content, and plant physiology [316]. Some authors have used synthetic (Triton X-100) or naturally produced biosurfactants (rhamnolipids) to enhance the water solubility and hence uptake of contaminants [317]. Once the compounds are taken up by the plant they can be metabolized, stored (often in the root system), or volatilized. Nitroreductases and laccases are the enzymes demonstrated so far to be involved in phytodegradation of nitroaromatics [318]. The root system can also play an active role in remediation; rhizofiltration refers to the adsorption and absorption of pollutants via this route [317]. Accelerated removal of TNT from an active rhizospheric zone has been observed with the use of prairie grass [319]. The secretion of sugars, alcohols, and acids by the plant promotes the growth of rhizospheric bacteria around the root system [320], and these enhance degradation of contaminants by humidification of the organics or by secreting enzymes such as peroxidases [320]. Scheidemann et al. [237] studied 11 plant species during an eight-week growing season for degrading TNT in contaminated soil. At low TNT mass concentrations (10 mg/L), *Medicago sativa* incorporated the highest level of nitroaromatics, but at 500 mg/L of soil contamination only one species (*Phaseolus vulgaris*) was able to grow. For the soils contaminated at 10 mg/L, more than 95 % of the original nitroaromatic was present as amino metabolites, but it was unclear whether this transformation occurred before or after uptake by the plants.

Root homogenate from poplar trees (*Populus deltoides* x *nigra* DN34, Imperial Carolina) stimulated perchlorate degradation in microcosms of soil and water samples collected at a perchlorate-contaminated site, the Longhorn Army Ammunition Plant (LHAAP), located outside Karnack, TX [321]. This study showed that root products were capable of promoting perchlorate degradation for pure culture bacteria and uncharacterized water samples. Perchlorate degradation occurred when using inocula with and without prior exposure to perchlorate.

Most phytoremediation studies have focused on the reduction products from TNT, notably 2-amino-4,6-DNT and 4-amino-2,6-DNT or their conjugates [322]. Oxidation products, which include transformation of the ring methyl group and hydroxylation catalyzed by monooxygenases, have been largely unstudied, even though they may account for more than 40 % of all products. Bhadra et al. [323] using *Myriophyllum aquaticum* were able to detect 6 TNT products including 2-amino-4,6-dinitrobenzoic acid (4.4 %), 2,4-dinitro-6-hydroxybenzyl alcohol (8.1 %), 2-*N*-acetoxyamino-4-6-dinitrobenzaldehyde (7.8 %), 2-methyl-3,5-dinitrophenol (15.6 %), and two binuclear metabolites (5.6 %). Many of the metabolic products of phytoremediation remain unidentified, making it difficult to assess their long-term fate and toxicity [324]. After a 5-day incubation of 49 mM TNT with *Myriophyllum spicatum*, Pavlostathis et al. [325] found that while all the TNT had disappeared from the culture medium, aminodinitrotoluenes and hydroxylaminodinitrotoluenes accounted for only 14 %, and 3 % was present in the extracted plant material. Sens et al. [326] studied the uptake and metabolism of ^{14}C-TNT in *Triticum aestivum* (wheat). Incorporated ^{14}C was distributed 43 % in the cytoplasm and 57 % in the cell wall, of the ^{14}C in the cell wall, 27 % was bound to lignin and 5 % to pectin. Three nonpolar and

10 polar metabolites were found but not identified. A full-scale project at the Iowa Army ammunition plant [327] involved the use of a constructed wetland, which was able to degrade up to 0.019 mg/L TNT per day. Plants used for remediation consisted of three aquatic species, pondweed, arrowroot, and coontail, and one nonaqueous species (poplar trees), which were planted around the edge of the wetland. Although plants are effective remediators due to their large amount of biomass, they are less efficient per unit of biomass than bacteria. French et al. [328] combined the strengths of both techniques by using genetically modified tobacco plants that expressed adenitrifying enzyme (pentaerythritol reductase). The transgenic line grew normally in a medium containing 0.05 mM TNT, whereas 0.025 mM was lethal to wild-type tobacco seedlings. The transgenic plants were able to denitrify TNT, producing dinitro and mononitro aromatics.

Several case studies have shown that constructed wetlands can remove mg/kg concentrations of benzoic acid, *p*-nitrotoluene, TNT, and their degradation products amino-di-nitrotoluene, phenol, benzene, toluene, 1,4-xylene, in systems planted with common reed (*Phragmites australis*) [329,330]. It is obvious that the only plants capable of confronting the longevity of recalcitrant soil pollutants in situ are long-living trees. Trees can meet the demands of sustainable phytoremediation, of long-term bioindication and of bioenergy production. Low nutrient and soil quality requirements and high tolerance to many soil pollutants enable trees to survive on real contaminated sites where most crop plants fail to grow [331a]. Four-year-old trees of hybrid willow (*Salix* sp., clone EW-20) and of Norway spruce (*Picea abies*) were cultivated in sand or ammunition plant soil (AP-soil) in wick-supplied growth vessels. Sixty days after [^{14}C]-TNT application, recovered [^{14}C]-TNT is accumulated in roots (70 % for sand variants, 34 % for AP-soil variant). 15 to 28 % of [^{14}C]-TNT remained in sand and 61 % in AP-soil. 3.3 to 14.4 % of [^{14}C]-TNT were located in above-ground tree portions. Above-ground distribution of ^{14}C differed considerably between the angiosperm *Salix* and the gymnosperm *Picea*. In *Salix,* nearly half of above-ground [^{14}C]-TNT was detected in bark-free wood, whereas in *Picea* older needles contained most of the aboveground [^{14}C]-TNT (54–69 %). TNT was readily transformed in tree tissue. Approximately 80 % of [^{14}C]-TNT was non-extractably bound in roots, stems, wood, and leaves, or needles. Only quantitatively less important stem-bark of *Salix* and *Picea* and May shoots of *Picea* showed higher extraction yields (up to 56 %) [331b].

Phytoremediation, like bioremediation, suffers from unpredictable climate variations, and is essentially inactive in the northern United States and Canada during the winter, making it difficult to predict degradation rates [332]. Against this, phytoremediation is more rugged than microbial bioreactors with respect to physical conditions and changes in contaminant loading. Work is actively in progress to develop strains of plant that can take up contaminants more actively (bioaccumulators) or to improve the ability of plants to grow under the adverse conditions of a contaminated soil. The latter approach is more promising for nitroaromatic compounds, which undergo principally phytodegradation rather than phytoextraction.

During the past decade, plants have been genetically modified to overcome the inherent limitations of plant detoxification capabilities, following a strategy similar to the development of transgenic crop. Bacterial genes encoding enzymes involved in the breakdown of explosives, such as nitroreductase and cytochrome P450, have been introduced in higher plants, resulting in significant enhancement of plant tolerance, uptake, and detoxification performances. Transgenic plants exhibiting biodegradation capabilities of microorganisms bring the promise of an efficient and environmentally friendly technology for cleaning up polluted soils [333].

3.1.7 Bioremediation of explosives-contaminated soil: A case study

Two bioremediation methods, namely, soil slurry reactor and land farming technique for the treatment of soil contaminated with explosives in the Louisiana Army Ammunition Plant (LAAP) in Minden, Louisiana were studied. The contaminated soil was collected from the LAAP in Minden, Louisiana, USA. The contaminant concentrations in the soil are given in Table 8. The TNT fraction in the soil ranged from 4000 to 10 000 mg/kg. The RDX fraction in the soil ranged from 800 to 1900 mg/kg. The

HMX fraction ranged from 600 to 900 mg/kg. The soil had a total organic matter content of 4 to 5 %, which included the contaminants, and an average pH of 6.5.

Table 8 Explosive concentrations in the contaminated soil.

Explosive	Mass fraction range (mg/kg of soil)
TNT	4000–10 000
RDX	800–1900
HMX	600–900

The concentrations of TNT in the slurry reactors are given in Fig. 14. The soil-TNT fraction in the no-carbon control reactor remained high around 7500 mg/kg of soil throughout the experiment. This observation suggests that the indigenous microflora from the contaminated site would not degrade TNT without the addition of nutrients or co-substrates. The soil-TNT fraction in the reactor that received molasses as co-substrate dropped gradually and fell below 50 mg/kg of soil on day 182 of the study.
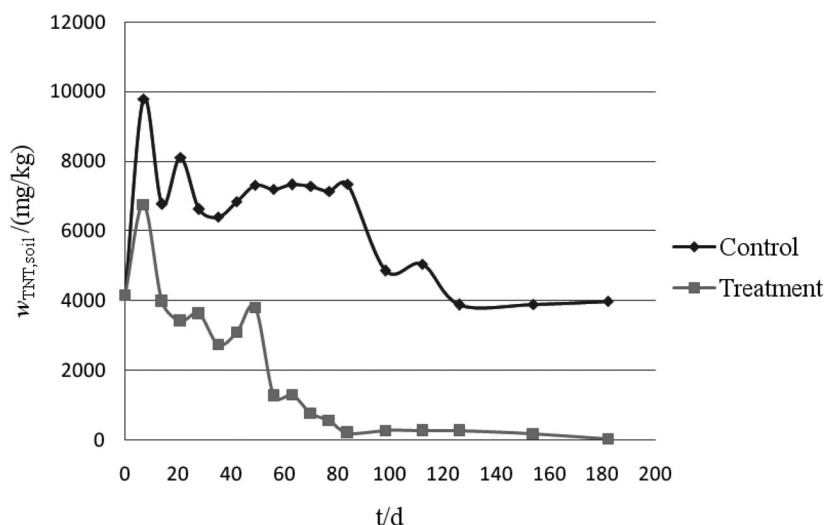

Fig. 14 TNT degradation in the soil slurry reactor over a period of 182 days.

