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Toxicity of 2,4-dinitrotoluene to terrestrial plants in natural soils

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ABSTRACT

The presence of energetic materials (used as explosives and propellants) at contaminated sites is a growing international issue, particularly with respect to military base closures and demilitarization policies. Improved understanding of the ecotoxicological effects of these materials is needed in order to accurately assess the potential exposure risks and impacts on the environment and its ecosystems. We studied the toxicity of the nitroaromatic energetic material 2,4-dinitrotoluene (2,4-DNT) on alfalfa (*Medicago sativa* L.), barnyard grass (*Echinochloa crusgalli* L. Beauv.), and perennial ryegrass (*Lolium perenne* L.) using four natural soils varying in properties (organic matter, clay content, and pH) that were hypothesized to affect chemical bioavailability and toxicity. Amended soils were subjected to natural light conditions, and wetting and drying cycles in a greenhouse for 13 weeks prior to toxicity testing to approximate field exposure conditions in terms of bioavailability, transformation, and degradation of 2,4-DNT. Definitive toxicity tests were performed according to standard protocols. The median effective concentration (EC_{50}) values for shoot dry mass ranged from 8 to 229 mg kg^{-1}, depending on the plant species and soil type. Data indicated that 2,4-DNT was most toxic in the Sassafras (SSL) and Teller (TSL) sandy loam soils, with EC_{50} values for shoot dry mass ranging between 8 to 44 mg kg^{-1}, and least toxic in the Webster clay loam soil, with EC_{50} values for shoot dry mass ranging between 40 to 229 mg kg^{-1}. The toxicity of 2,4-DNT for each of the plant species was significantly ($p \leq 0.05$) and inversely correlated with the soil organic matter content. Toxicity benchmark values determined in the present studies for 2,4-DNT weathered-and-aged in SSL or TSL soils will contribute to development of an Ecological Soil Screening Level for terrestrial plants that can be used for ecological risk assessment at contaminated sites.

1. Introduction

The nitroaromatic compound 2,4-dinitrotoluene (2,4-DNT), a toxic and recalcitrant chemical, is a by-product of 2,4,6-trinitrotoluene (TNT) manufacturing, and is used as a gelatinizing and waterproofing agent in the production of explosives. It is also used in dye processes, in smokeless gun powders and as an intermediate in the manufacture of polyurethanes (Agency for Toxic Substances and Disease Registry, 1998). 2,4-DNT is considered to be a possible carcinogen to humans by the International Agency for Research on Cancer—IARC (1996).

Limited information has been published on the environmental impact of 2,4-DNT to terrestrial plants (Kuperman et al., 2009). In previous studies, the toxicities of 2,4-DNT freshly amended or weathered-and-aged in Sassafras sandy loam (SSL) soil using alfalfa (*Medicago sativa* L.), barnyard grass (*Echinochloa crusgalli* L. Beauv.), and perennial ryegrass (*Lolium perenne* L.) with shoot growth EC_{50} values from 8 to 13 mg kg^{-1} soil were determined (Rocheleau et al., 2006). The toxicity of 2,4-DNT has also been assessed for wheat (*Triticum aestivum* L. var. Siria), mustard (*Sinapis alba* L. var Zlata), lettuce (*Lactuva sativa* L. var. Kral maje), lentil (*Lens culinaris* Med. Var. Laird) (Picka and Friedl, 2004), tomato (*Lycopersicum esculentum* var. Bellina RZ), and oats (*Avena sativa*) (Adema and Henzen, 1989). Other plant-related studies investigated the detoxification of 2,4-DNT using transgenic tobacco plants (Tognetti et al., 2007), the genotoxicity of 2,4-DNT as measured by the spiderwort *Tradescantia* micronucleus assay (Gong et al., 2003), and the toxicity of 2,4-DNT using fresh water green algae (*Pseudokirchneriella subcapitata*, formerly *Selenastrum capricornutum* Printz) (Dodard et al., 1999).

The bioavailability and toxicity of nitroaromatics to terrestrial plants can be affected by soil properties. Earlier studies have shown that organic matter (OM) and clay content are among the key constituents of soil that can sorb nitroaromatics (Upson and Burns, 2006; Roberts et al., 2007; Charles et al., 2008), and thus decrease their chemical availability and concomitant toxicity. In the present study, we build upon the results of our previous studies with SSL soil (Rocheleau et al., 2006) to determine the effects of 2,4-DNT on alfalfa, barnyard grass, and ryegrass in Teller sandy loam, Kirkland loam, and

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Table 1
Selected physico-chemical characteristics of the test soils.

Soil parameters	Sassafras sandy loam SSL2001	Teller sandy loam TSL2001	Kirkland loam KL2006	Webster clay loam WCL2001
pH	5.3 (0.03)	4.4 (0.03)	5.7 (0.09)	5.9 (0.03)
Organic matter (%)	1.3 (0.06)	1.4 (0.03)	1.5 (0.03)	5.3 (0.09)
Sand (%)	71 (1.0)	65 (1.0)	39 (1.6)	33 (0.6)
Silt (%)	18 (1.0)	22 (1.0)	42 (1.7)	39 (0.3)
Clay (%)	11 (0.0)	13 (0.0)	19 (0.1)	28 (0.7)
WHC (%)	18 (4.0)	13 (0.6)	20 (1.0)	23 (0.18)

Values are means ($n=3$) and standard error in parentheses. WHC: water holding capacity of the soil.

