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EVALUATION OF SOIL TOXICITY AT JOLIET ARMY AMMUNITION PLANT

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Abstract—Environmental toxicity testing and chemical analyses of soil were performed as part of an ecological risk assessment at the Joliet Army Ammunition Plant (JAAP), Joliet, Illinois. Soils were collected from an area where munitions were loaded, assembled, and packed (area L7, group 1), and from an area where waste explosives were burned on unprotected soil (area L2). Control samples were collected from an adjacent field. Soil toxicity was determined using early seedling growth and vigor tests, earthworm survival and growth tests, and Microtox® assays. Relative toxicity of soils was determined within each area based on statistical significance ($p = 0.05$) of plant and earthworm growth and survival, and the effective concentration at which luminescence of the bacterium *Photobacterium phosphoreum* was reduced by 50% (EC50) in the Microtox assay. Samples were designated as having high, moderate, or no significant toxicity. Soil that had significant toxicity according to at least one test, and representative samples showing no toxicity, were analyzed for munitions via HPLC. Chemical residues found in soils were 2,4,6-trinitrotoluene (TNT); 1,3,5-trinitrobenzene (TNB); 2,4-dinitrotoluene (2,4-DNT); 2,6-dinitrotoluene; 2-amino-4,6-DNT; 4-amino-2,6-DNT; 1,3,5-trinitro-1,3,5-triazine (RDX); and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). All soils with no significant toxicity were void of these chemicals. However, some soils void of munitions still showed toxicity that may have been caused by elevated levels of heavy metals. Linear regressions of toxicity test results vs. chemical concentrations showed that TNT and TNB accounted for most of the soil toxicity. Lowest-observable-effect concentrations (LOEC) of TNT were determined from these data. This study presents a simple, relatively inexpensive methodology for assessing toxicity of soils containing TNT, RDX, and other contaminants related to munitions production.

Keywords—Ecological risk assessment Explosives TNT RDX

INTRODUCTION

Joliet Army Ammunition Plant (JAAP) is a government-owned, contractor-operated installation currently maintained in nonproducing standby condition. Originally constructed in the 1940s, production ceased in 1977 [1]. The facility is divided into a manufacturing side, and a load, assemble, and pack (LAP) side. Currently, JAAP is on the National Priorities List. Remedial investigations have identified potential contamination of ground water, soil, sediment, and surface water by explosives and heavy metals [1,2]. However, the actual and potential impact of these contaminants on terrestrial ecosystems at JAAP has not been determined.

Toxicity tests can be used in risk assessments to characterize environmental conditions in and near hazardous-waste sites [3]. Site characterization involves the collection of data on hazardous substances and the assessment of potential toxicity effects at the site. Toxicity tests using contaminated matrices from the site can form an important part of the descriptive assessments required in the site characterization process. Additionally, toxicity test results, combined with a properly designed field testing program and chemical analyses, can be used to assess the extent of contamination and associated potential for toxic effects [3]. Previous experience has shown that it is important to choose a battery of tests representing different levels of biological organization to assess adequately the environmental toxicity of soil contaminants [4–6]. Therefore, plant (early seedling growth and vigor), earthworm (survival and weight change), and bacterial (Microtox® luminescence intensity) tests were chosen for this study. The present study was part of an ecological risk assessment conducted by the U.S. Army to determine the deleterious effects of chemical contamination on flora and fauna at JAAP.

The scope of this study was to (a) conduct toxicity tests with soils from two areas suspected of having high levels of contamination with explosives; (b) identify, quantify, and determine distribution of explosives in soils at the site; (c) correlate toxicity test results with soil explosives concentrations; and (d) estimate "no-effects" concentrations of the soil explosives.

MATERIALS AND METHODS

Soil sampling sites

Soil samples were collected from three sites at JAAP. The sites and number of samples collected from each were (a) Area L7, Group 1 (Fig. 1, LAP side, 31 samples) where munitions were loaded, assembled, and packed; (b) Area L2 burning ground (Fig. 2, LAP side, 33 samples) where waste explosives were burned on unprotected soil; and (c) an un-

