

PHYTOREMEDIATION OF NITROGLYCERIN
IN SMOKELESS POWDERS

A DISSERTATION
SUBMITTED TO THE GRADUATE SCHOOL
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE
DOCTOR OF EDUCATION IN SCIENCE

BY

NAVID ASBAGHI

DISSERTATION ADVISOR: DR. JOHN PICHTEL

BALL STATE UNIVERSITY

MUNCIE, INDIANA

JULY 2012

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APPROVED BY:

Committee Chairperson

Date

Committee Member

Date

Committee Member

Date

Committee Member

Date

Departmental Chairperson

Date

Dean of Graduate School

Date

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ABSTRACT

DISSERTATION: Phytoremediation of Nitroglycerin in Smokeless Powders

STUDENT: Navid Asbaghi

DEGREE: Doctor of Education in Science

COLLEGE: Sciences and Humanities

DATE: July 2012

PAGES: 138

This study evaluated the feasibility of rhizosphere-enhanced phytoremediation in the removal of nitroglycerin (NG), as applied in commercial smokeless powder from soil. Double base smokeless powder was applied to soil mesocosms at rates of 0, 1.0, 5.0 and 10% (w/w). Nitroglycerin-contaminated mesocosms were seeded with oats (*Avena sativa*) or planted with live sedge plants (*Carex vulpinoidea*). In addition, composted biosolids were used as a soil treatment. Mesocosms were sampled at 7, 14, 30, and 60 days after initial planting. Determination of residual NG in the samples was performed using gas chromatography with an electron capture detector. Additionally, the populations of soil-borne bacteria were determined for each treatment. Microbial activity in the plant rhizosphere was a major contributor to NG decomposition in the soil. Only modest quantities of NG removal could be accounted for by abiotic processes such as sorption. Nitroglycerin decomposition by photolytic processes was observed; however, this effect is considered to be a minor contribution to NG removal from soil. Soil

bacterial numbers remained relatively constant regardless of the rate of SP application (1% and 10%). The data also indicate that addition of CB amendment to soil imparted a positive effect in NG decomposition and/or removal from soil. Additional study is needed to determine which of the plants studied was/were superior in NG removal from soil.

DEDICATION

This dissertation is dedicated to my wonderful wife, Samila and my lovely son, Soroush.

ACKNOWLEDGMENTS

I would like to especially thank my adviser and committee chair Dr. John Pichtel for his advice, encouragement, and support. My appreciation also goes to my other committee members Dr. Brian Lepore, Dr. William Bock, Dr. John McKillip, and also the former committee member Dr. Klaus Neumann for their guidance and support. Dr. James Jones, Research Design Assistant Director at University Computing Services, is acknowledged for his guidance and consultation regarding statistical analysis. Stan Ross, John Taylor, Shari Grant, John Obrycki, Jackie Arroyo, Luke Schmid, Kelsey Bonhivert, and Kayla Coffin are acknowledged for their assistance in this research. I'd like to thank my loving parents Khairollah and Pouran, my sister Naghmeh, my father-in-law Rohoullah Charkharrin, my mother-in-law Farah Mahboubi, Mr. Farshid Mahboubi, Simin Charkharrin, Misagh Hakimian, Behrouz and Gwen Kousari, and Dr. Horst Siewert for their support and encouragement. My special thanks go to Dr. Marianne Walsh for her technical suggestions regarding the GC-ECD instrument.

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INTRODUCTION

Increasing population growth and industrialization over the past century have resulted in a range of soil and water pollution issues worldwide. Environmental contaminants may be classified as inorganic (e.g., heavy metals, radioactive elements) and organic (crude petroleum, refined fuels, petrochemicals, explosives, and others).

The United States is the largest producer of explosives and, therefore, of explosives wastes. Thousands of explosives-contaminated sites are known to occur in the U.S. for at least a half-century, so-called energetic materials such as nitroglycerin (NG) have been used extensively by both the US military and private firearms enthusiasts. As a result, numerous sites are currently contaminated with NG and its residues.

Several techniques are available which may detoxify and/or remove energetic compounds from soil including solidification/stabilization, incineration, and excavation combined with secure landfilling. Many of these technologies, however, are costly in terms of initial start-up and long-term operation. There are also concerns with generation of air pollutants and potentially toxic residues. Therefore, there is a need for an effective,

inexpensive and environmentally-friendly method to remove energetics residues from soil.

Bioremediation (i.e., the use of microorganisms to treat contaminated soil) is a microbially-based remediation strategy that has been used for decades for the removal of certain hydrocarbon wastes. This technology requires either the use of indigenous soil microbial populations or the introduction of specialized microbial types to the affected site. In the former case, native microbes may not be capable of completely decomposing the target compound. Additionally, toxic conditions may render indigenous microbes ineffective. In the latter case, introduced organisms may not be capable of successfully competing with native microbes, and additional treatments may be needed to enhance the decomposition process.

Phytoremediation (i.e., the use of green plants to treat soil contamination) is an inexpensive technology for the treatment of contaminated soil which may overcome some of the disadvantages of bioremediation. Phytoremediation is suited for large contaminated areas and requires relatively low maintenance. This technology does not alter soil physical and chemical properties. It can accelerate microbial reactions in the soil thereby more rapidly reducing contaminants below regulatory limits and it is environmentally-friendly.

Many large military and privately-owned sites which are contaminated with NG require effective and inexpensive methods of remediation. Phytoremediation may serve as a viable option. Numerous studies have been conducted using phytoremediation to remove explosives such as TNT and RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) from soil; however, relatively few investigations regarding phytoremediation of NG,

particularly occurring in sequestered forms such as smokeless powders, have been documented.

For a better understanding of NG phytoremediation, there is a need to assess its decomposition in soil as affected by various factors. Key areas requiring more detailed investigation include: the proper choice of plant species; the chemical transformations of NG in the plant rhizosphere (root zone), the rate of NG destruction as a function of soil treatment, and the key rhizosphere bacterial populations involved in NG decomposition.

Objectives

In the reported study, the feasibility of phytoremediation was assessed for the removal of nitroglycerin (NG) from soils under selected conditions in the greenhouse.

Specifically, the objectives were to:

- 1) compare the efficiency of smokeless-powder-derived NG decomposition in the rhizospheres of two plant species which require divergent nutrient and moisture regimes;
- 2) assess the influence of soil amendments (composted biosolids) on enhancing NG degradation rate; and
- 3) identify predominant microbial populations occurring in treatments undergoing the most efficient NG removal.

Literature Review

Classes of Energetic Compounds

Energetic compounds may be classified as either explosives or propellants.

Explosives are designed to combust at supersonic speeds, thus causing a detonation and generating a destructive shock wave. Explosives occur in three classes, i.e., primary, secondary and tertiary. Primary explosives are those that require only a small amount of energy for initiation. An example of a primary explosive is lead azide. The secondary class of explosive compounds comprises those that require a substantial amount of energy for initiation. Examples of secondary explosives are 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (Pichtel, 2012). Tertiary compounds require substantial initiation from a secondary (i.e., booster) explosive. Tertiary compounds include common blasting agents, for example, a physical mixture of ammonium nitrate and fuel oil (Explosives, 2011).

Propellant materials for guns, artillery, and mortars contain compounds that burn (deflagrate) at a controlled rate and produce gases that accelerate projectiles from a weapon (Jenkins et al., 2008b; Walsh et al., 2008). The primary energetic compounds used in guns, artillery, and mortars are nitro-containing organic compounds such as nitrocellulose (NC). Nitrocellulose is often combined with other energetic materials such as NG, nitroguanidine (NQ), or dinitrotoluenes (DNT) in order to control burn rate, limit barrel flash, and provide other practical advantages (Jenkins et al., 2007; Brannon and Pennington, 2002).

Solid propellant materials (i.e., smokeless powder) containing NC are divided into three classes based on presence of additive compounds (Table 1.1). Single-base

propellants contain NC as the primary energetic compound. Double-base propellants contain NC combined with an organic nitrate such as NG. Triple-base propellants include NC and NG mixed with nitroguanidine (NQ) (Juhasz and Naidu, 2008; Walsh et al., 2008). Other chemicals may be added to adjust burn rate. Also, binders or plasticizers are added to facilitate loading the propellant into the shell. Additional compounds can be added to enhance propellant stability during storage (Walsh et al., 1993). Smokeless powder can be manufactured in three different forms: thin and circular flakes; small rods (perforated and imperforated); and small circular or flattened spheres (Fig. 1.1) (Heramb and McCord, 2002).

Table 1.1. Composition of common energetic compounds formulations (Pichtel, 2012)

Name	Composition
Single base smokeless powder (M1; M6; M10)	NC, 2,4-DNT; NC, 2,4-DNT; NC, diphenylamine
Double base smokeless powder (M2, M5, M8)	NC, 2,4-DNT; NC, 2,4-DNT; NC, diphenylamine; NG
Triple base smokeless powder (M30, M31)	NC, NG, NQ, ethyl centralite

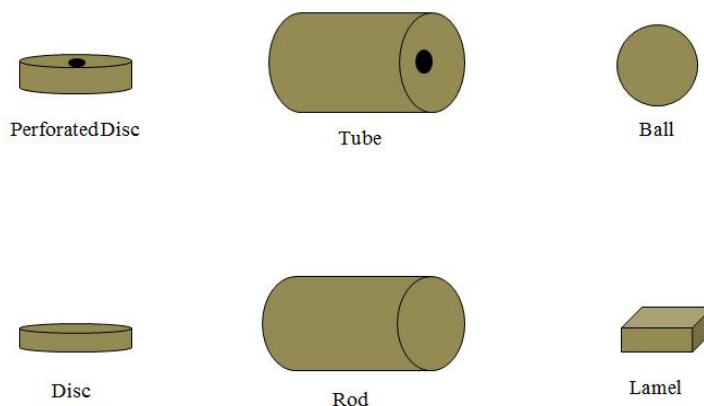


Fig 1.1. Physical forms of smokeless powders. (Heramb and McCord, 2002).

Commonly used military explosives include TNT, RDX, and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) (Leggett et al., 1977). A variety of formulations such as nitroglycerin (NG), nitroguanidine (NQ), nitrocellulose (NC), and 2,4-dinitrotoluene (DNT) have been used in missile, rocket, and gun propellants (Jenkins et al., 2007, Pennington et al., 2006 b). The chemical structures of some of these compounds appear in Figs. 1.2 and 1.3. Selected chemical and physical properties appear in Table 1.2.

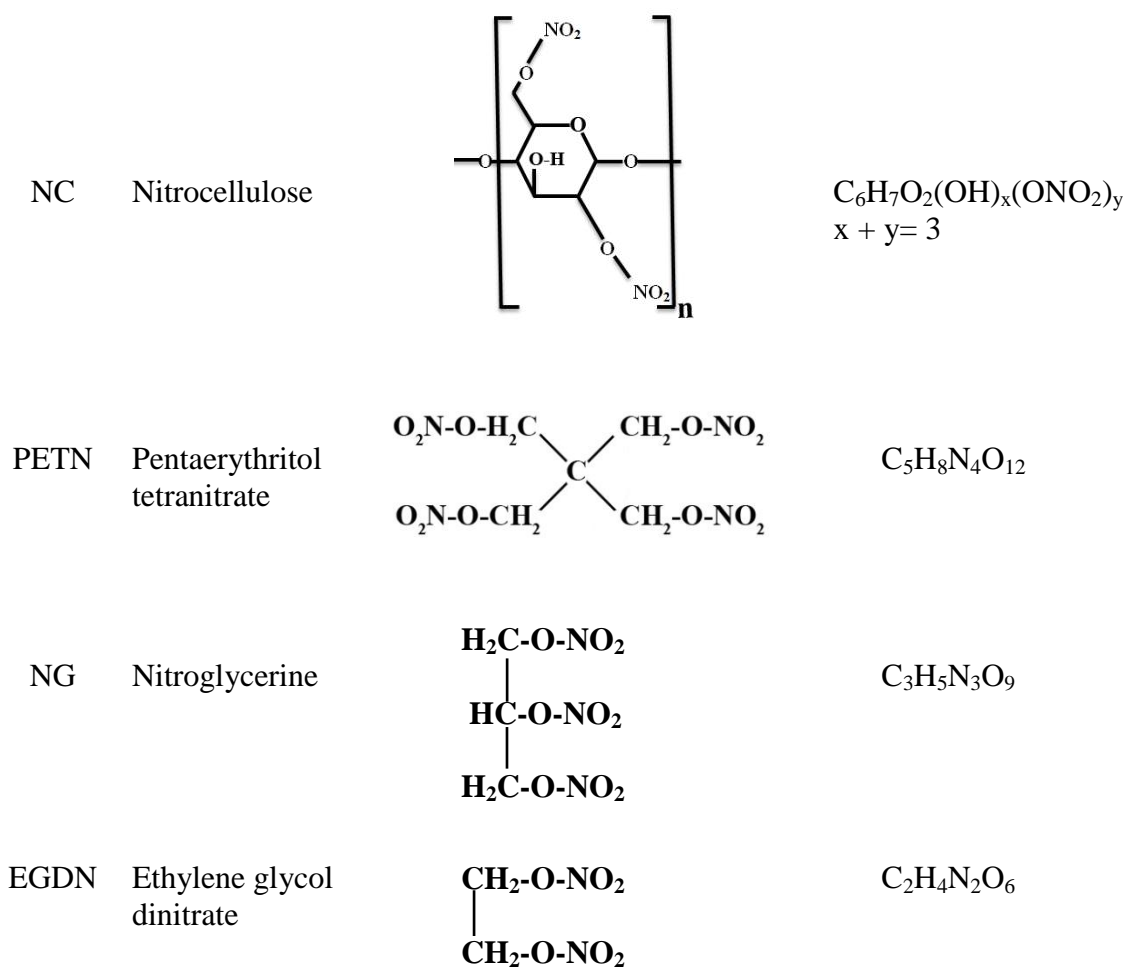


Fig. 1.2. Chemical structures of selected nitroesters (Sunahara, 2009).

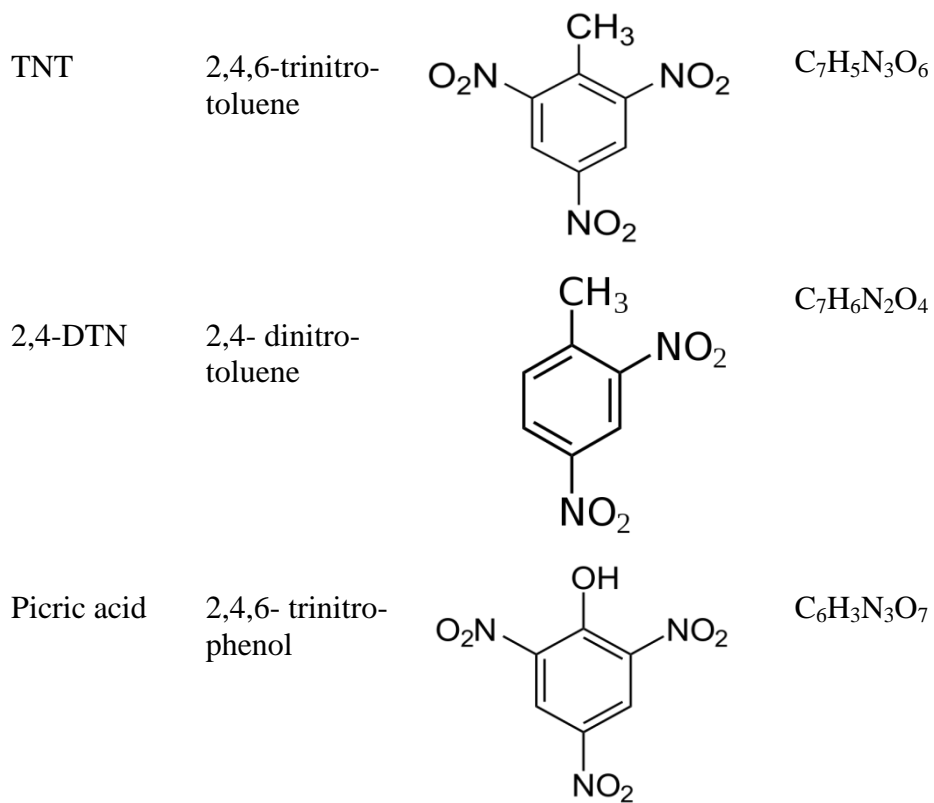


Fig. 1.3. Chemical structures of selected nitroaromatics (Sunahara, 2009).

Table 1.2. Physical and chemical properties of selected energetic compounds (Pichtel, 2012).

Energetic compound	TNT	RDX	HMX	NG	NQ	2,4-DNT
Chemical formula	$C_7H_5N_3O_6$	$C_3H_6N_6O_6$	$C_4H_8N_8O_8$	$C_3H_5(NO_3)_3$	CH_4N_4O	$C_7H_6N_2O_4$
Specific gravity	1.5-1.6	1.89	1.96	1.59	n/a	1.65
Melting point (°C)	80-82	204	276-280	13-14	239	64-66
Boiling point (°C)	240 (explodes)	(decomposes)	(decomposes)	50-60 (decomposes)	250	300 (decomposes)
MW	227.13	222.26	296.16	227	104.07	182.15

Soil Contamination by Energetics

From a chemical standpoint, most energetic compounds exist as either nitroesters or nitroaromatics, and both are considered worldwide environmental contaminants (Vanek et al., 2006). Nitroesters are alcohols containing one or more nitrate groups, i.e., $-ONO_2$ bound to a carbon atom. Nitroaromatics are aromatic compounds which contain one nitro group on the aromatic ring (Explosives, 2011) (Figs. 1.2 and 1.3).

Since World War I, energetics and their degradation products have contaminated thousands of sites around the world. Ammunition manufacturing facilities, firing ranges and impact zones, and open burning/open detonation (OB/OD) sites comprise some of most energetics-contaminated areas. Some energetic compounds such as TNT and RDX leach into groundwater (Ahmad and Hughes, 2002; Wang et al., 2002). Several energetics

are very resistant to subsequent biological degradation because they are often toxic to biota (TNT, RDX, and HMX) (Lachance et al., 1999). In some cases, solubility of these compounds is very low (Ro et al., 1996).

Nitroglycerin – General Information

In the military and in private use (e.g., firearms enthusiasts) NG is commonly employed as an ingredient in artillery and rocket propellants (Accashian et al., 1998), and in smokeless powders (Ahlner et al., 1991; Halasz, 2010), respectively. Nitroglycerin is a powerful propellant and is highly sensitive to shock and friction (Yinon, 1999). It is manufactured by direct nitration of glycerol with nitric acid using sulfuric acid as a catalyst (Marshall and White, 2001).

Nitroglycerin is toxic to humans; it can enter the body by dermal absorption, inhalation, and ingestion. Exposure to NG at high doses may be fatal, and at lower concentrations it can reduce blood pressure and cause headaches, nausea, and vomiting (Yinon, 1990). Nitroglycerin also is commonly used as a vasodilator in medicine to treat angina pectoris and other heart diseases (Accashian et al., 1998; Bhaumik et al., 1998).

Chemical and Physical Properties of Nitroglycerin

Nitroglycerin is a faint yellow, oily liquid or solid crystal with a sweet, burning taste (US Dept. of Labor, 2012). Its molecular weight is 227.11 g/mol; its boiling point is 50-60°C and it explodes at 218°C. The specific gravity of NG is 1.59 at 20°C; it has a vapor density of 7.84, a melting point of 13-14°C, and a vapor pressure at 20°C of 0.00026 mm Hg (Table 1.2).

Nitroglycerin is somewhat soluble in water (1.8 g/L at 20°C), which accounts for its ready mobility (Bleher et al., 1996), and it is capable of completely mixing with acetone, ether, benzene and other organic solvents (US Dept. of Labor, 2012). It may remain in solution at low pH levels. Nitroglycerin degradation products such as dinitroglycerin (DNG) ($C_3H_6NO_7$) and glycerol mononitrate (GMN) ($C_3H_7NO_5$), have higher solubility than does NG (Yost, 2004). When NG is mixed with other smokeless powder ingredients, the composite can remain in soil and aquatic ecosystems for much longer periods (Yost, 2004; Hewitt, 2002).

Potential Nitroglycerin Exposure Pathways and Health Effects

Toxic effects caused by nitrate esters differ with animal species (Podlipná et al., 2008). Nitrate esters can cause headaches and adverse cardiovascular effects in humans (Kanerva et al., 1991). Human exposure to nitroglycerin occurs via several pathways including inhalation, ingestion, eye or skin contact, and absorption through skin (Hathaway et al., 1991). Acute signs and symptoms of NG exposure include headaches, nausea, diarrhea, convulsions, paralysis, breathing difficulties, methemoglobinemia, circulatory collapse, and death (Sittig, 1991; Rom, 1992). Signs and symptoms of chronic NG exposure involve angina-like chest pains, severe headache, hallucinations, skin rashes, malaise, weakness, vomiting, dizziness, and death.

Studies of Nitrostat™ (NG capsule or spray), a stable parenteral component of nitroglycerin, have been conducted with mice, rats, rabbits and dogs. In general, no toxic impacts could be detected from intravenous infusion of Nitrostat™ (King and Fung, 1984). Single-dose i.v. resulted in LD₅₀ values of 17.3 and 18.2 mg/kg⁻¹ in male and

female mice, and 24.4 and 23.2 mg/kg⁻¹ in male and female rats, respectively. Subacute i.v. studies in rats and dogs at doses of 2.5, 5.0 and 10.0 mg/kg/day, and 1.0 and 3.0 mg/kg/day for two weeks, respectively, resulted in adverse reactions. Diminished weight gain and decreased food consumption were detected among treated rats. No drug-related clinical or pathological impacts were noted in dogs. Also, repeated intravenous administration of Nitrostat™ in rabbits did not show any significant local venous irritation (King and Fung, 1984).

A study was carried out to evaluate the potential cancerous effects of 10% nitroglycerin (NT-1 ointment) on New Zealand white rabbits. Pregnant rabbits were injected percutaneously with NT-1 ointment from day 6 to 18 of gestation at dose levels of 15, 60 and 240 mg/kg/day. All pregnant rabbits were sacrificed on day 29 of gestation, and the effects of NT-1 ointment on mothers and fetuses were studied. Erythema was noted during the treatment period on the treated dorsal skin. Although it disappeared rapidly after cessation of NT-1 injection, no apparent decrease in food consumption or body weight was detected in treated animals. Also, no apparent adverse effect was noted on the reproductive performance of mothers and the development of fetuses in treated animals (Imoto et al., 1986).

Although there has been some indication of mutagenic effects of NG in Ames assays, no significant correlations have been found indicating that NG could be teratogenic in test subjects (Yost, 2004). Some studies indicate formation of carcinogenic tumors in reproductive tissues of test subjects; however, the data is not conclusive (Ellis et al., 1978; 1984). Methemoglobin formation, however, was common in test subjects. In this illness, NG prevents hemoglobin from carrying sufficient oxygen to tissues.

Although this effect can disappear in 8-24 hours after dosing, continued dosing can cause cyanosis which is a discoloration of skin to blue or gray (Lee et al., 1976).

Nitroglycerin and EGDN have been considered environmental contaminants because of their recalcitrance to biodegradation and persistence in the environment (Podlipná et al., 2008). However, research has indicated that the toxicity of degradation by-products of NG tend to be less toxic than the original compounds. Nitrate esters also impart deleterious effects to some plants. For example, high concentrations of ethylene glycol dinitrate (EGDN), e.g., > 500 mg/l, can cause wilting, browning and subsequently be fatal to reed and rush plants (Podlipná et al., 2008).

Nitroglycerin is considered highly toxic to aquatic species (Bentley et al., 1978). Some algae have been shown to be highly sensitive to NG. Low NG concentrations reduced *Navicula pelliculosa* growth and its production of chlorophyll a. A 96-hour toxicity test has been carried out on different fish species. The results indicate that NG has a median lethal concentration (LC₅₀) ranging from 1.38-5.5 mg/l. Less susceptible fish species included fathead minnows and channel catfish.

Relevant Regulations

The Occupational Safety and Health Administration (OSHA) allows for a permissible exposure limit (PEL) of 0.2 parts per million (ppm) in air (2 mg/m³) as a ceiling limit for NG (US Dept. of Labor, 2012). The National Institute for Occupational Safety and Health (NIOSH) allows a short-term exposure limit (STEL) of 0.1 mg/m³ NG for periods not to exceed 15 min. Short-term exposures should not be repeated more than four times per day and require a waiting period of at least 60 min. between each

exposure. The American Conference of Governmental Industrial Hygienists (ACGIH) has assigned NG a threshold limit value (TLV) of 0.05 ppm (0.46 mg/m³) for an eight-hour work day for forty hours per week.

Certain precautions are necessary regarding NG storage. Nitroglycerin must be stored in a cool, dry environment in sealed containers labeled “Explosive, Poison, 0143” in accordance with DOT and OSHA requirements. Containers storing NG must be protected from physical damage, ignition sources, shock, and ultraviolet radiation. Containers should be also stored separately from strong acids or ozone (US Dept. of Labor, 2012).

Occurrence of Nitroglycerin in the Environment

Nitroglycerin has been documented as a significant contaminant of soil, surface water, and groundwater (Jenkins et al., 2001; 2002; Pennington et al., 2001; 2002; 2003). Waste streams of munitions manufacturing and pharmaceutical facilities have been found to be enriched with NG. Wastewaters saturated with NG have washed into lagoons and soakways, resulting in contamination of the surrounding environment (Accashian et al., 1998).

Development, testing, and fabrication of energetic components are necessary in NG manufacture (Gray and McGrath, 1996, Pantex, 2012). Soil contamination sources from NG manufacturing include machining, loading, improper storage practices, and improper disposal of contaminated wastewaters (Best et al., 1999; Pugh, 1982; Pichtel, 2012). The Pantex Plant (TX) was used by the US Army during World War II in order to load conventional ammunition shells and bombs. The US military used unlined

evaporation/percolation lagoons for disposal of wastewaters from energetics manufacturing processes. Therefore, many energetic compounds have accumulated at the surfaces of lagoons (Martel et al., 2008; Pantex, 2012). These areas are considered hazardous to the environment due to long-term soil and groundwater contamination as well as the potential for accidental detonation (Pennington et al., 2006b).

Several live-fire and demolition ranges have been evaluated at US and Canadian military bases in order to measure contamination by energetics (Jenkins et al, 2007; Pennington et al., 2006a; 2006b; Jenkins et al., 2006). Contaminated sites include anti-tank rocket, hand grenade, demolition, tank firing, mortar, artillery, and bombing ranges. A variety of energetic mixtures are used in military training operations. The primary source of energetics contamination at live-fire sites is from detonation of military munitions including mortar and artillery rounds, grenades, landmines, aerial bombs, missiles, and ordnance demolition charges (Jenkins et al, 2007; Pennington et al., 2006a; 2006b; Jenkins et al., 2006). Nitroglycerin concentrations of 3.6 mg/kg were measured in soil at the Cold Lake Weapons Range in Canada (Bordeleau et al., 2008). Nitroglycerin levels of 130 mg/kg were detected in the top 0.5 cm of soil at the Massachusetts Military Reservation (Yost, 2004).