Our previous study demonstrated that TNT removal in the soil slurry system was accomplished by a cometabolic process that required an additional carbon source such as molasses or succinate [242]. Molasses is a very effective carbon source that enhances the TNT degradation rate significantly over other carbon sources [242]. This study showed that the soil slurry reactors can effectively remediate TNT in the contaminated soil. The operation of laboratory-scale soil slurry reactors over 182 days showed that 99 % removal of TNT can be achieved. The soil slurry reactor also removed other explosives, namely, HMX and RDX (Figs. 15 and 16). However, the removal efficiency was not as high as TNT. This could be due to the complexity of molecules. HMX and RDX degradation can be achieved, but it will take a longer period of time as indicated by many other studies [141,334].
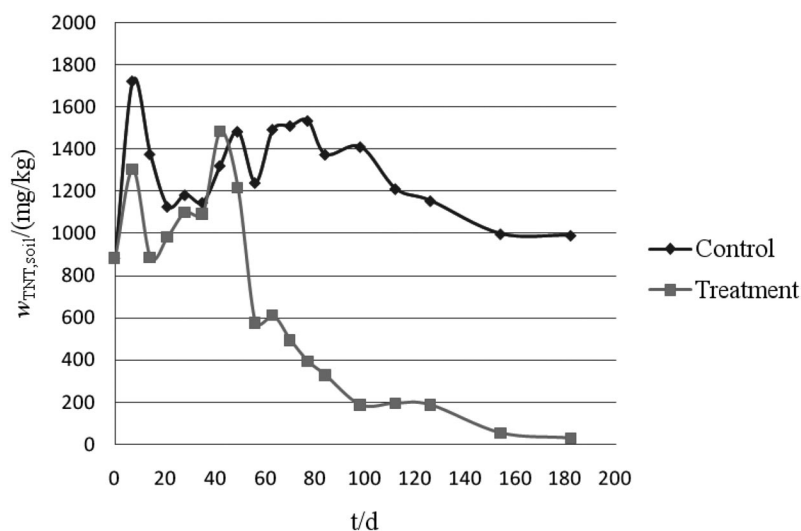
Fig. 15 RDX degradation in the soil slurry reactor over a period of 182 days.
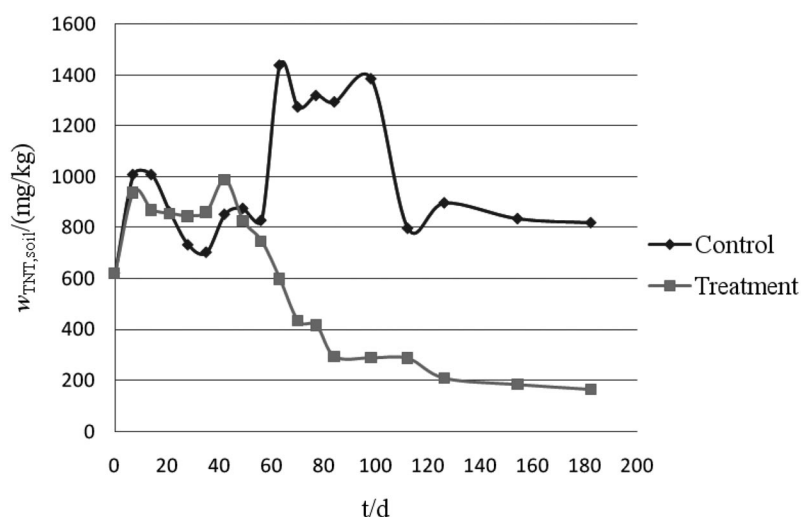

Fig. 16 HMX degradation in the soil slurry reactor over a period of 182 days.

The addition of radiolabeled TNT to the reactor biomass provided evidence for the mineralization of TNT. Of the original radiolabeled TNT, 23 % was converted to CO_2 and 24 % was used in making cellular materials. Radiotracer studies with the reactor biomass also revealed various intermediates, including 4-methyl-3,5-dinitroaniline, 2-methyl-3,5-dinitroaniline, 6-methyl-5-nitrobenzene-1,3-diamine, fatty acids, and an unidentified metabolite after three weeks of starting the radiolabeled studies. Extraction of soil with acetonitrile showed that 2 % of the TNT was adsorbed onto the soil. The rest of the TNT was accounted for as intermediates. The mass balance was reasonable with the recovery of 98 % of radiolabeled TNT. In the no-carbon control, 78 % of radiolabeled TNT was recovered as TNT, which did not undergo degradation. This radiolabeling study showed that the natural soil bacteria present in the contaminated soil can cause extensive degradation of TNT in a reasonable time under optimum conditions. Degradation was demonstrated by mineralization of radiolabeled TNT, metabolite for-

mation, and the presence of radioactivity in the cell biomass as trichloroacetic acid (TCA)-precipitable material.

The pH in the reactors was monitored throughout the experiment. The pH remained approximately neutral in the no-carbon control reactor. However, the molasses-containing reactor tended to be acidic with pH value of 5. Dissolved oxygen (d.o.) mass concentrations were monitored weekly in the soil slurry reactors. The d.o. mass concentration remained around 6.5 mg/L in the no-carbon control, and in the reactors with molasses the d.o. mass concentration was less than 1 mg/L.

Bacterial plate counts were performed several times over the course of the experiment. The bacterial plate counts in the reactor receiving molasses were consistently higher than those in the no-carbon control reactor (Table 9). This result also shows the value of molasses addition, which helps to increase the population of soil bacteria in the reactor. Molasses is the best among various substrates studied, such as succinate, glucose, acetate, and citrate [242], it is well balanced with nutrients including carbon, nitrogen, phosphorous, vitamins, and minerals for bacterial activity.

Table 9 Bacterial counts in the soil slurry reactors (colony-forming units (CFUs)/cm^3 of soil slurry).*

	Bacterial count (CMU/cm^3 of slurry) in the reactor	
Day	Control	Treatment
0	72×10^2	81×10^2
14	33×10^7	53×10^7
28	46×10^5	167×10^6
42	60×10^5	235×10^6
56	47×10^5	55×10^6
70	123×10^4	36×10^6
84	187×10^4	103×10^6
98	93×10^4	59×10^6
112	173×10^4	105×10^6
126	88×10^4	78×10^6
154	67×10^4	104×10^6
182	72×10^4	121×10^6

In the case of land farming, the TNT concentration in soil samples taken from pans 1 to 4 during the 182-day study is shown in Fig. 17. For each sampling date, three soil samples were collected from each pan, analyzed, and the mean concentrations were plotted. The TNT concentrations in the control pan remained at high levels over the course of the experiment. The treatment that included molasses solution was biologically active and showed removal of TNT. Starting from a high fraction of approximately 7000 mg/kg of soil, the average fraction of TNT after 182 days of treatment was less than 1250 mg/kg of soil, which was equal to 82 % removal of TNT. Very little RDX and HMX were removed in soil in both the control and treatment pans (data not shown). The degradation rates of RDX and HMX are extremely slow, and continuation of the experiment over a 300-day period might show significant reduction in HMX and RDX [297].
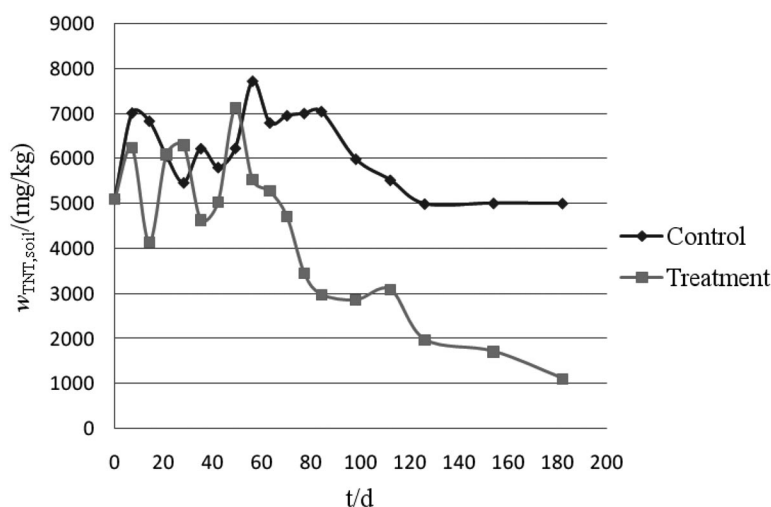
Fig. 17 TNT degradation in the land farming study over a period of 182 days.

The radiolabeled study used biomass taken from the pans and provided evidence for the mineralization of TNT. In pan biomass samples that received molasses, the proportion of the initial radiolabeled spike that was transformed to radiolabeled CO_2 was 6.5 %. In the control pans, the radiolabeled CO_2 was 1.2 %. This result clearly demonstrates that TNT was mineralized by the soil bacteria in the treatment that received molasses. The analysis of TCA-precipitable material showed that a significant amount of radiolabeled TNT was converted to cell biomass. There are various TNT metabolites present in varying degrees. The calculated mass balance was very reasonable, with recovery of 98 % of radiolabeled TNT in this study. This experiment showed that the control pans did not have an active biomass to convert TNT to CO_2 due to the lack of molasses as co-substrate.

Bacterial plate counts were performed several times over the course of the experiment on soil samples taken from each pan. The bacterial population densities in the soils receiving molasses solutions were consistently higher than those in the control pans (Table 10). This result also shows that the control pans consistently exhibited plate counts on the order of 10^4 colony-forming units/g of soil and thus were not strictly abiotic controls however, negligible biodegradation of TNT occurred in the control as TNT concentration in control remained high throughout the study.

Between the two different bioremediation methods, the soil slurry reactor system showed efficient removal of TNT for the LAAP soil in Minden, LA. The land farming method also removed TNT, but the removal rate was very slow. The land farming in the field should be done in a constructed cell with liner to prevent any leachate migrating to groundwater. Both methods showed that the native soil bacteria present at the contaminated site are capable of mineralizing TNT as demonstrated in the radiolabeled study. The advantage of soil slurry rector is its simple operating conditions. The method needs only mixing, supply of air and a carbon source. Molasses is an inexpensive carbon source that could be used in a large-scale operation at low cost. Based on this study, the soil slurry reactor can be used for effective and fast remediation of TNT at LAAP, Minden, LA. The removal of HMX and RDX in the soil can also be achieved with prolonged incubation.

Table 10 Bacterial counts in the land farming pans (CMU/g of soil slurry).*

	Bacterial count (CMU/g of soil) in the reactor	
Day	Control	Treatment
0	63×10^2	74×10^2
14	58×10^6	146×10^6
28	41×10^5	44×10^6
42	40×10^5	88×10^6
56	32×10^4	158×10^5
70	63×10^4	60×10^6
84	273×10^4	129×10^6
98	73×10^4	112×10^6
112	220×10^4	162×10^6
126	188×10^4	119×10^6
154	112×10^4	67×10^6
182	123×10^4	87×10^6

*The data represent an average of two plates.

3.2 Physical and chemical methods

The enormous environmental and financial ramification associated with remediating explosives-contaminated sites has provided a strong impetus to develop cost-effective and environmentally sound treatment technologies. While many researchers have taken a biological approach to solving HE contamination (bioremediation, phytoremediation, composting, etc.), there is also a large contingency that have tried physical or chemical approaches. Chemical/physical approaches are usually performed by adding one or more chemical reagents (reductant, oxidant) to the contaminated media or altering the physiochemical properties, i.e., pH, reduction potential (E_h) of the soil-water environment. These methods offer potential advantages to biological treatments because they are often faster, can treat highly contaminated environments, and are less sensitive to ambient conditions. The goal of a chemical/physical approach is to either transform the explosive into carbon dioxide, water, and mineral elements or structurally transform the parent compound into products that are more biodegradable (i.e., abiotic/biotic approach). This section provides a review of some of the more commonly used chemical and physical approaches for treating explosives-contaminated soil and water.