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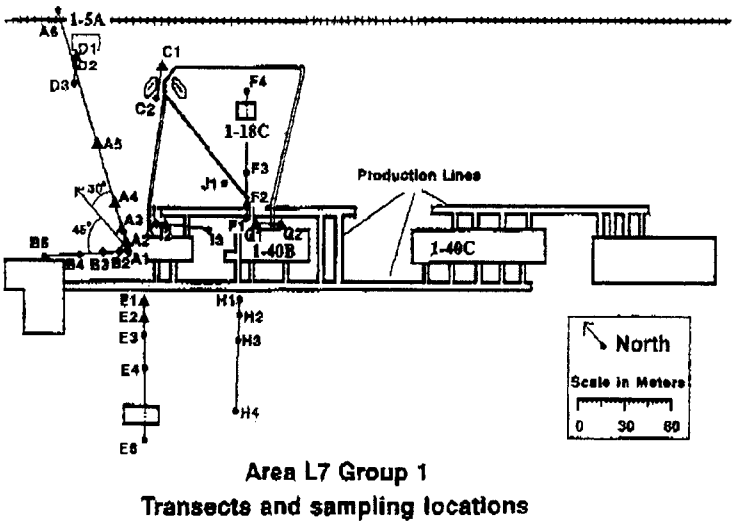

Fig. 1. Group 1 load, assemble, and pack area showing transects and soil-sampling locations with nontoxic response (●) or toxic response to at least one toxicity test (▲).

contaminated (control) site adjacent to the test sites (3 samples). Selection of these sites was based on a preliminary survey that indicated potential for contamination with explosives and their degradation products [2]. Samples were collected at varying distances, depending upon the estimated extent of contamination, along transects through potential "hot spots" of chemical contamination.

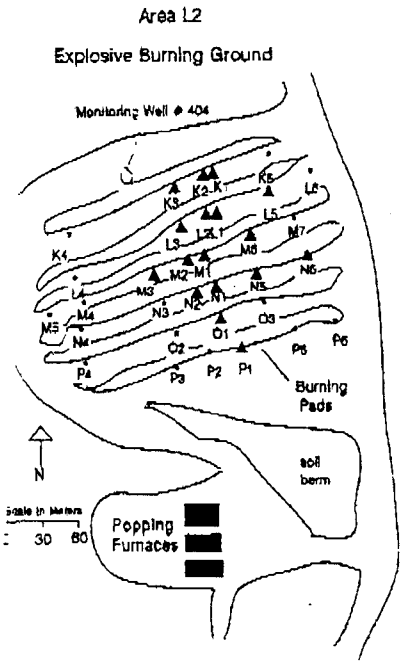

Fig. 2. Area L2 explosives burning ground showing transects and soil-sampling locations with nontoxic response (★) or toxic response to at least one toxicity test (▲).

Collection and preparation of soil samples

Each sample location was cleared of vegetation, surface organic matter, and debris. Soil was removed from an area approximately 20 to 25 cm in diameter and 15 cm deep in the A horizon, placed into a double plastic bag, and transported to Aberdeen Proving Ground (Edgewood, MD). Soil samples were sieved through a wire screen (5-mm^2 mesh), and the field moisture capacity was determined.

Phytotoxicity tests

Procedures for determining phytotoxicity of soils in this study were adapted from the U.S. Environmental Protection Agency's (EPA's) Early Seedling Growth and Vigor Toxicity Test [7]. Total plant heights and survival rates were used as end points to assess toxicity. For each soil tested, approximately 1,000 g of pea gravel was placed into a 150-mm (diameter) plastic pot. A single layer of cheesecloth was placed above the gravel and 800 g (dry wt.) of soil was added to the pot. Two pots of each soil sample were prepared for each species, cucumber (*Cucumis sativus* L. var. "strait eight") and radish (*Raphanus sativus* L. var. "cherry belle").

Twenty seeds of each species were planted in each pot and the soil was watered to field capacity. After germination, seedlings were thinned to the 10 most uniform per pot. Day 1 of the test period was determined when 50% of the total number of seeds had germinated. Plant height measurements recorded on day 14 were used as the measurement end points.

Statistical evaluations of plant data included a completely randomized analysis of variance (ANOVA) and Student–Newman–Keuls (SNK) pairwise comparison of means [8].

Earthworm toxicity tests

Test methods used for earthworm toxicity studies were adapted from Karnak and Hamelink [9] and Neuhauser et al. [10]. Survival rates and the differences between initial and final live weights were used as end points.

Earthworms (*Eisenia foetida*, Bert's Bait Farm, Irvine, KY) were bred in Styrofoam coolers. Two hundred grams of air-dried soil from each sampling location was brought to field moisture capacity with distilled water, mixed in a Waring blender for approximately 3 min, and placed into a 600-ml glass beaker (two beakers per sampling location).