Nitroglycerin residues are commonly found at the firing points of anti-tank rocket ranges due to the use of double-based energetic compounds in rockets. Residues have been found at distances up to 100 m in front of the muzzle (Pennington et al., 2006a). The most common locations of NG deposition were behind the firing line due to back blast. Contamination assessments have been performed at anti-tank rocket firing stations at Yakima Training Center (Pennington et al., 2006b; 2002), Fort Bliss (TX) (USA

CHPPM, 2004), CFB Gagetown (Thiboutot et al., 2004), CFB Valcartier (Jenkins et al, 2008a), and CFB Petawawa (Brochu et al, 2008). Results indicate that highest NG concentrations behind the firing line were due to back blast of shoulder-fired rockets. Low levels of NG residues were found in soil up to 25 m behind the firing line.

Nitroglycerin was the primary residue, with soil concentrations of hundreds to thousands of mg/kg, at different soil depths at firing points at US and Canadian bases. Concentrations of 788 mg/kg and 339 mg/kg were measured at 5–15 m and 15–25 m behind the firing line at CFB Valcartier (Jenkins et al., 2008a). Concentrations of 2400 mg/kg were measured 0–10 m behind the firing line at CFB Petawawa, while from 10–20 m behind the line NG concentrations measured 380 mg/kg (Brochu et al., 2008).

HMX predominated at the target at the Gagetown Training Area (Quebec, Canada), while NG was detected at very high levels close to the firing line (Thiboutot et al., 2004). Nitroglycerin concentrations were measured as high as 6560 mg/kg at 2 m behind the firing line and were detected to depths of 60 cm. Pennington et al. (2006 b) found that the majority of NG occurred in the top 11 cm (range of 6.5–11 mg/kg). Less than 1 mg/kg NG was detected below 11 cm. Possible fates of explosives and propellants appear in Fig. 1.4.

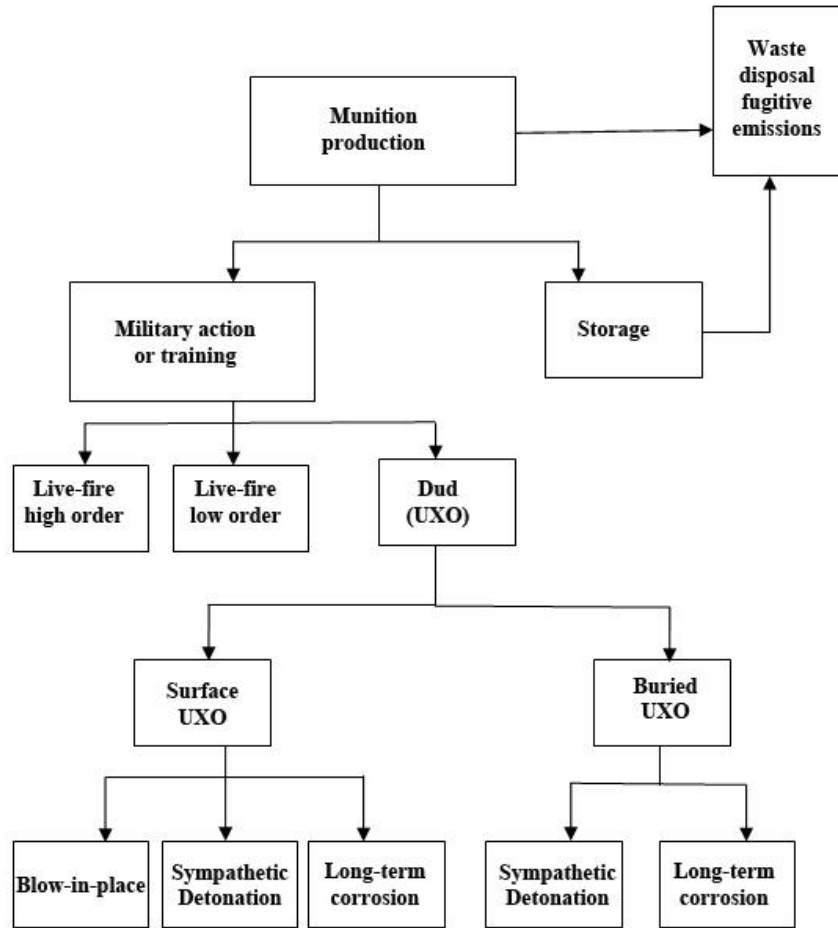


Fig. 1.4. Possible fates of energetic materials (Adapted from Kalderis et al., 2011; Brannon and Myers, 1997) (“UXO”= unexploded ordnance).

Based on the above and similar findings, efforts have been devoted to developing safe and cost-effective technologies for treating NG-contaminated soil, sediment, and groundwater (Accashian et al., 1998).

Environmental Fates of Energetic Materials

Following entry into the terrestrial environment both abiotic and biotic processes govern the fate of energetic compounds (Juhasz and Naidu, 2007; Brannon, and Pennington, 2002). The rate and extent of transport and transformation are influenced by the physicochemical properties of the compounds (e.g., solubility, vapor pressure, Henry's law constant), environmental factors (weather conditions, soil properties, pH, redox status), and biological factors (populations of energetics-degrading microorganisms). Processes that influence the environmental fate of explosive compounds may be divided into (1) influences on transport (dissolution, volatilization, adsorption) and (2) influences on transformation (photolysis, hydrolysis, reduction, and biological degradation) (Kalderis et al., 2011). Figure 1.5 illustrates the major fate and transport pathways for energetic materials in environment.

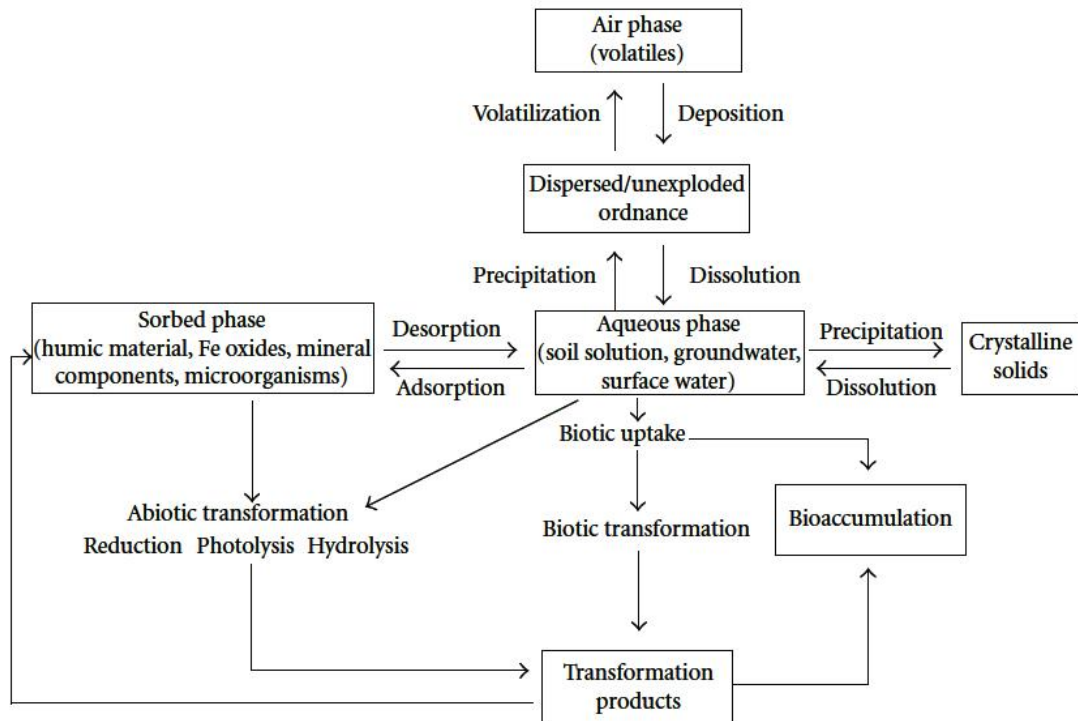


Fig. 1.5. Suggested fates of energetic compounds in the environment (Kalderis et al., 2011; Juhasz and Naidu, 2007; Townsend and Myers, 1996).

Relevant Remediation Technologies

Estimates for cleaning contaminated soil in the United States alone total approximately \$1.7 trillion (Kuiper et al., 1999). A range of biological, chemical and physical methods have been applied for remediation of energetic materials in soil and groundwater. These include solidification/stabilization, electrokinetic remediation, secure landfilling, incineration, and biological treatment (Accashian et al., 1998). Specific physicochemical methods of NG remediation include adsorption on activated carbon and reduction with inorganic chemicals or alkaline hydrolysis (Accashian et al., 1998).

Solidification/stabilization (S/S) is a combination of several processes used to immobilize contaminated soils. In the solidification process, soil is reacted with a fixative agent such as Portland cement, coal fly ash, or asphalt (bitumen). Solid monolith forms, which limits leaching and migration of contaminants by reducing the surface area exposed and/or by coating the material with low-permeability surfaces. In the stabilization process the leachability of contaminated soil is reduced resulting from chemical reactions such as precipitation and oxidation/reduction (USEPA, 2000b).

Electrokinetic remediation is a process in which heavy metals, radionuclides, and organic contaminants migrate through soil under the force of an applied electric current and are eventually removed down-gradient (USEPA, 1995). Electrode pairs are implanted in the ground on each side of the contaminated region and a low intensity direct current is applied across the electrodes. The current passes through the soil and causes electro osmosis and ion migration. As a result, contaminants are transported in the aqueous phase towards the respective electrodes depending on their charge. The hazardous materials are then extracted from the soil.

In the process of *in-situ* vitrification (ISV), a high voltage (> 2000 V) is used to heat and fuse (melt) soil, sludge, radioactive wastes, and sediments containing organic, inorganic, and metal wastes. Then the materials are allowed to cool to form a stable glass with a very low leaching capability (USEPA, 1994). Some conventional restoration techniques are not highly effective (Dixon, 1996), and some may impart adverse effects on the environment. For example, incineration of contaminated soil releases a range of air pollutants; leachates from landfills can pollute groundwater; and excavation of soil can generate toxic air emissions. Such methods also tend to be expensive. Many require

follow-up treatment of carbonaceous or nitrogenous products (Accashian et al., 1998). Therefore, there is an urgent need for less expensive, less labor-intensive, safe, and environmentally-friendly methods of restoring contaminated lands.

In recent years, federal and state environmental regulations have banned many intensive physical and chemical techniques because of concerns about potential adverse health and environmental effects. In the United States, the urgency of addressing land contamination has been made evident in recent legislation. Section 121(b) of the Comprehensive Environment Response, Compensation, and Liability Act (CERCLA) instructed US EPA to encourage using alternative remediation technologies that maximize the cleanup of hazardous materials from the environment. CERCLA also instructed the US EPA to discourage land disposal of untreated hazardous materials when alternative remediation technologies exist. Currently, environmentally-friendly biological remediation technologies are preferred and supported by federal and state agencies (USEPA, 1993a; 1993b).

Bioremediation of Energetics

Bioremediation is defined as the engineered use of microbes or other biological systems to degrade environmental contaminants (Caplan, 1993; Dua et al., 2002). Several advantages are associated with bioremediation. The process can be applied directly to contaminated soil *in situ* without physically moving soil and without drastically altering the soil matrix. In many cases, microbial degradation of contaminants usually results in complete mineralization of the contaminants (Heitzer and Saylor, 1993).

Different microorganisms including fungi and bacteria have been evaluated for their potential to degrade both explosive and propellant compounds. A variety of fungi such as *Agaricus aestivalia*, *Agrocybe praecox* and *Clitocybeodora* are able to mineralize TNT using specific extracellular enzymes such as lignin peroxidase, manganese peroxidase, and laccase (Kalderis et al., 2011; Juhasz et al., 2008). In cases of nitrogen deficiency, some fungi such as *Phanerochaete chrysosporium* are capable of mineralizing HMX (Fournier et al., 2004).

Clostridium bifermentans is a bacterial species that is capable of degrading RDX anaerobically (Regan and Crawford; 1994). *Morganella morganii*, *Enterobacteriaceae* and *Serratia marcescens* are other bacteria with the capability of biotransforming RDX anaerobically (Kitts et al., 1994; Young et al., 1997). Three strains of *Corynebacterium* are able to aerobically mineralize RDX (Binks et al., 1995; Bhushan et al., 2004). A limited number of bacterial species such as *Methylobacterium* sp. degrade HMX under aerobic conditions (Van Aken et al., 2004).

Biodegradation of Nitroglycerin

Degradation of NG can occur under both aerobic and anaerobic conditions using mixed or pure strains of bacterial species such as *Pseudomonas* spp., *Agrobacterium radiobacter* (*Rhizobium radiobacter*) and *Bacillus* spp. (Marshall and White, 2001; Meng et al., 1995; White et al., 1996; Wendt et al, 1978; Persari and Grasso, 1993). Several studies also suggest an active role of fungi in NG transformations (Bhaumik et al., 1997). The NG biodegradation process includes several denitration reactions and the formation of glycerol dinitrate (1,2-GDN and 1,3-GDN) and glycerol mononitrate (1-GMN and 2-

GMN) isomers, which can then be degraded as well (Figs. 1.6 and 1.7). The denitration process of NG favors production of 1,3-GDN and 1-GMN (Marshall and White, 2001; Bhaumik et al., 1998; Hempfling, 1997).

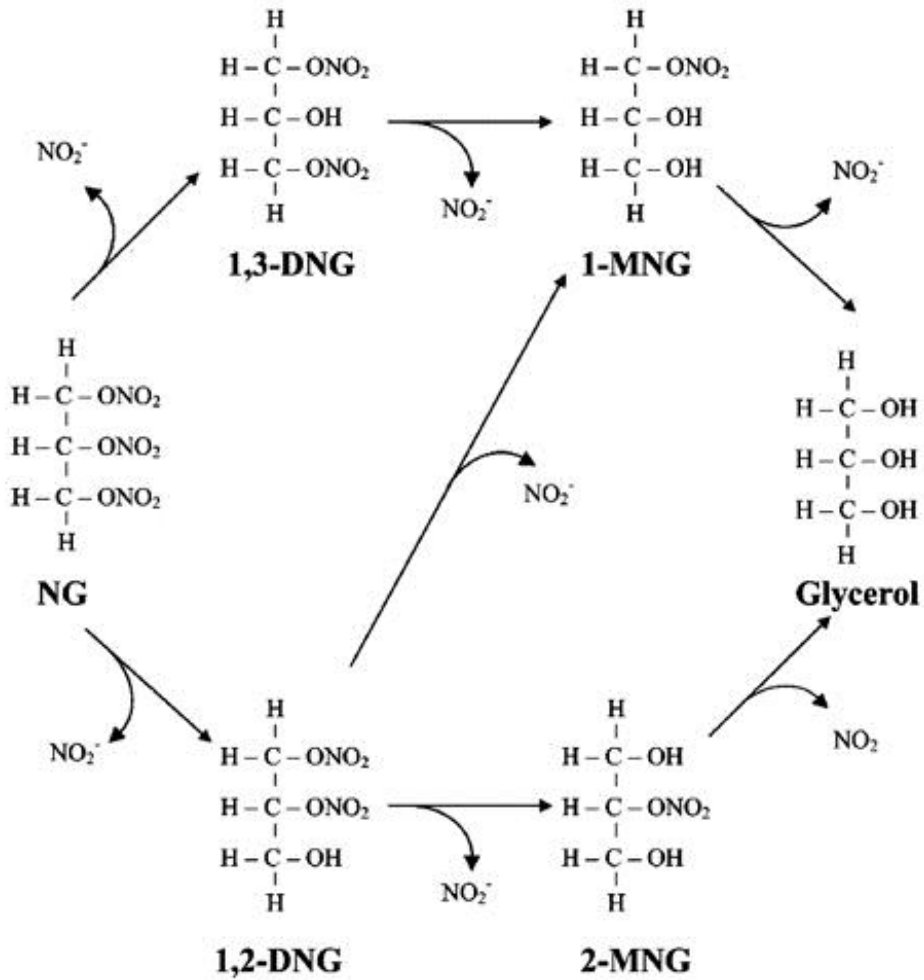


Fig. 1.6. Proposed pathways of biological degradation of NG (Oh et al.2004).

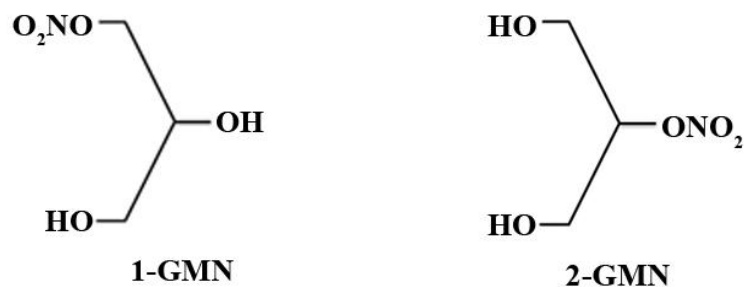


Fig. 1.7. Structures of glycerol mononitrates (GMN) (Lange et al. 2009).

Both *Pseudomonas putida* and *P. fluorescens*, isolated from NG-contaminated soils, have been shown to sequentially degrade NG to GDN and GMN isomers (Marshall and White, 2001; Blehert et al., 1997). Wendt et al. (1978) showed that without a carbon supplement, the ability of *Pseudomonas* to degrade NG is significantly reduced. Marshall and White (2001) showed that a wastewater disposal lagoon at a formerly used NG production plant contained *Arthrobacter ureafaciens*, *Klebsiella oxytoca*, and a *Rhodococcus*. These bacteria were capable of degrading NG and producing GDN and GMN isomers. Moreover, *Rhodococcus* species removed the final nitro group from GMN and thus achieved complete biodegradation of GTN.

One strain of *Agrobacterium radiobacter* was shown to denitrate NG with the formation of both GDN isomers and subsequent conversion to 1-GMN and 2-GMN (Husserl et al., 2010; Accashian et al., 1998; Blehert et al., 1997; White and Snape, 1993). Blehert et al. (1997) showed that NADPH-dependent GTN reductase enzymes isolated from *Pseudomonas putida* and *P. fluorescens* were capable of sequential reduction of GTN to GDNs. Cell extracts of *Bacillus thuringiensis/cereus* have

demonstrated complete denitration of GTN (Meng et al., 1995) when cell extracts were continuously added. Also, *Geotrichum candidum* fungi completely denitrated NG to 1-GMN and 2-GMN (Marshall and White, 2001; Ducrocq et al., 1989). The white rot fungus *Phanerochaete chrysosporium* has been reported to denitrate NG to 1,2-GDN and 2-GMN (Marshall and White, 2001; Servent et al., 1991; and 1992). Screening of several microorganisms and their enzymes revealed flavoproteins which are capable of denitrating NG. These nitrate ester reductases are closely related to NADPH dehydrogenase from *Saccharomyces carlsbergensis*, and are also found in a strain of *Enterobacter cloacae* (Williams et al., 2001).

Another bacterial genus, *Arthrobacter* sp. strain JBH1, was isolated from NG-contaminated soil which was found capable of degrading NG. The degradation process includes the conversion of NG to glycerol via 1,2-dinitrolycerin and 1-mononitrolycerin, with the simultaneous release of nitrite. Glycerol is then used as the source of carbon and energy (Williams et al., 2001).

The mold *Penicillium corylophilum* Dierckx, *Bacillus thuringiensis*, and *B. cereus* are the only known microbes capable of completely denitrating all NG esters to glycerol (Marshall and White, 2001; Meng, 1995). There are some concerns regarding use of *B. thuringiensis* in remediation, however, because these bacteria are both insect and mammalian pathogens (Yost, 2004).

Christodoulates et al. (1997) showed that mixed bacterial cultures in anaerobic microcosms mineralized NG more rapidly in the presence of a carbon source. NG mineralization was complete in 26 days with an addition of 2000 mg/L of glucose as compared with 114 days without a carbon source (Yost, 2004).

Marshall and White (2001) indicated that pure cultures of *Rhodococcus* spp. strains SP2-4 and SP3-4 achieved complete NG biodegradation. Both strains removed nitro groups from NG, dinitrates, and glycerol mononitrates, therefore achieving full and rapid biodegradation.

Blehart et al. (1996) reported the existence of at least six strains of bacteria isolated from soil capable of degrading NG. These included two strains of *Klebsiella oxytoca*, two strains of *Pseudomonas fluorescens*, and three strains of *P. putida*.

Recent research has revealed the existence of NG-degrading bacteria in environments such as activated sewage sludge, river water, and soils (White et al., 1995). These bacteria are capable of utilizing NG as a sole source of nitrogen (Binks et al., 1996; Blehart et al., 1997; Meng et al., 1995; White et al., 1996). Cometabolism has also been suggested as the mechanism for NG biotransformation (Accashian et al., 1998; Pesari and Grasso, 1993).

Mixed microbial cultures from aeration tank sludge are capable of metabolizing NG (Accashian et al., 1998; Wendt et al., 1978; Zhang et al., 1997). Aerobic microbial cultures have been shown to have the capacity to remove NG rapidly in the absence of a supplemental carbon source. Formation of the dinitrate and mononitrate isomers and complete denitration of NG was noted in aerobic batch cultures.

Phytoremediation

Phytoremediation is defined as the engineered use of green plants and their associated rhizospheric microorganisms for *in situ* treatment of contaminated soil and water (Vanek et al., 2006). Target plants include herbs, shrubs, vegetable crops,

agronomic crops, weeds and trees (Table 1.3). Phytoremediation is used to remove and/or decompose contaminants such as heavy metals, crude petroleum, refined petroleum products, and explosives (Table 1.4). Plants may remove contamination via several modes including phytoextraction, phytostabilization, phytodegradation, phytovolatilization, and rhizodegradation (US EPA, 1999) (Tables 1.5 and 1.6).

Table 1.3. Types of plants, contaminants, and media for phytoremediation (US EPA, 2001).

Type of Contaminant	Medium	Type of Plant												
		Alfalfa	alyssum	Bald cypress	Black locust	Cottonwood	Grasses	Hybrid poplars	Indian mustard	Pennycress	Red Mulberry	Stonewort	Sunflower	Water hyacinth
Organic	Soil			▲ PD RD			▲ RD	▲ PD RD			▲ RD	▲ PD		▲ PD RD
	Sediment			▲ PD RD			▲ RD	▲ PD RD			▲ RD	▲ PD		▲ PD RD
	Groundwater			▲ PD		▲ HC	▲ HC PD					▲ PD		▲ HC PD
Inorganic	Soil	▲ PV	▲ PE		▲ PV		▲ PS	▲ PS PV	▲ PE PS PV	▲ PE			▲ PE	
	Sediment	▲ PV	▲ PE		▲ PV		▲ PS	▲ PS PV	▲ PE PS PV	▲ PE			▲ PE	
	Groundwater					▲ HC	▲ HC	▲ RF					▲ RF	▲ RF

▲ Plant is effective for the type of contamination and medium shown.

HC: Hydraulic control PV: Phytovolatilization

PD: Phytodegradation RD: Rhizodegradation

PE: Phytoextraction RF: Rhizofiltration

PS: Phytostabilization

Table 1.4. Applications of phytoremediation (US EPA, 2000b).

Mechanism	Contaminant	Media	Plant	Staus
Degradation	Atrazine, nitrates	Surface Water	Poplar	Applied
Degradation	Landfill leachate	Groundwater	Poplar	Applied
Degradation	TCE	Groundwater	Poplar, cottonwood	Field demo
Degradation	TNT	Wetlands	Various	Field demo
Degradation	TPH	Soil	Grasses, crops	Field demo
Extraction- Concentration in shoot	Lead	Soil	Indian mustard	Field demo
Extraction- Concentration in root	Uranium	Surface water	Sunflower	Field demo
Extraction, Volatilization	Selenium	Soil, Surface Water	Various	Applied

Phytoextraction (i.e., phytoaccumulation) is a process in which contaminants are taken up from soil by plant roots and translocated into above-ground tissue (Tables 1.4 and 1.5). Phytostabilization is the process in which contaminants are restricted from moving in soil or groundwater by being absorbed by roots, adsorbed onto roots, or precipitated within the root zone. Phytodegradation is a process in which contaminants are decomposed through the metabolic reactions of plants. During phytovolatilization contaminants are taken up by plants, transformed to other products and then transported to leaves where they are transpired through the stomata to the atmosphere (US EPA, 1999).

Table 1.5. Overview of phytoremediation processes (US EPA, 2000b).

Mechanism	Process Goal	Media	Contaminants	Plants	Status
Phyto-extraction	Contaminant extraction and capture	Soil, sediment, sludges	Metals: Ag, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Zn; Radionuclides: ⁹⁰ Sr, ¹³⁷ Cs, ²³⁹ Pu, ²³⁸ U, ²³⁴ U	Indian mustard, pennycress, alyssum, sunflowers, hybrid poplars	Laboratory, pilot, and field applications
Rhizo-filtration	Contaminant extraction and capture	Groundwater, surface water	Metals, radionuclides	Sunflowers, Indian mustard, water	Laboratory and pilot-scale
Phyto-stabilization	Contaminant containment	Soil, sediment, sludges	As, Cd, Cr, Cu, Hs, Pb, Zn	Indian mustard, hybrid poplars, grasses	Field application
Rhizo-degradation	Contaminant destruction	Soil, sediment, sludges, groundwater,	Organic compounds (TPH, PAHs, pesticides chlorinated solvents, PCBs)	Red mulberry, grasses, hybrid poplar, cattail, rice	Field application
Phyto-degradation	Contaminant destruction	Soil, sediment, sludges, ground water , surface water	Organic compounds, chlorinated solvents, phenols, herbicides, munitions	Algae, stonewort, hybrid poplar, black willow, bald cypress	Field demonstration
Phyto-volatilization	Contaminant extraction from media and release to air	Groundwater, soil, sediment, sludges	Chlorinated solvents, some inorganics (Se, Hg, and As)	Poplars, alfalfa black locust, Indian mustard	Laboratory and field application
Hydraulic control	Contaminant degradation or containment	Groundwater, surface water	Water-soluble organics and inorganics	Hybrid poplar, cottonwood, willow	Field demonstration
Vegetative cover (evapotranspiration cover)	Contaminant containment, erosion control	Soil, sludge, sediments	Organic and inorganic compounds	Poplars, grasses	Field application
Riparian corridors (non-point source control)	Contaminant destruction	Surface water, groundwater	Water-soluble organics and inorganics	Poplars	Field application

Table 1.6. Phytoremediation mechanisms (Chappell, 1997).