3.2.1 Chemical reduction using zero-valent iron (Fe^0), ferrous iron (Fe^{+2}), and iron minerals
To date, the most demonstrated remediation technology for explosives-contaminated soils is incineration. Although incineration is effective, it is expensive, produces an unusable ash by-product, and has poor public acceptance due to safety concerns regarding air emissions [335]. Earlier work with zero-valent zinc demonstrated the utility of metals to treat soils contaminated with 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT) [336], methyl parathion [337], and polychlorinated biphenyls (PCBs) [338]. More recent research indicates the tremendous potential of Fe^0 to degrade HE [59,339–344].

From a historical perspective, zero-valent metals have been used to transform and synthesize organic chemicals since the late 1800s. The use of zero-valent metals in environmental research, however, began approximately 30 years ago when Sweeny [345], followed by Senzaki and Kumagai [346], reported that metallic iron could be used to degrade organic contaminants such as chlorinated solvents in water. The more recent idea that iron metal could be used for in situ remediation of subsurface contaminants grew primarily from research carried out at the University of Waterloo (Ontario, Canada). In a project involving sorption of organic compounds to well casing, it was noted that the concentration of the halogenated compound, bromoform, declined when in contact with steel and aluminum casing materials. This 1984 observation was attributed to a dehalogenation reaction, but the environmental sig-

nificance of this work was not realized until a few years later when the results were re-evaluated and published [347]. Today, the use of zero-valent metals has become an alternative to the common pump-and-treat and air-sparging technologies. Since these initial results were published, a flurry of research activity on the use of zero-valent metals in environmental research has ensued with more than 1000 publications currently available (<http://cgr.ese.ogi.edu/ironrefs/>). This heightened interest along with examples of field-scale deployment has helped to make Fe^0 the most widely studied chemical reductant for environmental applications [348].

One of the biggest obstacles to treating contaminated soils at former loading, packing, and manufacturing facilities is the sheer magnitude of contamination present in the impacted surface soils. It is not uncommon for surface soils to contain energetic compounds in percentage concentrations and approach detonation potential [224,344,349–351]. Because of the equilibrium relationship between the soil solution and the solid-phase explosive, remediating soils containing solid-phase HE will not only require treatments that demonstrate rapid destruction in solution but also those that continue to transform explosives as dissolution and desorption occurs from the soil matrix. To evaluate Fe^0 as a remedial treatment for RDX-contaminated soil, Singh et al. [340a] began by initially determining the effectiveness of zero-valent iron to remove or transform RDX in a near-saturated solution. Treating a 32 mg/L RDX solution (144 μM) with a solution containing Fe^0 in a mass concentration of 100 g/L resulted in complete RDX removal from solution within 72 h. Simultaneous tracking of ^{14}C in solution provided a carbon mass balance for the RDX. At Fe^0 mass concentration of 2 g/L or lower, ^{14}C activity remained unchanged, indicating that RDX transformation products produced from the Fe^0 treatment (measured as ^{14}C activity) were water-soluble and not strongly sorbed by the Fe^0. At a Fe^0 mass concentration of 100 g/L, 80 % of initial ^{14}C activity was lost from solution. More than 95 % of the ^{14}C lost, however, was recovered from the Fe^0 surface through a series of extraction and oxidation procedures. Oh et al. [352] treated RDX with scrap iron and high-purity iron under anaerobic conditions. They observed that RDX was readily transformed by both iron sources with no appreciable build-up of identifiable degradates. By measuring changes in total organic carbon (TOC) they also confirmed that RDX transformation products were not sorbed to the iron surface.

The emergence of the permeable reactive barriers (PRBs), consisting of scrap iron cuttings, has proven to be a highly cost-effective treatment for contaminated groundwater [353]. Although more than 150 PRBs have been installed worldwide [354], the majority of PRBs have been targeted for chlorinated compounds and only recently has research been aimed at using PRBs for environmental contaminants with multiple nitro groups (e.g., TNT, RDX, HMX) [60,339,354–360]. Laboratory-based research on the destruction of explosives has provided support for using zero-valent iron in PRBs for field-scale treatment. Recently, a PRB demonstration was performed at the Cornhusker Army Ammunitions Plant (Hastings, Nebraska) where RDX and TNT were successfully transformed by an iron barrier [361,362].

Examples of iron treatment of explosives-contaminated groundwater include Widman and Alvarez [341], who evaluated the potential benefits of an integrated microbial-Fe^0 system to intercept and treat RDX-contaminated groundwater. They found that a combined Fe^0-based bioremediation system offered significant advantages over Fe^0 or biodegradation. Specifically, anaerobic Fe^0 corrosion by water produces cathodic hydrogen, which can then serve as an electron donor for the biotransformation of RDX. Oh and Alvarez [342] used flow-through columns to evaluate the efficacy of PRBs to treat RDX-contaminated groundwater. They found that extensive RDX removal (higher than 99 %) occurred by Fe^0 columns for more than one year. Through a variety of treatments, they also showed that the Fe^0 could interact with indigenous aquifer microcosms and produce hydrogen gas and acetate, which subsequently facilitated RDX degradation. Column experiments with TNT have shown that permeable iron barriers can reduce TNT to triaminotoluene [363], which would be more prone to biotic oxidations (i.e., more biodegradable) in aerobic environments.

Although numerous reports now confirm that Fe^0 can effectively transform HE in solution and soil slurries [59,339–344], working with soil slurries is problematic for several reasons. The equipment required for continuous agitation is expensive and limits the volume of soil that can be treated at any

given time. Dewatering of treated soil is also required. A desirable alternative to slurry treatment would be in situ applications or on-site treatment in soil windrows. Using soil windrows allows much greater volumes of soil to be treated and is constrained by only the size of the windrows and acreages available [344]. However, for Fe^0 to be effective in static soil windrows, contaminant destruction must occur in the soil solution before the intermixed iron in the soil matrix becomes passivated by exposure to air. Initial laboratory work with RDX-contaminated soil from the Nebraska Ordnance Plant showed that Fe^0 intermixed with moist soil (0.3–0.4 kg water per kg soil) could transform RDX under static unsaturated conditions [33]. Results showed that a single addition of 5 % Fe^0 mass fraction transformed 57 % of the initial RDX (3600 mg/kg) following a 12-month incubation.

The effectiveness of Fe^0 to transform RDX in unsaturated soil opened the door for field-scale applications. But using zero-valent iron at the field scale requires the machinery that can thoroughly mix iron throughout the soil matrix. The importance of good mixing cannot be understated because unlike slurries where continual agitation would allow constant movement and contact with Fe^0, the radius of influence for Fe^0 in a static windrow is relatively stationary. The Microenfractionator® (Terry Horn, La Center, WA) is the trade name of a high-speed mixer that has been specifically augmented to mix windrows of soil (Fig. 19). A pull-behind-tractor version of this mixer has been successfully used to remediate pesticide-contaminated soil with Fe^0 at the field scale [364,365].

Zero-valent iron treatment of explosives-contaminated soil in static piles has also occurred at the pilot scale (70 kg). Comfort et al. [344] used contaminated soil containing RDX, TNT, and HMX from an outwash pond at Los Alamos National Laboratory (NM) and treated it with Fe^0 and some acidifying amendments. Zero-valent iron effectively removed 98 % of the RDX and TNT within 120 d under static unsaturated conditions [344]. Because HMX is considered less toxic than RDX [366,367], it was not initially considered a contaminant of concern. Further soil analysis, however, revealed that HMX was present at a very high mass fraction (larger than 30 000 mg/kg) and that this energetic compound was not effectively destroyed by the Fe^0 treatment. To determine if low solubility was responsible for the inability Fe^0 to transform HMX, Park et al. [59] attempted to increase HMX solubility with higher temperatures and surfactants. While higher temperatures increased the aqueous solubility of HMX (2 mg/L at 20 °C, 8 mg/L at 45 °C, 22 mg/L at 55 °C), increasing temperature did not increase HMX destruction by Fe^0 when RDX and TNT were also present in the soil slurry matrix. Furthermore, by conducting batch experiments with single and binary mixtures of RDX and HMX, Park et al. [59] showed that when RDX and HMX were present at equal molar concentrations, RDX was a preferential electron acceptor over HMX consequently, iron-based remedial treatments of RDX/HMX-contaminated soils may need to focus on removing RDX first. The rationale for using surfactants is typically to get more of the contaminant in solution so that it can be degraded. Park et al. [368] found that the cationic surfactants didecyl (didecyldimethyl ammonium bromide) and HDTMA (hexadecyltrimethyl ammonium bromide) could increase HMX solubility (about 200 mg/L) and that both RDX and HMX were effectively transformed by Fe^0 in the surfactant matrix.

Iron is an effective remediation tool because when placed in water, metallic iron (Fe^0) becomes an avid electron donor ($E° = -0.409$ V) and its oxidation can drive the reaction of many redox-sensitive contaminants. While contact with the iron surface is desired for HE destruction, it is also true that interstitial pore water both inside and downgradient of PRB will be laden with Fe^{+2}, Fe^{+3}, and associated minerals [369,370]. Moreover, when the hydraulic conductivities of the PRB no longer match regional groundwater velocity, as can occur with iron aging, secondary mineral precipitation can occur near the upgradient edge of the PRBs [353,362,371,372].

The formation of these secondary iron products (i.e., Fe^{+2} and hydroxides) can contribute to transforming explosive compounds because aqueous Fe^{+2} species surface-coordinated to iron oxides and iron (oxy)hydroxides surfaces are known to reduce explosive compounds [57,58,373–376]. The catalytic activity of Fe^{+2} coordinated to oxides is believed to be the result of complexation of Fe^{+2} with surface hydroxyl groups and the formation of inner-sphere bonds, which increases the electron density of the adsorbed Fe^{+2}.

Temporal monitoring of zero-valent iron surfaces during laboratory batch studies and field measurements of PRB have identified a wide range of iron minerals such as: akagenite, goethite, lepidocrocite, green rust, magnetite, ferrous sulfide, and maghemite [356,370,377]. Given that PRBs are emplaced at sites with widely differing groundwater chemistry, the suite of minerals that precipitate, as well as the rate of formation, can vary widely [372]. This heterogeneity makes it clear that explosives treated with Fe^0 could eventually encounter multiple components (i.e., reductants) rather than a single mineral phase and that these secondary minerals may aid in the removal of explosive compounds.