Seventy-five to 100 earthworms were quickly rinsed in tap water. Five earthworms were arbitrarily selected, quickly blotted with a paper towel, weighed collectively, and placed in each beaker. The beakers were covered with nylon screen and cheesecloth and placed within a temperature-controlled incubator (21.0 ± 0.2°C). Incubator lights were set for continuous operation to encourage the worms to burrow into the soil. Earthworms were removed on day 14, counted, weighed, and examined for changes in physical condition (e.g., color, texture, motility, etc.).

Earthworm data were subjected to analysis of covariance (ANCOVA) and *t* test pairwise comparison of least-squares means (means adjusted for the covariate) [8].

Microtox analysis

Photobacterium phosphoreum, a luminescent marine bacterium, was used in the Microtox assay. Luminescence of this organism is typically lowered in proportion to toxicity [11]. Analyses were performed on leachates of subsamples of soils collected at each site. Leachates were extracted by agitating 150 g of soil with 600 ml CO_2-saturated distilled water at 30 rpm in a rotary soil extractor for 48 h. The mixture was allowed to settle for 2 h then filtered (0.45 μm) and adjusted to pH 6 to 8 to obtain optimum conditions for the organisms' sensitivity and viability. Subsamples of leachates (approximately 3 ml) were centrifuged (11,000 rpm for 17 min at 4°C) to provide samples free of turbidity. The supernatant was stored at 5°C in the dark until used in the assay. Assays were performed within 72 h of extraction. Assays were conducted within the temperature-controlled (15°C) wells of a photometer (Microtox analyzer) at 5- and 15-min intervals and consisted of one blank and four sample dilutions, ranging from 11.3 to 91% of extract diluted by a factor of two. Lyophilized reagent (*P. phosphoreum*) was added directly to sample dilutions. These methods captured and maintained the bacteria in an optimum physiological state, provided consistent numbers of cells per trial, and ensured sensitivity and specificity of the test [12]. Resulting data were presented as the effective concentration at which luminescence was reduced by 50% (EC50). This value was used as the end point in this assay. The 15-min EC50 was used for comparisons; the 5-min EC50 was substituted if 15-min confidence factors were too large. All EC50s were expressed as 0 to 100% of the soil leachate to maintain the ability to test undiluted leachate should some samples have low toxicity (the standard assay tests up to a maximum of only 45.0% sample [11]).

Analytical methods for determining concentrations of explosives

Air-dried soil samples were extracted using acetonitrile containing 2.5 mg/L (ppm) of 1,3-dinitrobenzene (DNB) as an internal standard and 18 h of sonication at 20°C [13]. Extracts were then centrifuged at 3,900 rpm for 15 min, and analyzed by HPLC as described previously [13]. The quality-control program for this study assessed sample preparation, analyte recovery, and analytical precision and accuracy, and included as its basis the Quality Assurance Program of the U.S. Army Environmental Center (formerly U.S. Army Toxic and Hazardous Materials Agency) [14]. Respective criteria of detection were calculated and defined as the lowest certifiable limits for quantitation.

Regression analyses

Mean cucumber height, mean radish height, least-squares mean earthworm final live weight, and Microtox EC50 were singularly regressed against the natural log (ln) concentration of explosive residue found at each soil sampling location [8]. All bioassay results were then regressed against ln munition residue data using multiple regression techniques [8].

RESULTS

Toxicity tests

Data from plant, earthworm, and Microtox bioassays were summarized by site. Soil sampling locations where toxicity end points were significantly ($p = 0.05$) less than end points from tests performed on control soils were identified.

To assess the relative toxicity of each sample, soils were classified as being either highly (H), moderately (M), or not significantly (NS) toxic. Classifications were defined as:

H = survival rates were <30% and growth reduction as compared to controls was significant at $p = 0.05$ for both earthworm and phytotoxicity tests; EC50 was <30% for the Microtox test.

M = survival rates were 30 to 70% or growth reduction was significant at $p = 0.05$ for both earthworm and phytotoxicity tests; EC50 was 30 to 70% for the Microtox test.

NS = survival rates were >70% and growth reduction was not significant at $p = 0.05$ for both earthworm and phytotoxicity tests; EC50 was >70% for the Microtox test.