Treatment Method	Mechanism	Media
Rhizofiltration	Uptake of metals in plant roots	surface water and water pumped through troughs
Phytotransformation	Plant uptake and degradation of organics	surface water, groundwater
Plant-Assisted bioremediation	Enhanced microbial degradation in the rhizosphere	soils, groundwater within the rhizosphere
Phytoextraction	Uptake and concentration of metals via direct uptake into plant tissue with subsequent removal of the plants	soils
Phytostabilization	Root exudates cause metals to precipitate and become less bioavailable	soils, groundwater, mine tailings
Phytovolatilization	Plant evapotranspires selenium, mercury, and volatile organics	soils, groundwater
Removal of organics from the air	Leaves take up volatile organics	air
Vegetative caps	Rainwater is evapotranspired by plants to prevent leaching contaminants from disposal sites	soils

Rhizodegradation (rhizosphere biodegradation) is a process in which soil contaminants are degraded during microbial activities carried out in the root zone (Tables 1.3, 1.4, and 1.6). Degradation reactions are enhanced by the presence of the rhizosphere. This zone, directly adjacent to the root, is rich in plant exudates including enzymes, surfactants, carbohydrates, sugars, acids, and other organic molecules (US EPA, 1999). Certain of these compounds are exuded in order to solubilize potential plant nutrients while others are released to the soil as a result of root cell death. In this chemically and biochemically active region, rhizosphere microorganisms may metabolize numerous hydrocarbon contaminants into less harmful products (US EPA, 1999).

Phytoremediation has been successful at both Brownfield and U.S. superfund sites (Table 1.7)

Table 1.7. Phytoremediation at US Superfund sites.

Site Name, State	Date Planted	Plant	Contaminant/Matrix
Carswell Site, TX	Spring 1996	Eastern cottonwood tree	TCE/groundwater at 4-12 feet
Aberdeen Proving Grounds, MD	Spring 1996	Hybrid poplar trees	TCE/groundwater
Edward Sears Site, NJ	Fall 1996	Hybrid poplar trees	TCE/groundwater at 8 feet
Iowa Army Ammunition Depot, IA	Spring 1997	Wetland and terrestrial plants	TNT/soil and pond water
Fort Wainwright, AK	Spring 1997	Felt leaf willow	Pesticides/soil and groundwater
Kaufman & Minter, NJ	Spring 1997	Hybrid poplar trees	PCE/groundwater
Calhoun Park, SC	Fall 1998	Local landscaping plants	PAH/groundwater at 1-4 feet
Solvent Recovery Systems of New England, CT	Spring 1998	Hybrid poplar trees	Mixed solvents/groundwater
Twin Cities Army Ammunition Plant, MN	Spring 1998	Corn, Indian mustard	Metals/soil
Bofors-Nobel, MI	Planting scheduled	Various trees and wetland plants	Residual sludge in waste lagoons
Del Monte, HI	Spring 1998	Koa haole	Pesticides/soil and groundwater
INEEL, ID	Spring 1999	Kochia, willow	Cesium, mercury in soil

Rhizosphere Degradation of Energetic Compounds

The rhizosphere is a biologically and chemically active zone immediately adjacent (a few mm) to the plant root. Recent research has demonstrated the presence of different microbial groups in the rhizosphere as compared with non-vegetated, “bulk” soil. Additionally, total numbers of microorganisms are significantly greater in the rhizosphere compared with bulk soil (Campbell and Greaves, 1990; Glick, 2003; Yang,

2010). Furthermore, the range of metabolic activities (e.g., N fixation, S oxidation) is greater in this zone (Yang, 2010). Microorganisms isolated from the rhizosphere of some plants have been shown to not only degrade energetics but also enhance plant growth (Campbell and Greaves, 1990; Glick, 2003; and Yang, 2010).

Yang (2010) studied the degradation of TNT and RDX in the rhizosphere of two native grass species. TNT was degraded in both rhizosphere and control soil; however, degradation of TNT metabolites was greater in the rhizosphere soils compared to the control. Results of this study showed that 60.41% of initial C¹⁴-RDX concentration in EG (eastern gammagrass, *Tripsacum dactyloides*) soil and 50.21% in SW (switchgrass, *Panicum virgatum* L), soil were degraded, as compared to 16.25% in the control. RDX was degraded more rapidly in the rhizosphere compared to TNT. Travis et al. (2007) studied TNT degradation in the rhizosphere of transgenic tobacco (*Nicotiana tabacum*) plants. The transgenic plants, which overproduced a bacterial nitroreductase gene, degraded TNT by increasing microbial community biomass and metabolic activity in the rhizosphere of the transgenic plants compared with wild-type plants.

Wetland vegetation has been found to be beneficial in energetics remediation. In a study performed by the US Army Corps of Engineers Waterways Experiment Station (WES), submergent and emergent wetland plant species were evaluated for their ability to remove explosives from groundwater. The submergent plants included American pondweed (*Potamogeton epihydrus*) and coontail (*Ceratophyllum demersum* L.); emergent plants were water plantain (*Alisma triviale*), arrowhead (*Sagittaria latifolia*), fox sedge (*Carex stipata*), wool-grass (*Scirpus cyperinus*), spikerush (*Eleocharis palustris*), reed canarygrass (*Phalaris arundinacea* L.), and narrowleaf cattail (*Typha angustifolia* L.).

The study plants removed TNT by 94-100%. RDX removal by the plants was not as efficient as for TNT; RDX removal did not exceed 15% (Kiker et al, 2001).

The ability of aquatic and wetland plants to remove TNT and RDX from groundwater was studied by Best et al. (1996). Ten plant species were grown with explosive-contaminated groundwater for ten days. All plants removed TNT from groundwater efficiently. The most effective plant species for TNT removal included elodea (*Elodea canadensis*), sago pondweed (*Stuckenia pectinata*), Eurasian watermilfoil (*Myriophyllum spicatum* L.), stonewort (*Nitellopsis obtuse*), curlyleaf pondweed (*Potamogeton crispus* L.), waterstargrass (*Heteranthera dubia*), and reed canarygrass (*Phalaris arundinacea*). RDX removal was not as efficient as was TNT removal. There were some indications of involvement of facultative and/or obligate anaerobic microbes or the existence of non-aerotolerant enzymes in RDX removal (Best et al. (1996).

Phytoremediation Mechanisms for Nitroglycerin

The results of Williams et al. (2001) showed that *Enterobacter cloacae* PB2 used nitrate ester energetics such as pentaerythritol tetranitrate (PETN) and NG as the sole source of nitrogen for growth. In their study, a transgenic plant was produced by introducing PETN reductase, the enzyme initiating explosive degradation in *Enterobacter cloacae* PB2, into plants. This transgenic plant then could be used to degrade PETN and NG in contaminated soil (Williams et al., 2001).

Advantages and Disadvantages of Phytoremediation

Several advantages are associated with phytoremediation technology.

Phytoremediation takes advantage of microbial processes, which could be powerful and economic tools for eliminating environmental hazards (Williams et al., 2001), since the costs for cleaning contaminated soil with conventional techniques such as incineration and landfilling are often substantial (millions of dollars for large sites) (Kuiper et al., 2004). Phytoremediation is usually an inexpensive method of remediation. The method is also environmentally-friendly (Vanek et al., 2006).

One of the drawbacks of using phytoremediation technology is the time needed to completely remediate contaminated soil or water. There are also practical limitations such as root penetration depth, weather factors, and viability of specific plants in specific areas. The main disadvantage of using plants in remediation is the fact that plants lack the catabolic versatility which makes microorganisms capable of mineralizing a wide variety of xenobiotic compounds (Vanek et al., 2006) (Tables 1.8-1.10; Fig. 1.8).

Table 1.8. Advantages and limitations of phytoremediation (Chappell, 1997).

Advantages of Phytoremediation	Limitations of Phytoremediation
in situ	Limited to shallow soils, streams, and groundwater
Passive	High concentrations of hazardous materials can be toxic to plants
Solar-driven	Mass transfer limitations associated with other biotreatments
Costs 10% to 20% of mechanical treatments	Slower than mechanical treatments
Transfer is faster than natural attenuation	Only effective for moderately hydrophobic contaminants
High public acceptance	Toxicity and bioavailability of degradation products is not known
Fewer air and water emissions	Contaminants may be mobilized into the groundwater
Generate less secondary wastes	Potential for contaminants to enter food chain through animal consumption
Soils remain in place and are usable following treatment	Unfamiliar to many regulators

Table 1.9. Advantages and disadvantages of different phytoremediation systems (Pichtel, 2007).

Type of Phytoremediation	Advantages	Disadvantages
Phytoextraction by trees	High biomass production	Potential for off-site migration and leaf transportation of metals of surface Metals are concentrates in plant biomass and must eventually be disposed
Phytoextraction by grasses	High accumulation growth rate	Low biomass production; slow process Metals are concentrates in plant biomass and must eventually be disposed
Phytoextraction by crops	High biomass and increased growth rate	Potential threat to food chain through ingestion by herbivores Metals are concentrates in plant biomass and must eventually be disposed
Phytostabilization	No disposal of contaminated biomass required	Remaining liability issues, including maintenance for indefinite period of time
Phytodegradation in the rhizosphere	No disposal of contaminated soil	Limited to hydrocarbons only
Phytovolatilization	Limited application (As, Hg)	Possibility of releasing toxins to the atmosphere. Permit may be required

Table 1.10. Estimated cost savings through the use of phytoremediation rather than conventional treatment (USEPA, 2001).

Contaminant and Matrix	Phytoremediation		Conventional Treatment		Projected Savings
	Application	Estimated Cost	Application	Estimated Cost	
Lead in soil (1 acre)	Extraction, harvest, and disposal	\$150,000-\$250,000	Excavate and landfill	\$500,000	50-65 percent
Solvents in groundwater (2.5 acre)	Degradation and hydraulic control	\$200,000 for installation and initial maintenance	Pump and treat	\$700,000 annual operating cost	50 percent cost saving by third year
Total petroleum hydrocarbons in soil (1 acre)	In-situ degradation	\$50,000-\$100,000	Excavate and landfill or incinerate	\$500,000	80 percent

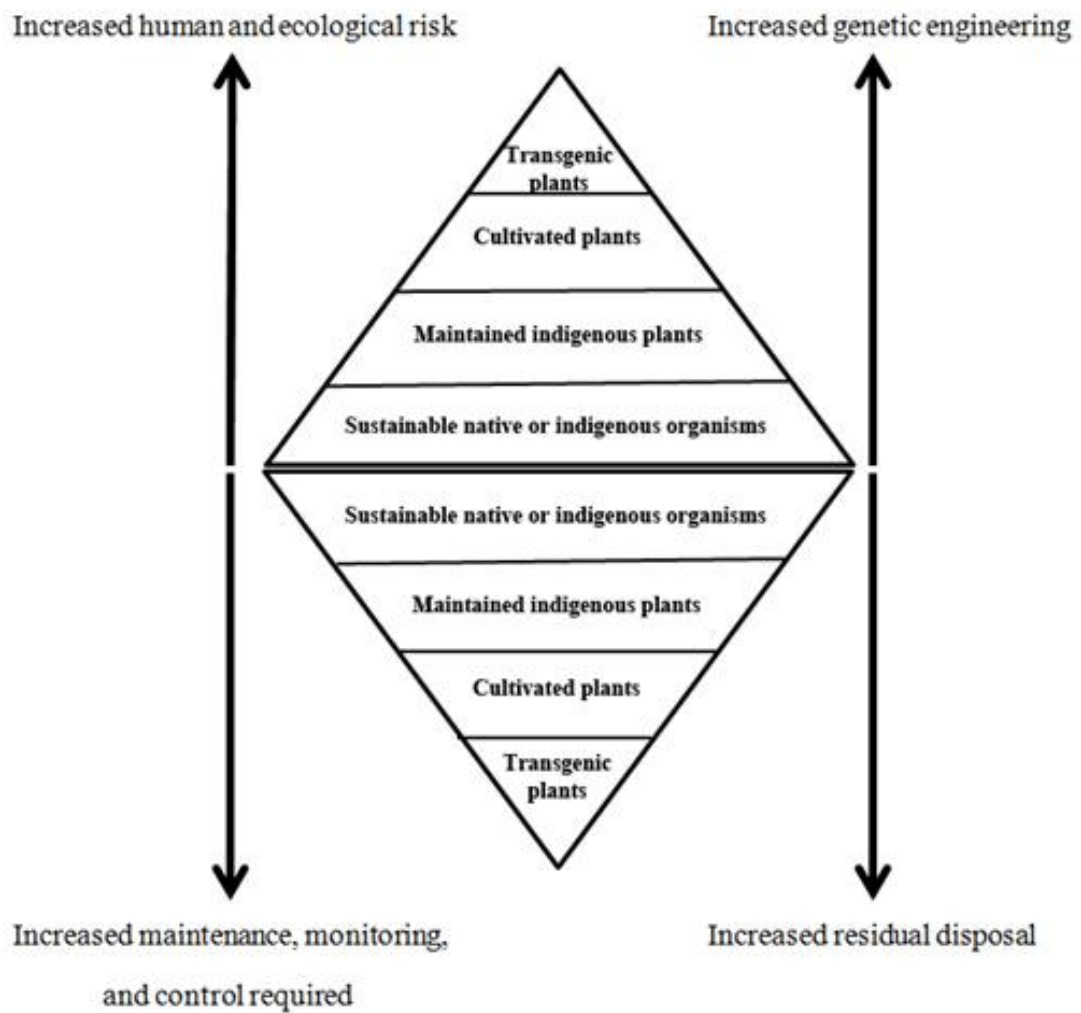


Fig. 1.8. Relationship between plant types and the advantages and disadvantages of use for phytoremediation (McCutcheon , 2003).

References

- Accashian J. V., R.T. Vinopal, B. J. Kim, and B. F. Smets. 1998. Aerobic growth on nitroglycerin as the sole carbon, nitrogen, and energy source by a mixed bacterial culture. *Applied and Environmental Microbiology*. 64: 3300-3304.
- Ahlner, J., R.G. Andersson, K. Torfgard, and K.L. Axelsson. 1991. Organic nitrate esters: Clinical use and mechanisms of actions. *Pharmacological Reviews*. 43: 351–423.
- Ahmad, F., and J. B. Hughes. 2002. Reactivity of partially reduced arylhydroxylamine and nitrosoarene metabolites of 2,4,6-trinitrotoluene (TNT) toward biomass and humic acids. *Environmental Science and Technology* 36: 4370-4381.
- Brannon, J.M and T.E. Myers. 1997. Review of fate and transport process of explosives. Technical Report. IRRP-97-2, U.S Army Engineer Waterways Experiment Station, Vicksburg MS, USA.
- Best, E.P., M.E. Zappi, H.L. Fredrickson, S. L. Sprecher, S. Larson, and J. L. Miller. 1996. Screening for removal of TNT and RDX by submersed and emergent plant species from contaminated ground water. Book of Abstracts, HSRC/WERC Joint Conference on the Environment. Albuquerque, NM.
- Bhaumik, S., C. Christodoulatos, B. W. Brodman, and N. Pal. 1998. Biodegradation of glycerol trinitrate by activated sludge: Cosubstrate requirements, inhibition, and kinetics. *Journal of Environmental Science and Health*. 4: 574-571.
- Binks, P. R., C. E. French, S. Nicklin, and N. C. Bruce. 1996. Degradation of pentaerythritol tetranitrate by *Enterobacter cloacae* PB2. *Applied and Environmental Microbiology*. 62: 1214–1219.

- Blehert, D. S., K. L. Knoke, B. G. Fox, and G. H. Chambliss. 1997. Regioselectivity of nitroglycerin denitration by flavoprotein nitroester reductases purified from two *Pseudomonas* species. *Journal of Bacteriology*. 179: 6912–6920.
- Blehert, D.S., K. Becker, and G.H. Chambliss. 1996. Isolation and characterization of bacteria that degrade nitroglycerin. Proceedings of the Tri-Service Environmental Technology Workshop. Enhancing Readiness through Environmental Quality Technology. ADP017715. Defense Technical Information Center, Hershey, PA. p. 197-204.
- Brochu, S., E. Diaz, and S. Thiboutot. 2008. Assessment of 100 years of military training in Canada: the case of Canadian force base petawawa. TR-2008-118. Defense Research Development Canada (DRDC-Valcartier), Quebec, Canada.
- Bordeleau, G., R. Martel, G. Ampleman, and S. Thiboutot. 2008. Environmental impacts of training activities at an air weapons range. *Journal of Environmental Quality*. 37:308-317.
- Brannon, J. M., and J.C. Pennington. 2002. Environmental fate and transport process descriptors for explosives,” ERDC/EL TR-02-10, U.S Army Engineer Research and Development Center. Vicksburg, MS.
- Bhushan, B., A. Halasz, S. Thiboutot, G. Ampleman, and J. Hawari. 2004. Chemotaxis mediated biodegradation of cyclic nitramine explosives RDX, HMX, and CL-20 by *Clostridium* sp. EDB2. *Biochemical and Biophysical Research Communications*. 316:816-821.

- Campbell, R., and M. Greaves. 1990. Anatomy and community structure of the rhizosphere. J. Lynch (Ed.), *The Rhizosphere*. John Wiley and Sons, Chichester, UK.
- Caplan, J. A. 1993. The worldwide bioremediation industry: Prospects for profit. *Tibtech*. 11: 320-323.
- Chappell, J. 1997. Phytoremediation of TCE using *Populus*. Status Report prepared for the US EPA Technology Innovation Office under a National Network of Environmental Management Studies Fellowship. Washington, DC.
- Dixon, B. 1996. Bioremediation is here to stay. *ASM News* 62: 527-528.
- Dua, M., N. Sethunathan, and A. K. Johri. 2002. Biotechnology and bioremediation: Successes and limitations. *Applied Microbiology and Biotechnology*. 59: 143-152.
- Ducrocq, C., C. Servy, and M. Lenfant. 1989. Bioconversion of glyceryl trinitrate into mononitrates by *Geotrichum candidum*. *FEMS Microbiology Letters*. 65:219–222.
- Ellis, H.V., C.D. Hong, C.C. Lee, J.C. Dacre, and J.P. Glenn. 1984. Subacute and chronic toxicity studies of trinitroglycerin in dogs, rats and mice. *Fundamental and Applied Toxicology* . 4: 248.
- Ellis, H.V., J.H. Hagenson, J.R. Hodgson, J.L. Minor, C.B. Hong, E.R. Ellis, J.D. Girvin, D.O. Helton, B.L. Herndon, and C.C. Lee. 1978. Mammalian Toxicity of Munitions Compounds. Phase III: Effects of Life-Time Exposure. Part II: Trinitroglycerin. Progress Report No. 8, AD A078746. Midwest Research Institute, Kansas City, MO.

- Explosives. Updated at 2011. Globalsecurity.org. [Online]. Available at:
<http://www.globalsecurity.org>, Accessed 18 May 2012.
- Fournier, D., A. Halasz, S. Thiboutot, G. Ampleman, D. Manno, and J. Hawari. 2004. “Biodegradation of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) by *Phanerochaete chrysosporium*: New insight into the degradation pathway,” *Environmental Science and Technology*. 38: 4130-4133.
- Glick, B.R. 2003. Phytoremediation: Synergistic use of plants and bacteria to clean up the environment. *Biotechnology Advances*. 21: 383-393.
- Halasz, A., S. Thiboutot, G. Ampleman, and J. Hawari. 2010. Microwave-assisted hydrolysis of nitroglycerine (NG) under mild alkaline conditions: New insight into the degradation pathway. *Chemosphere*. 79: 228-232.
- Hewitt, A.D. 2002. Analysis of nitroglycerine in soils and on mortar fins using GC-TID. US Army Corps of Engineers, Washington, DC.
- Hempfling, C. 1997. Ultraviolet/oxidation treatment of explosive wastewaters using a commercial process. *Environmental Progress*. 3:164-170.
- Heramb, R. M., and McCord, B. R. 2002. The Manufacture of Smokeless Powders and their Forensic Analysis: A Brief Review. *Forensic Science Communication*. 4(2): 7 pages.
- Hathaway, G. J., N. H. Proctor, J. P. Hughes, and M. L. Fischma. 1991. Proctor and Hughes' *Chemical Hazards of the Workplace* (3rd Edition). Van Nostrand Reinhold, New York, NY.
- Heitzer, A., and G. S. Sayler. 1993. Monitoring the efficacy of bioremediation. *Trends in Biotechnology*. 11: 334-343.

- Husserl, J., J.C. Spain, and J.B. Hughes. 2010. Growth of *Arthrobacter* sp. Strain JBH1 on nitroglycerin as the sole source of carbon and nitrogen. *Applied and Environmental Microbiology*. 76(5): 1689-1691.
- Imoto, S., M. Nakao, M. Kuramoto, M. Takeuchi, K. Shimpo, and T. Tanabe. 1986. Teratological test of 10% nitroglycerin (NT-1 ointment) in rabbits. *The Journal of Toxicological Sciences*. 2: 59-70.
- Jenkins, T. F., T.A. Ranney, A.D. Hewitt, M.E. Walsh, and K.L. Bjella. 2008a. Representative sampling for energetic compounds at an anti-tank firing range. ERDC/CRREL TR-04-7, US 28. Applied and Environmental Soil Science Army Engineer Research and Development Center. Hanover, NH.
- Jenkins, T. F., G.Ampleman, S.Thiboutot, S. R. Bigl, S.Taylor, M. R. Walsh, D. Faucher, R. Martel, I .Poulin, K. M Dontsova, M. E. Walsh, S. Brochu, A. D. Hewitt, G. Comeau, E. DiazM. A. , Chappell, J. L. Fadden, A. Marois, R. Fifield, B. Quémerais, J. Šimunek, N. M. Perron, A. Gagnon, T. Gamache, J. C. Pennington, V. Moors, D. J. Lambert, D. Gilbert, R. N. Bailey, V. Tanguay, C. A. Ramsey, L. Melanson, and M.C. Lapointe. 2008b. Characterization and fate of gun and rocket propellant residues on testing and training ranges: Final Report,” TR 08-01, Strategic Environmental Research and Development Program, Cold Regions Research and Engineering Laboratory, US Army Engineer Research and Development Center. Hanover, NH.
- Jenkins, T. F., J. C.Pennington, G.Ampleman, S.Thiboutot, M. R. Walsh, E. Diaz, K. M. Dontsova, A. D. Hewitt, M. E. Walsh, S. R. Bigl, S.Taylor, D. K. MacMillan, J. L. Clausen, D. Lambert, N. M. Perron, M. C. Lapointe, S. Brochu, M. Brassard,

- R. Stowe, R. Farinaccio, A. Gagnon, A. Marois, D. Gilbert, D. Faucher, S. Yost, C. Hayes, C. A. Ramsey, R. J. Rachow, J. E. Zufelt, C. M. Collins, A. B. Gelvin, and S. P. Saari. 2007. Characterization and fate of gun and rocket propellant residues on testing and training ranges: Interim Report 1," ERDC TR-07-01, Strategic Environmental Research and Development Program. Vicksburg, MS.
- Jenkins, T. F., A. D. Hewitt, C. L. Grant et al. 2006. Identity and distribution of residues of energetic compounds at army livefire training ranges. *Chemosphere*. 63 (8): 1280–1290.
- Jenkins, T.F., M.E. Walsh, P.H. Miyares, A.D. Hewitt, N.H. Collins, and T.A. Ranney. 2002. Use of snow covered ranges to estimate explosives residues from high-order detonations of army munitions. *Thermochimica Acta*. 384: 173-185.
- Jenkins, T.F., J.C. Pennington, T.A. Ranney, T.E. Berry, P.H. Miyares, M.E. Walsh, A.D. Hewitt, N.M. Perron, L.V. Parker, C.A. Hayes, and E.G. Wahlgren. 2001. Characterization of explosives contamination at military firing ranges. Technical Report ERDC TR-01-05. US Army Engineer Research and Development Center, Vicksburg, MS.
- Juhasz, A. L., and Naidu, R. 2007. Explosives: fate, dynamics, and ecological impact in terrestrial and marine environments. *Reviews of Environmental Contamination and Toxicology*. 191: 163–215.
- Juhasz, A. L., and Naidu, R. 2008. Explosives: Fate, dynamics, and ecological impact in terrestrial and marine environments. *Reviews in Environmental Contamination and Toxicology*. 191:163-215.

- Kalderis, D., A.L. Juhasz, R. Boopathy, and S. Comfort. 2011. Soils contaminated with explosives: Environmental fate and evaluation of state-of-the-art remediation processes (IUPAC Technical Report). *Pure Applied Chemistry*. 83 (7):1407-1484.
- Kanerva, L., R. Laine, R. Jolanki, K. Tarvainen, T. Estlander, and I. Helander. 1991. Occupational allergic contact dermatitis caused by nitroglycerin. *Contact Dermatitis*. 24(5): 356-62.
- Kiker, J., S. Larson, D.D. Moses, and R. Sellers. 2001. Use of engineered wetlands to phytoremediate explosives contaminated surface water at the Iowa Army Ammunition Plant, Middletown, Iowa. *International Containment and Remediation Technology Conference And Exhibition, Orlando, FL.*[Online]. Available at: <http://www.containment.fsu.edu/cd/content/pdf/416.pdf>, Accessed 09 September 2011.
- King, S.Y. and H. L. Fung. 1984. Rapid microbial degradation of organic nitrates in rat excreta. Re-examination of the urinary and fecal metabolite profiles of pentaerythritol tetranitrate in the rat. *Drug Metabolism and Disposition*. 12: 353-357.
- Kuiper, I., E. L. Legendijk, G. V. Bloemberg, and B. J. Lugtenberg. 2004. Rhizoremediation: A beneficial plant-microbe interaction. *Molecular Plant Microbe Interaction*. 17: 6-15.
- Lachance, B., P. Y. Robideux, J. Hawari, G. Ampleman, S. Thiboutot, and G. I. Sunahara. 1999. Cytotoxic and genotoxic effects of energetic compounds on bacterial and mammalian cells in vitro. *Mutation Research*. 444: 25-39.
- Lee, C.C., H.V. Ellis, J.J. Kowalski, J.R. Hodgson, S.W. Hwang, R.D. Short,

- J.C. Bhandari, J.L. Sanyer, T.W. Redding, J.L. Minor, and D.O. Helton. 1976. Mammalian toxicity of munitions compounds. Phase II: Effects of multiple doses. Part I. Report No. DAMD-17-74-C-4073, AD A047067. Midwest Research Institute, Kansas City, MO.
- Leggett, D.C., T. F. Jenkins, and R.P. Murrmann. 1977. Composition of Vapors Evolved From Military TNT as Influenced by Temperature, Solid Composition, Age and Source. Special Report 77-16. Cold Regions Research and Engineering Laboratory, Hanover, NH.
- Lange, K., A. Koenig, C. Roegler, A. Seeling, J. Lehmann. 2009. NO donors. Part 18: Bioactive metabolites of GTN and PETN—Synthesis and vasorelaxant properties . *Bioorganic & Medicinal Chemistry Letters*. 19(11): 3141–3144.
- Marshall, S. J., and G. F. White. 2001. Complete denitration of nitroglycerin by bacteria isolated from a washwater soakaway. *Applied and Environmental Microbiology*. 67: 2622-2626.
- McCutcheon, S.C., and J.L. Schnoor. 2003. Phytoremediation: Transformation and control of contaminants. Hoboken, New Jersey. Wiley-Interscience, Inc.
- Meng, M., W.-Q. Sun, L. A. Geelhaar, G. Kumar, A. R. Patel, G. F. Payne, M. K. Speedie, and J. R. Stacy. 1995. Denitration of glycerol trinitrate by resting cells and cell extracts of *Bacillus thuringiensis/cereus* and *Enterobacter agglomerans*. *Applied and Environmental Microbiology*. 61: 2548–2553.
- Oh, S-Y, D.K. Cha, B.J. Kim, and P. Chiu. 2004. Reduction of nitroglycerin with elemental iron: pathway, kinetics, and mechanisms. Department of Civil and

- Environmental Engineering, University of Delaware, Newark, Delaware 19716, and U.S. Army Engineering Research and Development Center Champaign, IL.
- Pantex. [Online]. Available at: <http://www.pantex.com>, Accessed 18 May 2012.
- Podlipna, R., Z. Fialova, and T. Vanek. 2008. Toxic effect of nitroesters on plant tissue cultures. *Plant Cell, Tissue and Organ Culture*. 94(3): 305-311.
- Pennington, J. C., T. F. Jenkins, G. Ampleman, S. Thiboutot, J. M. Brannon, A. D. Hewitt, J. Lewis, S. Brochu, E. Diaz, M. R. Walsh, M. E. Walsh, S. Taylor, J. C. Lynch, J. Clausen, T. A. Ranney, C. A. Ramsey, C. A. Hayes, C. L. Grant, C. M. Collins, C. R. Bigl, S. Yost, and K. Dontsova. 2006a. Distribution and fate of energetics on DOD test and training ranges: Final Report. ERDC TR-06-13, US Army Corps of Engineers Engineer Research and Development Center. Vicksburg, MS.
- Pennington, J. C., T. F. Jenkins, G. Ampleman, S. Thiboutot, A. D. Hewitt, S. Brochu, J. Robb, E. Diaz, J. Lewis, H. Colby, R. Martel, K. Poe, K. Groff, K. L. Bjella, C. A. Ramsey, C. A. Hayes, S. Yost, A. Marois, A. Gagnon, B. Silverblatt, T. Crutcher, K. Harriz, S., Bigl, S. R. Heisen, Jr. T. E Berry, J. Muzzin, D. J. Lambert, M. J. Bishop, B. Rice, M. Wojtas, M. E. Walsh, M. R. Walsh, and S. Taylor. 2006b. Distribution and fate of energetics on DOD test and training ranges: Interim Report 6," Strategic Environmental Research and Development Program, TR 06-12. US Army Corps of Engineers Engineer Research and Development Center. Vicksburg, MS.
- Pennington, J.C., T.F. Jenkins, G. Ampleman, S. Thiboulet, J.M. Brannon, J. Lewis, J. DeLaney, J. Clausen, A.D. Hewitt, M.A. Hollander, C.A. Hayes, J.A. Stark, A.