3.2.2 Chemical reduction of explosives using in situ redox manipulation

In situ redox manipulation (ISRM) is a technology that injects a chemical reductant (sodium dithionite buffered at high pH) into an aquifer. Because dithionite is a strong reductant, particularly in alkaline solutions (reduction potential of –1.12 V), it chemically dissolves and abiotically reduces amorphous and some crystalline Fe^{+3} oxides [378–380], leaving behind several possible Fe^{+2} species such as structural Fe^{+2}, adsorbed Fe^{+2}, $FeCO_3$ precipitates, and FeS. The simple reaction describing the reduction of iron by dithionite is

$$S_2O_4^{2-} + 2Fe^{3+} + 2H_2O = 2Fe^{2+} + 2SO_3^{2-} + 4H^+ \tag{1}$$
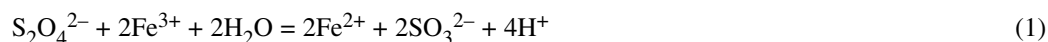
Once the aquifer solids are reduced, subsequent oxidation of the adsorbed and structural ferrous iron in the reduced zone (i.e., redox barrier) occurs passively by the inflow of d.o. and additionally by contaminants that can serve as electron acceptors (i.e., RDX, Cr^{+6}, tetrachloroethylene). The longevity of the reduced sediment barrier is dependent on the flux of electron acceptors. In relatively uncontaminated aquifers, d.o. in water is the dominant oxidant. Although oxidation of Fe^{+2} occurs relatively quickly at alkaline pH, slower rates of oxidation are likely for surface Fe^{+2} phases [380b].

Considerable research on ISRM has been conducted with chlorinated solvents and Cr^{+6}, but only recently has this technology been investigated for HE. Boparai et al. [381] determined the efficacy of dithionite-reduced sediments from the perched Pantex Aquifer (Amarillo, Texas) to abiotically degrade the explosives RDX, HMX, and TNT. Results showed that reduced Pantex sediments were highly effective in transforming all three HE (Fig. 18) with little to no adsorption of the transformation products (Fig. 18B) [381]. As observed with Fe^0 treatment of RDX [31], dithionite-reduced sediments also produced nitroso derivatives of RDX, but these degrades were further reduced into ring fragments that were not strongly adsorbed (Fig. 18B) [381].

Earlier biodegradation studies of the transformed products produced from ISRM showed that the RDX degrades were readily biodegradable under aerobic conditions, with approximately 50 % of the initial ^{14}C recovered as $^{14}CO_2$ after 100 d [382]. Consequently, abiotic reduction of explosive compounds by a redox barrier followed by biodegradation of the transformed products may result in a variable treatment scenario for groundwater contaminated with explosives.
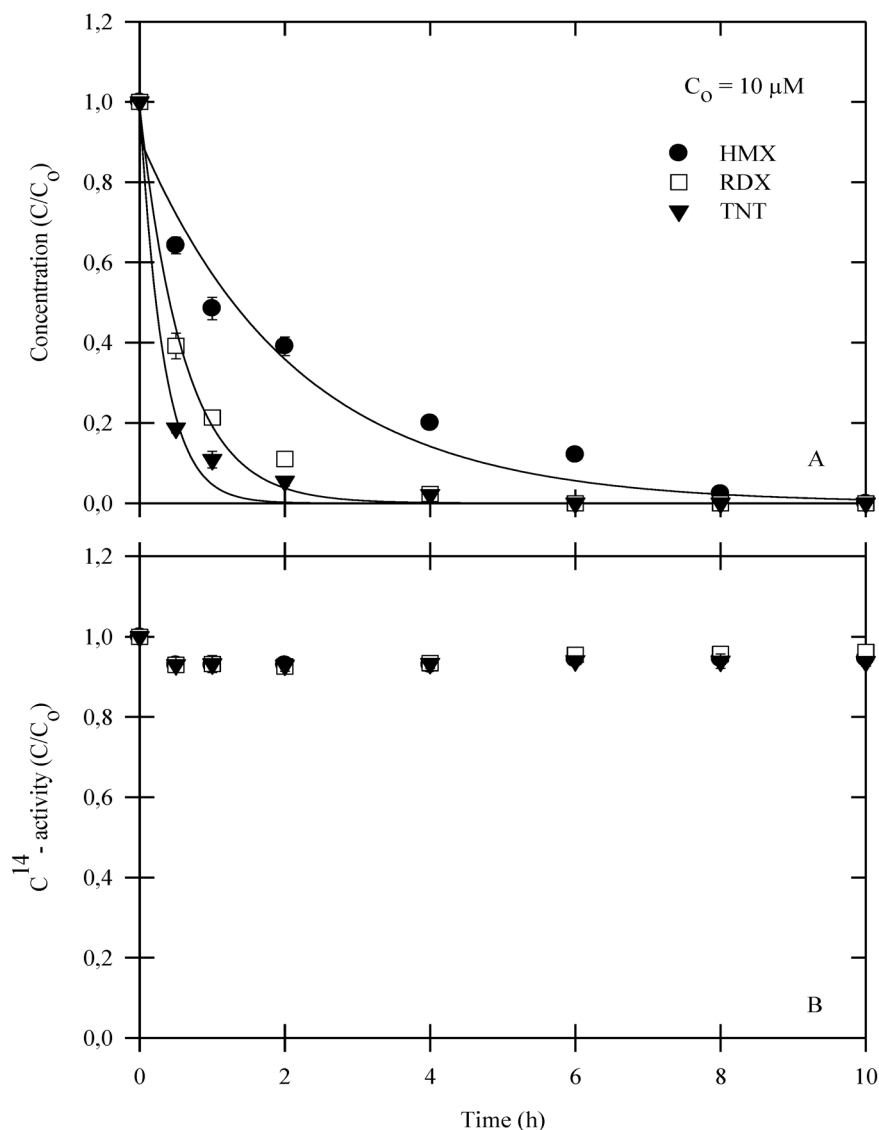
Fig. 18 Changes in aqueous HE concentrations (A) and ^{14}C (B) concentrations after treating an equal molar solution containing 10 µM with 2 g reduced sediments (100 mM dithionite + 400 mM K_2CO_3). Part A was originally printed in *Chemosphere* **71**, 933 (2008).

3.2.3 Electrochemical treatment of explosives

Electrolysis, the use of electrical energy to drive an otherwise unfavorable chemical reaction, is a technology that has been used to remediate industrial wastes and recently applied to explosives for wastewater treatment [313,383–385]. Some potential advantages of electrochemical treatment include the low cost of electricity compared with the cost of chemical treatments, relatively low capital costs, modular design, operations under ambient conditions, and the possibility of higher energy efficiency than thermal or photolysis treatments [313]. Rodgers and Bunce [313] demonstrated electrochemical reduction of TNT at a reticulated vitreous carbon cathode while Pascale et al. [385] utilized a cascade of divided flow through reactors and showed that an aqueous solution of RDX (48 mg/L) was completely

degraded by a current of 10 mA after flowing through three reactors. The major degradation pathway involved reduction of RDX to the mononitroso derivative (MNX) followed by ring cleavage to yield formaldehyde and methylenedinitramine, which underwent further reduction and/or hydrolysis [385]. Doppalapudi et al. [384] also demonstrated that RDX (10 mg/L) could be degraded under anoxic and oxic conditions by electrolysis while Meenakshisundaram et al. [383] found that RDX degradation increased with increasing current (about 20–50 mA) and stir rate (630–2040 rounds per minute). Khodadoust et al. [386] combined enhanced electrokinetic remediation with cyclodextrin as a solubility enhancer to remediate two soils contaminated with 2,4-DNT. Their results showed that when a clay soil with no organic matter (kaolin) was treated, about 94 % of the 2,4-DNT was transformed and that the cyclodextrin improved removal rates. However, when a glacial soil that was more representative of contaminated sites was treated, only 20 % of the 2,4-DNT was transformed and cyclodextrin did not significantly enhance removal rates.

In a treatment combining both electrolysis and alkaline hydrolysis, Gent et al. [387] created an in situ electrolytic and alkaline hybrid treatment zone in a sand column for treatment of RDX-contaminated water. An upgradient cathode and downgradient anode created alkaline-reducing conditions followed by oxic, acid conditions. Results showed that about 95 % of the RDX was transformed by this process with approximately 75 % of the RDX transformed near the cathode by electrolysis and 23 % decomposed downgradient of the cathode by alkaline hydrolysis.

3.2.4 Lime treatment of explosives-contaminated soil

Military testing and training grounds provide vital lands for preparing military troops for combat and maintaining readiness. While an important resource for military exercises, site commanders must delicately balance these lands so that training operations proceed without the environmental consequences of repeated release of energetic compounds. Unfortunately, decades of continuous discharge of live ammunition from small arms, artillery mortar fire, and explosive detonations have contaminated surface soils at several locations. Given the multitude of training operations that have occurred at testing grounds, surface soil contamination has been documented to be widespread and very heterogeneous [388]. While the use of Fe^0 has been shown to be effective in transforming explosives in highly contaminated soils, this treatment can be costly and not practical for training facilities that encompass thousands of acres. Remedial techniques for training ranges must be inexpensive, easily applied to remote locations, and due to the possibility of UXO, environmentally non-intrusive [48]. The use of chemical amendments to increase the pH of the soil has been found to meet these objectives. Based on previous observations that explosive compounds can be degraded under alkaline conditions [48,51,389–391], 3 % lime mass fraction was sufficient to raise the soil pH value higher than 10 in a variety of test soils and drive alkaline hydrolysis of most HE common to training ranges. Solid-phase or precipitated materials from UXO were also found to be eventually degraded by the lime treatment. TNT and RDX were most prone to hydrolysis while HMX and TNT degradation products (i.e., 6-methyl-5-nitrobenzene-1,3-diamine and 4-methyl-3,5-dinitroaniline) were more recalcitrant and required a higher pH value of 12 for effective removal [48].

3.2.5 Chemical oxidation for in situ remediation of soils and groundwater

In situ chemical oxidation (ISCO) is a class of remediation technologies that delivers oxidants on-site and in-place to groundwater or the vadose zone. While municipal and industrial companies have routinely used chemicals to oxidize organic contaminants in drinking and wastewater, it is the ability to treat contaminated field sites that has fueled ISCO popularity, especially when bioremediation is inadequate or where treatment time is considered a factor [392]. Increased interest and research in ISCO has caused significant developments and application changes since the mid-1990s.

