

Phytoremediation of nitroglycerin in smokeless powders

Navid Asbaghi, John Pichtel

Ball State University, Natural Resources and Environmental Management, Muncie, IN 47306, U.S.A.

Corresponding author: John Pichtel, Voice: 765 285 2182, Fax: 765 285 2606, E-mail: jpichtel@bsu.edu

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ABSTRACT

The reported study evaluated the feasibility of rhizosphere-enhanced phytoremediation in the removal of nitroglycerin (NG), as applied in commercial smokeless powder (SP), from soil. Double base smokeless powder was applied to soil mesocosms at rates of 0, 1, 5 and 10% (w/w). The mesocosms were seeded with oats (*Avena sativa*) or planted with live sedge plants (*Carex vulpinoidea*). Composted biosolids (20% w/w) were used as a soil treatment. Mesocosms were sampled at 7, 14, 30, and 60 days after initial planting. Determination of residual soil NG was performed using gas chromatography with an electron capture

detector. Both plant species were capable of modest NG uptake (146.0 and 87.5mg·kg⁻¹ for sedge and oat, respectively at the 10% SP rate). Only modest quantities of NG removal were accounted for by abiotic processes such as soil sorption. Soil bacterial numbers remained relatively constant regardless of rate of SP application. Microbial activity in the plant rhizosphere was concluded to be the major contributor to NG solubilization and decomposition. Addition of composted biosolids to soil imparted a positive effect in NG decomposition and/or removal from soil. Additional study is needed to determine long-term decomposition of smokeless powder and subsequent NG reactions in soil.

INTRODUCTION

Nitroglycerin (NG; glycerol trinitrate; C₃H₅N₃O₉) is commonly employed by military forces as an ingredient in artillery and rocket propellants (Accashian et al. 1998). In private application (e.g., firearms enthusiasts) NG is a component of smokeless powders (Ahlner et al. 1991; Halasz 2010). Nitroglycerin is additionally known to possess medicinal benefits – it is used for treatment of high blood pressure and heart disease (Harvard 1997).

Nitroglycerin has been documented as a significant contaminant of soil, surface water, and groundwater resulting from both military conflict and munitions manufacturing and testing (Jenkins et al. 2001, 2002; Pennington et al. 2001, 2002, 2003). A range of chemical, physical and biological methods have been applied for remediation of NG and other

so-called energetic materials in soil and groundwater, including sorption to activated carbon, reduction with inorganic chemicals, Fenton reaction, alkaline hydrolysis and bioremediation (Accashian et al. 1998; Kalderis et al. 2011). In the United States, environmentally-friendly remediation technologies such as bioremediation are encouraged by federal and state agencies (USEPA 1993a, b).

Degradation of NG can occur under both aerobic and anaerobic conditions using mixed or pure strains of bacterial species (Marshall and White 2001; Meng et al. 1995; Pesari and Grasso 1993; Wendt et al. 1978; White et al. 1996). Recent research has revealed the existence of NG-degrading bacteria in activated sewage sludge, river water, and soils (Wendt et al. 1978; White et al. 1995; Zhang et al. 1997). Bacteria are capable of utilizing NG as a sole source of nitrogen (Binks et al. 1996; Blehert 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). Aerobic microbial cultures have been shown to have the capacity to remove NG rapidly in the absence of a supplemental carbon source.

Bioremediation 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 (van Veen et al. 1997). Phytoremediation (i.e., the use of green plants to treat soil contamination) is an inexpensive technology which may overcome some of the disadvantages of bioremediation. Phytoremediation is suited for large contaminated areas and requires relatively low maintenance (Frick et al. 1999). The 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 (Pichtel 2007).