Webster clay loam to test the hypothesis that the toxicity of 2,4-DNT is correlated inversely with the soil OM or clay content, and to develop phytotoxicity benchmark data for 2,4-DNT that can be used for ecological risk assessment (ERA) at contaminated sites (USEPA, 2005).

2. Materials and methods

2.1. Chemicals and reagents

The 2,4-DNT (CAS: 121-14-2; purity: 97%) was obtained from Sigma-Aldrich Canada Ltd. (Oakville, Ontario, Canada). 2-Amino-4-nitrotoluene (2-ANT) and 4-amino-2-nitrotoluene (4-ANT), which are degradation products of 2,4-DNT, were supplied from AccuStandard, New Haven, Connecticut. Boric acid was used as a positive control for the plant toxicity tests, and was obtained from BDH Chemicals (VWR International, Montreal, Quebec, Canada). Acetonitrile and 1,3-dinitrobenzene (1,3-DNB; an internal standard solution) used for the soil extractions were obtained from EMD Chemicals (Gibbstown, New Jersey) and Fluka Chemical (Milwaukee, Wisconsin), respectively. American Society for Testing and Materials (ASTM) type I water (ASTM, 2004) was obtained using the Super Q water purification system (Millipore®, Nepean, Ontario, Canada) and was used throughout the studies.

2.2. Test soils

The toxicity of 2,4-DNT was assessed in four natural upland soils with a relatively wide range of physico-chemical characteristics, including 1.3–5.3% OM, 33–71% sand, 18–42% silt, 11–28% clay, and pH range of 4.4–5.9 (Table 1). Standard methods were used to measure soil pH, organic matter, and particle size distribution (Gee and Bauder, 1986; ISO, 1994; Schulte, 1995). Sassafras sandy loam (SSL2001), a fine-loamy, siliceous, semiactive, mesic Typic Hapludult soil, was obtained from Aberdeen Proving Ground, Maryland. Teller sandy loam (TSL2001), a fine-loamy, mixed, active, thermic Udic Argiustoll soil, and Kirkland loam (KL2006), a fine, mixed, superactive, thermic Udertic Paleustoll soil, were obtained from Payne County, Oklahoma. Webster clay loam (WCL2001), a fine-loamy, mixed, superactive, mesic Typic Endoaquoll soil, was obtained from Story County, Iowa. The qualitative "relative bioavailability scores" for organic chemicals in natural soils were considered "high" for SSL or TSL, and "medium" for KL or WCL soil, according to the Ecological Soil Screening Level (Eco-SSL) criteria (USEPA, 2005). The vegetation and organic layers were removed from all soils, and the top 15 cm of the A horizon was then collected. Soil