- Marois, S. Brochu, H.Q. Dinh, D. Lambert, A. Gagnon, M. Borchard, R.B. Martel, P. Patrick, M. Nancy R. LeFebure, W. Davis, T.A. Ranney, C. Ganther, S. Taylor, and J.M. Ballard. 2003. Distribution and fate of explosives on DoD test and training ranges: Interim report 3. Technical Report ERDC TR-03-2. US Army Engineer Research and Development Center. Vicksburg, MS.
- Pennington, J. C., J. M. Brannon, and J. E. Mirecki. 2002. Distribution and fate of energetics on DoD test and training ranges: Interim report 2. Technical Report ERDC TR-02-8. US Army Engineer Research and Development Center, Vicksburg, MS.
- Pennington, J.C., T.F. Jenkins, J.M. Brannon, J. Lynch, T.A. Ranney, T.E. Berry, C.A. Hayes, P.H. Miyares, M.E. Walsh, A.D. Hewitt, N. Perron, and J.J. Delfino. 2001. Distribution and fate of energetics on DoD test and training ranges: Interim report 1. Technical Report ERDC TR-01-13. US Army Engineer Research and Development Center, Vicksburg, MS.
- Pesari, H., and D. Grasso. 1993. Biodegradation of an inhibitory nongrowth substrate (nitroglycerin) in batch reactors. *Biotechnology and Bioengineering*. 41: 79–87.
- Pichtel, J. 2007. *Fundamentals of Site Remediation for Metal- and Hydrocarbon-Contaminated Soils, Second Edition*. Government Institutes, Inc., Rockville, MD.
- Pichtel, J. 2012. Distribution and fate of military explosives and propellants in soil: A review. *Journal of Applied and Environmental Soil Science*. Volume 2012.
- Pugh, D. L. 1982. Milan army ammunition plant contamination survey. USATHAMA Report DRXTH-FR-8213, US Army Toxic and Hazardous Materials Agency,

Aberdeen, MD.

- Ro, K. S., A. Venugopal, D. D. Adrian, D. Constant, K. Qaisi, K. T. Valsaraj, L. J. Thibodeaux, and D. Roy. 1996. Solubility of 2,4,6-trinitrotoluene (TNT) in water. *Journal of Chemical and Engineering Data*. 41: 758-761.
- Rom, W. N. 1992. *Environmental and Occupational Medicine* (2nd edition). Brown and Company, Boston, MA.
- Sunahara, G. I. 2009. *Ecotoxicology of Explosives*. CRC Press, Boca Raton, FL.
- Servent, D., C. Ducrocq, Y. Henry, C. Servy, and M. Lenfant. 1992. Multiple enzymatic pathways involved in the metabolism of glycerol trinitrate by *Phanerochaete chrysosporium*. *Biotechnology and Applied Biochemistry*. 15: 257–266.
- Servent, D., C. Ducrocq, Y. Henry, A. Guissani, and M. Lenfant. 1991. Nitroglycerin metabolism by *Phanerochaete chrysosporium*: Evidence for nitric oxide and nitrite formation. *Biochimica et Biophysica Acta* . 1074: 320–325.
- Sittig, M. 1991. *Handbook of Toxic and Hazardous Chemicals*, Third Edition. Noyes Publications, Park Ridge, NJ.
- Smets, B. F., R. T. Vinopal, D. Grasso, K. A. Strevett, and B.-J. Kim. 1995. Nitroglycerin biodegradation: Theoretical thermodynamic considerations. *Journal of Energetic Materials*. 13: 385–398.
- Thiboutot, S., G. Ampleman, A. Marois, A. Gagnon, M. Bouchard, A. D. Hewitt, T. F. Jenkins, M. Walsh, K. Bjella, C. Ramsey, and T. A. Ranney. 2004. *Environmental Conditions of Surface Soils, CFB Gagetown Training Area: Delineation of the Presence of Munitions-Related Residues (Phase III, Final Report)*,” DREV-TR-2004-205, Defence Research and Development Canada-

Valcartier, Quebec, Canada, <http://cradpdf.drdc.gc.ca/PDFS/unc57/p522641.pdf>,
2004.

Townsend, D.M and T.E. Myers. 1996. Recent development in formulating model
descriptors for subsurface transformation and sorption of TNT, RDX, and HMX.
Technical Report. IRRP-96-1. U.S. Army Engineer Waterways Experiment
Station, Vicksburg, MS, USA.

Travis, E.R., N.K. Hannik, C.J. Van der gast, I.P. Thompson, S.J. Rosser, and N.C.
Bruce. 2007. Impact of transgenic tobacco on trinitrotoluene (TNT) contaminated
soil community. *Environmental Science and Technology*. 41: 5854-5861.

US Department of Labor. Occupational Safety and Health Guideline for Nitroglycerin.
[Online]. Available at:
<http://www.osha.gov/SLTC/healthguidelines/nitroglycerin/recognition.html>,
Accessed 03 April 2012.

US Environmental Protection Agency. 2001. Brownfields Technology Primer: Selecting
and Using Phytoremediation for Site Cleanup. EPA 542-R-01-006. Office of
Solid Waste and Energy Response. Washington, DC.

US Environmental Protection Agency. 2000a. Introduction to Phytoremediation.
EPA/600/R-99/107. National Risk Management Research Laboratory. Office of
Research and Development. Cincinnati, OH.

US Environmental Protection Agency. 2000b. Solidification/Stabilization Use at
Superfund Sites. Office of Solid Waste and Emergency Response. EPA-542-R-
00-010. Cincinnati, OH.

- US Environmental Protection Agency. 1999. Phytoremediation Resources Guide. EPA 512-b-99-003. Office of Solid Waste and Energy Response. Washington, DC.
- U.S. Environmental Protection Agency. 1995. In Situ Remediation Technology: Electrokinetics EPA542-K-94-007. Office of Solid Waste and Emergency Response. Technology Innovation Office. Washington, DC.
- U.S. Environmental Protection Agency. 1994. Engineering Bulletin: In Situ Vitrification Treatment. EPA/540/S-94/504. Office of Emergency and Remedial Response. Washington, DC.
- US Environmental Protection Agency. 1993a. Guide for Conducting Treatability Studies Under CERCLA: Biodegradation Remedy Selection. EPA/540/R-93-519b. Office of Emergency and Remedial Response.
- U.S. Environmental Protection Agency. 1993b. BiogenesisTM Soil Washing Technology. Innovative Technology Evaluation Report. EPA/540/R-93/510. Office of Research and Development. Cincinnati, OH.
- Van Aken, B., J.M. Yoon, C.L. Just, and J.L. Schnoor. 2004. Metabolism and mineralization of hexahydro-1,3,5-trinitro-1,3,5-triazine inside poplar tissues (*Populus deltoids* × *nigra* DN-34). Environmental Science and Technology. 38:4572-4579.
- Vanek, T., A. Nepovim, R. Podlipna, A. Hebner, Z. Vavrikova, A. Gerth, H. Thomas, and S. Smreck. 2006. Phytoremediation of explosives in toxic wastes. Soil and Water Pollution Monitoring, Protection and Remediation. 3: 23.
- Walsh, M. R., M.E. Walsh, A.D. Hewitt, and C.M. Collins. 2008. Field-expedient disposal of excess artillery propellants. SERDP & ESTCP's Partners in

Environmental Technology Technical Symposium & Workshop. Washington, DC.

Walsh, M. E., C. M. Collins, R. N. Bailey, and C. L. Grant. 1997. Composite sampling of sediments contaminated with white phosphorus. Special Report 97-30. US Army Cold Regions Research and Engineering Laboratory, Hanover, NH, USA.

Walsh, M. E., T.F. Jenkins, P.S. Schnitker, J.W. Elwell, and M.H. Stutz. 1993. Evaluation of SW846 Method 8330 for Characterization of Sites Contaminated with Residues of High Explosives. CRREL Report 93-5, US Cold Regions Research and Engineering Laboratory, Hanover, NH.

Wang, C. J., S. Thiele, and J. M. Bollag. 2002. Interaction of 2,4,6-trinitrotoluene (TNT) and 4-amino-2,6-dinitrotoluene with humic monomers in the presence of oxidative enzymes. *Archives of Environmental Contamination and Toxicology* 42: 1-8.

Wendt, T. M., J. H. Cornell, and A. M. Kaplan. 1978. Microbial degradation of glycerol nitrates. *Applied and Environmental Microbiology*. 36: 693–699.

White, G. F., and J. R. Snape. 1993. Microbial cleavage of nitrate esters: defusing the environment. *Journal of General Microbiology*. 139: 1947–1957.

White, G. F., J.R. Snape, and S. Nicklin, S. 1996. Bacterial biodegradation of glycerol trinitrate. *International Biodeterioration and Biodegradation*. 38:77-82.

White, G. F., J. R. Snape, and S. Nicklin. 1995. Bacterial degradation of nitrate ester explosives. In *Proceedings of the 26th International Annual Conference of ICT, Pyrotechnics: basic principles, technology, application*. DWS Werbeagentur und Verlag GmbH, Karlsruhe, Federal Republic of Germany.

- Williams, R.E., D. A. Rathbone, P. C. Moody, N. S. Scrutton, and N. C. Bruce. 2001. Degradation of explosives by nitrate ester reductases. Biochemical Society symposium. 68: 143-153.
- Yang, H. 2010. Enhanced rhizodegradation of munitions explosives and degradates by selected native grass species. MS Thesis. University of Missouri, Columbia, MO.
- Yinon, J. 1999. Forensic and Environmental Detection of Explosives. John Wiley and Sons, New York, NY.
- Yinon, J. 1990. Toxicity and Metabolism of Explosives. CRC Press, Boca Raton, FL.
- Yost, S. 2004. Effects of redox potential and pH on the fate of nitroglycerin in a surface and aquifer soil. MS Thesis. Louisiana State University, Baton Rouge, LA.
- Zhang, Y. Z., S. T. Sundaram, A. Sharma, and B. W. Brodman. 1997. Biodegradation of glyceryl trinitrate by *Penicillium corylophilum* Dierckx. Applied and Environmental Microbiology. 63:1712–1714.

MATERIALS AND METHODS

Characterization of Soil and Composted Biosolids

Glynwood soil (Fine, illitic, mesic Aquic Hapludalf) was collected from the top 30 cm from an agricultural field in Albany, IN (N 40 18.436 , W 85 13.929). The soil material was composited, air-dried, gently ground with an agate mortar and pestle, and sieved through a 2.0-mm mesh sieve. The soil was mixed with a commercial potting soil (Infinity Fertilizers INC, Milan, IL) in a 3:1 ratio using a small steel shovel.

Composted sewage sludge (biosolids) (CB) was used as a soil treatment. The biosolids were obtained from the Southwesterly Compost Facility, Columbus, OH. Biosolids were thoroughly mixed into the soil at a 20% (w/w) rate.

Total organic carbon (TOC) content of both the soil and biosolids was determined by loss on ignition at 360°C (ASTM, 1988). Solids pH was measured using a standardized AB15 Accumet pH meter on a 1:2 solids:deionized H₂O slurry. The percentage of carbon (C), hydrogen (H) and nitrogen (N) was analyzed on a Perkin Elmer

Series II CHNS/O Analyzer 2400 (Shelton, CT). Acetanilide, Lot #MKAA0338, Date 04/07/2010, was the standard used. Nitrate was measured using Szechrome reagents (Polysciences, no date) in a BioteK PowerWave XS2 microassay system (Winooski, VT). Soil ammonium concentrations were determined by the method of Sims et al. (1995) which uses a modified indophenol blue technique. The method was adapted for the BioteK PowerWave system. Potassium concentrations were determined after extraction by 1.0 N ammonium acetate, pH 7.0, followed by analysis using a Perkin Elmer AAnalyst 2000 flame atomic absorption spectrometer (FAAS) set in emission mode (Knudsen, 1982). Phosphorous was measured using Bray-1 extractant combined with a microplate method (PowerWave XS2 Microplate Spectrophotometer) (Olsen, 1982). Metal (Cd, Cr, Cu, Fe, Ni, Pb, and Zn) concentrations were analyzed using DTPA extraction followed by FAAS. Samples were extracted with diethylene triamine pentaacetic acid (DTPA) solution (0.05 M) for 2 h on an oscillating shaker. The mixtures were filtered through Whatman no. 2 filter paper and analyzed using FAAS (Sposito, 1982).

All glassware was washed with Alconox™ detergent and rinsed with deionized water.

Germination Study

Seeds of oats (*Avena sativa*) (two varieties), wheat (*Triticum aestivum*), ryegrass (*Phleum pratense*), maize (*Zea mays* L.) (two varieties), Indian mustard (*Brassica*

juncea), sunflower (*Helianthus annuus*), white clover (*Trifolium repens*), redclover (*Trifolium pratense*), green bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) were obtained commercially and assessed for their relative ability to germinate in the presence of NG in smokeless powder.

A sheet of Kimwipe™ was placed on the bottom of a Petri dish and the SP was added in a mixture with 25 g washed sand. Sterilized tweezers were used to add five seeds to each Petri dish. Tap water (approx. 10 ml) was added to each dish. Petri dishes were incubated for 7 days in the dark. In order to maintain a moist state, tap water was added as needed. On Day 7 the seeds were checked for germination. Total numbers of germinated seeds were recorded. The Petri dishes were incubated for additional 7 days. On Day 14, the total germinated seeds were recorded.

Growth of initial oat shoot; first, second, and third root were analyzed using a WinRhizo scanner equipped with ImageJ™ software (Burton et al., 2012).

Nitroglycerin Incubation Study

Plastic pots measuring 12.5 cm tall by 20 cm diameter were filled with 1 kg soil. Half the pots contained soil only and the remainder contained soil plus CB in an 80:20 mixture. The pots were amended with 0, 1, 5 and 10% (w/w) double base smokeless powder (Alliant, Radford, VA). Smokeless powder was applied to the top 5-10 cm of the soil and mixed manually using a stainless steel spatula.

Seeds of oat (*Avena sativa*) were obtained from Seedville (Massillon, OH). A total of 10 seeds were planted per pot. After germination, five plants were removed. Live fox sedge plants (*Carex vulpinoidea*) were purchased from Spence Restoration Nursery (Muncie, IN). The sedges were planted into pots lined with plastic to maintain a saturated condition. No supplemental nutrients were added to the pots. Both aerobic and anaerobic controls were prepared. There were four replicates for each treatment for a total of 96 pots. Plants were cultivated in the Ball State University greenhouses (Muncie, IN). Light settings of 12 h light and 12 h dark were used. Plants were watered with tap water as needed to maintain pots in a moist state. The plants were watered 30-45 min before collecting soil and leachate samples in order to prevent evaporative losses of leachate.

Initial growth (up to 7 days) of the oat plants was poor for 5% and 10% SP rates; therefore, an additional 3-5 seeds were sown into each pot.

Soil and Leachate Analyses

Soil and leachate samples were collected after 7, 14, 30, and 60 days of incubation. Soil samples were collected from the root zone using a stainless steel rod, placed immediately in paper bags, and brought to the laboratory. Leachate samples were collected from the plastic pot saucer for each of the oat and control pots. Free liquids were collected directly from within the sedge pots using a pipette with disposable tip. Leachate samples were placed into washed Nalgene™ plastic bottles, transported to the laboratory and stored at 4°C.

Soil samples (5 g) were extracted by shaking with 25 ml 92% ethanol for 30 min on a reciprocating shaker. The soil suspensions were filtered using Whatman no. 2 filter paper. Leachate samples were likewise filtered using Whatman no. 2 filter paper. Both soil extracts and filtered leachate were stored at 4°C until analysis.

Gas chromatographic (GC) analysis of the soil extracts and filtered leachate was conducted using a Perkin Elmer Clarus 500 gas chromatograph with an electron capture detector (ECD) and a Programmed On-Column (POC) Inlet System. The system included a 6 m Perkin Elmer fused silica capillary column measuring 0.53 mm ID with a 1.5 µm film thickness. Samples measuring 1 µl were injected into the column. The GC oven was temperature-programmed as follows: 130°C for 1 min, 10°C /min ramp to 160°C, 30°C /min ramp to 285°C hold for 1 min. The carrier gas was helium at 7.0 ml/min flow rate. The ECD temperature was set to 300°C and the makeup gas was nitrogen at a 30 ml/min flow rate. A 1000 mg/L nitroglycerin (NG) standard in ethanol was obtained from AccuStandard, Inc., New Haven, CT. A total of five NG standards were prepared, i.e., 0.05, 0.1, 0.2, 0.5, and 1 mg/L. The TotalChrom™ Navigator Application (v. 6.3) (Perkin Elmer, Shelton, CT) was used to process, record and report the chromatographic results. A sample chromatogram appears in Fig. 2.1.

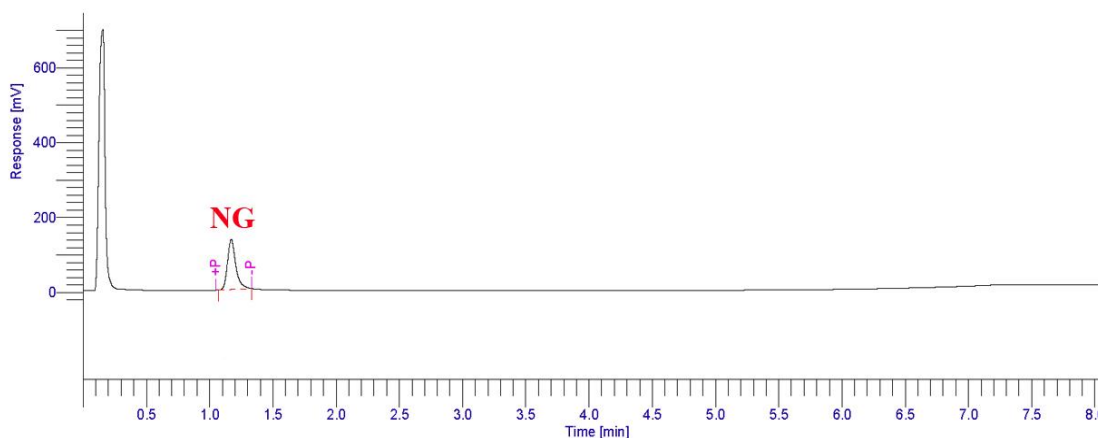


Fig. 2.1. Chromatogram of soil extract showing NG peak (0.9 mg/l).

Plant Uptake of NG

Harvested plants were placed in a freezer until analysis. Five grams of tissue were extracted with 25 ml 92% ethanol, ground in a commercial blender, filtered through Whatman no. 2 filter paper, and analyzed by GC-ECD as described above.

NG Column Leaching Study

PVC columns measuring 30.5 cm tall with a 3.2 cm inner diameter were packed with a mixture of Glymwood soil and washed sand. In one set of treatments, raw field soil was used; in the other, ground soil was used. The soil was ground using an agate mortar and pestle and was capable of passing through a 0.84-mm mesh sieve. For both treatments, a 1:1 mixture of soil:sand was prepared and five replicates were established. Smokeless powder (SP) was added at a rate of 0.1% (w/w) to the top 5-7 cm of each column. A Masterflex™ peristaltic pump was used to pass deionized H₂O through the columns at a rate of 1 ml/h. Leachates were collected after each 200 ml input and stored

at 4°C until analysis. The leachates were analyzed for NG on GC-ECD as described above.

NG Batch Adsorption Study

Twenty soil samples (25g each) were prepared for a batch adsorption study. Ten samples were studied in a raw condition and the other ten were autoclaved. Of the raw soils, five were prepared with Glynwood soil and sand (1:1 ratio) and the other five with ground Glynwood soil and sand (1:1 ratio). Soil materials were ground as described in the previous section. In the A soils, ten soil:sand mixtures were ground and then autoclaved using a Tomy autoclave SS-325E at 121°C for 20 minutes.

Samples were placed into beakers and moistened with deionized H₂O. Smokeless power was added at a rate of 0.1% (w/w) and mixed with the soil/sand mixture. Samples were incubated in the dark for 30 days at ambient temperature (approx. 25°C). Five grams of each sample were extracted with 25 ml of 92% ethanol after 30 d. Soils were incubated for an additional 30 d and then extracted with ethanol. All soil extracts were analyzed for NG concentrations on GC-ECD.

Microbial Identification and Enumeration

Populations of total bacteria were counted using the spread plate technique (Capuccino and Sherman, 1998). Plate Count Agar was used for bacterial identification. Sterilization of the agar was carried out in a Tomy autoclave SS-325E at 121°C for 15 min. Agar was then poured into sterile polystyrene Petri dishes and stored at 4°C. There were four replicates of all treatments. Soil dilutions (10^{-3} to 10^{-7}) were spread onto Petri dishes with

a sterilized L-shaped bent glass rod. Petri dishes were then incubated in the inverted position at room temperature for two days. Microbial enumeration was subsequently carried out in a Darkfield 3330 Quebec® colony counter.

Photolytic Decomposition Study

Soluble NG, dissolved in 92% ethanol, was placed in glass vials and subjected to artificial lighting (approx. 1275 lumens). Vials were maintained at approx. 21 °C. Vials were sampled at 4, 8, 24, 48, and 96h and were analyzed for NG via GC-ECD.

Statistical Analysis

All data obtained for NG decomposition as a function of treatment were tested for statistical significance using three-way analysis of variance (ANOVA). Tests showing significance at $\alpha = 0.05$ were analyzed using a Pairwise Comparisons Test. SPSS™ and MS Excel were used on a Windows-based PC for all statistical analyses.

References

- ASTM Standard C33. 1988. ASTM D7348 - 08 Standard Test Methods for Loss on Ignition (LOI) of Solid Combustion Residues. ASTM International, West Conshohocken, PA.
- Burton, A.L., M. Williams, J.P. Lynch, K.M. Brown. 2012. RootScan: Software for high-throughput analysis of root anatomical traits. *Plant and Soil*. [Online]. Available at <http://roots.psu.edu/en/node/972>, Accessed at 12 June 2012.
- Cappuccino, J.G., and N. Sherman. 1998. *Microbiology: A Laboratory Manual*. Experiment 53: Microbial populations in soil: Enumeration. Addison Wesley Longman, New York, NY.
- Knudsen, D., A. Peterson, and P.F. Pratt. 1982. Potassium. In Page, A.L., R.L. Miller, and D.R. Keeney (eds.). *Methods of Soil Analysis, Part 2*. American Society of Agronomy, Madison, WI.
- Olsen, S.R., and L.E. Sommers. 1982. Phosphorus, p. 403-430. In Page, A.L., R.L. Miller, and D.R. Keeney (eds.). *Methods of Soil Analysis, Part 2*. American Society of Agronomy, Madison, WI. Polysciences, Inc. No date. Szechrome reagents. Technical Data Sheet 239. Warrington, PA.

Sims, G.K., T.R. Ellsworth, and R.L. Mulvaney. 1995. Microscale determination of inorganic nitrogen in water and soil extracts. *Communications in Soil Science and Plant Analysis*. 26(1-2): 303-316.

Sposito, G., L.J. Lund, and A.C. Chang. 1982. Trace metal chemistry in arid-zone field soils amended with sewage sludge: I. Fractionation of Ni, Cu, Zn, Cd and Pb in solid phases. *Soil Science Society of America Journal*. 46: 260-264.

Watanabe K. 2001. Microorganisms relevant to bioremediation. *Current Opinions in Biotechnology*. 12: 237-41.

RESULTS AND DISCUSSION

Characterization of Soil, Composted Biosolids and Smokeless Powder

The Glynwood soil was slightly acidic in reaction (pH 6.7) and had moderate levels of organic carbon (3.9%) and extractable metals. Concentrations of chloride (Cl^-), nitrate (NO_3^-) and sulfate (SO_4^{2-}) were high (Table 3.1). The soil was collected from an agricultural field and it is possible that the high Cl^- , NO_3^- , and SO_4^{2-} concentrations are the result of recent application of commercial fertilizer. Soil phosphate concentrations were low. The soil texture is classified as silt loam (Dominguez-Rosado et al., 2004).

The composted biosolids (CB) were near-neutral in reaction (pH = 7.2) and contained high levels of organic carbon (57.2%) and total N (3.2%), resulting in a C:N ratio of 17.9. Approximately 3.2% of the total N was composed of NH_4^+ . Phosphate concentrations were high. Concentrations of extractable metals were very low (Table 3.2). Based on the above properties, the CB should serve as an excellent soil amendment for both plant and microbial growth.

Table 3.1. Selected chemical and physical properties of the study soil.