Area L7, Group 1 soil-sampling locations are shown in Figure 1. Table 1 compares the results of all four bioassays at the Group 1 site. High toxicity for all bioassays was found at A2, D1, E1, G1, G2, I1, and I2. Moderate to high toxicity was found for one or more of the tests at sampling locations A3–A5, B5, C1, and E2. Results of toxicity testing at all other locations were not significant at $p = 0.05$. The ANOVA of the mean plant heights of cucumbers and radishes indicated a significant ($p = 0.0001$) difference between soil-sampling locations. Survival rates of cucumber were ≥80% for all but seven soils. Survival rates of radish were ≥80% for all but seven soils (see Table 1).

Earthworm test results indicated both lethal and sublethal effects at certain locations within Group 1. Results of ANCOVA showed a significant ($p = 0.01$) difference in live weight change among soils. Survival rate was >75% for all but seven soils (see Table 1).

The Microtox test for Group 1 had EC50 values <30% for soils A2, A4, B5, C1, D1, E1, G1, G2, I1, and I2. All other soils produced EC50s ≥100% (see Table 1).

Table 1. Toxicity of Group 1 soils using four bioassays

Sampling location	Toxicity levels[a]			
	Earthworm	Cucumber	Radish	Microtox
A2	H	H	H	H
A3	NS	NS	NS	M
A4	NS	NS	NS	H
A5	H	NS	NS	NS
B5	NS	NS	NS	H
C1	M	H	M	H
D1	H	H	H	H
E1	H	H	H	H
E2	NS	H	M	NS
G1	H	H	H	H
G2	H	H	H	H
I1	H	H	H	H
I2	H	H	H	H

No significant toxicity for all tests: A6, B4, C2, D2, D3, E3, E4, E5, F1, F3, F4, H1, H2, H3, H4, I3, J1, Control.

[a]Toxicity levels:

H = high (earthworm and plants: <30% survival and growth reduction at $p = 0.05$; Microtox: EC50 < 30%).

M = moderate (earthworm and plants: 30–70% survival or growth reduction at $p = 0.05$; Microtox: EC50 30–70%).

NS = no significant decrease in survival rate, growth rate, or EC50 at $p = 0.05$.

Table 2. Toxicity of Area L2 soils using four bioassays

Sampling location	Toxicity levels[a]			
	Earthworm	Cucumber	Radish	Microtox
K1	H	H	H	H
K2	H	H	H	H
K3	H	H	H	H
L1	H	H	H	H
L2	H	H	H	H
L3	H	H	H	H
L5	H	M	H	H
M1	H	H	H	H
M2	H	H	H	H
M3	H	H	H	H
M6	NS	NS	NS	M
N1	H	H	H	H
N2	H	H	H	H
N5	NS	NS	NS	H
N6	NS	M	NS	H
O1	M	NS	NS	NS
P1	H	H	H	H

No significant toxicity for all tests: K4, K5, L4, L6, M4, M5, M7, N3, N4, O2, O3, P2, P3, P4, P5, P6, Control.

[a]Toxicity levels:

H = high (earthworm and plants: <30% survival and growth reduction at $p = 0.05$; Microtox: EC50 < 30%).

M = moderate (earthworm and plants: 30–70% survival or growth reduction at $p = 0.05$; Microtox: EC50 30–70%).

NS = no significant decrease in survival rate, growth rate, or EC50 at $p = 0.05$.

Sampling locations at the Area L2 site are shown in Figure 2. Overall toxicity was greatest in the central portion of Area L2, including soil-sampling locations K1–K3, L1–L3, M1–M3, N1, and N2, as indicated by the results of the four toxicity tests (Fig. 2, Table 2). Toxicity generally decreased with increasing distance from this centralized area. Samples collected at the perimeter of this area (L5, M6, N5, N6, and O1) had variable toxicity among tests, whereas soils outside the perimeter of this area were not significantly ($p = 0.05$) toxic except for P1, which was highly toxic for all tests.

The ANOVA of the mean plant heights of cucumbers and radishes grown in Area L2 soils indicated a significant ($p = 0.0001$) difference between sampling locations. Survival rates for cucumber were 100% for all but 12 soils. Survival rates for radish were ≥80% for all but 12 soils (see Table 2).