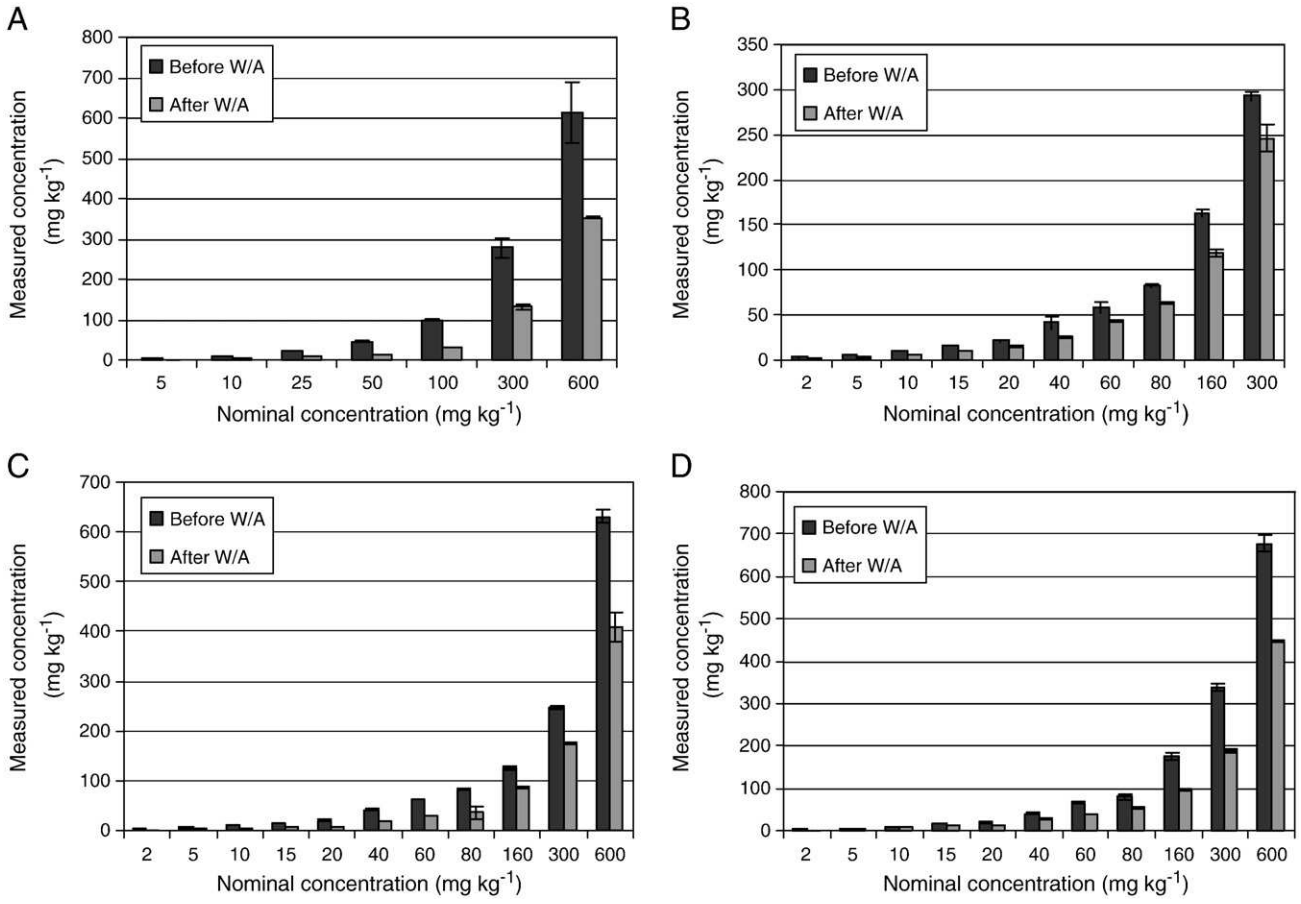

Fig. 1. Analytically determined concentrations of 2,4-dinitrotoluene before and after weathering-and-aging (W/A) in Sassafras sandy loam (A), Teller sandy loam (B), Kirkland loam (C), and Webster clay loam (D) soils. Values are means and standard errors ($n=3$).

analyses showed that no 2,4-DNT or its degradation or transformation products were present above analytical detection limits. Soil batches were separately amended with 2,4-DNT dissolved in an acetone carrier, which was allowed to volatilize for a minimum of 18 h in darkness in a chemical hood. Batches were separately mixed overnight (18 ± 2 h) using a three-dimensional rotary soil mixer, and then hydrated with ASTM type I water to a level equivalent to 60% of the water holding capacity (WHC) of each soil type. An experimental method for soil WHC (Earl, 2003) was modified to include evaporation and drainage, using three sub-samples of soil below the surface at depth-midpoints within free drainage containers (conventional 8″ pots; nominal 20 cm diameter, and height) as a function of time (days); soil moisture content was gravimetrically determined (FAO, 2007), and the WHC reported as the average of the resulting steady state soil moisture contents. Amended soils were subjected to natural light conditions, and wetting and drying cycles in a greenhouse for 13 weeks, as described in Rocheleau et al. (2006) and Kuperman et al. (2005), to approximate field exposure conditions for plants in terms of bioavailability, transformation and degradation of 2,4-DNT prior to toxicity testing. At the end of the 13-week weathering-and-aging period, each soil batch was hydrated with ASTM type I water to 75% of the WHC prior to the initiation of the plant toxicity tests.

2.3. Plant toxicity tests

Alfalfa (*Medicago sativa,* Canada no. 1) and barnyard grass (*Echinochloa crusgalli,* Common no. 1) were purchased from William Dam Seeds (Dundas, Ontario, Canada) and Labon Inc. (Boucherville, Quebec, Canada), respectively, whereas perennial ryegrass (*Lolium perenne,* Express) was obtained from Pickseed Canada Inc. (St-Hyacinthe, Quebec, Canada). In order to obtain optimal growth conditions, alfalfa seeds were inoculated with nitrogen-fixing bacteria (*Rhizobium meliloti*) prior to sowing. Plant definitive toxicity tests were performed according to ASTM (2002) and USEPA (1996) methods with the following modifications. Twenty seeds were sown in 10-cm wide pots containing 200 g dry soil, and incubated in sealed plastic bags to maintain soil moisture (USEPA, 1996) for the duration of the test. Plant toxicity tests were performed in a temperature and light controlled growth chamber. Plants were incubated in the dark for the first two days and then exposed to a diurnal photoperiod cycle afterwards. The growth chamber conditions were set as follows: light intensity at 5000 ± 500 lux, light for 16 h at 25 °C, dark for 8 h at 20 °C. The luminosity level was measured weekly using a photometer, and luminosity was readjusted as needed. Nominal concentrations of 2,4-DNT included 2, 5, 10, 15, 20, 40, 60, 80, 160, 300, and 600 mg kg^{-1} soil dry weight. Control treatments included negative (ASTM type I water), carrier (acetone), and positive controls (boric acid at concentrations of 0, 175, 200, 230, 260, and 290 mg kg^{-1} for alfalfa; 0, 65, 110, 175, 260, 350, and 450 mg kg^{-1} for barnyard grass; 0, 50, 80, 110, 150, and 200 mg kg^{-1} for ryegrass). All treatments were carried out using four replicates. Results from control treatments complied with quality control requirements (USEPA, 1996; ASTM, 2002). Seedling emergence was determined after 5 d for alfalfa and barnyard grass, and after 7 d for ryegrass. Shoot growth (fresh mass and dry mass) was determined after 16 d for alfalfa and barnyard grass, and after 19 d for ryegrass. Both fresh mass-based and dry mass-based toxicity endpoints were included in the studies because both are acceptable for derivation of Eco-SSL values by USEPA. Shoots were cut just above the soil line, and fresh mass was determined immediately to minimize moisture loss. Dry mass was determined after drying the shoot tissue at 70 °C for 24 h.