Parameter	
pH	6.7
TOC* %	3.9
Extractable metals (mg/kg)	
Cd	2.55
Cr	0.09
Cu	0.22
Fe	32.4
Ni	0.31
Pb	0.50
Zn	9.80
Soluble anions (mg/kg)	
Cl ⁻	2323.7
NO ₃ ⁻	85.5
PO ₄ ³⁻	0.54
SO ₄ ²⁻	1097.8
Total N (%)	0.36
Bray-1 P (mg/kg)	13
NH ₄ ⁺ (mg/kg)	8.2
K (mg/kg)	86.9
Texture, %	
Sand	28
Silt	51
Clay	21

*TOC= Total organic carbon

Table 3.2. Selected chemical properties of the CB amendment.

Parameter	
pH	7.3
TOC* %	57.2
Extractable metals (mg/kg)	
Cd	0.29
Cr	0.12
Cu	1.40
Fe	11.3
Ni	0.70
Pb	0.73
Zn	0.78
Total N (%)	3.2
NO ₃ ⁻ (mg/kg)	26.5
NH ₄ ⁺ (mg/kg)	1063
Bray-1 P (mg/kg)	799
K (mg/kg)	1747.2

*TOC= Total Organic Carbon

Mean NG concentration in the SP was 255,150 mg/kg (data not tabulated). This value is comparable to that for other commercial smokeless powders. Nitroglycerin concentrations vary with manufacturer. For example, several Accurate™ powders contain 7-23 % NG (Western, 2007), and Ramshot Zip™ double-base smokeless powder contains from 8-42% NG (Western, 2010)

Germination Study

Ryegrass, maize 1, white clover, redclover and soybean germinated at rates of \geq 90% at the 0.1% SP rate (Table 3.3). Oats 1, wheat, maize 2, mustard and green bean all experienced \geq 50% germination rate. Germination of sunflower was poor (8%).

At the 1% SP rate maize 1, redclover and soybean germinated at \geq 90%. Germination of most test crops decreased markedly at the 5% SP rate; however, maize 2 and soybean experienced high germination rates (85 and 93%, respectively). Regardless, however, shoots and roots for all plants were stunted at this SP rate. Green bean and soybean seeds germinated at 53 and 44%, respectively, at the 10% SP application rate.

Growth of initial oat shoot; first, second, and third root; and plant wet weight declined substantially with even modest SP application rates (Figs. 3.1-3.4). Data for all these parameters followed a curvilinear relationship, with R^2 values \geq 0.9.

Table 3.3. Germination success of test plants as a function of SP application rate.

SP rate (%)	Plant species											
	Oats	Oats 2	Wheat	Rye- grass	Maize 1	Maize 2	Indian Mustard	Sun- flower	White Clover	Red Clover	Green Bean	Soy- bean
	Number of Germinated Seeds (%)											
0	63	52	63	75	99	95	83	21	89	97	87	87
0.1	52	28	53	96	100	55	75	8	91	91	77	96
1	35	4	63	72	96	63	57	7	84	96	76	97
5	0	0	15	11	24	85	23	0	17	48	11	93
10	0	0	0	5	0	19	3	0	21	9	53	44

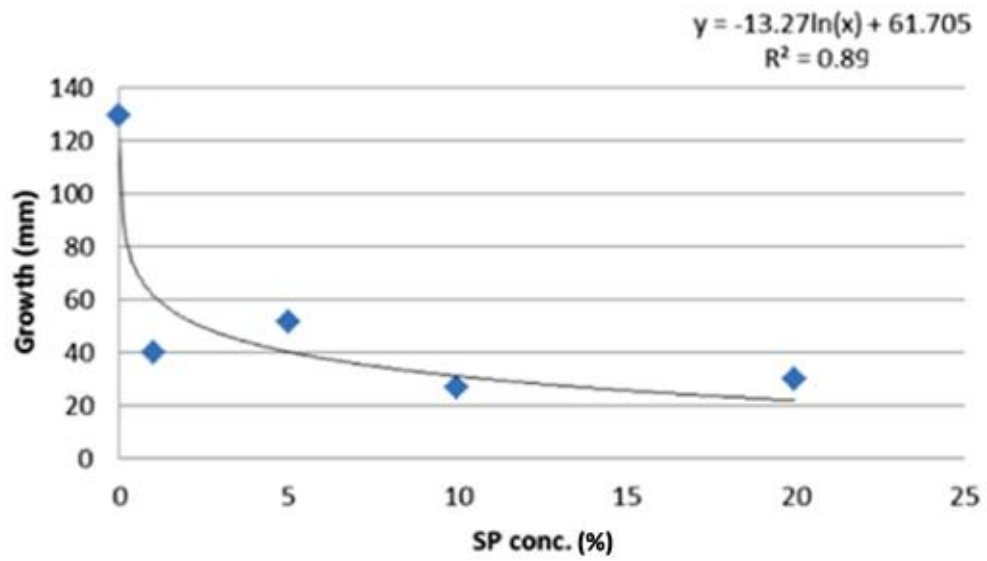


Fig. 3.1. Growth of oat shoots immediately following germination as affected by SP rate.

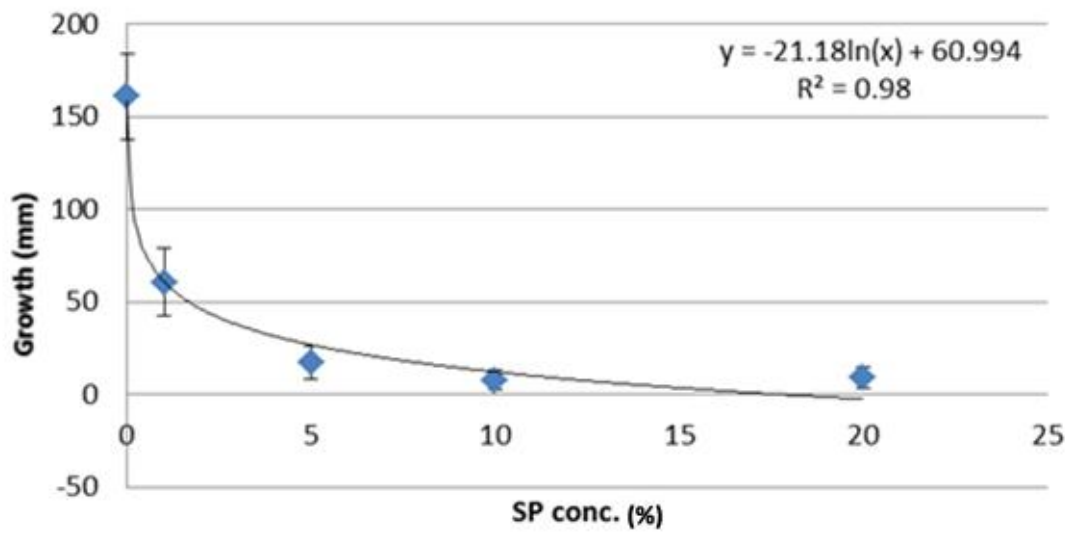


Fig. 3.2. Growth of primary root immediately following germination as affected by SP rate.

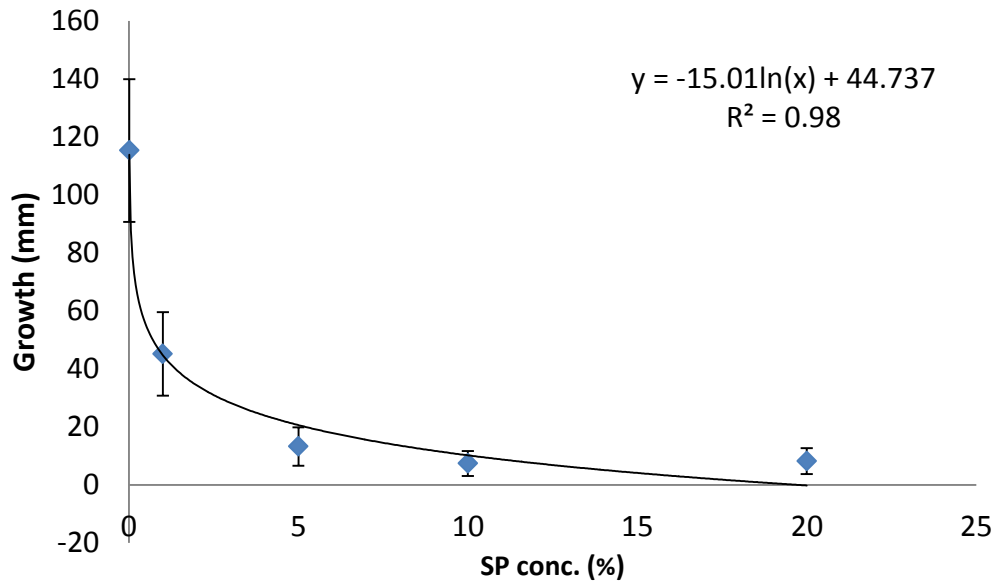


Fig. 3.3. Growth of second root immediately following germination as affected by SP rate.

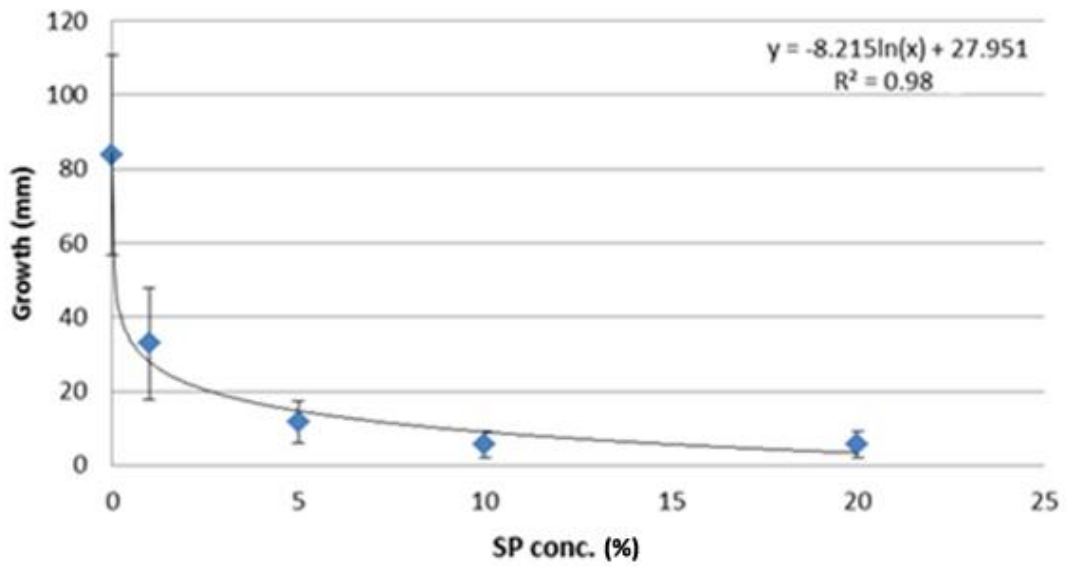


Fig. 3.4. Growth of third root immediately following germination as affected by SP rate.

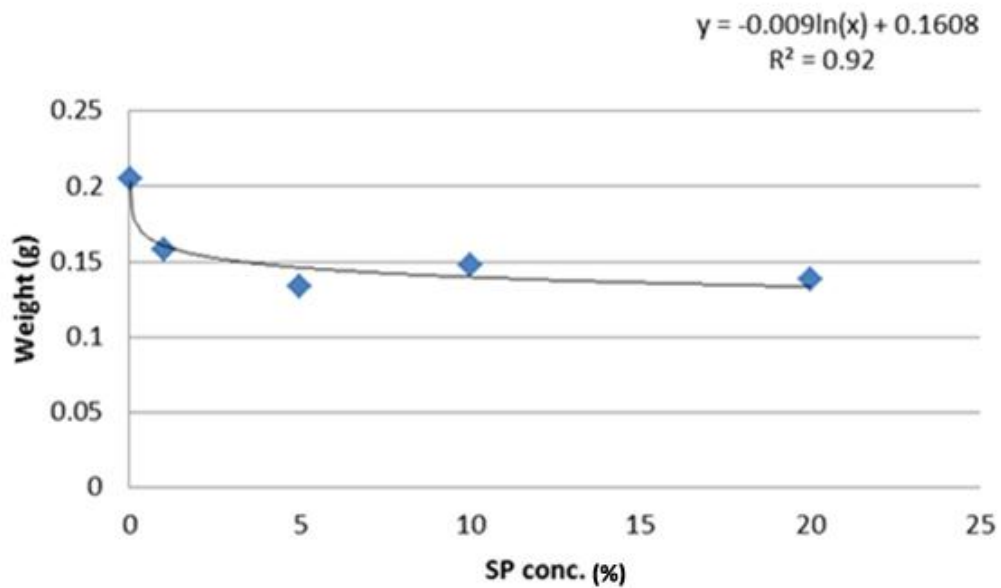


Fig. 3.5. Plant wet weight immediately following germination as affected by SP rate.

At the 1% SP rate shoot growth declined from 130 mm to 40 mm (Fig. 3.1). At the 20% SP rate shoot growth declined to 32 mm. Likewise, growth of the primary root declined from 140 mm to 60 mm with 1% SP (Fig. 3.2). At the 5, 10 and 20% SP application rates these plant growth parameters were virtually unchanged. At the 20% SP rate primary root length decreased to 10 mm. From these data it can be inferred that even modest SP application rates hinder oat growth and development; however, higher rates do not impart a significantly greater effect.

Plant Growth and Development

Growth of oat, oat-CB, sedge, and sedge-CB were all adversely affected by application of SP (Fig. 3.6). Sedge plants experienced a 73% decrease in biomass production with the addition of 1% SP (from 29.93 g to 8.08 g/pot). At the 5 and 10% SP levels growth of the sedge plants stabilized and increased slightly, though the increases are not statistically significant ($p > 0.05$). Biomass production decreased by 50% from the 0% rate (29.9 g/pot) to the 10% rate (14.5 g tissue/pot).

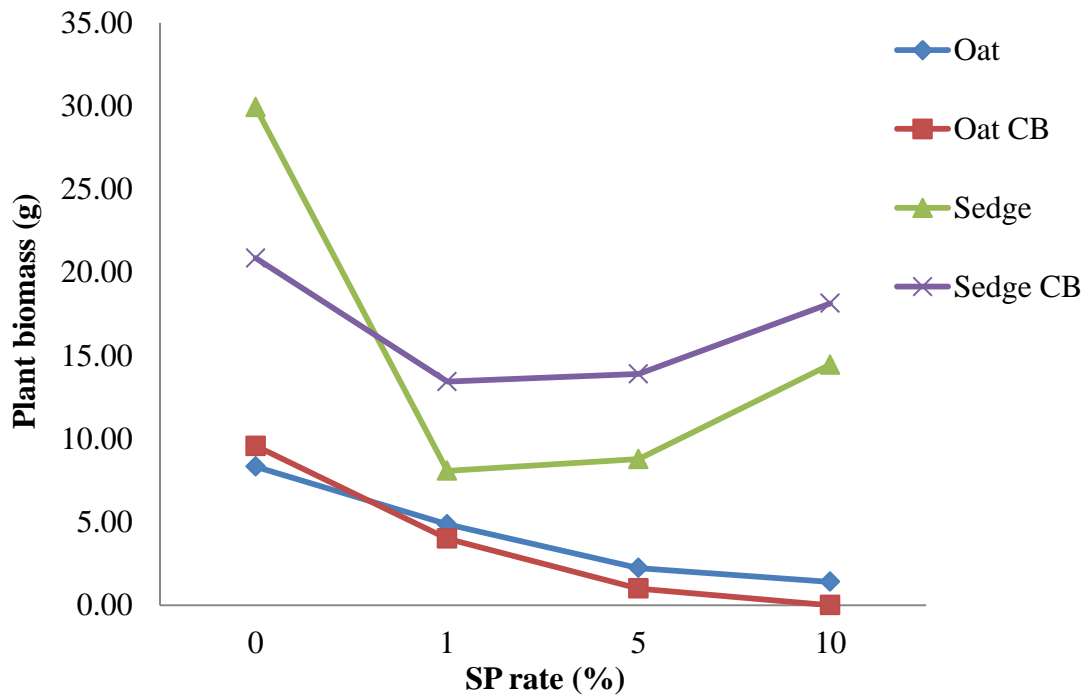


Fig. 3.6. Oat and sedge biomass after 60 days as a function of SP application rate.

Oats produced substantial biomass at low levels of SP (4.9 g/pot at 1% SP) but biomass was reduced to 1.4 g/pot at the 10% SP rate. In many cases oat plants at the 5 and 10% SP application rates did not fully mature (e.g., set seed). Visual assessment of the plants indicated no obvious symptoms of toxicity at the 5 and 10% levels of SP. These data are similar to those of Best et al., (2008), where biomass of *Lolium perenne* significantly decreased with increase in soil TNT concentration. From the data it is apparent that sedges were more tolerant of the presence of SP than were oats. Chang et al. (2004) studies phytoremediation of TNT- contaminated soil (120 mg TNT/kg) using Indian mallow (*Abutilon avicennae*). After 50 days of incubation the growth of Indian mallow roots decreased by 32.4% and shoots by 34.3%.

Nitroglycerin Incubation Study

At Day 7 soil NG concentrations at the 1% SP rate were relatively low (range of 65-152 mg/kg), indicating minimal dissolution of the SP pellets (Table 3.5). In the Control pots the 1% SP rate released maximum NG concentrations at Day 14 (1337 mg/kg for Control and 721.1 mg/kg for Control CB) (Table 3.4 and Fig. 3.7), which were significantly ($p < 0.05$) different. After Day 14, NG concentrations declined. The lower NG concentrations in the Control CB may be a result of the significantly higher TOC levels in this treatment (Table 3.2). The Glywood soil contains 3.9 % TOC (Table 3.1). When mixed with 20% CB the overall TOC content increases to approximately 15.4%.

Sorption of NG by the organic matter occurring in the CB may be significant. In the current discussion, sorptive surfaces include those of humic substances and microorganisms. Sorption reactions may include hydrophobic partitioning, hydrogen

bonding, ion exchange, and chemisorption (Kalderis et al., 2011; Juhasz and Naidu, 2007; Singh et al., 1998; Pennington and Patrick, 1990). The extent of partitioning between solute and sorbent is a function of their physicochemical properties as well as environmental conditions (Kalderis et al., 2011; Juhasz and Naidu, 2007).

Table 3.4. Soil NG concentrations at the 1% SP application rate.

Sampling Dates	Oat	Oat CB	Sedge	Sedge CB	Control	Control CB	Control-AN*	Control-AN-CB
	mg/kg							
7	134.5±115.5	121.1±106.2	120.2±65.9	65.1±40.1	151.6±80.2	83.6±38.6	108.3	39.3
14	1571.0±1418.2	1819.1±1878.1	1240.5±474.9	1365.0±558.3	1336.9±1123.6	721.1±409.7	3596.1	434.3
30	1620.6±1513.8	3488.4±3272.5	1092.5±524.0	1027.8±554.4	1180.0±505.7	690.1±153.7	724.3	63.6
60	810.5±273.3	2466.2±1864.0	1623.1±1181.7	852.6±213.4	829.7±387.0	690.1±153.7	1360.6	40.8

$n = 4$; values indicate mean \pm standard deviation.

*AN = anaerobic

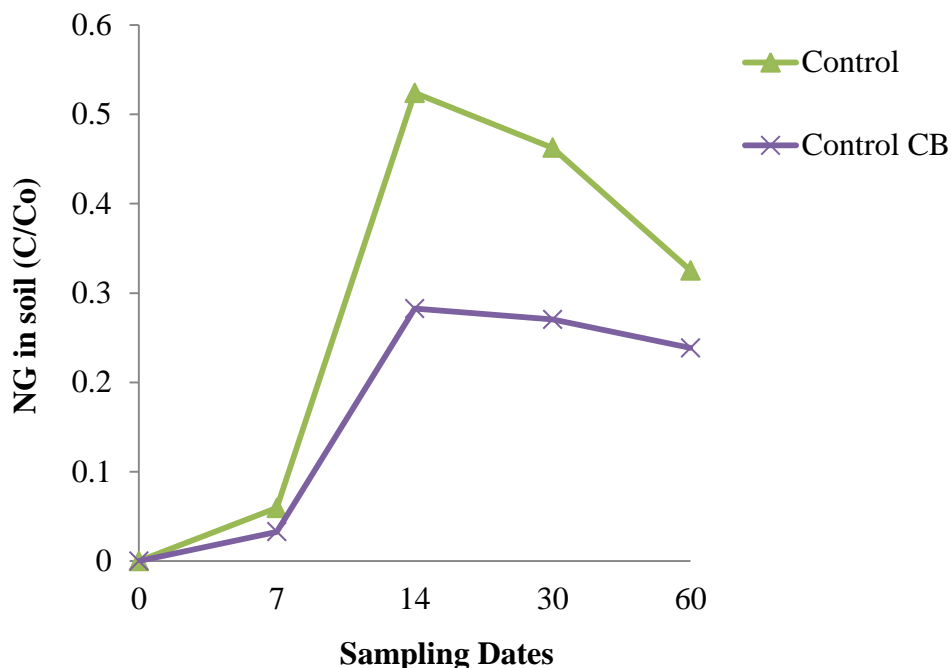


Fig. 3.7. Soil NG concentrations (C/C₀) Control and Control-CB at the 1% SP rate.

The organic carbon fraction in soil plays a significant role in sorption of energetic compounds. Yamamoto et al. (2004) determined that sorption coefficients (*K_d* values) for TNT, RDX, and 2,4-DNT were dependent on quantity of soil organic carbon. In studies by Brannon and Pennington (2002) and Price et al. (1998) significantly more TNT than RDX was associated with soil organic matter.

Jenkins et al. (2007) found that NG and NQ were retained only slightly in low organic carbon soils. In studies by Brannon et al. (2004) NQ partition coefficients were very low, ranging from 0.15 to 0.43 L/Kg. Haag et al. (1990) reported a value of <0.1 L/Kg. In soil at small-arms ranges *K_d* values for 2,4-DNT, 2,6-DNT, and NG ranged from 0.1 to 21.3, 0 to 18.2, and 0 to 7.3 L/kg, respectively (Clausen et al., 2010). Mean

K_d values were 3.2, 2.6, and 0.9 L/kg, respectively. Soil properties (e.g., organic carbon content, cation exchange capacity) imparted a significant effect on *K_d*. Desorption experiments revealed that a portion of these propellants were irreversibly bound (Clausen et al., 2011).

For the 5% SP treatments, NG concentrations were highest in the Control at 14 days (9267.1 mg/kg). In the Control CB the NG release appeared to be delayed, and on Day 30 reached maximal concentrations (6181.8 mg/kg) (Table 3.5 and Fig. 3.8). At the 10% SP rate, maximal dissolution of the SP pellets did not occur until Day 30 -- highest NG concentrations were 7899 mg/kg in the Control pots, and 6181.8 mg/kg NG in the Control CB (Table 3.7 and Fig. 3.8).

Table 3.5. Soil NG concentrations at the 5% SP application rate.

Sampling Dates	Oat	Oat CB	Sedge	Sedge CB	Control	Control CB	Control-AN	Control-AN-CB
	mg/kg							
7	322.6 \pm 122.8	627.6 \pm 284.1	393.2 \pm 122.6	372.5 \pm 85.5	1114.6 \pm 833.4	831.0 \pm 474.5	557.6	375.9
14	8761.4 \pm 3371.2	8009.1 \pm 4820.4	7720.4 \pm 373.8	6149.1 \pm 861.4	9267.0 \pm 4219.8	5990.5 \pm 703.5	5606.7	7150.1
30	10861.0 \pm 7728.7	6015.2 \pm 4256.2	6815.6 \pm 2307.5	9965.3 \pm 5449.8	7264.1 \pm 2283.2	6181.7 \pm 1900.2	3670.5	29792.0
60	5981.2 \pm 2221.7	5820.3 \pm 4204.0	4268.6 \pm 595.1	3674.7 \pm 800.3	7898.8 \pm 2114.4	4717.1 \pm 2658.5	3864.9	3707.1

$n = 4$; values indicate mean \pm standard deviation.

*AN = anaerobic

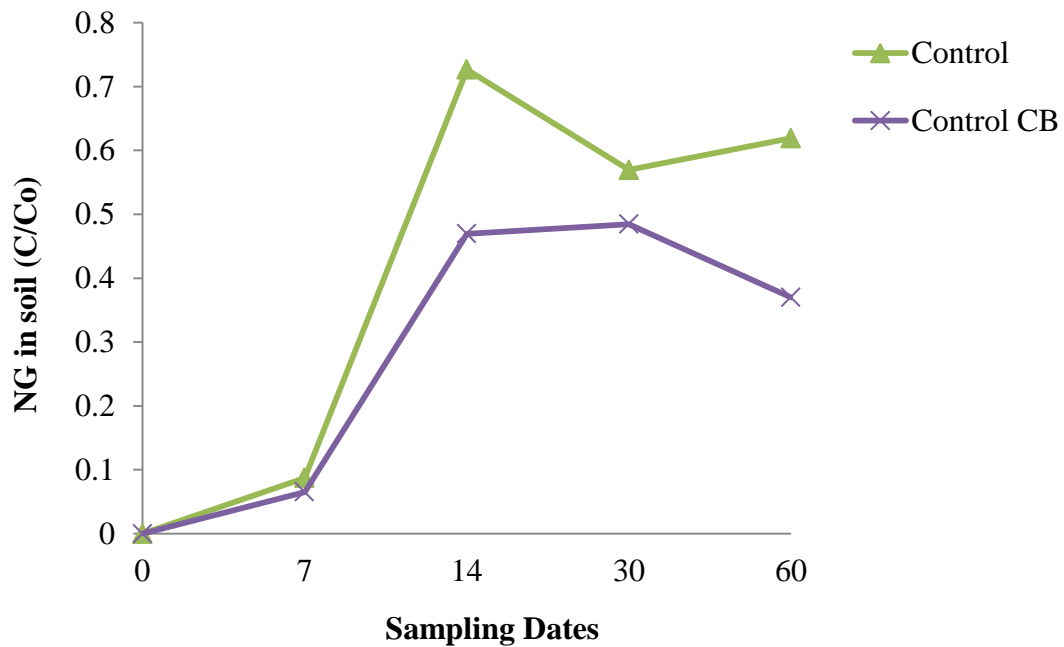


Fig. 3.8. Soil NG concentrations (C/C_0) Control and Control-CB at the 5% SP rate.

Double-base propellants contain NC impregnated with an organic nitrate such as NG (Juhasz and Naidu, 2007; Walsh et al., 1993; NFSTC, 2011). Additional ingredients include compounds that modify burn rate, binders or plasticizers that facilitate loading the propellant into the shell, and compounds that enhance propellant stability during storage (Walsh et al., 2008). Energetic residues often occur in soil as solid particles and chunks resulting from low-order detonation or as partially fragmented unexploded ordnance (UXO). Dissolution in water is the primary mechanism for their transport and dispersion in the biosphere (Pennington et al., 2006a, 2006b; Kalderis et al., 2011). Nitroglycerin is rather mobile in soil in part due to its moderate solubility (1,250 to 1,950 mg/l) (Sullivan et al., 1979); however, NG introduced to soil during military training at

small arms ranges did not leach (Clausen et al., 2011). The degree to which NG is available for release is a function of the degree of deterioration of the nitrocellulose (NC) encapsulation in the propellant mix (Clausen et al., 2011; Windholz, 1979).