Much of the groundwork for ISCO applications can be traced back to 20 years or more of research conducted on advanced oxidative processes (AOPs), which employ reactive oxidizing agents such as hydrogen peroxide (H_2O_2) or O_3, with or without additional catalysts or photolysis, to generate short-lived chemical species of high oxidation power. Past studies specific to the treatment of explosives

include oxidative systems such as H_2O_2/O_3, H_2O_2/ultraviolet light (UV), O_3/UV, or Fenton's reagent for rapid destruction of nitroaromatic and nitramine compounds [393,394]. Examples of this research include Ho [395] who demonstrated that photooxidation of 2,4-DNT by an H_2O_2/UV system resulted in a side-chain oxidation converting 2,4-DNT to 1,3-dinitrobenzene followed by hydroxylation and cleavage of the benzene ring to produce carboxylic acids and aldehydes. Fleming et al. [396] used a 1:1 mixture of O_3 and H_2O_2 at a pH value of higher than 7 (peroxone) to generate hydroxyl radicals and reported that RDX, HMX, and several nitroaromatics in groundwater from the Cornhusker Army Ammunitions Plant were degraded by at least 64 %, with a destruction efficiency of 90 % for RDX. Bose et al. [397] also conducted a detailed evaluation of oxidative treatments for RDX using a combination of O_3, UV, and H_2O_2 and showed that side-chain oxidation and elimination of nitro radicals or nitrous oxide equivalents occurred followed by cleavage of the heterocyclic ring, which resulted in the formation of urea and formamide. A pilot-scale assessment of UV/photolysis at the Nebraska Ordnance Plant for treatment of a RDX plume showed that RDX mass concentrations (5–78 µg/L) were typically reduced below 5 µg/L [398].

Successfully implementing ISCO requires that the oxidant react with the contaminants of concern, and an effective means of dispersing the oxidant to the subsurface is achieved. Technology advances in this regard include delivery processes such as deep soil mixing, hydraulic fracturing, multi-point vertical lancing, horizontal well recirculation, and vertical well recirculation [399]. Because of their high oxidation potential, the four oxidants commonly employed include hydrogen peroxide (H_2O_2, 1.78 V) either alone or in the form of Fenton's reagent ($H_2O_2 + Fe^{2+}$), O_3 (2.07 V), permanganate (MnO_4^-, 1.68 V), and persulfate ($S_2O_8^{2-}$). Specific examples illustrating the use of these oxidants for treating explosives-contaminated soil and water follow.

3.2.5.1 Permanganate

Chemical oxidation using permanganate has been widely used for treatment of pollutants in drinking water and wastewater for more than 50 years [399]. Commonly manufactured and sold as a solid ($KMnO_4$) or liquid ($NaMnO_4$), permanganate is an oxidizing agent with a strong affinity for organic compounds containing carbon–carbon double bonds, aldehyde groups, or hydroxyl groups. Site-specific issues are always a concern, and the Office of Environmental Management concluded that ISCO using $KMnO_4$ is applicable for the destruction of dissolved organic compounds in saturated permeable zones with hydraulic conductivities higher than 10^{-4} cm/s, low organic carbon contents (below 0.5 %), and a pH range between 3 and 10 (optimum, 7 to 8) [399].

Like most of the abiotic treatments discussed thus far, permanganate has been primarily used for treating chlorinated solvents and only recently have permanganate treatments been directed toward treating explosives. Research with chlorinated solvents has shown that permanganate is attracted to the negative charge associated with the π-electrons of chlorinated alkenes such as tetrachloroethene, trichloroethene, dichloroethene, and vinyl chloride [400]. Although the chemical structure of RDX and HMX does not readily lend itself to reaction with permanganate, IT and Stroller Corporation [401] initially demonstrated effective RDX destruction by $KMnO_4$ treatment. Based on favorable laboratory results, a single-well push/pull test was also conducted at the Pantex site. In this test, permanganate was injected (push) into a single well, allowed to react, and then extracted (pull). Significant degradation of all HE compounds was observed with RDX half-life estimated at about 7 d at a $KMnO_4$ mass concentration of 7000 mg/L [402].

In a follow-up to the observations of IT and Stroller [401], Adam et al. [403] subsequently treated ^{14}C-RDX with $KMnO_4$. They found that a solution of RDX at a concentration of 2.8 mg/L, when treated with 20 000 mg/L $KMnO_4$, was decreased to 0.1 mg/L within 11 d with cumulative mineralization continuing for 14 d until 87 % of the labeled carbon was trapped as [14]CO_2. Moreover, they showed lower $KMnO_4$ concentrations (1000–4000 mg/L) also produced slow (weeks) but sustainable RDX destruction (Fig. 19) [403]. Treatment parameters such as initial RDX concentration (1.3–10.4 mg/L)
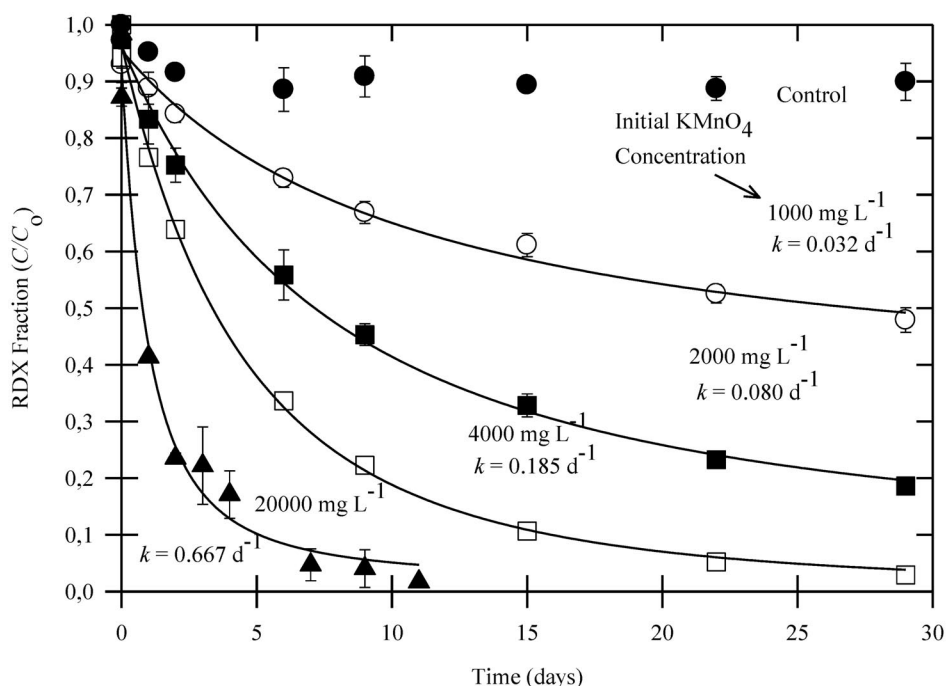
Fig. 19 Loss of RDX from an aquifer slurry treated with varying $KMnO_4$ concentrations. Bars on symbols represent standard deviations of means ($n = 4$); where absent, bars fall within symbols. Originally printed in *J. Environ. Qual.* **33**, 2165 (2004).

or pH (4.1–11.3) had no significant effects on reaction rates. Microcosm studies also demonstrated that RDX products produced by permanganate were more biodegradable than parent RDX.

Chokejaroenrat [404] recently studied the RDX-MnO_4^- reaction and concluded that the first step in the RDX-MnO_4^- reaction is rate-limiting. Based on the products identified, two possible degradation pathways were proposed. The first mechanism is favored at neutral pH and involves removal of a hydride from the methylene carbon followed by hydrolysis and decarboxylation. The second mechanism is similar to a previously reported hydrolysis pathway where proton abstractions from the methylene hydrogens occurs causing a nitro group to be released and a double bond formed on the triazinane ring. Regardless of the pathway, it is clear that permanganate is capable of transforming and mineralizing RDX [382,404]. To scale up these observations, Albano [405] recently conducted a pilot-scale test using permanganate on a test section of RDX-contaminated groundwater at the Nebraska Ordnance Plant. The pilot-scale ISCO was performed by using an extraction/injection well configuration to create a curtain of permanganate between two injection wells. RDX destruction was then quantified as the RDX-permanganate plume migrated downgradient through a monitoring well field. Electrical resistivity imaging (ERI) was used to identify the location of the permanganate after injection. Despite problems in getting the permanganate evenly distributed throughout the well screen, pilot-scale results showed that RDX concentrations decreased in wells closest to the injection wells by 70–80 % [405].

3.2.5.2 Fenton reaction
The Fenton reaction [406] is recognized as one of the oldest and most powerful oxidizing reactions available. This reaction has been used to decompose a wide range of refractory synthesized or natural organic compounds [407,408]. The Fenton reagent is a mixture of H_2O_2 and ferrous iron (Fe^{2+}), which produces OH radicals [409].

$$H_2O_2 + Fe^{2+} = Fe^{3+} + \cdot OH + OH^- \qquad\qquad (2)$$

The hydroxyl radical is second only to fluorine as an oxidizing agent and is capable of nonspecific oxidation of many organic compounds. If a sufficient concentrations of •OH are generated, the reaction can continue to completion, ultimately oxidizing organic compounds to CO_2, H_2O, and low-molecular-weight mono- or di-carboxylic acids.

The Fenton reaction has been effective in treating volatile organic compounds (VOCs), light and dense nonaqueous phase liquids (LNAPL, DNAPL), petroleum hydrocarbons, PCBs, and nitroaromatic explosives [410]. A significant advantage of using the Fenton reaction for treatment of explosives is that destruction is rapid. Zoh and Stenstrom [411] investigated Fenton treatment of both RDX and HMX and reported 90 % removal of RDX from a solution within 70 min, with HMX removal one-third as rapid. Most researchers have found that the Fenton reaction works best between pH 3 and 5, but destruction has been observed across a wider pH range (3–7). High subsurface pH can limit the effectiveness of the reaction, especially when free-radical scavengers are present, such as carbonate [402].

Bier et al. [412] found that the Fenton treatment readily oxidized RDX under a wide range of conditions. They performed experiments with baseline RDX mass concentrations ranging from 4.4 to 28 mg/L and controlled reaction variables such as pH (2.0–7.5), ferrous ion mass concentrations (0 to 320 mg/L), and H_2O_2 (0–4 %). Results showed a 100 % transformation of all baseline RDX mass concentrations was achieved at pH 3 with H_2O_2 content larger than 0.5 % and ferrous ion mass concentration larger than 8.2 mg/L. More relevant to aquifer treatments, Bier et al. [412] also showed 80 % transformation of RDX at pH 7.5. Bier et al. [412] found formic acid, nitrate, ammonium were formed as intermediate or final oxidation products and presented evidence that methylene dinitramine was a product of Fenton oxidation of RDX. A nitrogen mass balance indicated that 80 % of the nitrogen from RDX was accounted for by nitrate and ammonium. Recently, Liou et al. [413] investigated Fenton and photo-Fenton processes for treatment of a wide variety of explosives in wastewater. Their results showed that RDX and HMX were more difficult to destroy than TNT, but oxidation rates significantly increased with increasing ferrous iron concentrations and illumination with UV.