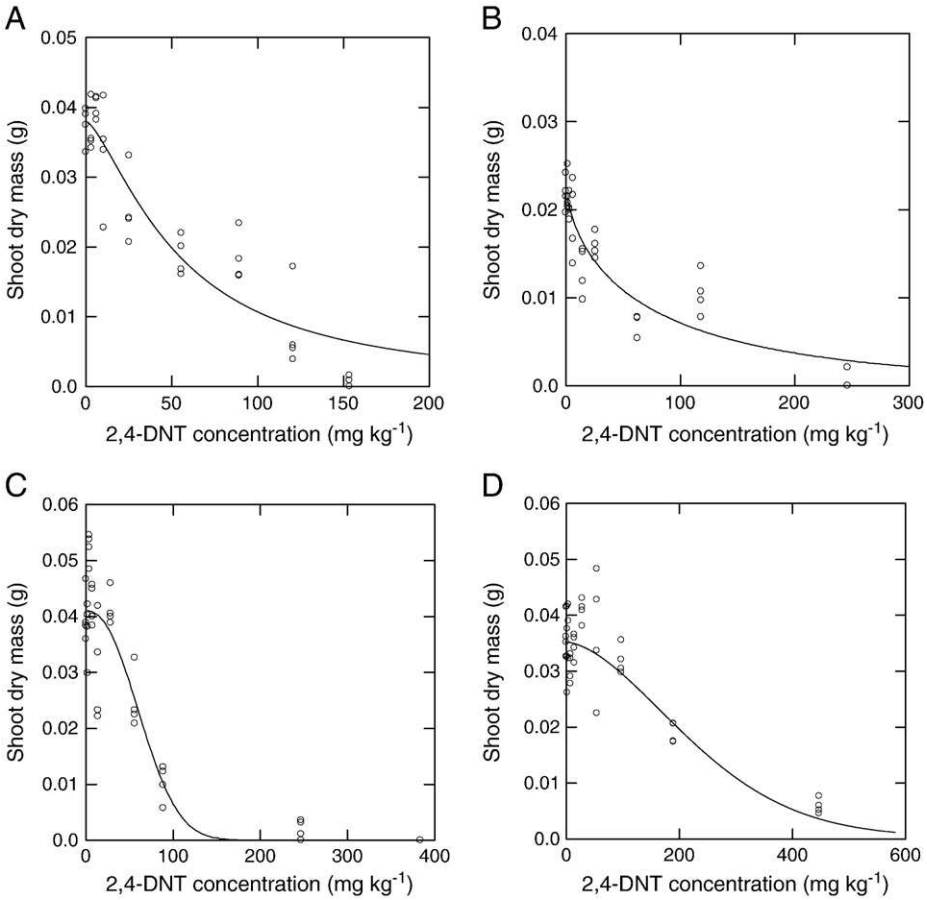

Fig. 2. Effects of 2,4-dinitrotoluene (2,4-DNT), weathered-and-aged in soil for 13 weeks, on alfalfa *Medicago sativa* in Sassafras sandy loam (SSL2001) (A), Teller sandy loam (TSL2001) (B), Kirkland loam (KL2006) (C), and Webster clay loam (WCL2001) (D) soils. Regression models used had the best fit of the data and are indicated in Table 2.

2.4. Chemical extractions and analytical determinations

Soil samples taken prior to the weathering-and-aging of soil treatments and at the beginning of the toxicity tests were extracted and analyzed using USEPA Method 8330A (USEPA, 2007) with some modifications. Acetonitrile extraction was selected to measure the quantity of 2,4-DNT in soil to comply with Eco-SSL Guidance stipulations that standard methods be used to determine best estimates of total concentrations in soil (USEPA, 2005). Soil was subsampled (2 g, dry weight basis) in triplicate from each treatment. Acetonitrile (10 mL) and 100 µL of internal standard solution (1,3-DNB, 50 mg L^{-1}) were added to each soil subsample in individual glass tubes, vortexed for 1 min, then sonicated in darkness for 18 ± 2 h at 20 °C. Five mL of the sonicated sample was then transferred to another tube, to which 5 mL of 5 g L^{-1} $CaCl_2$ solution was added. Supernatant was filtered through a 0.45 µm Millex-HV cartridge. Soil extracts were analyzed and quantified using a high performance liquid chromatography (HPLC) system (Waters, Milford, Massachussets). Separation was made on a Discovery C18 column (25 cm, 4.6 mm, 5 µm; Supelco, Oakville, Ontario) maintained at 35 °C. An acetonitrile/water gradient was run at a flow rate of 1.2 mL min^{-1}. The initial solvent composition was 30% acetonitrile and 70% water, which was held for 8 min. A linear gradient was run from 30% to 70% acetonitrile over 7 min, and a second linear gradient was then run from 70% to 80% acetonitrile over 3 min. This solvent ratio was held for 7 min and then changed to the initial conditions over 2 min. The initial conditions were then held for another 8 min, for a total run time of 35 min. The detector was set to scan from 200 to 500 nm, and chromatograms were analyzed at a wavelength of 230 nm. The HPLC detection limit was 0.01 mg L^{-1} for 2,4-DNT, and 0.005 mg L^{-1} for both 2-ANT and 4-ANT; and the quantification limits in soil were 0.1 mg kg^{-1}, 0.05 mg kg^{-1}, and 0.05 mg kg^{-1}, respectively. Extraction was repeated if the 1,3-DNB internal standard recovery was less than 90%.