For a better understanding of NG phytoremediation, there is a need to assess its decomposition in soil as affected by various factors. In addition, although limited work has been documented regarding the decomposition of NG in soil, little is known with respect to the reactions of smokeless powder and its consequent release of NG. In the reported study, the feasibility of phytoremediation was assessed for the removal of NG in smokeless powder from soils. Specifically, the objectives were to: (1) compare the efficiency of smokeless- powder-derived NG dissolution and decomposition in the rhizospheres of two plant species which require divergent nutrient and moisture regimes; and (2) assess the potential of composted biosolids mixed with soil to enhance degradation of applied NG.

MATERIAL AND METHODS

Characterization of soil, biosolids and smokeless powder

Glynwood soil (Fine, illitic, mesic Aquic Hapludalf) was collected from the top 30cm from an agricultural field. The soil material was composited, air-dried, gently ground with an agate mortar and pestle, and sieved through a 2.0mm mesh sieve. Composted sewage sludge (biosolids) (CB) was used as a soil treatment and was obtained from the Southwesterly Compost Facility, Columbus, OH.

For the soil and CB amendment, pH was measured using a standardized AB15 Accumet pH meter on a 1:2 solids:deionized H₂O slurry. Total organic carbon (TOC) and total nitrogen (N) were analyzed on a Perkin Elmer Series II CHNS/O Analyzer 2400 (Shelton, CT). Acetanilide, Lot #MKAA0338, was the standard used.

Nitrate was measured using Szechrome reagents (Polysciences, no date) in a BioTeK PowerWave XS2 microassay system

(Winooski, VT). 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 et al. 1982). Phosphorous was measured using Bray-1 extractant combined with a microplate method (PowerWave XS2 Microplate Spectrophotometer) (Olsen and Sommers 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 (0.05M) for 2h on an oscillating shaker. The mixtures were filtered through Whatman no. 2 filter paper and analyzed using FAAS (Sposito et al. 1982).

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

Nitroglycerin incubation

Plastic pots measuring 12.5cm tall by 20cm diameter were filled with 1kg air-dried soil. Half the pots contained soil only and the remainder contained soil plus CB in an 80:20 (air-dry, w/w) mixture. Experimental pots ($n=96$) were amended with 0, 1, 5 and 10% (w/w) double base smokeless powder (SP). The SP was mixed manually with soil 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. Experimental treatments not planted to either species are hereafter referred to as "control" and "control-CB". Both aerobic and anaerobic controls, i.e., without vegetation, were prepared. The experimental design was a split-split plot with four replicates of each treatment. Plants were cultivated in the greenhouse and watered twice weekly with tap water. Moisture content of the oat treatments was maintained at 50% of field capacity; sedge treatments were maintained in a continuously wet state using closed pots. Day/night temperatures were set at 22°C/18°C, respectively.

Soil samples were collected after 7, 14, 30, and 60 days of incubation. Samples were collected from the root zone using a stainless steel rod, placed immediately in plastic bags, and brought to the laboratory. Five grams of each sample were extracted by shaking with 25ml 92% ethanol for 30min on a reciprocating shaker. The soil suspensions were filtered using Whatman no. 2 filter paper and stored at 4°C until analysis.

Samples (5g each, four replicates) of raw smokeless powder were extracted using the procedure above, and measured for total NG concentration.

Gas chromatographic (GC) analysis of soil, SP extracts, and raw SP 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 6m Perkin Elmer fused silica capillary column measuring 0.53mm ID with a 1.5 μ m film thickness. Samples measuring 1 μ l were injected into the column. The GC oven was temperature-programmed as follows: 130°C for 1min, 10°C·min⁻¹ ramp to 160°C, 30°C·min⁻¹ ramp to 285°C hold for 1min. The carrier gas was helium at a 7.0ml·min⁻¹ flow rate. The ECD temperature was set to 300°C and the makeup gas was nitrogen at a 30ml·min⁻¹ flow rate. A 1000mg·l⁻¹ nitroglycerin standard in ethanol was obtained from AccuStandard, Inc., New Haven, CT. The TotalChrom™ Navigator Application (v. 6.3) (Perkin Elmer, Shelton, CT) was used to process, record and report the chromatographic results.