Table 3.6. Soil NG concentrations at the 10% SP application rate.

Sampling Dates	Oat	Oat CB	Sedge	Sedge CB	Control	Control CB	Control-AN*	Control-AN-CB
mg/kg								
7	1154.7±463.7	952.8±436.6	797.2±301.8	803.3±283.5	1046.0±537.5	1433.8±799.1	2377.0	2011.8
14	26835.8±26103.3	15033.5±8683.9	13796.3±6397.7	11038.4±3830.9	10641.9±2900.9	8596.4±3148.8	13859.0	21955
30	34663.9±11654.0	21225.2±16402.6	10868.6±2288.1	10463.3±1677.6	21934.7±4328.5	16949.3±11487.8	149.3	20127.0
60	24958.9±10968.0	10551.9±6433.7	13537.9±8685.1	12829.6±9678.3	11973.0±4736.7	13990.0±6151.0	14544.0	12928.0

n = 4; values indicate mean ± standard deviation.

*AN = anaerobi

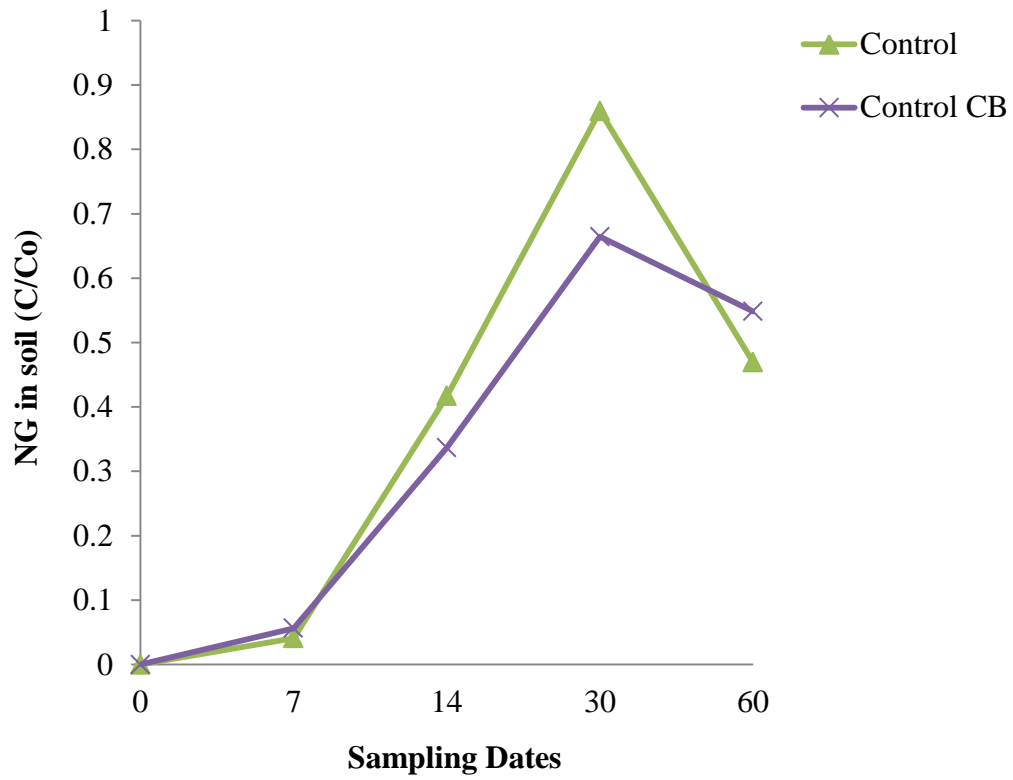


Fig. 3.9. Soil NG concentrations (C/C_0) Control and Control-CB at the 10% SP rate.

At the 1% SP rate soil NG concentrations were low in the oat and oat-CB, indicating minimal dissolution of the SP pellets (Fig. 3.10). Over the 60-day incubation period NG concentrations attained maximal concentrations of 3488 mg/kg (Oat-CB, Day 30) and 2466 mg/kg (Oat-CB, Day 60) (Table 3.4 and Fig. 3.10).

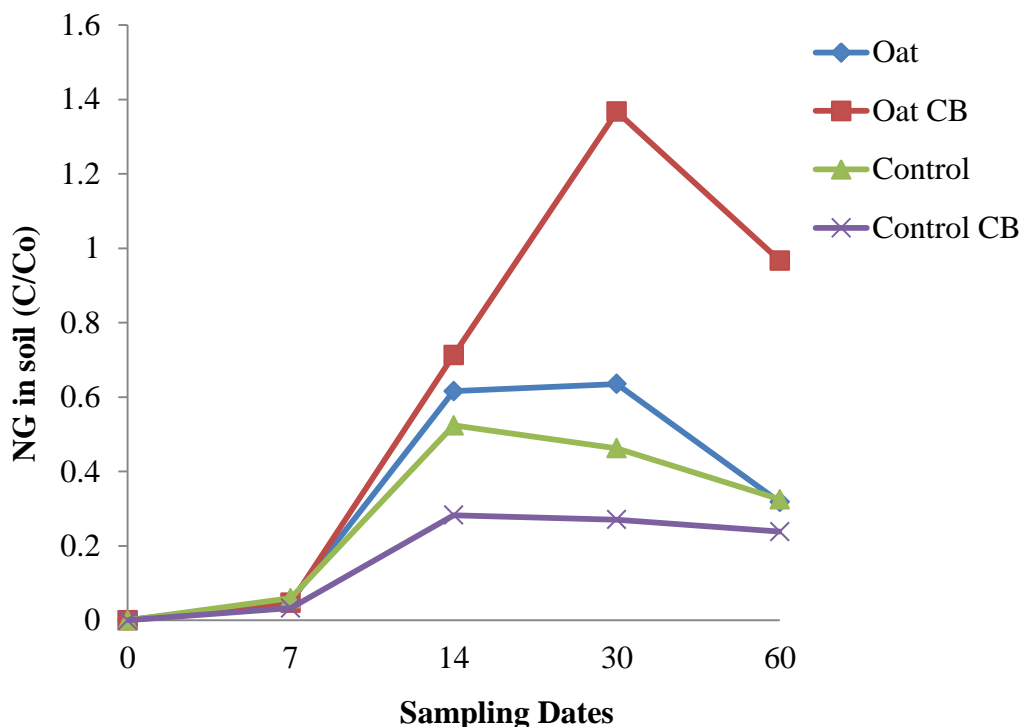


Fig. 3.10. Soil NG concentrations (C/C_0) for oat treatment at the 1% SP rate.

On Days 14 and 30 significantly ($p < 0.05$) greater NG occurred in oat- and oat-CB-treated soils compared to the Control and Control-CB treatments. This effect may be due to the so-called “rhizosphere effect” (Pichtel, 2007), i.e., a highly diverse and active microbial population will colonize and potentially dissolve soil contaminants, including SP pellets.

Nitroglycerin levels at the 1% rate in sedge- and sedge-CB soils followed those of the Control and Control-CB rather closely (Table 3.4 and Fig. 3.11). At Day 60, however, NG concentrations in the sedge-CB increased to 1623 mg/kg.

In most cases in the 1% SP pots, soil NG concentrations in the oat and oat-CB were significantly ($p < 0.05$) greater than those in the sedge and sedge-CB treatments

(Table 3.5 and Figs. 3.10-3.11). On Day 30 mean NG in oat-CB was 240% greater than that of sedge-CB; likewise, on Day 60 mean NG in oat-CB was 189% greater than that in sedge-CB.

Aerobic decomposition of NG is reported as being more rapid than anaerobic processes (Christodoulatos et al., 1997). Decomposition of smokeless powder may be greater in pots incubated aerobically (i.e., to oats) than in anaerobic (sedge) soil pots. Double base smokeless powders typically contain nitrocellulose, dibutyl phthalate, diphenylamine, ethyl centralite, waxes and other hydrocarbon-based additives (Western, 2007). All should be amenable to beta-oxidation as carried out by aerobic soil heterotrophic bacteria in the plant rhizosphere. Once these compounds are acted upon by microorganisms, the soil NG is released. It is estimated, however, that approximately 14 times greater energy is derived by microorganisms during aerobic, as compared to anaerobic decomposition of hydrocarbons (Pichtel, 2007).

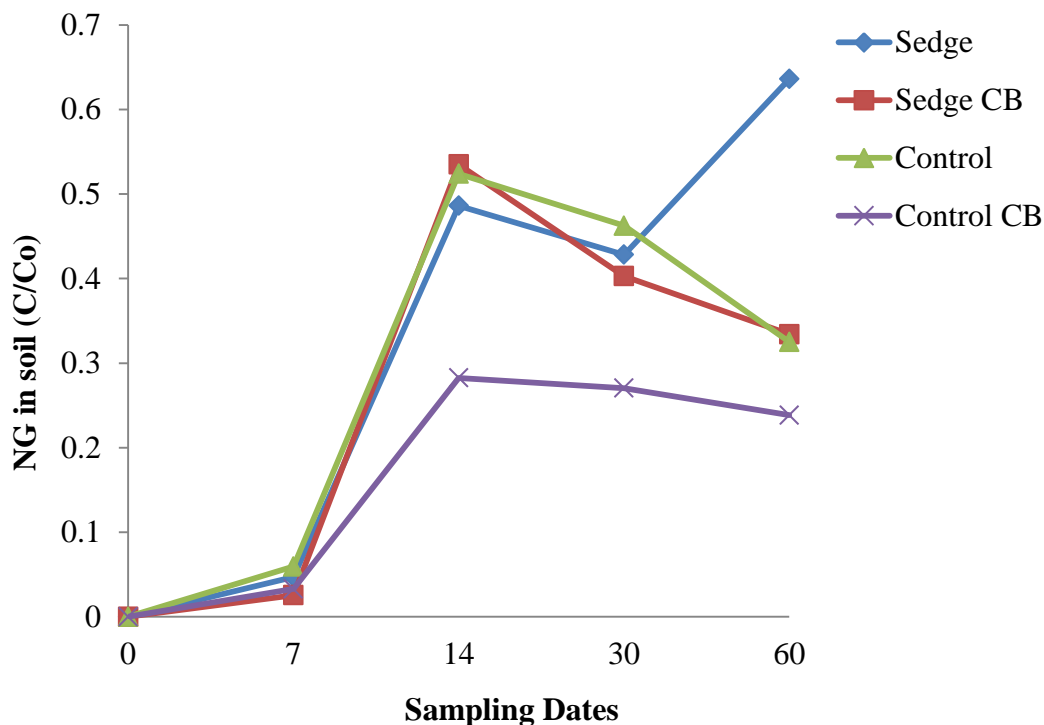


Fig. 3.11. Soil NG concentrations (C/C_0) for sedge at the 1% SP rate.

At the 5% SP rate, most treatments released maximal quantities of NG to the soil by Day 14, typically not exceeding 9300 mg/kg (Table 3.6 and Figs. 3.12-3.13). The only exceptions were oat at Day 30 (10,861 mg/kg) and sedge-CB at Day 30 (9,965 mg/kg). By Day 60, however, most soil NG concentrations had declined substantially and did not exceed 6,000 mg/kg. Control treatments did not exceed 7,900mg/kg over the 60-day incubation period (Figs. 3.12-3.13).

In most cases in the 5% SP pots, soil NG concentrations in the oat and oat-CB were significantly ($p < 0.05$) greater than those in the sedge and sedge-CB treatments (Table 3.6 and Figs. 3.12-3.13). On Day 30 mean NG in oat was 59% greater than that of sedge; similarly, on Day 60 mean NG in oat was 40% greater than that in sedge.

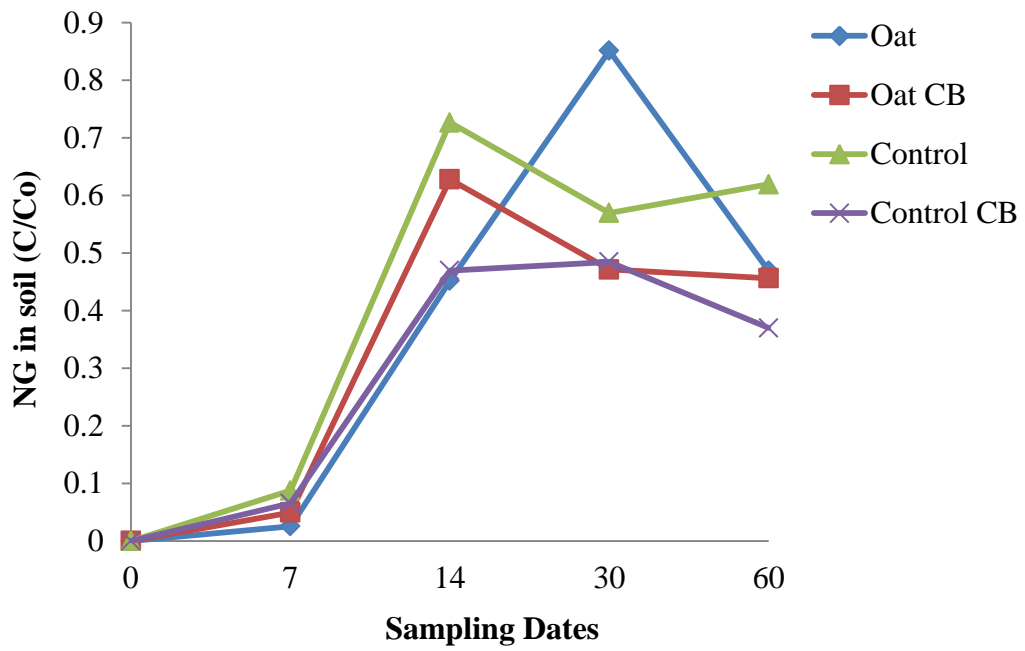


Fig. 3.12. Soil NG concentrations (C/C₀) for oat treatment at the 5% SP rate.

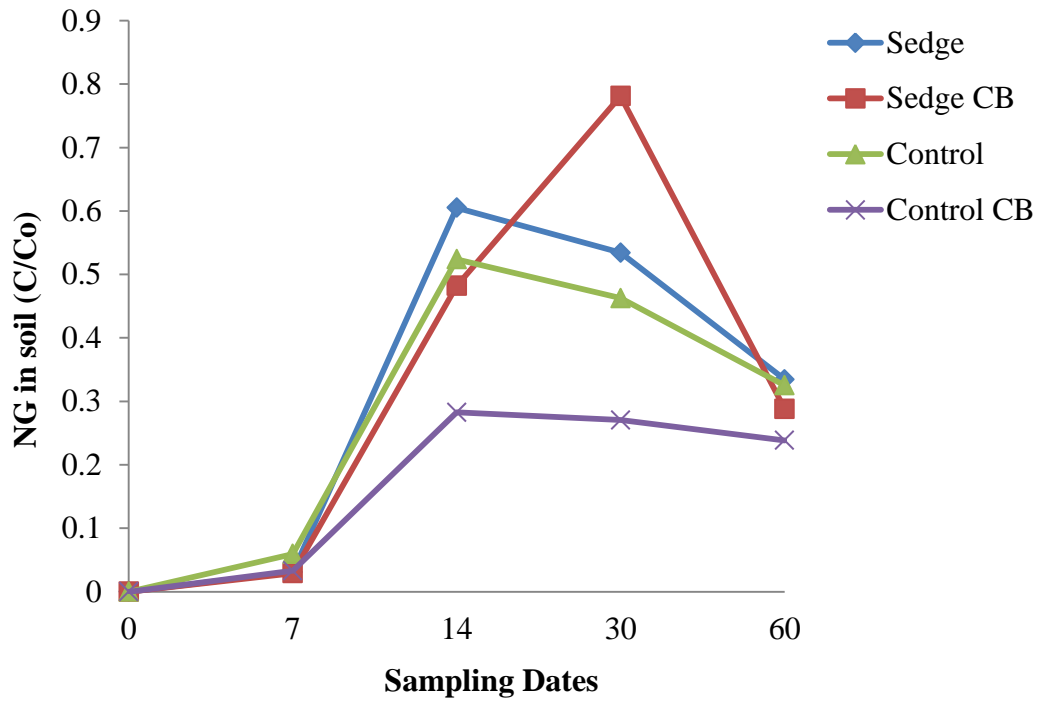


Fig. 3.13. Soil NG concentrations (C/C_0) for sedge treatment at the 5% SP rate.

At the 10% SP rate the oat treatments released maximal quantities of NG to the soil at Day 30 (Table 3.6 and Figs. 3.14-3.15). The highest NG concentrations occurred in oat at Day 30 (34,664 mg/kg). By Day 60 most soil NG concentrations had declined substantially and attained 24,959 mg/kg. Control treatments did not exceed 14,000 mg/kg over the 60-day incubation period (Table 3.6).

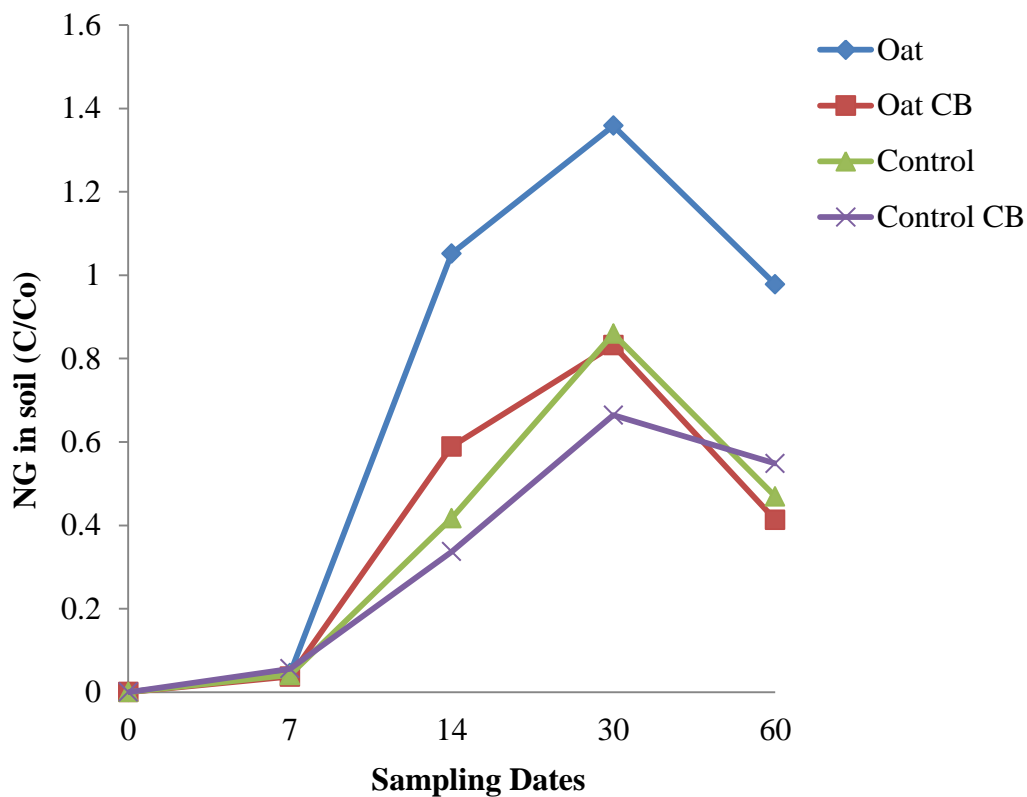


Fig. 3.14. Soil NG concentrations (C/C_0) for oat treatment at the 10% SP rate.

Nitroglycerin levels at the 10% SP rate in sedge- and sedge-CB-grown soils followed those of the Control and Control-CB rather closely (Table 3.6, Figs. 3.9 and 3.15). At Day 60, however, NG concentrations in the sedge and sedge-CB increased to 13,538 and 12,830 mg/kg, respectively. This effect is very similar to that of the sedge treatment at the 1% SP rate (Table 3.4 and Fig. 3.11).

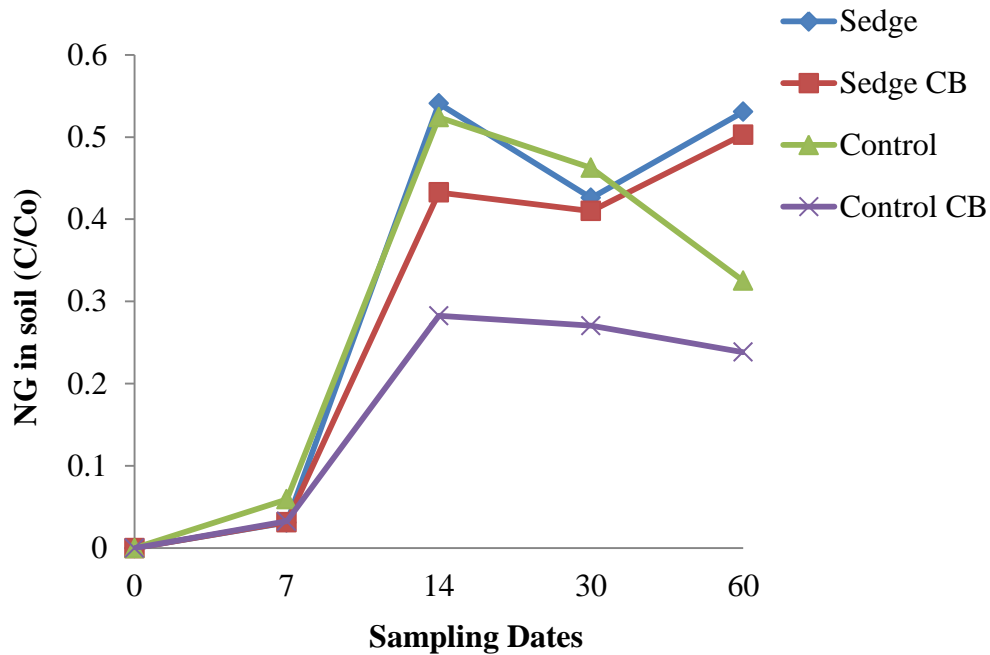


Fig. 3.15. Soil NG concentrations (C/C_0) for sedge treatment at the 10% SP rate.

The current results are consistent with those of Ouyang et al. (2005), who found soil TNT concentrations to decline to 87% of original levels after 30 d incubation with poplar trees (*Populus deltoides*). The authors suggested that some TNT was taken up by roots, and other portions were biodegraded in soil. This explanation could suit current trends for NG data as well. Ouyang et al. (2005) also reported the importance of initial soil explosive concentration and the depth of soil in which the explosive had been added.

Plant Uptake of NG

There was a general positive trend between rate of SP application and NG content of plant tissue in all treatments (Fig. 3.16). Over most SP rates the sedge took up more NG than did the oats. At the 10% SP rate the sedge-CB contained 146 mg NG/kg tissue, and the sedge contained 134 mg/kg. The oat treatment contained 87.5 mg/kg, which is 53% lower than that of the sedge. Very little oat-CB tissue was produced at the 10% SP rate; therefore, it was not possible to adequately measure NG uptake for that treatment.

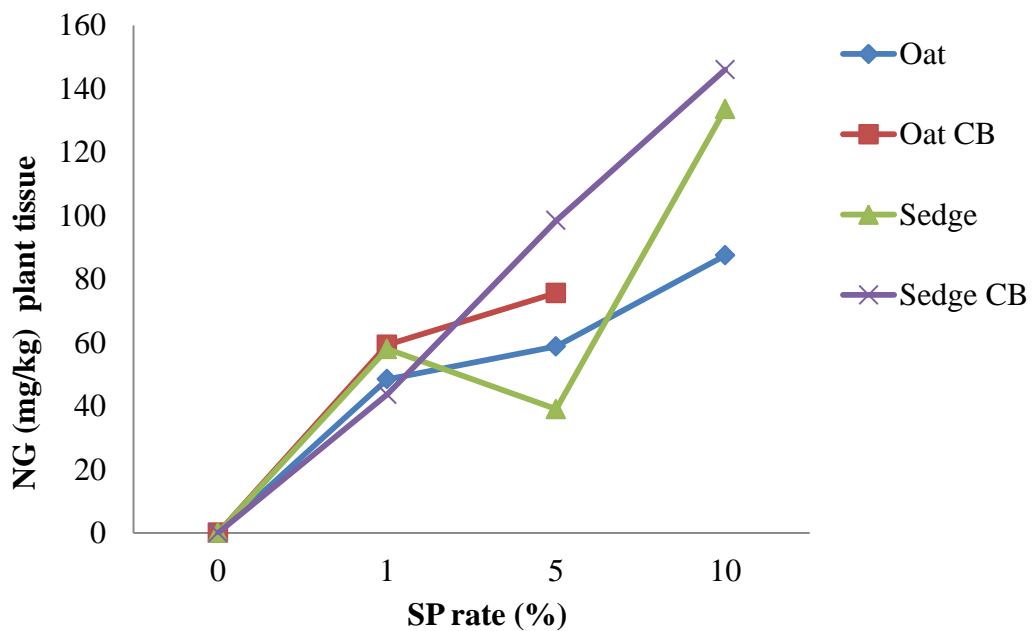


Fig. 3.16. Uptake of NG by oat and sedge plant treatments.

RDX was found to accumulate in leaf and shoot tissues of plants grown in contaminated soils (Harvey et al. 1991; Rivera et al. 1998). Johnson et al. (1999) observed that up to 60% of supplied RDX was translocated and accumulated in leaf tissue of the poplar. In HMX uptake studies, 70% of supplied HMX was translocated and accumulated in the leaves (Yoon et al. 2002). TNT tends to accumulate in the roots of plants with limited transport to above-ground biomass. In a study with TNT-spiked soil (10 mg/kg) and bush beans, Cataldo et al. (1989) reported TNT concentrations of <0.6, 9.0, 24.0, and 104 mg/kg in the seeds, leaves, stems, and roots, respectively. Poplar trees may also take up significant concentrations of TNT with most of the contaminant residing in the roots (Thompson et al., 1998).

The above data for plant uptake (Fig. 3.16) is, overall, a minor contribution to NG disappearance from the study soils. Mean NG concentration in the SP was 255,150 mg/kg (data not tabulated); therefore, the removal indicated in Fig. 3.16 comprises only a very small fraction (<< 1%) of total soil NG. Chang et al. (2004) studied phytoremediation of TNT-contaminated soil using Indian mallow (*Abutilon avicennae*). After 50 days of incubation, 77% of TNT was removed in the columns with plants, while 52% was removed in the columns with no plants. It is important to note that only 0.2% of removed TNT was recovered in the shoot and root extracts. Therefore, TNT removal by Indian mallow may be attributed to enhanced microbial activity which resulted in TNT transformation.

Leachate NG Recovery

Leachate samples were collected only from pots with 10% SP (100g). Nitroglycerin recovery in leachate reached a peak at 30 days (142.2 mg/l), following which it declined to 92.0 mg/l (Figs. 3.17-3.18). In the Control treatment, NG continued to leach from the pots over 60 days (max. 160 mg/l at Day 60) (Fig. 3.16). These data indicate the importance of plant roots and/or microbial activity in taking up and/or decomposing the NG. By Day 60, a total of 8946 mg NG was measured in the sedge leachate, compared with 19542 mg in the oat. A total of 15062 mg was measured in the sedge-CB leachate, compared with 20042 mg in the oat-CB, thus indicating greater uptake and/or decomposition of NG in the sedge pots.