2.5. Data analyses

Phytotoxicity data were analyzed using the appropriate regression models selected from among those described in Environment Canada Guidance Document (EC, 2005). During the model selection process, compliance with the normality assumptions and homoscedasticity of the residuals were determined by examining the stem-and-leaf graphs and histograms of the residuals. The best fit was evident when the regression lines generated by the models were closest to the data points, the regression coefficients for point estimates were the greatest, the residuals were homoscedastic (i.e., had most random scattering), and the means, standard errors, and variances of the residuals were the smallest. These models were:

$$\text{Logistic Gompertz model}: Y = a \times e\left(\left[\log(1-p)\right] \times \left[C/\mathrm{EC}_p\right]^b\right)$$

Logistic Hormetic model :

$$Y = a \times [1 + hC] / \left\{1 + [(p + hC) / (1-p)] \times \left[C/\mathrm{EC}_p\right]^b\right\}$$

$$\text{Exponential model}: Y = a \times e\left(\left(\left[\log(1-p)\right] / \mathrm{EC}_p\right) \times C\right) + b,$$

where Y is the number of emerged seedlings or the shoot mass, a is the y-intercept (i.e., the control response), e is the exponent of the

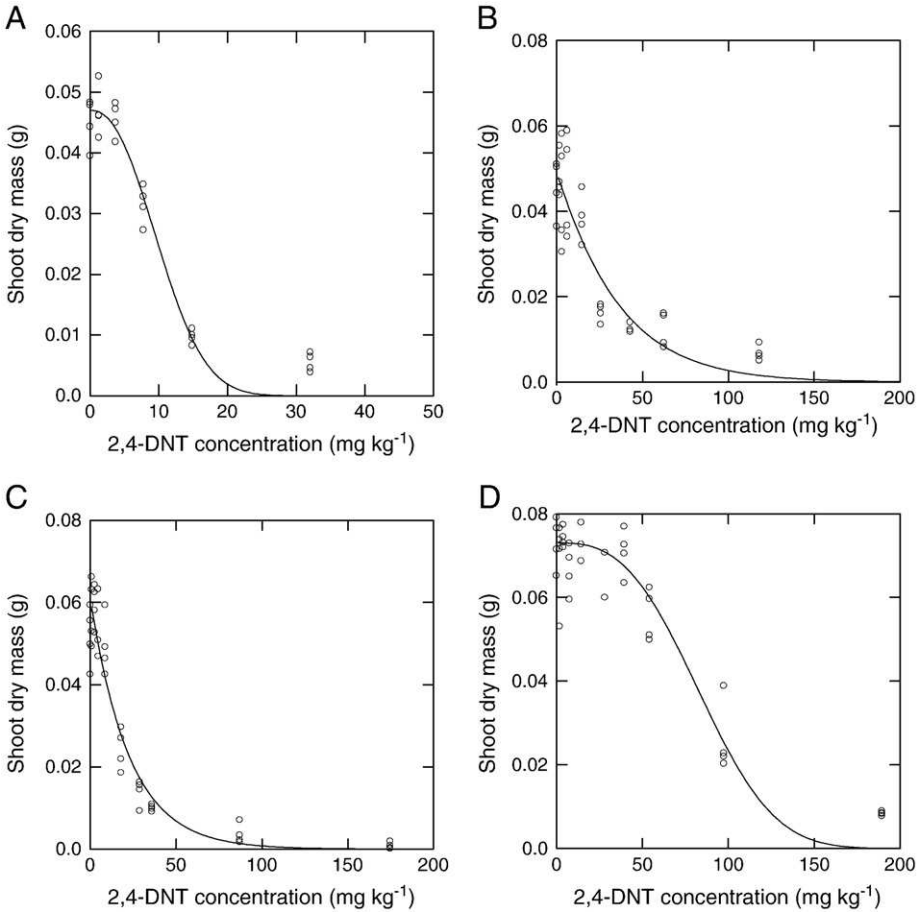

Fig. 3. Effects of 2,4-dinitrotoluene (2,4-DNT), weathered-and-aged in soil for 13 weeks, on barnyard grass *Echinochloa crusgalli* in Sassafras sandy loam (SSL2001) (A), Teller sandy loam (TSL2001) (B), Kirkland loam (KL2006) (C), and Webster clay loam (WCL2001) (D) soils. Regression models used had the best fit of the data and are indicated in Table 2.

base of the natural logarithm, p is the desired value for 'p' effect (e.g., 0.5 for EC_{50}), C is the exposure concentration in test soil, EC_p is the estimate of effect concentration for a specified percent effect, h is the hormetic effect parameter, and b is the scale parameter that defines the shape of the equation.