Plant response and uptake of NG

After 60days incubation plants were harvested by clipping 1-2cm above ground surface and the fresh weight measured. To determine NG uptake, aliquots of fresh harvested plants were placed in a freezer until analysis. Five grams of tissue were extracted with 25ml 92% ethanol, ground in a commercial blender, filtered through Whatman no. 2 filter paper, and analyzed by GC-ECD as described above.

Column leaching

PVC columns measuring 30cm tall with a 3.2cm inner diameter were packed with a mixture of Glynwood soil and washed sand. In one set of treatments, raw 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 0.84mm 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 5-7cm of each column. A Masterflex™ peristaltic pump was used to pass deionized H₂O through the columns at a rate of 1ml·h⁻¹. Leachates were collected in Nalgene® plastic bottles and stored at 4°C until analysis. The leachates were analyzed for NG with GC-ECD as described above.

Batch adsorption

Twenty samples of Glynwood soil and sand in a 1:1 ratio were prepared for an adsorption study. Ten samples were studied in a raw (untreated) condition and the other ten were autoclaved. Of the ten raw soils, half were ground to pass through a 0.84mm mesh sieve as described in the previous section. The remaining ten soil:sand mixtures (half as raw solids and half as ground) were autoclaved using a Tomy autoclave SS-325E at 121°C for 20 minutes.

Samples were placed into beakers and moistened to field capacity with deionized H₂O. Smokeless powder was added at a rate of 0.1% (w/w) and mixed with the soil/sand

mixtures. Samples were covered and incubated in the dark at 22°C. Five grams of each sample were extracted with 25ml of 92% ethanol after 30 days. Soils were incubated for an additional 30 days and extracted with ethanol. All soil extracts were analyzed for NG concentrations on GC-ECD.

Bacterial enumeration

Populations of total bacteria were counted using the spread plate technique. Plate Count Agar was used for bacterial identification. There were four replicates of each treatment. Petri dishes were incubated at room temperature 22°C for 7 days and subsequently counted.

Statistical analysis

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 Least Significant Difference Test. SPSS™ and MS Excel were used on a Windows-based PC for all statistical analyses.

RESULTS AND DISCUSSION

Characterization of soil, biosolids and smokeless powder

The Glynwood soil was near-neutral in pH (pH 6.7) and had moderate levels of organic carbon (3.9%) and low concentrations of extractable metals (Table 1). 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

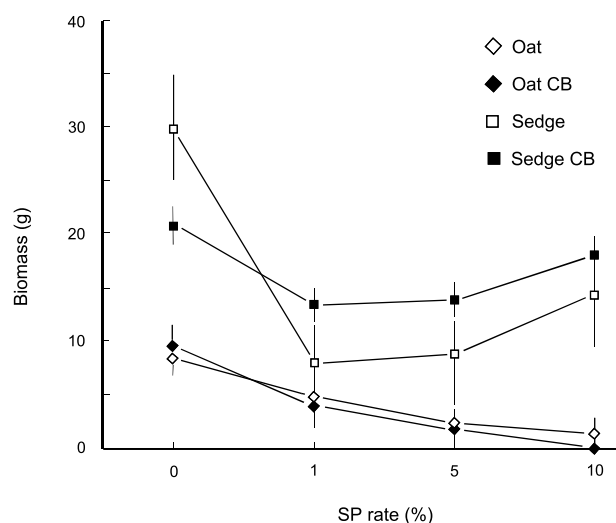


Figure 1. Oat (soil and soil plus CB (composted sewage sludge)) and sedge (soil and soil plus CB) biomass after 60 days as a function of smokeless powder (SP) application rate. Vertical bars represent standard error.

the total N was composed of NH_4^+ . Phosphate concentrations were high and concentrations of extractable metals were low (Table 2). Mean NG concentration in the raw smokeless

powder was $255,150\text{mg}\cdot\text{kg}^{-1}$ (data not shown). This value is comparable to those for other commercial smokeless powders (Western 2007, 2010).