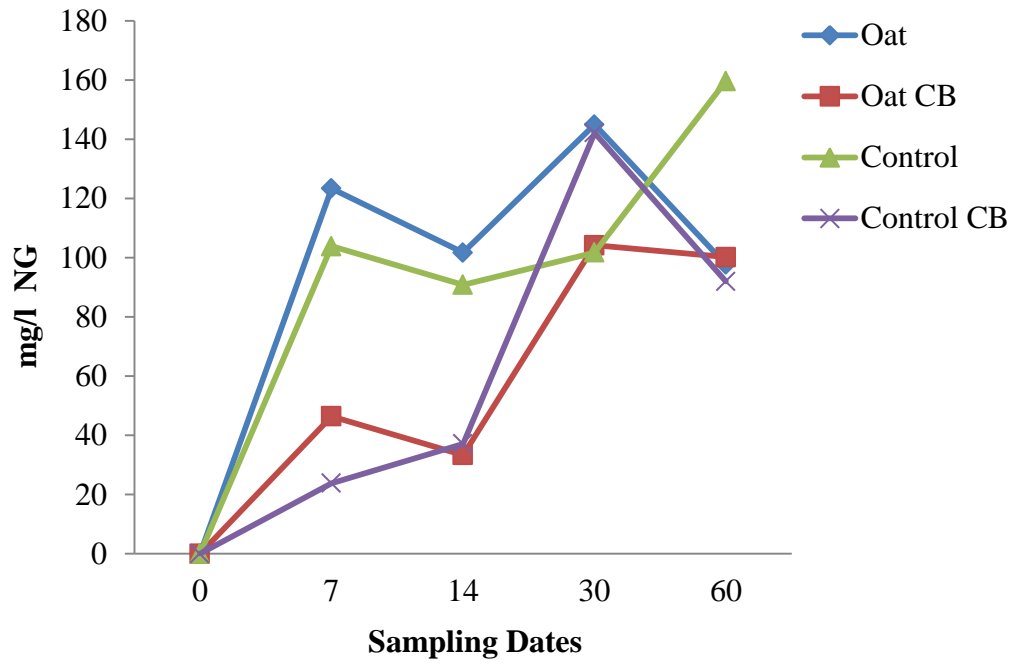


Fig. 3.17. Average NG concentrations (mg/l) for oat treatment at 10% SP rate in leachate samples.

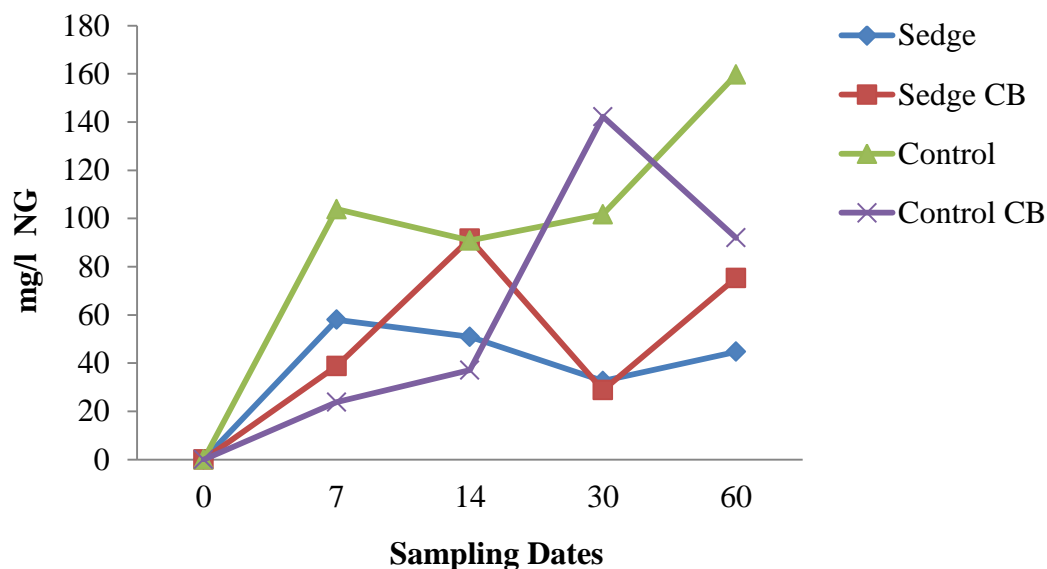


Fig. 3.18. Average NG concentrations (mg/l) for sedge treatment at 10% SP rate in leachate samples.

Nitroglycerin is an uncharged molecule; therefore it will not readily sorb to charged soil particle surfaces such as those occurring in silicate clays and organic matter. It is possible, however, that a portion of the NG is either immobilized (i.e., sorbed to nonpolar components of soil organic matter) and/or decomposed by soil microorganisms. A significant portion of soil organic matter may be nonpolar (Stevenson, 1992).

Soil Bacterial Counts

Numbers of total bacteria in Control and Control-CB samples were higher than those for other treatments (3.39×10^5 and 2.44×10^5 CFU/g, respectively) (Fig. 3.19). Bacterial numbers in 10% SP samples were higher than many in the 1% SP samples; however, these differences are not significantly different ($p < 0.05$). For example, total

bacteria in the sedge treatment at 1% SP were 1.78×10^6 CFU/g, while those at the 10% SP rate were 2.18×10^6 CFU/g. These bacterial numbers are comparable to those for a typical, undisturbed Indiana agricultural soil and indicate that, at least among those organisms that can be detected via plate count methods, soil bacteria are highly tolerant of SP and its various ingredients.

Numbers of total bacteria were consistently higher in the sedge soils compared with oat soils (Fig. 3.19). Sedges are wetland plants and the sedge soil during this study was consistently saturated; therefore, anaerobic conditions existed in those pots. The higher bacterial numbers may have improved overall nutrient uptake potential and stress tolerance of the sedges (Weyens et al., 2009a, 2009b; Ghanem et al., 2011).

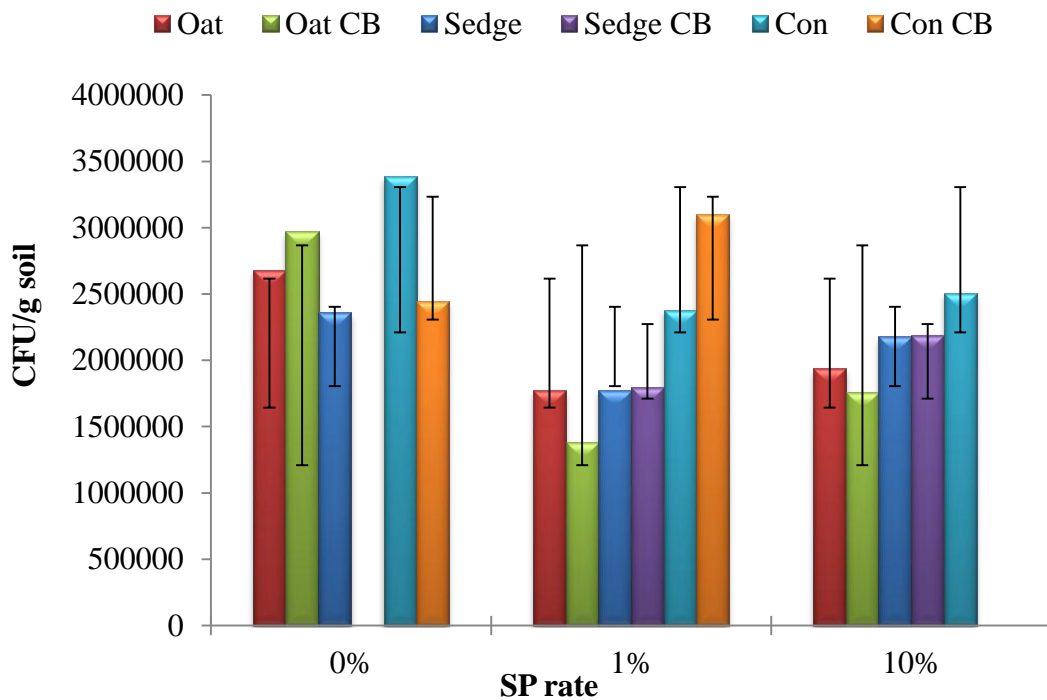


Fig. 3.19. Numbers of total bacteria (CFU/g soil) for the soils (mean_±SD).

Batch Incubation Study

The raw soil contained the lowest concentrations of NG at both Day 30 and Day 60 among other soils (1141.1 and 1413.4 mg/kg, respectively) (Fig. 3.20). By Day 60 NG concentrations in the raw autoclaved and ground autoclaved soils were highest (2603.1 and 2688.8 mg/kg, respectively). These results indicate the contribution of microbial populations in decomposing a portion of soil NG.

Numerous investigations of the fate of energetic compounds in soils and sediments have used weathered clays, silts, and sands in column and incubation studies (Thorn et al., 2002; Singh et al., 2008; Haag, 1990; Price et al., 1997). The behavior of aged mineral surfaces differs markedly from that of newly created surfaces generated by fracturing from detonations. Douglas et al. (2009) found that fractured soil particles exhibited greater transformation rates for nitroaromatic and nitramine compounds than did weathered soil particles.

The current results may be caused either by enhanced adsorption to the fractured surfaces or by accelerated transformation processes (chemical and/or biological).

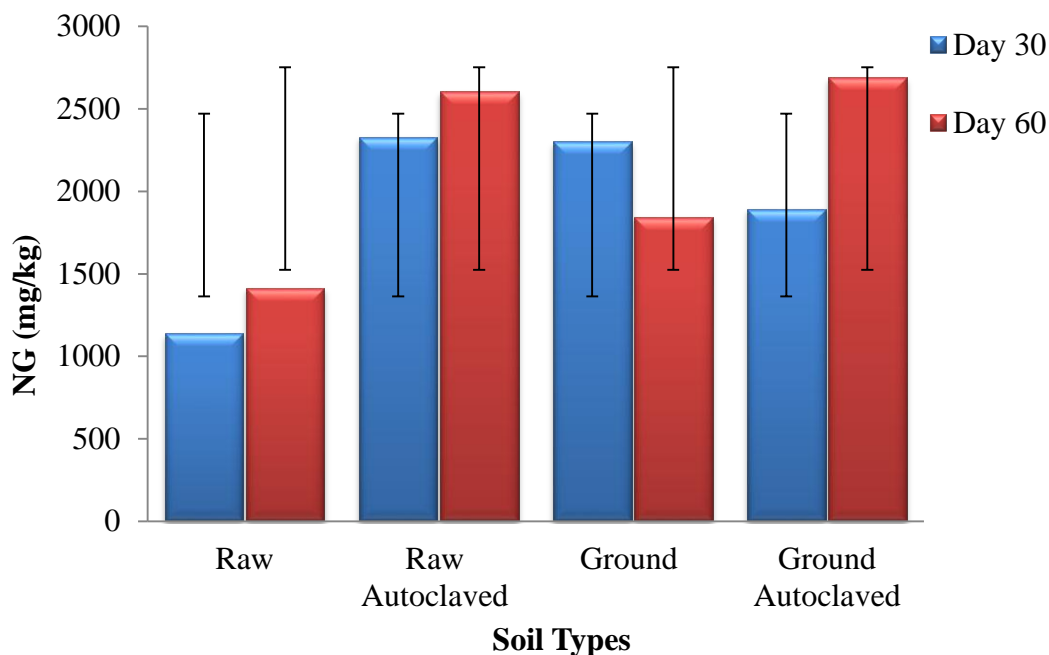


Fig. 3.20. Average NG concentrations in natural and ground soil after 30 and 60 days incubation for both autoclaved and non-autoclaved soil (mean \pm SD).

Column Leaching Study

Nitroglycerin leaching was substantial during the first leaching (200 ml) for both raw and ground soils (Fig. 3.21). The second leaching, however, had low NG concentrations (2.0 and 0.07 mg/l for the raw and ground soil leachates, respectively). This effect might be explained by a delay in SP decomposition followed by an eventual, greater release of NG at leaching 3.

Overall, more total NG (57%) was leached from the raw soil as compared with ground soil. The results may be due to enhanced adsorption to the fractured surfaces or by accelerated transformation processes (chemical and/or biological).

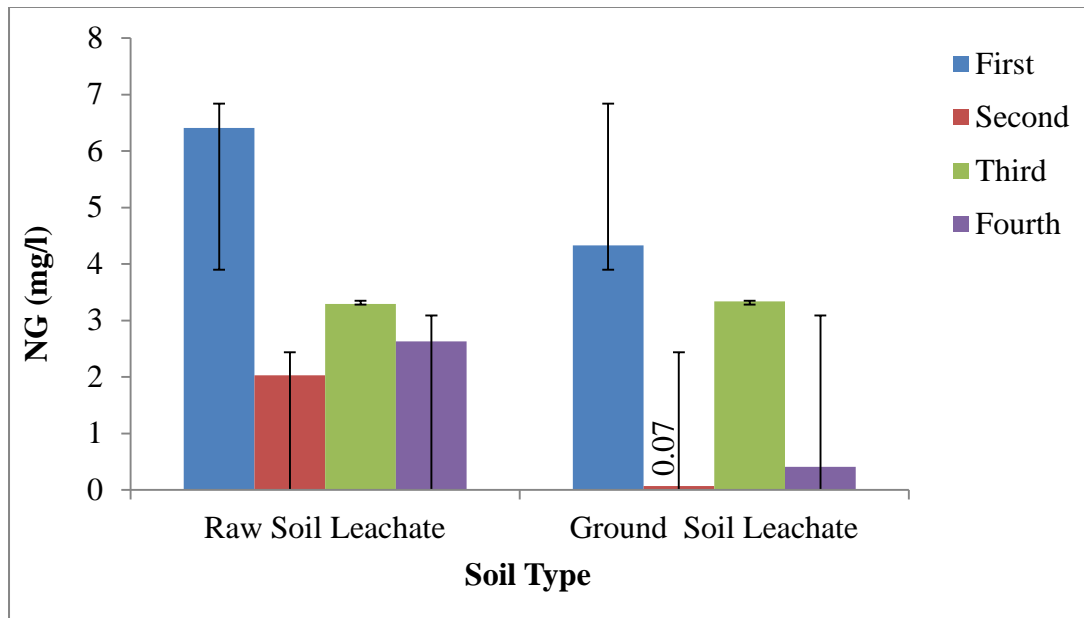


Fig. 3.21. Average NG concentrations in leachate for both natural and ground Glynnwood soils.

Numerous studies have addressed the dissolution mechanisms of energetic compounds in soil; many of these, have addressed dissolution of individual explosive and propellant formulations (Douglas et al., 2010; Taylor et al., 2009; Ro et al., 1996; Brannon et al., 1992; Verschueren, 1983; Spanggord, 1983). Results may have limited applicability for dissolution of residues on soils at impact zones or firing ranges because propellants are typically formulated with binders, waxes, stabilizers, and other compounds during manufacture. Binders and waxes decrease dissolution rates of individual explosive compounds (Dontsova et al., 2009; Phelan et al., 2002; Lynch et al., 2002)

The current study demonstrates that dissolution may proceed more slowly than predicted on the basis of solubility of the pure compound (Pennington et al., 2006).

Photolytic reactions

Over 96 h the concentration of NG in solution declined by 31%, i.e., from 1.0 mg/l to 0.72 mg/l (Fig. 3.22). During the first 8 h decomposition was negligible.

No documentation of NG decomposition via photolysis has been located in the published literature.

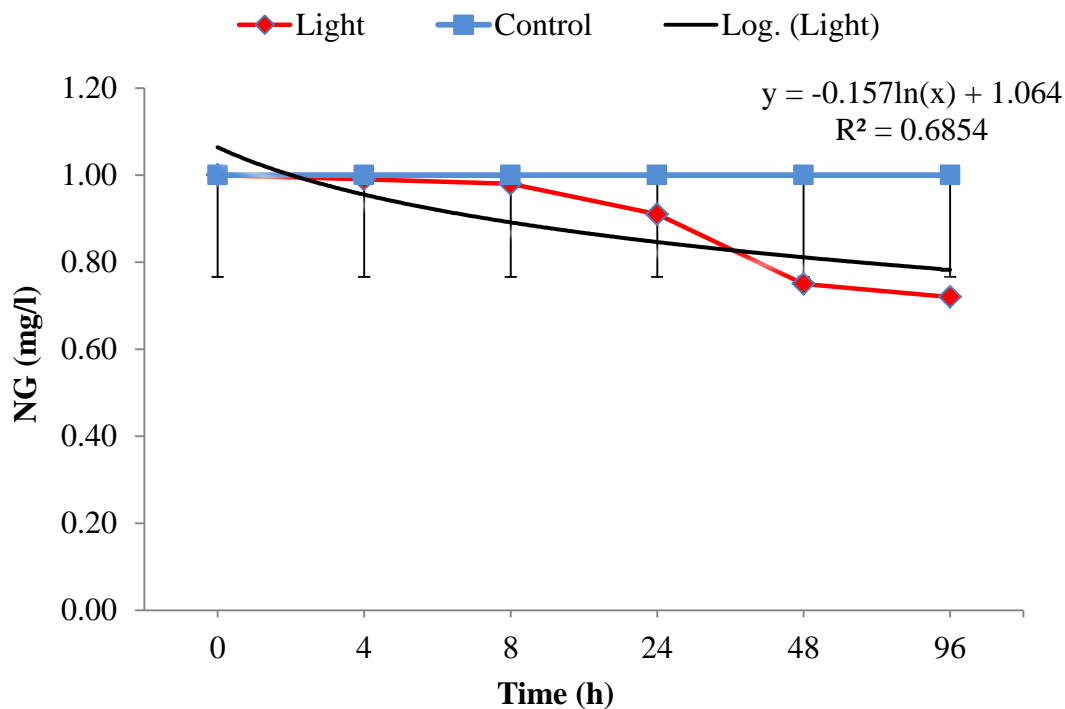


Fig. 3.22. Average NG concentration in solution as affected by exposure to artificial light.

Photolysis has been identified as one of the major processes affecting the transformation of energetic compounds in waste streams and surface water bodies (McGrath, 1995). Alteration of an energetic molecule may occur as a result of direct absorption of light energy as influenced by wavelength and intensity or via transfer of energy from a photosensitized compound (e.g., peroxide, humic compounds) (Juhasz and Naidu, 2007; Glover and Hoffsommer, 1979).

Burlinson et al. (1973) identified 20 products of TNT photolysis from laboratory irradiation. About 45-50% of the photodecomposition products of TNT were recovered in solution with the remainder present as insoluble residues which were not identified (Burlinson et al., 1973). The rate of TNT photolysis over a 16 h period of irradiation was relatively rapid (Pennington et al, 2006b). Photolysis was faster when TNT was mixed with soil. Limited data are available to describe photolytic half-lives of other energetics. The use of ultraviolet light as a decomposition tool for the remediation of some explosives-containing wastes has been explored, typically in combination with oxidizing agents (Larson et al., 1996, Lipczynska- Kochany, 1992; Wang and Kotal, 1995).

It must be emphasized that losses by photolysis will occur in test soils only at the uppermost levels of soil (< 1 cm) (Juhasz and Naidu, 2007). Therefore, such losses will contribute only a minor amount to the total NG disappearance from soil. The only exception, however, is if the soil should be regularly tilled, thus exposing more NG to the action of sunlight.

Volatilization

At ambient temperatures (approximately 0° – 40°C) most energetic compounds exist as crystalline solids with vapor pressures ranging from 10^{-8} to 10^{-17} atm (Table 1.2) (Juhasz and Naidu, 2007; Brannon et al., 2002). Therefore, sublimation (i.e., direct transformation from solid to vapor phase) is insignificant. Likewise, few energetic compounds volatilize from the aqueous phase. Compounds with Henry's law constant (KH) values $>10^{-5}$ atm-m³/mol may volatilize from aqueous solutions. With KH values of 10^{-7} to 10^{-15} atm-m³/mol, TNT, RDX, HMX, NG, 2,4-DNT, and 2,6-DNT do not readily volatilize in the aqueous phase (Juhasz and Naidu, 2007; Brannon et al., 2002). Volatilization of NG is therefore a negligible source of disappearance in this study.

Summary and Conclusions

Phytoremediation is an inexpensive means to treat contaminated soil which overcomes some of the disadvantages of bioremediation alone.

The reported study evaluated rhizosphere-enhanced phytoremediation of NG originally present in smokeless powders in soil with the support of soil amendments (composted biosolids). Degradation of NG using two plant species (oat, *Avena sativa*, and sedge, *Carex vulpinoidea*) was compared.

From the data it can be inferred that microbial activity has been a major contributor to NG decomposition in the study soils. This was apparent by observing NG data in the soils of actively-growing oat and sedge, and in batch incubation studies (raw versus autoclaved soils). In addition, recovered soil bacterial densities remained relatively constant regardless of the rate of SP application.

Addition of CB amendment to the soils provided additional organic material, which may serve several practical purposes; first, it may sorb some free NG and eventually incorporate it into humic-like substances; second, it is a source of microbial biomass; third, it provides physical protection for soil microorganisms; finally, CB amendments are a source of both micro- and macronutrients for plants and soil microorganisms.

From the data, it is, unfortunately, difficult to conclude which plant species was “superior” to the other in terms of degree of NG decomposition – during the 90-day incubation process NG continued to be released from decomposing SP pellets. Future studies may investigate the fate of a single addition of nitroglycerin (i.e., without smokeless powder). However, the purpose of the current study was to better assess the fate of NG added in SP pellets in order to simulate processes in soil at an actual firing range.

Photolysis of NG in solution was substantial; a 28% decline in soluble NG concentration was noted after 96 h incubation. This is the first report of NG decomposition via photolysis. However, this effect will only be significant for surface-deposited NG. Based on published data, NG volatilization is expected to be negligible.

Recommendations for Further Study

Phytoremediation of explosive contaminants is a new field of study. The published literature provides few studies specifically devoted to remediation of NG. For better understanding of NG phytoremediation, there is a need to study its decomposition in soil as affected by various factors.

Recommendations for future studies in NG phytoremediation are as follows:

1. The standard procedure for NG extraction from soil (US EPA Method 8095; USEPA , 2007) calls for using acetonitrile. This extractant is toxic and is probably not necessary. Given the high water-solubility of NG, and given the EPA's goal of reducing hazardous waste generation (40 CFR 259), it would be worthwhile to submit to the US EPA a revised method listing concentrated ethanol as the soil extractant.
2. Use gas chromatography-mass spectrometry (GC-MS) to identify the degradation products of NG in soil and leachate samples. This procedure is necessary to assess any rate-limiting steps in the biodegradation process. Furthermore, it is important to determine whether or not any potentially toxic by-products accumulate.
3. Use high performance liquid chromatography (HPLC) as an analytical tool for the determination of extractable NG. Compare the HPLC-derived data with that of GC-ECD.
4. In order for rhizosphere-enhanced biodegradation of NG to succeed, it is necessary to determine the microbial populations responsible for the decomposition. Extensive microbial assays using PCR followed by genetic assays would be essential to fulfill this need.
5. A study can introduce individual or combinations of known hydrocarbon-degrading microorganisms in order to evaluate their performance in NG degradation.

6. Conduct a study employing a wide range of plant types (e.g., leguminous crops; prairie grasses; herbaceous weeds; young trees) for NG decomposition. Such a study would serve as an important baseline survey of potentially valuable plants and rhizospheric microorganisms.
7. Study the effect of a combination of plants in mesocosms and compare the results with single plant species. In the field, such potentially synergistic effects must be taken into account.
8. Possible future directions include determination of NG in different compartments of plants grown in NG-contaminated soil. Ouyang et al. (2005) found TNT metabolites in root cells of plants grown on TNT-enriched soils.
9. Field-scale studies, for example at firing points in artillery ranges, are essential to know with certainty the effectiveness of rhizosphere-enhance biodegradation of NG. The reported study took place under the controlled conditions of a university greenhouse. It is essential to assess the effect of oats, sedges and other potential phytoremediation species under field conditions where rainfall, temperature, solar irradiance, humidity, pest infestations and other variables come into play.
10. Assess the influence of other common (and inexpensive) soil amendments to increase plant/microbial performance in NG degradation. Some of these amendments are currently considered wastes; however, many have shown promise as a soil conditioner (e.g., powerplant fly ash, papermill sludge; (Pichtel et al., 1994). Others may include blast furnace slag and water treatment sludge.

References

- Best, E. P. H., T. Smith, F. L. Hagen, J. Dawson, and A. J. Torrey. 2008. TNT and RDX phytoremediation capacities of candidate herbaceous plants for phytoremediation of energetics on ranges. ERDC TR 08-8. U.S. Army Engineer Research and Development Center, Vicksburg, MS, USA.
- Brannon, J. M., C. B. Price, S. L. Yost, C. Hayes, J. E. Mirecki, and B. Porter. 2004. Fate and transport parameters for firing range residues. In *Distribution and Fate of Energetics on DoD Test and Training Ranges: Interim Report 4* ERDC TR-04-4. US Army Engineer Research and Development Center, Vicksburg, MS, USA.
- Brannon J. M., and J. C. Pennington. 2002. Environmental fate and transport process descriptors for explosives. ERDC/EL TR-02-10. U.S Army Engineer Research and Development Center, Vicksburg, MS, USA.
- Burlinson, N. E., L. A. Kaplan, and C. E. Adams. 1973. Photochemistry of TNT: investigation of the pink water problem. NOLTR 73-172. Naval Ordnance Laboratory, Silver Spring, MD, USA.
- Cataldo, D.A., S. Harvey, R. Fellows, R.M. Bean, and G.D. McVeety. 1989. An evaluation of the environmental fate and behavior of munitions materials (TNT, RDX) in soil and plant systems. U.S. Department of Energy. Pacific Northwest Laboratory Final report. PNL-7370 UC-402, Richland, WA, USA.
- Chang, Y.Y., Y.S. Kwon, S.Y. Kim, I.S. Lee, and B. Bae. 2004. Enhanced degradation of 2,4,6-trinitrotoluene (TNT) in a soil column planted with Indian mallow (*Abutilon avicennae*). *Journal of Bioscience and Bioengineering*. 97: 99–103.

- Christodoulatos, C., S. Bhaumik, and B. W. Brodman. 1997. Anaerobic biodegradation of nitroglycerin. *Water Research*. 31 (6): 1462-1470.
- Clausen, J. L., C. Scott, and I. Osgerby. 2011. Fate of nitroglycerin and dinitrotoluene in soil at small arms training ranges. *Soil and Sediment Contamination*. 20: 649-671.
- Clausen, J. L., C. Scott, N. Mulherin. 2010. Sorption/desorption measurements of nitroglycerin and dinitrotoluene in Camp Edwards, Massachusetts soil. ERDC/CRREL TR-10-1. Cold Regions Research and Engineering Laboratory. US Army Engineer Research and Development Center, Hanover, NH, USA.
- Code of Federal Regulations. Vol. 40 Part 259. Protection of the Environment. US Government Printing Office, Washington, DC, USA.
- Dominguez-Rosado, E., J. Pichtel, and M. Coughlin. 2004. Phytoremediation of soil contaminated with used motor oil: I. Enhanced microbial activities from laboratory and growth chamber studies. *Environmental Engineering Science*. 21:157-168.
- Douglas, T. A., M. E. Walsh, C. J. McGrath, C. A. Weiss, A. M. Jaramillo, and T. P. Trainor. 2010. Desorption of nitramine and nitroaromatic explosive residues from soils detonated under controlled conditions. *Environmental Toxicology and Chemistry*. 30 (2): 345-353.
- Douglas, T. A., M. E. Walsh, C. J. McGrath, and C. A. Weiss. 2009. Investigating the fate of nitroaromatic (TNT) and nitramine (RDX and HMX) explosives in fractured and pristine soils. *Journal of Environmental Quality*. 38 (6): 2285-2294.