The EC estimates and 95% confidence intervals (CI) associated with the point estimates included the 2,4-DNT concentration producing 20% (EC_{20}) or 50% (EC_{50}) reduction in the measurement endpoint compared with the carrier control. Analysis of variance (ANOVA) was used to determine the chemical concentration associated with the statistically significant change compared with the results in carrier control, and to establish the No-Observable-Effect-Concentration (NOEC), the Lowest-Observable-Effect-Concentration (LOEC), and the Lowest-Observable-Adverse-Effect-Concentration (LOAEC) values. Means separations were done using Fisher's-Least-Significant-Difference (FLSD) tests (SPSS Inc., 1997). The relationships among the selected soil parameters and toxicity data were determined using Pearson's correlation analysis and uncorrected probabilities (SPSS Inc., 1997). A significance level of $p \leq 0.05$ was accepted for all statistical analyses.

3. Results and discussion

3.1. Analytical determination of 2,4-DNT in soils

The concentrations of 2,4-DNT decreased significantly in all four amended soils during the 13-week weathering-and-aging procedure (Fig. 1). The resulting concentrations were representative of 2,4-DNT concentrations found in contaminated soils at some former ammunition plants (Simini et al., 1995) and military training ranges (Hewitt et al., 2007; Jenkins et al., 2006; Walsh et al., 2007). Concentrations after the weathering-and-aging procedure corresponded to the amount of 2,4-DNT measured in soil at the beginning of the definitive toxicity tests. The decrease in 2,4-DNT recovered from the soils, which was relative to the initial concentrations in freshly amended soil, ranged between 42 and 72% in SSL2001, between 16 and 48% in TSL2001, between 30 and 59% in KL2006, and between 28 and 44% in WCL2001. Traces of 2-ANT and 4-ANT were detected in the SSL and TSL soils after the 13-week weathering-and-aging period. The decrease in recovery of 2,4-DNT was the greatest in the SSL2001 soil (up to 72%) compared to the other three tested soils. In our studies, neither soil OM ($r = 0.641$; $p = 0.359$) nor clay content ($r = 0.658$; $p = 0.342$) were significantly correlated to the percent decreases of 2,4-DNT (data not shown); therefore, neither OM nor clay content could explain completely the decreased recoveries of 2,4-DNT in the four tested soils. Although the results of the studies presented here only partially explain the relationships that may exist among soil properties and the decrease in recoveries of 2,4-DNT as a result of weathering-and-aging, the alteration of 2,4-DNT bioavailability in soil would be most directly and reliably based on measurements of biological uptake, which was indirectly assessed by our toxicity studies. Studies with a greater number of soils will be required to confirm trends determined in our present investigations.

3.2. Phytotoxicity of 2,4-DNT

The concentration ranges selected for the definitive studies with the three plant species were sufficient to determine phytotoxicity benchmarks for 2,4-DNT on the basis of concentration–response relationships (Figs. 2–4). Generally, the trend among phytotoxicity benchmark values indicated that shoot fresh mass was a more sensitive

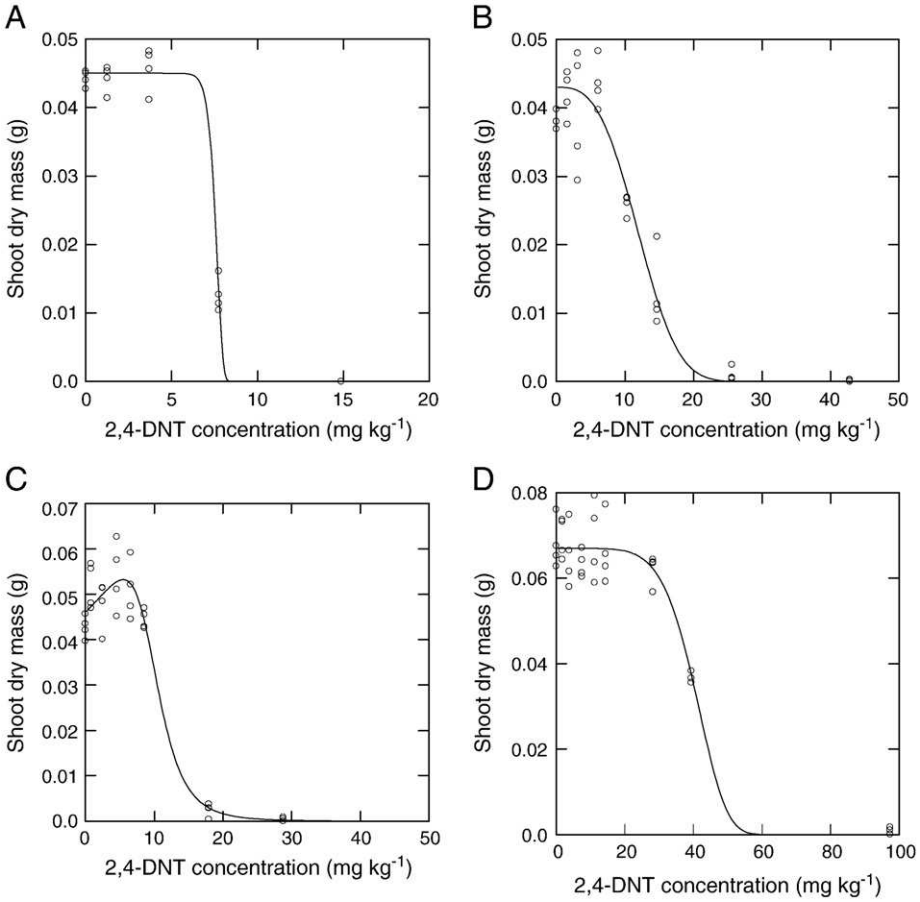

Fig. 4. Effects of 2,4-dinitrotoluene (2,4-DNT), weathered-and-aged in soil for 13 weeks, on ryegrass *Lolium perenne* in Sassafras sandy loam (SSL2001) (A), Teller sandy loam (TSL2001) (B), Kirkland loam (KL2006) (C), and Webster clay loam (WCL2001) (D) soils. Regression models used had the best fit of the data and are indicated in Table 2.