Although the majority of research with the Fenton reaction has been directed at treating wastewaters and groundwater, examples of soil treatments are available [408,414–419]. Many soils contain enough iron to initiate the Fenton's reactions, but those with insufficient iron require the additional step of adding a source of Fe^{2+} [420]. Bier et al. [412] also conducted oxidation tests with soil slurries, using soils from the Nebraska Ordnance Plant that had RDX fractions larger than 900 mg/kg. In one set of experiments, contaminated soil was washed with water and the wash solutions treated with Fenton's reagent. RDX in the wash solution was oxidized but not as rapidly as pure aqueous solutions due to the scavenging effects of soil organic matter, carbonates, or other oxidizable materials. To enhance solubilization of explosives from soils, use of cyclodextrins as flushing agents has been proposed [151,181]. Yardin and Chiron [421] performed photo-Fenton treatment of TNT-contaminated soil extracts obtained by flushing with cyclodextrin solutions. They found that methylated-β-cyclodextrin (MCD) performed better than hydroxypropyl-β-cyclodextrin in removing TNT and that the MCD had a beneficial effect on destruction rates during photo-Fenton treatment. Combining soil flushing with Fenton oxidation at the field scale would require full-scale soil-washing equipment, perhaps like that used by Brice Environmental (Fairbanks, AK), which has developed a portable above-ground soil washing process that reduces the volume of contaminated soil by concentrating the fine soil fraction. This technology was adapted from the mining industry and essentially offers a physical approach to treating contaminated soils, but when in combination with a chemical treatment offers an innovative physical/chemical approach.

Commercial applications of the Fenton reaction to treat soils include pilot and field-scale studies where Fenton oxidation of contaminated soil slurries (primarily associated with manufactured gas plants) has been combined with biodegradation [422]. Another unique example is the direct spraying of

50 % H_2O_2 onto soils by the Microenfractionator soil mixer (Fig. 19) as it self-propels through windrows, thus providing an ex situ treatment for unsaturated soils [423].

Several commercial firms also specialize in using the Fenton reaction as well as other chemical oxidants to treat contaminated groundwater. While specific applications are site-dependent, ISCO treatments using Fenton's reagent typically include H_2O_2 volume fractions between 5 to 50 %, and where native iron is lacking or unavailable, ferrous sulfate is commonly added in mM concentrations [402]. In some cases, acetic or mineral acids are added to reduce the pH. Potassium phosphate (KH_2PO_4) is sometimes added to prevent premature decomposition of H_2O_2 in soil systems [424]. Delivery systems have included common groundwater wells or specialized injectors with compressed air or deep soil mixing equipment [402]. Not all sites are appropriate for ISCO treatment with Fenton's reagent. Suitable groundwater characteristics for ISCO treatment using Fenton reagent typically include: pH lower than 7.8, alkalinity of 400 mg/L (as $CaCO_3$) or lower, depth to groundwater of 5 ft below grade, and hydraulic conductivity higher than 10^{-6} cm/s.

Examples of Fenton treatment of explosive plumes in the field are limited but Geo-Cleanse International conducted a test program at a former munitions production facility that had contaminated groundwater (Pueblo Chemical Depot, Pueblo, Colorado). In this field test program, 16 600 L of 12.5 % H_2O_2 was injected with catalysts into a test plot over two days. After 26 d, HMX was completely removed and RDX concentrations had decreased by 60 %. Decreases in the nitroaromatic compounds also present decreased by 72–100 % (<http://www.geocleanse.com>).

3.2.5.3 Ozone

O_3 was first discovered in 1840 and used as a disinfectant at the end of the 19th century [425a]. Commonly used in treating drinking water, O_3 has been more recently applied to treat organic contaminants in groundwater and the vadose zone. Chemically, O_3 can be represented as a hybrid of four resonance structures that present negatively and positively charged oxygen atoms. The high reactivity of O_3 is attributed to this electronic configuration where the absence of electrons in one of the terminal oxygen atoms imparts electrophilic character while excess negative charge in other oxygen atoms imparts nucleophilic character [425b]. This dual character allows O_3 to react through two different mechanisms, namely, direct and indirect ozonation. Direct ozonation involves degradation of organics by the O_3 molecule under acidic conditions, while indirect ozonation results in the formation of hydroxyl radicals and occurs under basic conditions [426,427]. O_3 is also similar to permanganate in that it has a strong affinity for organic compounds containing carbon–carbon double bonds by forming unstable ozonide intermediates.

Slightly soluble in water, O_3 is a very reactive reagent in both air and water. O_3 is a gas that is highly reactive and must be produced on-site. It can be vented into a soil profile for remediation purposes and has been studied as an alternative for unsaturated soils contaminated with compounds resistant to soil vapor extraction [428–431]. In porous geologic media, hydroxyl radical production will occur in the vadose zone through catalytic reactions of O_3 with iron oxides and organic material. The OH radicals produced should in turn be able to transform HE present in the soil. While much is known regarding the destructive mechanisms of O_3 on chlorinated solvents and HE in groundwater, far less is known regarding how O_3 attacks and breaks down explosive compounds in unsaturated soils (vadose zone).

Adam et al. [432] conducted treatability studies with O_3 on RDX-contaminated soils obtained from the vadose zone of the U.S. Department of Energy's Pantex Plant. In a feasibility study, vadose zone soil from the Pantex site (about 6–10 m deep) was placed in soil columns under varying soil water contents and treated with O_3. Soils initially had background fractions of RDX (about 1–2 mg/kg) but were augmented with [14C]-RDX to quantify mineralization. O_3 generated from O_2 was then passed through the soil columns (26–30 mg/L) at about 125 mL/min and subsequently through two midget bubblers containing 0.5 M NaOH to trap emitted $^{14}CO_2$. Unsaturated soil columns treated with O_3 revealed that 50 % RDX mineralization was achieved within 1 day and more than 80 % within 7 days. Soil water volume fraction of 11–28 % had little effect on cumulative mineralization. They also

observed that O_3-generated RDX degradation products were much more biodegradable than untreated RDX in aerobic microcosms [432].

3.2.5.4 Persulfate

Persulfate (peroxydisulfate) is a chemical oxidant that has gained in popularity over the last few years. Persulfate ($S_2O_8^{2-}$) is a sulfate peroxide with the structure

$$[O_3S\text{–}O\text{–}O\text{–}SO_3]^{2-} \tag{3}$$

The sulfate moieties substituted for hydrogen significantly increase the stability of $S_2O_8^{2-}$ compared to H_2O_2. In addition to direct oxidation, $S_2O_8^{2-}$ can be induced to form sulfate radicals, which is a stronger oxidizer than H_2O_2, permanganate, and O_3. This is similar to the Fenton reaction where H_2O_2 must react with a metal to initiate the reaction, $S_2O_8^{2-}$ must also be activated to form radical species. $S_2O_8^{2-}$ is generally activated by four initiators according to the general reaction

$$S_2O_8^{2-} + \text{initiator} = 2SO_4^{\bullet-} \tag{4}$$

$S_2O_8^{2-}$ activators or initiators commonly used include: heat, Fe^{2+}, H_2O_2, and alkaline pH [433,434].

While $S_2O_8^{2-}$ has been used at numerous sites contaminated with chlorinated solvents, to date there are few citations on the use of $S_2O_8^{2-}$ for treating explosive compounds. Waisner and Hoag [435a] reported that heat-activated $S_2O_8^{2-}$ was effective in destroying RDX, HMX, and TNT but found little degradation with iron-activated $S_2O_8^{2-}$. Waisner et al. [435b] also evaluated lime and $S_2O_8^{2-}$ individually and in combination to treat soils from a former burning ground that was contaminated with TNT, 2,4-DNT, 2,6-DNT, and PCBs. They found the lime treatment removed 98 % of the TNT, 75 % of the DNT, and 80 % of the PCBs. Similar rates of removal were found when $S_2O_8^{2-}$ was used alone or following the lime treatment. Results from the University of Nebraska showed that alkaline-activated $S_2O_8^{2-}$ could rapidly transform RDX but as observed with lime treatments, alkaline pH alone was sufficient to cause loss of RDX (Fig. 20). However, when experiments were performed with ^{14}C-labeled RDX, results showed that $S_2O_8^{2-}$ and alkaline-activated $S_2O_8^{2-}$ were effective in mineralizing RDX (i.e., oxidation to CO_2) under laboratory conditions (Fig. 21). The kinetics of RDX mineralization was relatively slow and sustained when 35 mM $Na_2S_2O_8$ was used (Fig. 21). However, when this same $S_2O_8^{2-}$ concentration was activated by heat (55 °C), complete RDX transformation was observed within 3 d with more than 95 % mineralization (Fig. 22). Waisner and Hoag [435a] similarly observed much faster mineralization rates of RDX when heat-activated $S_2O_8^{2-}$ was used.