Table 1. Selected chemical and physical properties of the soil used during the experimental trials.

Parameter	
pH	6.7
TOC* (%)	3.9
Extractable metals ($\text{mg}\cdot\text{kg}^{-1}$)	
Cd	0.26
Cr	0.09
Cu	0.22
Fe	32.4
Ni	0.31
Pb	0.50
Zn	9.80
Soluble anions ($\text{mg}\cdot\text{kg}^{-1}$)	
Cl^-	2323.7
NO_3^-	85.5
PO_4^{3-}	0.54
SO_4^{2-}	1097.8
Total N (%)	0.36
NH_4^+ ($\text{mg}\cdot\text{kg}^{-1}$)	8.2
Bray-1 P ($\text{mg}\cdot\text{kg}^{-1}$)	13
K ($\text{mg}\cdot\text{kg}^{-1}$)	86.9
Texture (%)	
Sand	28
Silt	51
Clay	21

*TOC = Total organic carbon

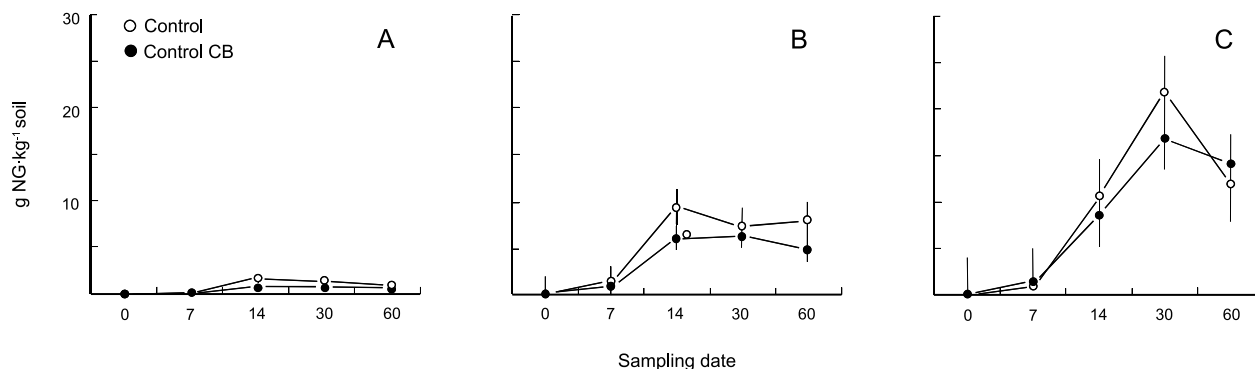


Figure 2. Soil nitroglycerin (NG) concentrations in Control (soil) and Control-CB (soil plus composted sewage sludge). (A) 1% smokeless powder (SP); (B) 5% SP; (C) 10% SP. Vertical bars represent standard error.

Plant response

Oats produced $4.9\text{g}\cdot\text{pot}^{-1}$ at the 1% SP rate but biomass was reduced to $1.4\text{g}\cdot\text{pot}^{-1}$ at the 10% SP rate (Figure 1). In many cases oat plants at the 5 and 10% SP application rates did not fully mature. Sedge plants experienced a 73% decrease in biomass production (from

29.9g to $8.1\text{g}\cdot\text{pot}^{-1}$) with addition of 1% SP (Figure 1). At the 5 and 10% SP levels sedge biomass production increased slightly, though the increases were not statistically significant ($p>0.05$). At the 10% SP rate biomass production was reduced by 50% ($14.5\text{g}\cdot\text{pot}^{-1}$) compared to the 0% SP rate.