- Dontsova, K. M., J. C. Pennington, C. Hayes, J. Simunek, and C. W. Williford. 2009. Dissolution and transport of 2,4-DNT and 2,6-DNT from M1 propellant in soil. *Chemosphere*.77 (4): 597-603.
- Ghanem, M., I. Hichri, A. Smigocki, A. Albacete, M. L. Fauconnier, E. Diatloff, C. Martinez-Andujar, S. Lutts, I. C. Dodd, F. Pérez-Alfocea. 2011. Root-targeted biotechnology to mediate hormonal signalling and improve crop stress tolerance. *Plant Cell Reports* 30: 807–823.
- Glover, D. J., and J. C. Hoffsommer.1979. Photolysis of RDX. Identification and reactions of products. Technical. Report. NSWC TR-79-349. Naval Surface Weapons Center, Silver Spring, MD, USA.
- Haag, W. R., R. Spanggord, T. Mill et al. 1990. Aquatic environmental fate of nitroguanidine. *Environmental Toxicology and Chemistry*. 9 (11): 1359-1367.
- Harvey, S.D., R.J. Fellows, A. Cataldo, and R.M. Bean. 1991. Fate of the explosive hexahydro-1,3,5-trinitro-1,2,5-triazine (RDX) in soil and bioaccumulation in bush bean hydroponic plants. *Environ. Environmental Toxicology and Chemistry*.10: 845-855.
- Jenkins, T. F., J. C. Pennington, G. Ampleman et al. 2007 Characterization and fate of gun and rocket propellant residues on testing and training ranges: interim report 1. Technical Report. ERDC TR-07-01. Strategic Environmental Research and Development Program, Vicksburg, MS, USA.
- Johnson M.S., L.S. Franke, R.B. Lee, and S.D. Holladay. 1999. Bioaccumulation of 2,4,6-trinitrotoluene and polychlorinated biphenyls through two routes of

- exposure in a terrestrial amphibian: is the dermal route significant? *Environmental Toxicology and Chemistry*.18: 873–878.
- Juhasz, A. L., and R. Naidu.2007. Explosives: fate, dynamics, and ecological impact in terrestrial and marine environments. *Reviews of Environmental Contamination and Toxicology*.191: 163-215.
- Kalderis, D., A. L. Juhasz, R. Boopathy, and S. Comfort. 2011. Soils contaminated with explosives: environmental fate and evaluation of state-of-the-art remediation processes (IUPAC technical report). *Pure and Applied Chemistry*. 83 (7): 1407-1484.
- Larson, R. A., P. L. Miller, and T. O. Crowley. 1996. Borohydride photoreduction of nitroaromatic compounds related to military ordnance constituents. *Environmental Science and Technology*. 30: 1192-1197.
- Lipczynska-Kochany, E. 1992. Degradation of nitrobenzene and nitrophenols by means of advanced oxidation processes in a homogeneous phase: Photolysis in the presence of hydrogen peroxide versus the Fenton Reaction. *Chemosphere*. 24: 1369-1380.
- Lynch, J. C., J. M. Brannon, and J. J. Delfino. 2002. Effects of component interactions on the aqueous solubilities and dissolution rates of the explosive formulations octol, composition B, and LX-14. *Journal of Chemical and Engineering Data*. 47 (3): 542-549.
- McGrath, C. J. 1995. Review of formulations for processes affecting the subsurface transport of explosives. Technical Report. IRRP- 95-2. US Army Corps of Engineers, Waterways Experiment Station, Vicksburg, Miss, USA.

- National Forensic Science Technology Center. 2011. Propellants. Firearm Examiner Training. [Online]. Available at http://projects.nfstc.org/firearms/module05/firm05_t04_01.htm, Accessed 10 June 2012.
- Ouyang, Y., D. Shinde, and L. Q. Ma. 2005. Simulation of phytoremediation of a TNT-contaminated soil using the CTSPAC Model. *Journal of Environmental Quality*. 34: 1490-1496.
- Pennington, J. C., T. F. Jenkins, G. Ampleman et al. 2006a. Distribution and fate of energetics on DOD test and training ranges: final report. ERDC TR-06-13. US Army Corps of Engineers Engineer Research and Development Center, Vicksburg, MS, USA.
- Pennington, J. C., T. F. Jenkins, G. Ampleman et al. 2006b. Distribution and fate of energetics on DOD test and training ranges: interim report 6, TR 06-12. Strategic Environmental Research and Development Program. US Army Corps of Engineers Engineer Research and Development Center, Vicksburg, MS, USA.
- Pennington J. C., and W. H. Patrick Jr. 1990. Adsorption and desorption of 2,4,6-trinitrotoluene by soils. *Journal of Environmental Quality*. 19 (3): 559-567.
- Phelan, J. M., J. V. Romero, J. L. Barnett, and D. R. Parker. 2002. Solubility and dissolution kinetics of composition B explosive in water. SAND Report. SAND2002-2420. Sandia. National Laboratories, Albuquerque, NM, USA.
- Pichtel, J. 2007. *Fundamentals of Site Remediation for Metal- and Hydrocarbon-Contaminated Soils*, Second Edition. Government Institutes, Inc., Rockville, MD.

- Pichtel, J., W.A. Dick, and P. Sutton. 1994. Comparison of amendments and management practices for long-term reclamation of abandoned mine lands. *Journal of Environmental Quality*. 23: 766-772.
- Price, C. B., J. M. Brannon, S. L. Yost et al. 1998. Transformation of RDX and HMX under controlled Eh/pH conditions. Technical. Report. IRRP-98-2. US Army Engineering Waterways Experimental Station, Vicksburg, MS, USA.
- Price, C. B., J. M. Brannon, and C. A. Hayes. 1997. Effect of redox potential and pH on TNT transformation in soil-water slurries. *Journal of Environmental Engineering*. 123 (10): 988-992.
- Rivera, R., V.F. Medina, S.L. Larson, and S.C. McCutcheon. 1998. Phytotreatment of TNT contaminated groundwater. *Journal of Soil Contamination*. 7 (4): 511-520.
- Ro, K. S., A. Venugopal, D. D. Adrian et al. 1996. Solubility of 2,4,6-trinitrotoluene (TNT) in water. *Journal of Chemical and Engineering Data*. 41 (4): 758-761.
- Singh, N., D. Hennecke, J. Hoerner, W. Koerdel, and A. Schaeffer. 2008. Sorption-desorption of trinitrotoluene in soils: effect of saturating metal cations. *Bulletin of Environmental Contamination and Toxicology*. 80 (5): 443-446.
- Singh, J., S. D. Comfort, L. S. Hundal, and P. J. Shea. 1998. Longterm RDX sorption and fate in soil. *Journal of Environmental Quality*. 27 (3): 572-577.
- Spanggord, R. J., R. W. Mabey, T. W. Chou, P.L. Alferness, D.S. Tse, and T. Mill. 1983. Environmental fate studies of HMX, phase II, detailed studies, final report, SRI International, Menlo Park, California, USA.
- Stevenson, F.J. 1992. *Humus Chemistry: Genesis, Composition, Reactions*. John Wiley and Sons, New York, NY.

- Sullivan, J. H., H. D. Puttman, M. A. Keirn, B. C. Pruitt, J.C. Nichols, and J. T. McClave. 1979. A summary and evaluation of aquatic environmental in relation to establishing water quality criteria for munitions unique compounds: Part 2. Nitroglycerin, Water and Air Research. US Army Medical Research and Development Command, Gainesville, FL, USA.
- Taylor, S., J. H. Lever, J. Fadden, N. Perron, and B. Packer. 2009. Simulated rainfall-driven dissolution of TNT, Tritonal, Comp B and Octol particles. *Chemosphere*.75 (8): 1074-1081.
- Thorn, K. A., J. C. Pennington, and C. A. 2002. Hayes, 15N NMR investigation of the reduction and binding of TNT in an aerobic bench scale reactor simulating windrow composting. *Environmental Science and Technology*. 36 (17): 3797-3805.
- Thompson, P.L., L.A. Ramer, and J.L. Schnoor. 1998. Uptake and transformation of TNT by hybrid poplar trees. *Environmental Science and Technology*. 32: 975–980.
- U.S. Environmental Protection Agency, 2007. Method 8095. Explosives by gas chromatography. [Online]. Available at <http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/8095.pdf>, Accessed 15 June 2012.
- Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemical. 2nd edition. D. Van Nostrand Reinhold Company, New York, NY, USA.
- Walsh, M. R., M. E. Walsh, A. D. Hewitt, and C. M. Collins.2008. Field-expedient disposal of excess artillery propellants, in Proceedings of the SERDP & ESTCP's

Partners in Environmental Technology Technical Symposium and Workshop,
Washington, DC, USA.

Walsh, M. E., T. F. Jenkins, P. S. Schnitker, J. W. Elwell, and M. H. Stutz. 1993.

Evaluation of SW846 Method 8330 for characterization of sites contaminated
with residues of high explosives. CRREL Report 93-5. US Cold Regions
Research and Engineering Laboratory, Hanover, NH, USA.

Wang, Z., and C. Kotal. 1995. Photocatalytic mineralization of 2,4,6-trinitrotoluene in
aqueous suspensions of titanium dioxide. *Chemosphere*.

30: 1125-1136.

Western Powders, Inc. 2010. Material Safety Data Sheet. Ramshot Double-base

Smokeless Powders. Miles City, MT. [Online]. Available at

<http://www.ramshot.com/wp-content/uploads/2010/12/msds1.pdf>, Accessed 09
June 2012.

Western Powders, Inc. 2007. Material Safety Data Sheet. Accurate Double-base

smokeless powder, propellant. Miles City, MT. Online]. Available at

<http://207.65.1.181/data/AccurateDoubleBasePropellant.pdf>, Accessed 09 June
2012.

Weyens, N., D. van der Lelie, S. Taghavi, L. Newman, J. Vangronsveld. 2009a.

Exploiting plant–microbe partnerships to improve biomass production and
remediation. *Trends in Biotechnology* 27: 591–598.

Weyens, N., D. van der Lelie, S. Taghavi, J. Vangronsveld. 2009b. Phytoremediation:

plant–endophyte partnerships take the challenge. *Current Opinion in
Biotechnology* 20: 248–254.

Windholz. M. 1979. The Merck Index, 9th edition. Merck and Company, Rahway, NJ, USA.

Yamamoto, H., M. C. Morley, G. E. Speitel, and J. Clausen. 2004. Fate and transport of high explosives in a sandy soil: adsorption and desorption. *Soil and Sediment Contamination*. 13 (5): 459-477.

Yoon, J.M., B-T. Oh, C.L. Just, and J.L. Schnoor. 2002. Uptake and leaching of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine by hybrid poplar trees. *Environmental Science and Technology*. 36: 4649-4655.

APPENDICES

Appendix A. Average NG Concentrations for Leachate Samples.

Appendix B. Pairwise Comparisons Test Results (P-Values) for Soil Samples on Day7.

Appendix C. Pairwise Comparisons Test Results (P-Values) for Soil Samples on Day14.

Appendix D. Pairwise Comparisons Test Results (P-Values) for Soil Samples on Day 30.

Appendix E. Pairwise Comparisons Test Results (P-Values) for Soil Samples on Day 60.

Appendix F. Pairwise Comparisons Test Results (P-Values) for Leachate Samples on Day 14.

Appendix G. Pairwise Comparisons Test Results (P-Values) for Leachate Samples on Day 30.

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Appendix I. Batch Study Results.

Appendix J. Column Study Results.

Appendix K. Pairwise Comparisons Test Results (P-Values) for Plant Tissue Samples.

Appendix L. Mann-Whitney Test Results (P-Values) for Plant Tissue Samples.

Appendix M. Average and standard deviation for Plant Biomass.

Appendix A: Average NG Concentrations for Leachate Samples

Table A1. Average NG concentrations for the 10% SP rate in leachate samples.

Sampling Dates	Oat	Oat-CB	Sedge	Sedge-CB	Control	Control-CB
	mg/l					
7	123.3	46.4	58.0	38.8	103.8	23.8
14	101.7+29.1	33.3+2.0	50.8+21.3	91.5+36.5	90.8+38.1	37.1+15.4
30	144.9+82.8	104.2+17.7	32.5+12.0	28.8+3.3	101.7+44.1	142.2+28.5
60	97.7+45.9	100.2+27.2	44.7+17.7	75.3+26.4	159.6+45.7	92.0+24.6

Appendix B: Pairwise Comparisons Test Results (P-Values) for Soil Samples on Day7.

Table B1. Pairwise Comparisons Test between plant treatments.

Compared Plant Treatments	P-Value
None and Oats	<0.05
None and Sedges	<0.01
Oats and Sedges	0.249

Table B2. Pairwise Comparisons Test between Soil Treatments.

Compared Soil Treatments	P-Value
Biosolids and None	0.945

Table B3. Pairwise Comparisons Test between SP rates.

SP Rate (g)	P-Value
0g and 10g	0.238
0g and 50g	< 0.01
0g and 100g	< 0.01
10g and 50g	< 0.01
10g and 100g	< 0.01
50g and 100g	< 0.01

Table B4. Pairwise Comparisons Test between plant treatments at different SP rates.

SP Rate (g)	Plant Treatment	P-Value
0	None and Oats	1.000
	None and Sedges	1.000
	Oats and Sedges	1.000
10	None and Oats	0.950
	None and Sedges	0.880
	Oats and Sedges	0.831
50	None and Oats	<0.01
	None and Sedges	<0.01
	Oats and Sedges	0.575
100	None and Oats	0.260
	None and Sedges	<0.01
	Oats and Sedges	0.126

Table B5. Pairwise Comparisons Test between soil treatments at different SP rates.

SP Rate (g)	Soil Treatment	P-Value
0	None and CB	1.000
10	None and CB	0.734
50	None and CB	1.000
100	None and CB	0.633

Table B6. Pairwise Comparisons Test between soil treatments and plant treatment at different SP rates.

SP Rate (g)	Soil Treatment	Plant Treatment	P-Value
0	CB	None and Oats	1.000
		None and Sedges	1.000
		Oats and Sedges	1.000
	None	None and Oats	1.000
		None and Sedges	1.000
		Oats and Sedges	1.000
10	CB	None and Oats	0.871
		None and Sedges	0.937
		Oats and Sedges	0.809
	None	None and Oats	0.941
		None and Sedges	0.893
		Oats and Sedges	0.952
50	CB	None and Oats	0.384
		None and Sedges	0.052
		Oats and Sedges	0.275
	None	None and Oats	<0.01
		None and Sedges	<0.01
		Oats and Sedges	0.761
100	CB	None and Oats	<0.05
		None and Sedges	<0.01
		Oats and Sedges	0.520
	None	None and Oats	0.640
		None and Sedges	0.287
		Oats and Sedges	0.128

Appendix C: Pairwise Comparisons Test Results (P-Values) for Soil Samples on Day14.

Table C1. Pairwise Comparisons Test between plant treatments.

Compared Plant Treatments	P-Value
None and Oats	<0.05
None and Sedges	0.699
Oats and Sedges	0.093

Table C2. Pairwise Comparisons Test between Soil Treatments.

Compared Soil Treatments	P-Value
Biosilids and None	0.137

Table C3. Pairwise Comparisons Test between SP rates.

SP Rate (g)	P-Value
0g and 10g	0.448
0g and 50g	< 0.01
0g and 100g	< 0.01
10g and 50g	< 0.01
10g and 100g	< 0.01
50g and 100g	< 0.01

Table C4. Pairwise Comparisons Test between plant treatments at different SP rates.

SP Rate (g)	Plant Treatment	P-Value
0	None and Oats	1.000
	None and Sedges	1.000
	Oats and Sedges	1.000
10	None and Oats	0.828
	None and Sedges	0.929
	Oats and Sedges	0.898
50	None and Oats	0.805
	None and Sedges	0.818
	Oats and Sedges	0.634
100	None and Oats	<0.01
	None and Sedges	0.362
	Oats and Sedges	<0.01

Table C5. Pairwise Comparisons Test between soil treatments at different SP rates.

SP Rate (g)	Soil Treatment	P-Value
0	None and CB	1.000
10	None and CB	0.974
50	None and CB	0.457
100	None and CB	<0.05

Table C6. Pairwise Comparisons Test between soil treatments and plant treatment at different SP rates.

SP Rate (g)	Soil Treatment	Plant Treatment	P-Value
0	CB	None and Oats	1.000
		None and Sedges	1.000
		Oats and Sedges	1.000
	None	None and Oats	1.000
		None and Sedges	1.000
		Oats and Sedges	1.000
10	CB	None and Oats	0.800
		None and Sedges	0.882
		Oats and Sedges	0.916
	None	None and Oats	0.957
		None and Sedges	0.982
		Oats and Sedges	0.939
50	CB	None and Oats	0.641
		None and Sedges	0.971
		Oats and Sedges	0.667
	None	None and Oats	0.907
		None and Sedges	0.718
		Oats and Sedges	0.807
100	CB	None and Oats	0.140
		None and Sedges	0.573
		Oats and Sedges	0.357
	None	None and Oats	<0.01
		None and Sedges	0.467
		Oats and Sedges	<0.01

Appendix D: Pairwise Comparisons Test Results (P-Values) for Soil Samples on Day 30.

Table D1. Pairwise Comparisons Test between plant treatments.

Compared Plant Treatments	P-Value
None and Oats	<0.05
None and Sedges	0.200
Oats and Sedges	<0.01

Table D2. Pairwise Comparisons Test between Soil Treatments.

Compared Soil Treatments	P-Value
Biosilids and None	0.129

Table D3. Pairwise Comparisons Test between SP rates.

SP Rate (g)	P-Value
0g and 10g	0.334
0g and 50g	< 0.01
0g and 100g	< 0.01
10g and 50g	< 0.01
10g and 100g	< 0.01
50g and 100g	< 0.01

Table D4. Pairwise Comparisons Test between plant treatments at different SP rates.

SP Rate (g)	Plant Treatment	P-Value
0	None and Oats	1.000
	None and Sedges	1.000
	Oats and Sedges	1.000
10	None and Oats	0.550
	None and Sedges	0.963
	Oats and Sedges	0.582
50	None and Oats	0.527
	None and Sedges	0.539
	Oats and Sedges	0.986
100	None and Oats	<0.01
	None and Sedges	<0.01
	Oats and Sedges	<0.01

Table D5. Pairwise Comparisons Test between soil treatments at different SP rates.

SP Rate (g)	Soil Treatment	P-Value
0	None and CB	1.000
10	None and CB	0.543
50	None and CB	0.676
100	None and CB	<0.01

Table D6. Pairwise Comparisons Test between soil treatments and plant treatment at different SP rates.

SP Rate (g)	Soil Treatment	Plant Treatment	P-Value
0	CB	None and Oats	1.000
		None and Sedges	1.000
		Oats and Sedges	1.000
	None	None and Oats	1.000
		None and Sedges	1.000
		Oats and Sedges	1.000
10	CB	None and Oats	0.466
		None and Sedges	0.930
		Oats and Sedges	0.521
	None	None and Oats	0.908
		None and Sedges	0.982
		Oats and Sedges	0.890
50	CB	None and Oats	0.965
		None and Sedges	0.325
		Oats and Sedges	0.304
	None	None and Oats	0.349
		None and Sedges	0.907
		Oats and Sedges	0.293
100	CB	None and Oats	0.266
		None and Sedges	0.094
		Oats and Sedges	<0.01
	None	None and Oats	<0.01
		None and Sedges	<0.01
		Oats and Sedges	<0.01

Appendix E: Pairwise Comparisons Test Results (P-Values) for Soil Samples on Day 60.

Table E1. Pairwise Comparisons Test between plant treatments.

Compared Plant Treatments	P-Value
None and Oats	0.217
None and Sedges	0.704
Oats and Sedges	0.108

Table E2. Pairwise Comparisons Test between Soil Treatments.

Compared Soil Treatments	P-Value
Biosolids and None	0.119

Table E3. Pairwise Comparisons Test between SP rates.

SP Rate (g)	P-Value
0g and 10g	0.331
0g and 50g	< 0.01
0g and 100g	< 0.01
10g and 50g	< 0.01
10g and 100g	< 0.01
50g and 100g	< 0.01

Table E4. Pairwise Comparisons Test between plant treatments at different SP rates.

SP Rate (g)	Plant Treatment	P-Value
0	None and Oats	1.000
	None and Sedges	1.000
	Oats and Sedges	1.000
10	None and Oats	0.666
	None and Sedges	0.807
	Oats and Sedges	0.851
50	None and Oats	0.848
	None and Sedges	0.274
	Oats and Sedges	0.366
100	None and Oats	<0.05
	None and Sedges	<0.05
	Oats and Sedges	<0.05

Table E5. Pairwise Comparisons Test between soil treatments at different SP rates.

SP Rate (g)	Soil Treatment	P-Value
0	None and CB	1.000
10	None and CB	0.899
50	None and CB	0.451
100	None and CB	<0.05

Table E6. Pairwise Comparisons Test between soil treatments and plant treatment at different SP rates.

SP Rate (g)	Soil Treatment	Plant Treatment	P-Value
0	CB	None and Oats	1.000
		None and Sedges	1.000
		Oats and Sedges	1.000
	None	None and Oats	1.000
		None and Sedges	1.000
		Oats and Sedges	1.000
10	CB	None and Oats	0.537
		None and Sedges	0.935
		Oats and Sedges	0.592
	None	None and Oats	0.995
		None and Sedges	0.792
		Oats and Sedges	0.787
50	CB	None and Oats	0.714
		None and Sedges	0.729
		Oats and Sedges	0.477
	None	None and Oats	0.524
		None and Sedges	0.230
		Oats and Sedges	0.570
100	CB	None and Oats	0.255
		None and Sedges	0.700
		Oats and Sedges	0.450
	None	None and Oats	<0.01
		None and Sedges	0.603
		Oats and Sedges	<0.01

Appendix F: Pairwise Comparisons Test Results (P-Values) for Leachate Samples on Day 14.

Table F1. Pairwise Comparisons Test results between plant treatments.

Compared Plant Treatments	P-Value
None and Oats	0.809
None and Sedges	0.607
Oats and Sedges	0.800

Table F2. Pairwise Comparisons Test results between soil treatments.

Compared Soil Treatments	P-Value
Biosolids and None	<0.05

Table F3. Pairwise Comparisons Test results between soil treatments and plant treatments at 10% SP rate.

SP Rate (g)	Soil Treatment	Plant Treatment	P-Value
100	CB	None and Oats	0.859
		None and Sedges	<0.05
		Oats and Sedges	<0.05
	None	None and Oats	0.585
		None and Sedges	0.057
		Oats and Sedges	<0.05

Appendix G: Pairwise Comparisons Test Results (P-Values) for Leachate Samples on Day 30.

Table G1. Pairwise Comparisons Test results between plant treatments.

Compared Plant Treatments	P-Value
None and Oats	0.900
None and Sedges	<0.01
Oats and Sedges	<0.01

Table G2. Pairwise Comparisons Test results between soil treatments.

Compared Soil Treatments	P-Value
Biosolids and None	0.937

Table G3. Pairwise Comparisons Test results between soil treatments and plant treatments at 10% SP rate.

SP Rate (g)	Soil Treatment	Plant Treatment	P-Value
100	CB	None and Oats	0.207
		None and Sedges	<0.01
		Oats and Sedges	<0.05
	None	None and Oats	0.154
		None and Sedges	<0.05
		Oats and Sedges	<0.01

Appendix H: Pairwise Comparisons Test Results (P-Values) for Leachate Samples on Day 60.

Table H1. Pairwise Comparisons Test results between plant treatments.

Compared Plant Treatments	P-Value
None and Oats	0.127
None and Sedges	<0.01
Oats and Sedges	<0.05

Table H2. Pairwise Comparisons Test results between soil treatments.

Compared Soil Treatments	P-Value
Biosolids and None	0.405

Table H3. Pairwise Comparisons Test results between soil treatments and plant treatments at 10% SP rate.

SP Rate (g)	Soil Treatment	Plant Treatment	P-Value
100	CB	None and Oats	0.723
		None and Sedges	0.473
		Oats and Sedges	0.289
	None	None and Oats	<0.05
		None and Sedges	<0.01
		Oats and Sedges	<0.05

Appendix I. Batch Study Results.

Table II. Average NG concentrations in leachate for both natural and ground Glynwood soils

	Autoclaved Natural Glynwood Soil	Autoclaved Ground Glynwood Soil	Non-Autoclaved Natural Glynwood Soil	Non-Autoclaved Ground Glynwood Soil
	mg/kg			
Day 30	2330.50818	1894.10764	1141.08464	2302.46252
Day 60	2603.11575	2688.767375	1413.40602	1844.72978

Appendix J. Column Study Results.

Table J1. Average NG concentrations in Natural Soil and Ground soil for Autoclaved and Non- Autoclaved Soil after Incubation in 30 and 60 Days.

Sampling Date	Natural Glynwood leachate	Ground Glynwood leachate
	mg/L	
3/22/2012	6.41	4.33
3/29/2012	2.03	0..07
4/16/2012	3.29	3.34
4/25/2012	2.63	0.41

Appendix K. Pairwise Comparisons Test Results.

Table K1. Pairwise Comparisons Test results between SP rates for plant tissue samples.

SP rate (g)	P-Value
0 and 10	0.074
0 and 50	<0.05
0 and 100	<0.05
10 and 50	0.53
10 and 100	<0.05
50 and 100	<0.05

Appendix L. Mann-Whitney Test Results.

Table L1. Mann-Whitney Test Results for plant tissue samples between plant treatments and soil treatments at different SP rates.

SP Rate (g)	Soil Treatment	Plant Treatment	P-Value
0	CB	Oats and Sedges	1.000
	None	Oats and Sedges	1.000
10	CB	Oats and Sedges	0.064
	None	Oats and Sedges	0.248
50	CB	Oats and Sedges	0.480
	None	Oats and Sedges	0.564
100	CB	Oats and Sedges	No Data
	None	Oats and Sedges	1.000

Appendix M. Average and standard deviation for Plant Biomass.

Table M1. Average and standard deviation for Plant Biomass.

SP rate (%)	Oat	Oat-CB	Sedge	Sedge-CB
	g			
0	8.33±1.8	9.57±1.4	29.93±8.0	20.86
1	4.85±2.0	3.995±3.3	8.075±4.7	13.43±4.8
5	2.23±1.5	1.0±0.5	8.78±6.5	13.895±0.3
10	1.4±0.7	0	14.45±1.6	18.14±2.1