3.2.5.5 Hydrothermal oxidation/reduction

Subcritical water is hot water (above 100 °C) under enough pressure to maintain the liquid state. It is an environmentally friendly and inexpensive solvent that exhibits a wide range of properties that render it very effective in solvating and decomposing moderately polar or nonpolar substances from a wide range of environmental matrices. Laboratory [436] and pilot-scale research [437] focused on the destruction of TNT, HMX, and RDX on contaminated soil using subcritical water under non-oxidative conditions, As far as TNT was concerned, it was found that near complete (more than 99.9 %) degradation was achieved at 250 °C and 30 min residence time. The results from the pilot-plant scale experiments agreed well with those obtained at laboratory scale. When the contaminated soil was heated at 275 °C for 1 h, in the presence of water, more than 99 % destruction of TNT was achieved. Similar results were obtained with respect to HMX and RDX. A mass transfer limitation was observed in the degradation of TNT on aged, contaminated soils, which controlled the rate of degradation. Additionally, the soil matrix or any organic substances that exist in it acted catalytically to some extent.
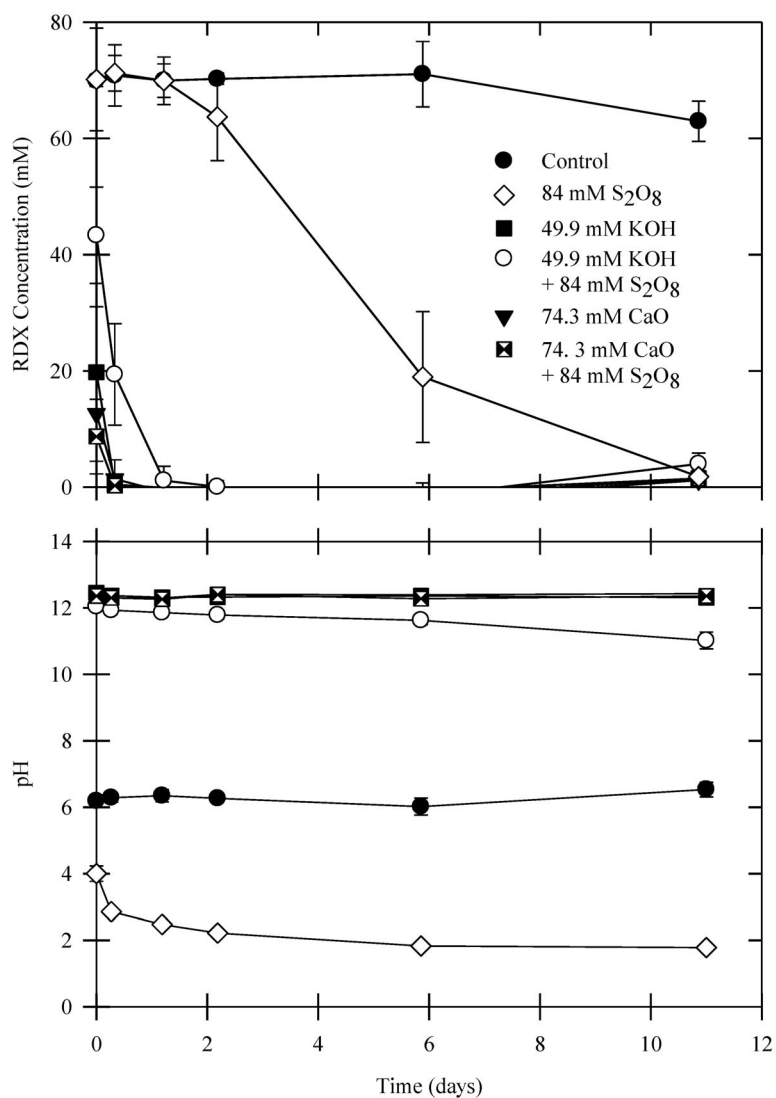
Fig. 20 Effect of alkaline (KOH, CaO) and $S_2O_8^{2-}$ amendments alone and in combination on temporal changes in RDX concentrations and pH.
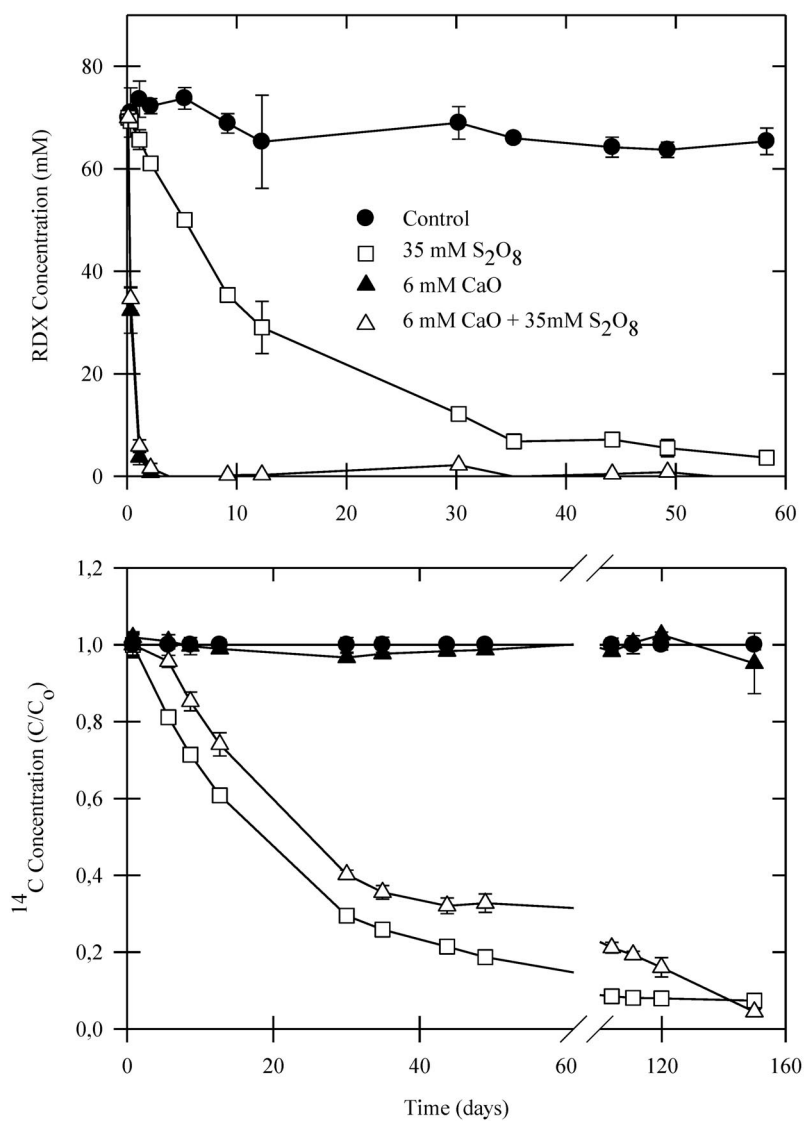
Fig. 21 Changes in RDX and ^{14}C concentrations following treatment with CaO, $S_2O_8^{2-}$, and CaO/$S_2O_8^{2-}$.
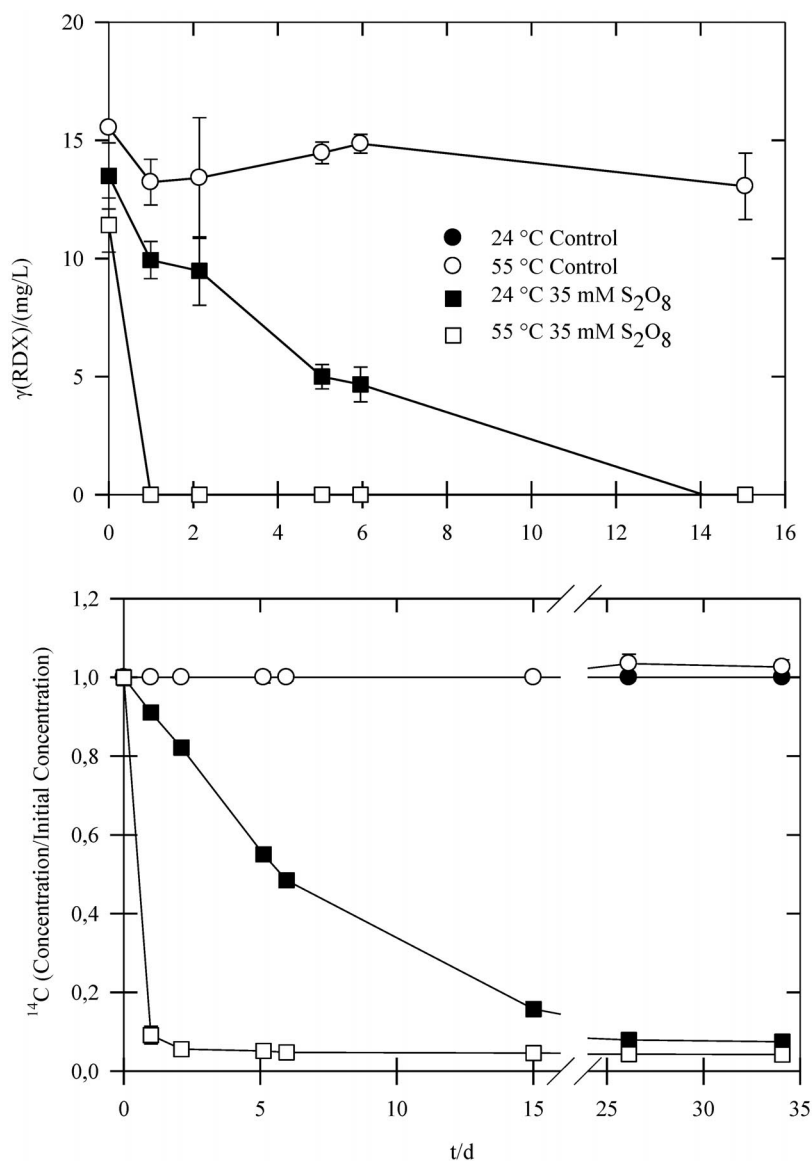
Fig. 22 Changes in RDX and ^{14}C concentrations following treatment with heat-activated $S_2O_8^{2-}$.

3.3 Thermal methods

Thermal methods are commonly used for the deactivation of explosive substances and to a lesser extent, for the treatment of explosives-contaminated soil.

3.3.1 Hot gas decontamination

Hot gas decontamination is a technology still in the pilot scale of development that can be used for decontamination of explosives-contaminated masonry or metallic structures. The method involves sealing and insulating the structures, heating with hot gas stream to 260 °C (500 °F) for a prescribed period of time, volatilizing the explosive contaminants, and destroying them in an afterburner. Operating conditions are site-specific. Contaminants are completely destroyed.

3.3.2 Incineration

Incineration processes can be used to treat the following waste streams: explosives-contaminated soil and debris, explosives with other organic or metals, initiating explosives, some bulk explosives, UXO, bulk explosive waste, and pyrophoric waste. In addition, incineration can be applied to sites with a mixture of media, such as sand, clay, water, and sludge, provided the media can be fed to the incinerator and heated for a sufficient period of time. With the approval of the U.S. Department of Defense (DOD) Explosives Safety Board, the U.S. Army considers incineration of materials containing less than 10 % explosives by weight to be a nonexplosive operation. Soil with less than 10 % explosives by weight has been shown by the U.S. Army Environmental Center (USAEC) to be nonreactive; that is, not to propagate a detonation throughout the mass of soil. (The military explosives to which this limit applies are secondary explosives such as TNT and RDX and their manufacturing by-products).

The U.S. Army primarily uses three types of incineration devices: the rotary kiln incinerator, deactivation furnace, and contaminated waste processor. The rotary kiln incinerator is used primarily to treat explosives-contaminated soils. In rotary kiln incineration, soils are fed into a primary combustion chamber, or rotary kiln, where organic constituents are destroyed. The temperature of gases in the primary chamber ranges from 427 to 649 °C (800–1200 °F), and the temperature of soils ranges from 316 to 427 °C (600–800 °F). Retention time in the primary chamber, which is varied by changing the rotation speed of the kiln, is approximately 30 min. Off-gases from the primary chamber pass into a secondary combustion chamber, which destroys any residual organics. Gases from the secondary combustion chamber pass into a quench tank where they are cooled from approximately 2000 to 200 °C (3600–400 °F). From the quench tank, gases pass through a Venturi scrubber and a series of baghouse filters, which remove particulates prior to release from the stack. The treated product of rotary kiln incineration is ash (or treated soil), which drops from the primary combustion chamber after organic contaminants have been destroyed. This product is routed into a wet quench or a water spray to remoisturize it, then transported to an interim storage area pending receipt of chemical analytical results.