Results of the earthworm toxicity test from Area L2 indicated both lethal and sublethal effects at certain sampling locations. Results of ANCOVA showed a significant ($p = 0.01$) difference in live weight change among soils. Survival rate was >80% for all but 12 soil samples (see Table 2). Microtox analysis showed soils K1–K3, L1–L3, L5, M1–M3, N1, N2, N5, and N6 had EC50s below 22% and were considered highly toxic (the EC50 for L3 was derived from a 5-min assay); M6 had an EC50 of 58%. All other soils in this area had EC50s ≥100% (see Table 2).

Residues of explosives in soils

Soil samples that tested positive to any of the toxicity tests were analyzed for explosives residues. Thirteen samples (seven from L2 and six from Group 1) that had no toxicity according to all assays, as well as three samples from the uncontaminated site, were also analyzed. Detectable levels of 2,4,6-trinitrotoluene (TNT); 1,3,5-trinitrobenzene (TNB); 2,4-dinitrotoluene (2,4-DNT); 2,6-dinitrotoluene (2,6-DNT); 2-amino-4,6-dinitrotoluene (2-am-DNT); 4-amino-2,6-dinitrotoluene (4-am-DNT); 1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) were found in both Area L2 and Group 1 soils (Table 3).

Both TNT and TNB were the most abundant chemicals, in terms of percent of total samples having detectable levels of explosives, at Group 1 (91% TNT, 75% TNB) and at Area L2 (87% TNT, 93% TNB) (Table 3). In terms of concentration, TNT was greatest with up to 87,000 mg/kg in Group 1 and up to nearly 20,000 mg/kg in Area L2. Both RDX and HMX were highly concentrated (>100 mg/kg) in only eight total samples (Table 3).

Regression analyses

Coefficients of determination (R^2) from singular linear-regression analyses of bioassay end points vs. ln (soil explosives concentrations) are shown in Table 4. Both TNT and TNB consistently had the greatest R^2 values when compared to all bioassay end points at all sites. The Group 1 data set had relatively high (0.53–0.88) R^2 values for 2-am-DNT and 4-am-DNT (Table 4); however, most soil concentrations of these chemicals were below the lowest certifiable limit (Table 3). RDX, HMX, and 2,6-DNT had the weakest relationships with bioassay end points (Table 4). The Microtox test had consistently lower R^2 values compared with the other tests (Table 4).

A stepwise forward selection multiple-regression technique [8] was used starting with TNT concentration vs. each

Table 3. Concentrations (mg/kg) of explosives in Group 1 and Area L2 soils

Site and sample	TNT	TNB	2,4-DNT	2,6-DNT	2-am-DNT	4-am-DNT	RDX	HMX
Group 1								
A2	655	22	<	2*	4*	2*	<	<
A3	<	<	<	<	<	<	<	<
A4	3*	<	<	<	<	<	<	<
A5	<	<	<	<	<	<	<	<
A6	<	<	<	<	<	<	<	<
B4	<	<	<	<	<	<	<	<
B5	<	<	<	<	<	<	<	<
C1	7	8	<	<	4*	9*	<	<
D1	1,066	17	2*	<	3*	7*	<	<
E1	7,114	49	7	<	19	20	1,509	315
E2	15	1*	<	<	2*	7*	3,101	572
E3	<	<	<	<	<	<	<	<
G1	9,123	28	22	<	7*	11*	<	<
G2	2,092	30	3*	<	6*	12*	<	<
H2	<	<	<	<	<	<	<	<
I1	10,680	22	19	<	4	12*	80	<
I2	87,052	24	117	4*	12*	8*	25	24
I3	17	<	<	<	<	<	<	<
Area L2								
K1	19,990	15	40	<	10*	8*	<	<
K2	519	12	8	4*	8*	5*	<	<
K3	4,594	200	<	<	<	<	7	5*
K5	<	<	<	<	<	<	<	<
L1	4,518	39	1*	<	6*	8*	6*	2*
L2	1,435	58	1*	<	8*	5*	3*	12*
L3	355	52	<	<	<	<	157	3*
L5	31	9	<	<	<	<	<	<
L6	<	<	<	<	<	<	<	<
M1	2,417	7	3*	3*	7*	3*	2*	4*
M2	6,025	161	13	8	14*	5*	1*	6*
M3	266	25	<	<	11*	4*	<	<
M5	<	<	<	<	<	<	<	<
M6	19	<	<	<	4*	6*	<	<
N1	2,655	145	3*	4*	12*	5*	3,574	433
N2	1,158	188	3*	1*	<	<	9	3,055
N3	<	<	<	<	<	<	<	<
N5	<	1*	<	<	6*	4*	<	1*
N6	<	1*	2*	<	<	6*	7	26
O1	<	<	<	<	<	<	<	<
O2	<	<	<	<	<	<	<	<
P1	<	<	<	<	<	<	<	<
Control								
A	<	<	<	<	<	<	<	<
B	<	<	<	<	<	<	<	<
C	<	<	<	<	<	<	<	<