Nitroglycerin Incubation

At Day 7 soil NG concentrations at the 1% SP rate ranged from 65-152mg·kg⁻¹, indicating minimal dissolution of SP pellets (Figure 2a). In the control (non-vegetated) pots the 1% SP rate released maximum NG at Day 14 (1336.9mg·kg⁻¹ for control and 721.1mg·kg⁻¹ for control-CB). The significantly ($p < 0.05$) lower NG concentrations in the control-CB may be a result of the higher TOC level with addition of CB amendment, as the Glynwood soil contained 3.9% TOC (Tables 1 and 2). When mixed with CB the overall soil TOC content increased to 15.4%. Jenkins et al. (2007) found that NG was retained only slightly in low organic carbon soils. In soil at small-arms ranges soil/water partitioning coefficients (K_d values) for NG ranged from 0.0 to 7.3l·kg⁻¹ (Clausen et al. 2010), and mean K_d values were 0.9l·kg⁻¹. Soil organic carbon content and other properties (e.g., cation exchange capacity) imparted a significant effect on K_d .

In the 5% SP treatments, NG concentrations were highest in the control at Day 14 (9267.1mg·kg⁻¹). In the control-CB the NG release was delayed and on Day 30 reached maximal concentrations (6181.8mg·kg⁻¹) (Figure 2b). At the 10% SP rate maximal dissolution of SP pellets did not occur until Day 30 – highest NG concentrations were 21934mg·kg⁻¹ in the control pots and 16949mg·kg⁻¹ in the control-CB (Figure 2c). Dissolution in water is the primary mechanism for NG transport and dispersion in the biosphere (Kalderis et al. 2011; Pennington et al. 2006a, b). Nitroglycerin is rather mobile in soil in part due to its moderate solubility (1,250 to 1,950mg·l⁻¹) (Sullivan et al. 1979); however, the degree to which NG is available for release is a function of the degree of deterioration of the NC encapsulation in the pellet mix (Clausen et al. 2010; Windholz 1979).

Table 2. Selected chemical properties of the CB amendment.

Parameter	
pH	7.3
TOC* (%)	57.2
Extractable metals (mg·kg ⁻¹)	
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 ⁻¹)	26.5
NH ₄ ⁺ (mg·kg ⁻¹)	1063
Bray-1 P (mg·kg ⁻¹)	799
K (mg·kg ⁻¹)	1747.2

*TOC = Total organic carbon

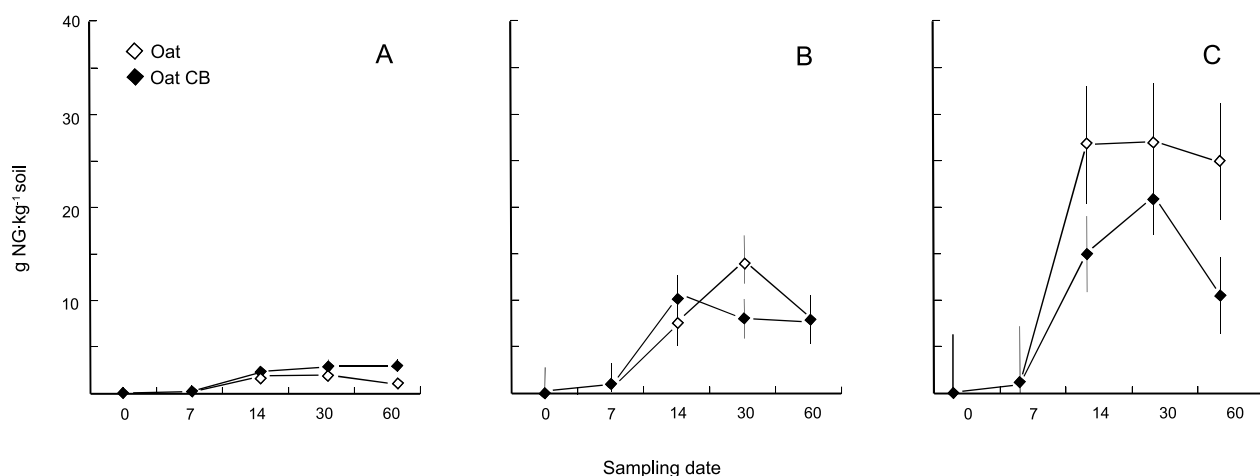


Figure 3. Soil nitroglycerin (NG) concentrations for oat treatment. (A) 1% smokeless powder (SP); (B) 5% SP; (C) 10% SP. Vertical bars represent standard error.