The deactivation furnace is also referred to as U.S. Army Peculiar Equipment (APE) 1236 because it is used almost exclusively by the U.S. Army to deactivate large quantities of small arms cartridges, and 50-caliber machine gun ammunition, mines, and grenades. The deactivation furnace is similar to the rotary kiln incinerator except it is equipped with a thick-walled primary combustion chamber capable of withstanding small detonations. Deactivation furnaces do not have secondary combustion chambers because they are intended not to completely destroy the vaporized explosives but to render the munitions unreactive. Most deactivation furnaces are equipped with air pollution control equipment to limit lead emissions. The operating temperature of deactivation furnaces is approximately 650–820 °C (1200–1500 °F).

The contaminated waste processor handles materials, such as surface-contaminated debris, that are lighter and less reactive than those processed in the deactivation furnace. Contaminated waste processors are thin-walled, stationary ovens that heat contaminated materials to about 600 °C (1100 °F) for 3–4 h. The purpose of this process is not to destroy contaminated debris but to sufficiently lower contaminant levels through volatilization to meet U.S. Army safety standards. USAEC currently is helping to develop standardized time and temperature processing requirements to meet these safety standards.

OB and OD operations are conducted to destroy unserviceable, unstable, or unusable munitions and explosive materials. In OB operations, explosives or munitions are destroyed by self-sustained combustion, which is ignited by an external source, such as flame, heat, or a detonation wave. In OD operations, detonable explosives and munitions are destroyed by a detonation initiated by a disposal charge. OB/OD operations require regulatory permits. These permits must be obtained from the appropriate regulatory agency on a case-by-case basis.

OB/OD operations can destroy many types of explosives, pyrotechnics, and propellants. OB areas must be able to withstand accidental detonation of any or all explosives being destroyed, unless the characteristic of the materials involved is such that orderly burning without detonation can be ensured.

Personnel with this type of knowledge must be consulted before any attempt is made at OB disposal, especially if primary explosives are present in any quantity. OB and OD can be initiated either by electric or burning ignition systems. In general, electric systems are preferable because they provide better control over the timing of the initiation. In an electric system, electric current heats a bridge wire, which ignites a primary explosive or pyrotechnic, which in turn ignite or detonate the material slated to be burned or detonated. If necessary, safety fuses, which consist of propellants wrapped in plastic weather stripping, are used to initiate the burn or detonation. The following factors may limit the applicability and effectiveness of the process:

- Minimum distance requirements for safety purposes mean substantial space is required for open processes.
- OB/OD operations emissions are difficult to sufficiently capture for treatment and may not be permitted in areas with emissions limitations, although subsurface processes minimize emission release.
- In OB/OD operations, prevailing winds must carry sparks, flame, smoke, and toxic fumes away from neighboring facilities. OB/OD operations are never conducted during sand, snow, or electrical storms strong enough to produce static electricity, which might cause premature detonation.
- OB/OD requires special permits in many countries.
- In addition, with growing OB/OD restriction, the DOD's ability to treat energetic wastes is diminishing and energetics disposal, through OB/OD, may be eliminated.

The environmental impacts of traditional OB and OD can be drastically reduced using controlled incineration techniques in combination with high-pressure water washout. This enables the explosive contents to be separated from the casing, and simultaneously the explosive is transformed to a nonsensitive water-based slurry [438].

4. CONCLUSIONS AND RECOMMENDATIONS

With respect to their environmental fate, TNT degrades the most rapidly, followed by RDX and HMX. In semi-arid and arid environments, HE have the ability to persist for long periods of time, on the order of decades, especially when buried beneath the surface soil layer. In environments of greater rainfall, or where there is an additional water supply from, as an example, an effluent discharge or a washout lagoon, HE are able to mobilize and transport through the soil profile into shallow and relatively deep groundwater, where both the primary parent explosive and decomposition products have been documented.

The optimal remediation strategy for nitroaromatic compounds depends on many site-specific factors. Remediation strategies must be considered on a site-by-site basis. For example, the toxicity of nitroaromatics may limit the applicability of some biological methods when concentrations are high, or the treatment process may produce recalcitrant reaction by-products. Conversely, energy-intensive chemical treatments such as incineration may be too expensive at low concentrations, or may cause other environmental problems such as NOx emissions.

Composting and the use of reactor systems lend themselves to treating soils contaminated with high levels of explosives (e.g., at former ammunition production facilities, where areas with a high contamination level are common). Compared to composting systems, bioreactors have the major advantage of a short treatment time, but the disadvantage of being more labor-intensive and more expensive. Phytoremediation, although not as widely used as other bioremediation methods, has the potential to become an important strategy for the remediation of soil and water contaminated with explosives. It is best suited where contaminant levels are low (e.g., at military sites where pollution is rather diffuse) and where larger contaminated surfaces or volumes have to be treated. In addition, phytoremediation can be used as a polishing method after other remediation treatments, such as composting or bioslurry, have taken place. This in situ treatment method has the advantage of lower treatment costs, but has the dis-

advantage of a considerably longer treatment time. In order to improve the cost-efficiency, phyto-remediation of nitroaromatics (and other organic xenobiotics) could be combined with bioenergy production. This requires, however, detailed knowledge on the fate of the contaminants in the plants as well as the development of efficient treatment methods for the contaminated biomass that minimize the spreading of the contaminants into the environment during post-harvest treatment. However, with respect to soil treatment phytoremediation suffers from drawbacks that follow most in situ bioremediation technology, mainly sorption/desorption phenomena. Sorbed explosive compounds and their metabolites must move from the bulk soil to the zone of influence near roots for phytoremediation to occur. Phytoremediation has one advantage in that the planting of vegetation is relatively well accepted by the public. Both bioremediation and phytoremediation methods suffer from unpredictable climate variations, and are essentially nonapplicable in colder climates during the winter, making it difficult to predict degradation rates.

Of the chemical reduction methods, the cost of hydrogenation catalysts is substantial, and it is essential to find catalysts such as the recently developed Ni/zeolite Y that avoid the use of precious metals. Likewise, electrochemical reduction can become competitive only if inexpensive and long-lasting electrode materials can be developed. Additionally, chemical reduction converts the nitroaromatic contaminants to amines, which must be further treated. Hydrothermal oxidation of nitroaromatics has a higher installation and operational cost due to the difficulty of raising and maintaining high temperatures under non-batch conditions. Like incineration, hydrothermal treatment is extremely energy-intensive and hence unlikely to be economic.

Future research in this area will range from clean-up directives for explosives manufacturing and munitions development to sustaining military readiness by appropriately managing training and testing ranges in an environmentally responsible manner. Assessing the potential for explosives contamination and the potential for exposure of environmental and human receptors resulting from various military activities will be necessary. Research will be needed to refine environmental and human health risk assessment methods and develop tools for effective management of necessary military training operations to minimize adverse environmental and human health effects. Additionally, novel, "green" explosive substances need to be developed in order to reduce the post-war environmental impact in areas of military conflict. Finally, the bioavailability of explosives is an important aspect requiring attention, especially factors influencing soil/sediment aging, desorption of energetic compounds from varying soil and sediment types, methods for modeling/predicting energetic bioavailability, development of biomarkers of energetic exposure or effect, and the impact of bioavailability on ecological risk assessment.

LIST OF ACRONYMS

1-NO-HMX	1-nitroso-3,5,7-trinitro-1,3,5,7-tetrazocane
2,4,6-TNBA	2,4,6-trinitrobenzoic acid
2,4-DANT	2,4-diamino-6-nitrotoluene
2,4-DNBA	2,4-dinitro-benzoic acid
2,4-DNP	2,4-dinitrophenol
2,4-DNT	2,4-dinitrotoluene
2,6-DANT	2,6-diamino-4-nitrotoluene
2,6-DNT	2,6-dinitrotoluene
2-A-4,6-DNBA	2-amino-4,6-dinitro-benzoic acid
2-M-3,5-DNA	2-methyl-3,5-dinitroaniline
4-ADNT	4-amino-2,6-dinitrotoluene
4-M-3,5-DNA	4-methyl-3,5-dinitroaniline
AOP	advanced oxidation process
AP	ammunition plant
APE	Army Peculiar Equipment

ATSDR	Agency for Toxic Substances and Disease Registry
CA	chromosomal aberration
CFU	colony-forming unit
d.o.	dissolved oxygen
DDT	1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane
DNAPL	dense nonaqueous-phase liquid
DNX	1,3-dinitroso-nitro-1,3,5-triazinane
DOD	U.S. Department of Defense
EOD	Explosive Ordnance Disposal
ERI	electrical resistivity imaging
ESTCP	Environmental Security Technology Certification Program
HADNT	4-hydroxylamino-2,6-dinitrotoluene
HE	high explosives
HMX	1,3,5,7-tetranitro-1,3,5,7-tetrazocane
ISCO	in situ chemical oxidation
ISRM	in situ redox manipulation
LAAP	Louisiana Army Ammunition Plant
LC-MS	liquid chromatography-mass spectrometry
LHAAP	Longhorn Army Ammunition Plant
LNAPL	light nonaqueous-phase liquid
LOEC	lowest observed effective concentration
MAG	Mines Advisory Group
MCD	main charge disrupter
MCD	methylated-β-cyclodextrin
MNX	1-nitroso-3,5-dinitro-1,3,5-triazinane
NB	nitrobenzene
NFESC	Naval Facilities Engineering Service Center
NG	nitroglycerine (1,3-dinitrooxypropan-2-yl nitrate)
NOEC	no observed effective concentration
OB	open burning
OD	open detonation
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PETN	[3-nitrooxy-2,2-bis(nitrooxymethyl)propyl]nitrate
PMSO	peat moss plus soybean oil
PRB	permeable reactive barrier
RDX	1,3,5-trinitro-1,3,5-triazinane
SPE	solid-phase extraction
TCA	trichloroacetic acid
tetryl	*N*-methyl-*N*,2,4,6-tetranitroaniline
TNT	2,4,6-trinitrotoluene
TNX	1,3,5-trinitroso-1,3,5-triazinane
USAEC	U.S. Army Environmental Center
UXO	unexploded ordnance
VOC	volatile organic compound
WP	white phosphorus

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