measurement endpoint than shoot dry mass, but differences were not significant based on corresponding confidence intervals. However, both shoot fresh and dry masses were more sensitive measurement endpoints than seedling emergence, based on corresponding confidence intervals (Table 2). Because shoot dry mass is considered to be a robust measure of plant growth (Natr and Lawlor, 2005), it may be used as the primary indicator of 2,4-DNT toxicity for comparison of data within our studies. The toxicity of 2,4-DNT was greater in the SSL2001, TSL2001, and KL2006 soils, with shoot dry mass EC_{50} values (mg kg^{-1}) ranging from 8 to 42 in SSL2001, 12 to 44 in TSL2001, and 11 to 65 in KL2006, respectively, than in the WCL2001 clay soil, in which the EC_{50} values ranged from 40 to 229 mg kg^{-1} (Table 2). Picka and Friedl (2004) obtained similar toxicity data for 2,4-DNT using wheat, mustard, lettuce, and lentil exposed in a low organic carbon content (0.86%) soil, equivalent to 1.90% OM (Ranney, 1969), which is comparable to the OM of SSL2001, TSL2001, and KL2006 soils. These authors determined the EC_{50} values (and 95% CI; mg kg^{-1}) for shoot dry mass to be 25 (23–28) for wheat, 38 (35–41) for mustard, 21 (19–23) for lettuce, and 75 (71–79) for lentil. In a different study, Adema and Henzen (1989) assessed the toxicity of 2,4-DNT in a loam soil (1.4% OM) and a humic sand (3.7% OM) using lettuce, tomato, and oats. The respective EC_{50} values established for shoot dry mass were 6, 5,

Table 2
Summary of phytotoxicological benchmarks for 2,4-dinitrotoluene weathered-and-aged in natural soils.

	Seedling emergence			Shoot fresh mass			Shoot dry mass		
Species Soil type	LOEC (mg kg^{-1})	EC_{20} (mg kg^{-1})	EC_{50} (mg kg^{-1})	LOEC (mg kg^{-1})	EC_{20} (mg kg^{-1})	EC_{50} (mg kg^{-1})	LOEC (mg kg^{-1})	EC_{20} (mg kg^{-1})	EC_{50} (mg kg^{-1})
Alfalfa									
SSL2001	121	104a	115a	10	7a	30a	10	15a	42a
p or 95% CI	<0.001	(91–117)	(109–121)	<0.001	(2–11)	(20–40)	0.011	(9–21)	(29–56)
Model		Hormetic	Hormetic		Gompertz	Gompertz		Hormetic	Hormetic
R^2		0.989			0.976			0.979	
TSL2001	62	30b	94a	6	5a	26a	15	7a	44a
p or 95% CI	<0.001	(4–55)	(57–130)	0.024	(1–8)	(16–37)	<0.001	(0–15)	(22–66)
Model		Gompertz	Gompertz		Gompertz	Gompertz		Gompertz	Gompertz
R^2		0.959			0.973			0.964	
KL2006	89	71ab	141a	14*	21a	46a	14*	40b	65a
p or 95% CI	0.001	(51–91)	(117–164)	<0.001	(7–35)	(31–60)	0.005	(25–55)	(54–77)
Model		Gompertz	Gompertz		Gompertz	Gompertz		Gompertz	Gompertz
R^2		0.987			0.942			0.962	
WCL2001	447	258c	541b	54	64b	157b	189	120c	229b
p or 95% CI	<0.001	(154–362)	(378–703)	0.009	36–91	122–192	<0.001	74–166	177–280
Model		Gompertz	Gompertz		Gompertz	Gompertz		Gompertz	Gompertz
R^2		0.987			0.980			0.975	
Barnyard grass									
SSL2001	90*	86	>90	4	4ab	7a	8	6a	10a
p or 95% CI	<0.001	(ND)		0.015	(2–5)	(5–8)	<0.001	(5–8)	(9–12)
Model		Hormetic			Gompertz	Gompertz		Gompertz	Gompertz
R^2		0.994			0.982			0.989	
TSL2001	62*	112	>118	15	9a	15b	26	9a	26b
p or 95% CI	0.018	(59–166)		<0.001	(5–13)	12–19	<0.001	(2–15)	(17–35)
Model		Gompertz			Gompertz	Gompertz		Gompertz	Gompertz
R^2		0.987			0.961			0.953	
KL2006	175	>175	>175	1	3b	11b	18	5a	16ab
p or 95% CI	<0.001			0.015	(2–4)	(9–12)	<0.001	(4–7)	(11–20)
Model					Exponential	Exponential		Exponential	Exponential
R^2					0.962			0.971	
WCL2001	189	>189	>189	28	32c	56c	54	57b	84c
p or 95% CI	0.783			0.008	(26–38)	(51–62)	0.003	44–69	75–93
Model					Gompertz	Gompertz		Gompertz	Gompertz
R^2					0.988			0.984	
Ryegrass									
SSL2001	8	>8	>8	8	5a	7a	8	2a	8
p or 95% CI	0.014			<0.001	(4–7)	(6–8)	<0.001	(0–4)	ND
Model					Gompertz	Gompertz		Gompertz	Gompertz
R^2					0.992			0.990	
TSL2001	15	15a	19a	10	6a	11b	10	8b	12a
p or 95% CI	0.004	(14–17)	(18–20)	<0.001	(5–8)	(10–12)	<0.001	(6–11)	(10–14)
Model		Gompertz	Gompertz		Gompertz	Gompertz		Gompertz	Gompertz
R^2		0.994			0.984			0.964	
KL2006	18	14a	16a	9*	7a	9ab	18	10b	11a
p or 95% CI	<0.001	(8–19)	(14–19)	0.001	(6.5–8)	(8–10)	<0.001	(9–11)	(10–13)
Model		Gompertz	Gompertz		Hormetic	Hormetic		Hormetic	Hormetic
R^2		0.995			0.981	9ab		0.989	
WCL2001	97	60b	72b	28	22b	33c	39	34c	40b
p or 95% CI	<0.001	(41–79)	(57–88)	<0.001	(18–25)	(31–35)	<0.001	(30–37)	(39–42)
Model		Gompertz	Gompertz		Gompertz	Gompertz		Gompertz	Gompertz
R^2		0.997			0.994			0.993	