At the 1% SP rate soil NG concentrations were low in the oat and oat-CB, indicating limited dissolution of SP pellets (Figure 3a). Over the 60-day incubation period NG concentrations attained 2488mg·kg⁻¹ (Oat-CB, Day 30) and 1621mg·kg⁻¹ (Oat, Day 30). 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 (Figure 2 and 3). This effect may be due to the so-called rhizosphere effect (Cheng 2009), i.e., a highly diverse and active microbial population will colonize and potentially dissolve organic compounds such as SP pellets.

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 (Figure 3 and 4). 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. Decomposition of the SP formulation may be greater in pots incubated aerobically (i.e., to oats) than in anaerobic (sedge) soil pots.

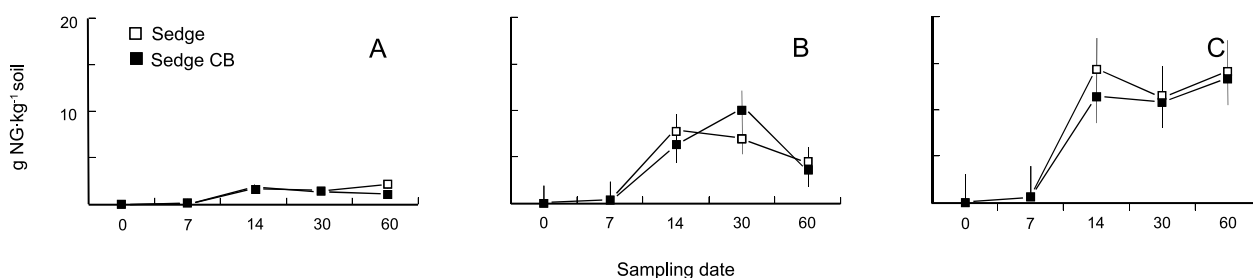


Figure 4. Soil nitroglycerin (NG) concentrations for sedge. (A) 1% smokeless powder (SP); (B) 5% SP; (C) 10% SP. Vertical bars represent standard error.

Plant uptake of NG

There was a positive correlation between SP application rate and NG content of plant tissue in all treatments (r^2 ranged from 0.77-0.99) (Figure 5). Over most SP rates sedge took up more NG than did oats. At the 10% SP rate the sedge-CB contained 146mg NG·kg⁻¹ tissue, and the sedge contained 134mg·kg⁻¹. The oat treatment contained 87.5mg·kg⁻¹. Observed values for plant uptake were, overall, a minor contribution to NG disappearance from the study soils. Mean NG concentration in the SP was 255,150 mg·kg⁻¹; therefore, the NG removal indicated in this study comprises only a small fraction ($<<1\%$) of total soil NG.

Bacterial enumeration

The mean bacterial count was numerically greater in the 10% SP compared with the 1% SP treatments (Figure 6); however, these differences were not significantly different ($p>0.05$). Total bacteria in the sedge treatment at 1% SP were $1.78 \cdot 10^6$ CFU·g⁻¹, while those at the 10% SP rate

At the 5% SP rate most treatments released maximal quantities of NG to the soil by Day 14, typically not exceeding 10861mg·kg⁻¹ (Figure 3b). By Day 60 most soil NG concentrations declined substantially. In several 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 (Figure 3b and 4b). On Day 30 mean NG in oat was 59% greater than that of sedge; on Day 60 mean NG in oat was 40% greater than that in sedge.

At the 10% SP rate the treatments released maximal quantities of NG at Day 30 (oat and oat CB) (Figure 3c). Highest NG concentrations occurred in oat at Day 30 (27,100mg·kg⁻¹). By Day 60 most soil NG concentrations had declined substantially. For comparison, control treatments did not exceed 22,000mg·kg⁻¹ over the 60-day incubation period (Figure 2c).