<Below the detection limit (0.1 mg/kg).
*Value is below the lowest certifiable limit by U.S. Army Toxic and Hazardous Materials Agency QA program [14].

end point. Chemical variables were added if their inclusion in the model significantly ($p = 0.05$) improved R^2 values. The R^2 values were not improved significantly by addition of the other chemical variables for any of the toxicity tests. Therefore, the multiple-regression model was not included in this study.

Bioassay end points were plotted against ln(TNT concentration) at each site to delineate the range of concentrations at which toxicity first appeared. Group 1 linear-regression curves of end points vs. ln(TNT concentrations) are shown in Figure 3. The R^2 values for cucumber, radish, earthworm, and Microtox assays were 0.748, 0.864, 0.759, and 0.590, respectively. The data point at 100% Microtox EC50 and 0 mg/kg TNT (Fig. 3) represents seven sampling sites. All results were statistically significant at $p = 0.05$. Area L2 linear-regression curves of end points vs. ln(TNT concentrations) are shown in Figure 4. The R^2 values for cucumber, radish, earthworm, and Microtox assays were 0.764, 0.846, 0.911, and 0.678, respectively. The data point at 100% Microtox EC50 and 0 mg/kg TNT (Fig. 4) represents eight sampling sites. All results were statistically significant at $p = 0.05$.

Table 4. R^2 values from linear regression analysis of toxicity tests vs. soil explosives at Group 1 and Area L2

Site and toxicity test	Explosive (ln mg/kg)							
	TNT	TNB	2,4-DNT	2,6-DNT	2-am-DNT	4-am-DNT	RDX	HMX
Group 1 (n = 18)								
Cucumber	0.748	0.816	0.393	0.070	0.820	0.796	0.195	0.142
Radish	0.864	0.885	0.548	0.108	0.876	0.839	0.201	0.125
Earthworm	0.759	0.773	0.613	0.082	0.691	0.691	0.077	0.036
Microtox	0.590	0.713	0.364	0.047	0.605	0.533	0.007	0.0001
Area L2 (n = 23)								
Cucumber	0.764	0.710	0.348	0.190	0.310	0.204	0.183	0.192
Radish	0.846	0.761	0.521	0.254	0.324	0.146	0.142	0.155
Earthworm	0.911	0.814	0.358	0.293	0.383	0.201	0.246	0.232
Microtox	0.678	0.671	0.298	0.183	0.390	0.384	0.194	0.241

Values used for regression analyses were mean height (cm) for cucumber and radish, least-squares mean final weight (g) for earthworm, EC50 for Microtox, and natural log of the concentration (mg/kg) for explosives.

DISCUSSION

Data from the bioassays, HPLC analyses, and regression analyses were correlated to determine the extent of contamination in relation to environmental toxicity in soils at Area L2 burning ground and Group 1. Area L7. Toxicity testing resulted in the identification of toxic regions of soil within L2 and Group 1. The HPLC analyses confirmed contamination by explosives in samples that showed a highly toxic response in one or more of the bioassays. Results of regression analyses exhibited a strong relationship between the bioassays and soil TNT levels. Furthermore, an analysis of the scatter plots (Figs. 3, 4) indicated boundary sectors marking the extent of contamination and level(s) of toxicity within each site.

We were able to characterize the extent of toxic regions at each site by using transect sampling. Group 1 soils close to loading docks of buildings and to storage bunkers were found to be moderately to highly toxic. Area L2 had a fairly well-defined, highly toxic section in the central region. Radiating out from this central portion, toxicity levels decreased. The phytotoxicity and earthworm tests were most highly correlated with soil explosives concentrations in this study. Sensitivity varied slightly with site. The Microtox assay was the least correlated with explosives concentrations.