*LOAEC: Lowest-Observable-Adverse-Effect-Concentration.
95% Confidence Intervals (CI) are presented in parentheses.
R^2: regression coefficient (reported only in the EC_{20} column; applies to both EC_{20} and EC_{50} for each respective pairing).
ND: could not be determined.
EC_{20} or EC_{50} values with different letters designate statistically significant difference (based on 95% CI) among soils for each plant species.

Table 3
Pearson correlation coefficients for key soil properties and phytotoxicity based on the EC_{50} benchmarks for shoot growth (dry mass).

Soil property	Alfalfa	*p*-value	Barnyard grass	*p*-value	Ryegrass	*p*-value
Organic matter	0.997**	0.003	0.983*	0.017	0.996**	0.004
Clay	0.940	0.060	0.887	0.113	0.915	0.085
pH	0.918	0.082	0.804	0.196	0.860	0.140

Pearson correlation coefficients with corresponding probabilities (*p*-values) were determined using data from the definitive toxicity tests with alfalfa, barnyard grass, and ryegrass exposed to 2,4-dinitrotoluene weathered-and-aged in Sassafras sandy loam, Teller sandy loam, Kirkland loam, and Webster clay loam soils.
* Correlation significant ($p \leq 0.05$).
** Correlation highly significant ($p \leq 0.01$).

and 46 mg kg^{-1} in the loam soil, and 13, 10, and 35 mg kg^{-1} in the humic sand. The differences in phytotoxic sensitivity among plant species in our studies were most evident in the WCL2001 soil with EC_{50} values (mg kg^{-1}) and corresponding 95% CI of 40 (39–42) for ryegrass, 84 (75–93) for barnyard grass, and 229 (177–280) for alfalfa (Table 2). These findings showed that the phytotoxicity data determined in our studies were similar to those reported in the literature for 2,4-DNT effects on terrestrial plants.

The toxicity of 2,4-DNT to all three plant species, based on the EC_{50} benchmarks for shoot dry mass, correlated inversely ($r \geq 0.983$) and significantly ($p \leq 0.017$) with the soil OM content (EC_{50} increased as OM content increased; Table 3). Inverse correlations ($r \geq 0.804$) for phytotoxicity of 2,4-DNT and the soil clay content or pH were also determined, but these were not statistically significant ($p > 0.05$) (Table 3). We could not partition the contributions of individual soil constituents to the toxicity of 2,4-DNT due to inter-correlations among the soil OM, clay, and pH characteristics. In a recent study, Charles et al. (2008) reported a stronger sorption of 2,4-DNT in soil with greater OM and clay contents (2.1 to 3.87% OM and 31% clay) compared to that in soil with lower OM and clay contents (0.59 to 2.69% OM, and 25 to 27% clay).

4. Conclusions

The toxicity of 2,4-DNT to all three plant species inversely and significantly correlated with the soil OM content, based on the EC_{50} values for shoot dry mass. Differences in sensitivity among the plant species described herein were most evident in the Webster clay loam soil, in which 2,4-DNT was the least phytotoxic. The 2,4-DNT weathered-and-aged in soil was generally more toxic in the Sassafras and Teller sandy loam soils compared with clay loam soils. The EC_{20} toxicity benchmark values determined in the present studies for 2,4-DNT weathered-and-aged in SSL and TSL soils will contribute to development of an Ecological Soil Screening Level for terrestrial plants that represents exposure conditions at Superfund and similar contaminated sites (USEPA, 2005).

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