Biomass production in the oat and oat-CB correlated with soil NG concentrations ($r^2 = -0.74$ and -0.91 , respectively); however, there was poor correlation between soil NG and sedge and sedge CB biomass ($r^2 = -0.28$ and 0.11 , respectively).

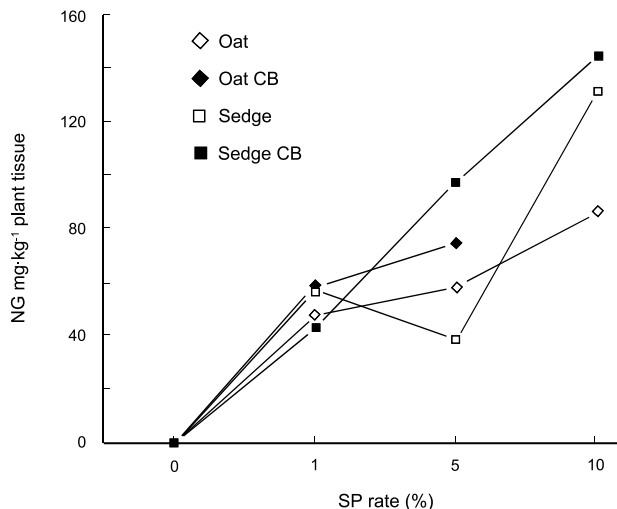


Figure 5. Uptake of nitroglycerin (NG) by oat and sedge plant treatments.

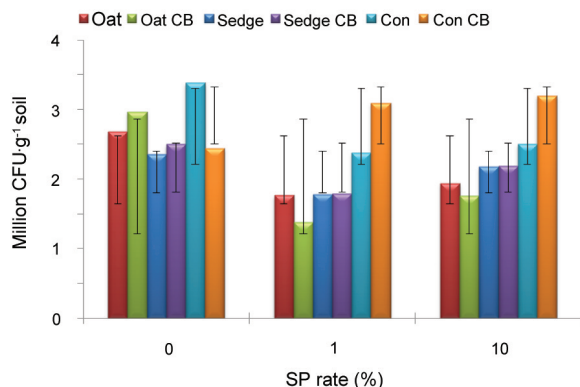


Figure 6. Number of total bacteria (CFU·g⁻¹ soil) for the soils (mean±SD) 60 days after seeding to oat (*Avena sativa*), transplanting to sedge (*Carex vulpinoidea*) and unplanted control soil (Con).

were $2.18 \cdot 10^6$ CFU·g⁻¹. These numbers are comparable to those for Midwestern US agricultural soils (Brady and Weil 2009) and indicate that, at least among those organisms that can be detected via plate count methods, soil bacteria are tolerant of SP ingredients. It should be noted that plate count agar will only give an indication of bacteria capable of growing on the agar, but not necessarily degrading NG.

Numbers of total bacteria were consistently higher in sedge-grown SP-treated soils compared with oat-grown soils (Figure 6). High numbers may have improved overall nutrient uptake by sedges (Weyens et al. 2009a, b).

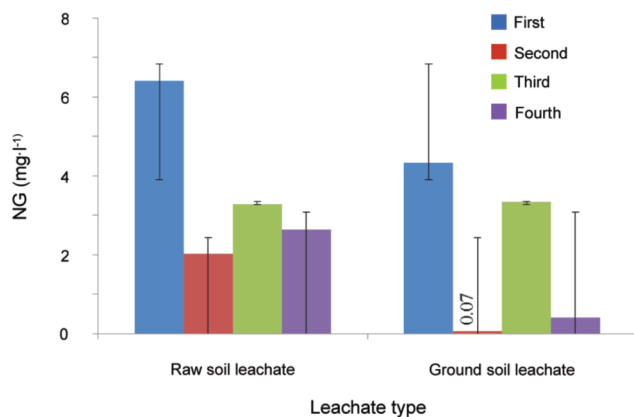


Figure 7. Average nitroglycerin (NG) concentrations in leachate for both raw and ground Glynwood soil treated by surface-applied NG in smokeless powder at a rate of 0.1% (w/w) and leached with deionized H₂O. Vertical bars represent standard error.

Column leaching

Nitroglycerin leaching was substantial during the first leaching for both raw and ground soils (Figure 7). The second leaching, however, had low NG concentrations (2.0 and 0.07mg·l⁻¹ for the raw- and ground-soil leachates, respectively). Overall, more total NG (57%) was leached from the raw soil as compared with ground soil. The results may be due to enhanced sorption to newly exposed, fractured surfaces or by accelerated transformation processes (chemical and/or biological) (Douglas et al. 2010).

Numerous studies have addressed the dissolution mechanisms of energetic compounds in soil; however, most have addressed dissolution of individual explosive and propellant formulations (Brannon et al. 1992; Ro et al. 1996; Spangord et al. 1983; Taylor et al. 2009; Verschuere 1983). Results may have limited applicability for dissolution of residues in 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; Lynch et al. 2002; Phelan et al. 2002). The current study demonstrates that dissolution may proceed more slowly than predicted on the basis of solubility of the pure compound.

Batch adsorption

The raw soil contained the lowest concentrations of NG at both Day 30 and Day 60 among other soils (1141.1 and 1413.4mg·kg⁻¹, respectively) (Figure 8). By Day 60 NG concentrations in the autoclaved soils, both raw and ground, were highest (2603.1 and 2688.8mg·kg⁻¹, respectively). These results indicate the contribution of microbial activity in decomposing soil NG.

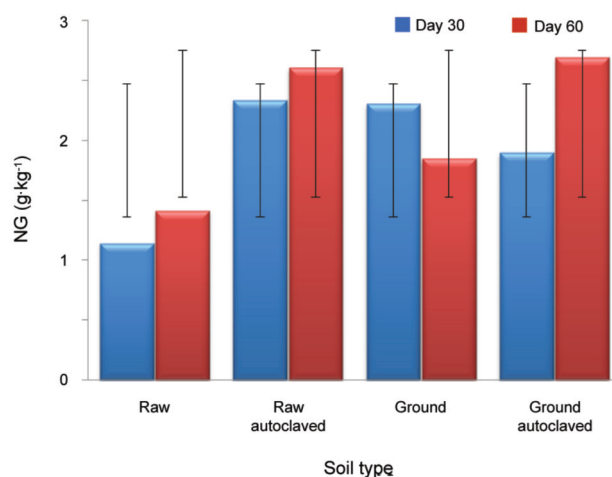


Figure 8. Average nitroglycerin (NG) concentrations in raw and ground soil after 30 and 60 days incubation for both autoclaved and non-autoclaved samples (mean±SD).

Numerous investigations of the fate of energetic compounds in soils and sediments have used weathered clays, silts, and sands in column and incubation studies (Haag et al. 1990; Price et al. 1997; Singh et al. 2008; Thorn et al. 2002). 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 increased sorption to fractured surfaces or by more rapid chemical and biological transformation of the NG.

SUMMARY AND CONCLUSIONS

In the reported study the dissolution of smokeless powder varied between non-vegetated soils and those cultivated to oat, *Avena sativa*, and sedge, *Carex vulpinoidea*. Microbial activity in the rhizosphere of oat and sedge is considered a key contributor to both solubilization and decomposition of NG released from smokeless powder. Addition of CB amendment to the soils provided additional organic material which may serve several practical purposes; first, it is a source of microbial biomass; second, it provides physical protection for soil microorganisms; finally, CB amendments are a source of both micro- and macronutrients for plants and microorganisms.

It is, unfortunately, difficult to conclude which plant species was best in terms of degree of NG decomposition – during the 60-day incubation period, NG continued to be released from decomposing SP pellets.

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