Few data exist to explain the fate and bioavailability of nitroaromatic explosive compounds in soil and their effects on terrestrial ecosystems. Cataldo et al. [15] found that TNT absorption by plants was inversely related to the amount of organic matter present in the soil. Therefore, plants grown

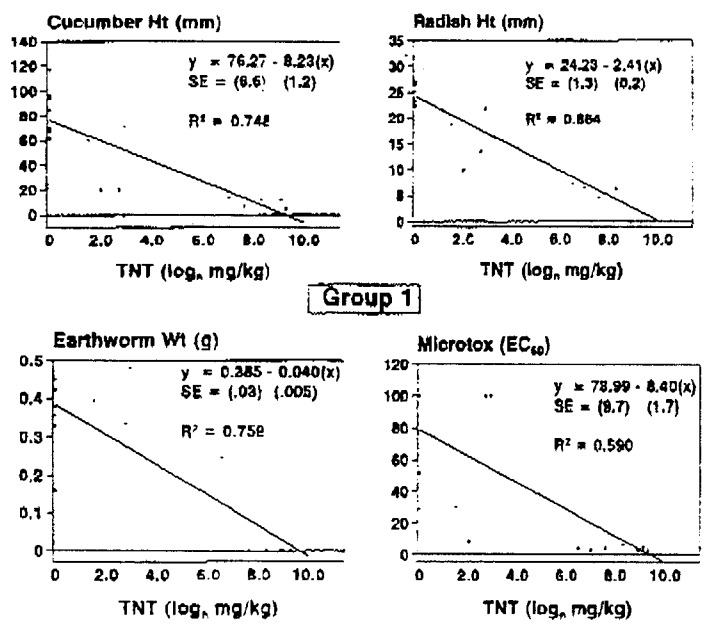

Fig. 3. Linear regression of Group 1 bioassay end points and soil TNT concentrations. SE = standard error.

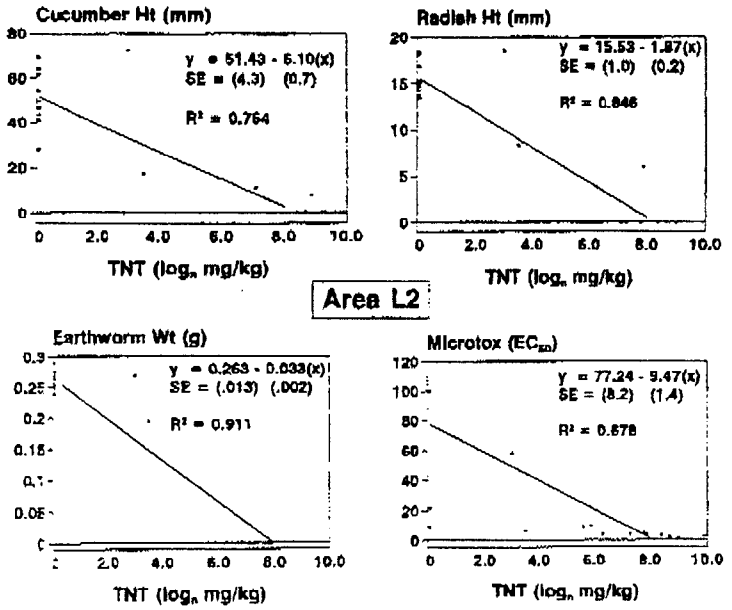

Fig. 4. Linear regression of Area L2 bioassay end points and soil TNT concentrations. SE = standard error.

in different soil types, containing varying amounts of organic matter, would absorb different amounts of TNT. Palazzo and Leggett [16] found that shoot and root growth of yellow nutsedge (hydroponically grown) was inhibited at 5 mg/L TNT. Our plant studies showed that growth reduction, germination rates, and survivability of plants grown in field soils can be used to define toxic areas at the sites used in the present study.

The soil explosive found in the greatest concentration and frequency, TNT, was used as an indicator of soil contamination in this study because it had the best correlation with the bioassay end points. In general, toxicity intensified as TNT concentrations increased. However, samples A3, A4, A5, B5, N5, N6, O1, and P1 had little or no detectable levels of explosives (see Table 5), yet were toxic to one or more of the bioassays (see Tables 1 and 2). To determine if toxicity may have been attributable to heavy metal contamination, these samples, excluding A5 and B5 (insufficient material), were analyzed for copper, zinc, and lead by atomic absorption spectroscopy (Perkin-Elmer model 3030 AAS, Norwalk, CT) as previously described [17]. Samples A3, A4, and N5 were toxic to *P. phosphoreum* (Microtox) but not to the other test organisms. Copper concentrations in these samples ranged from 22 mg/kg (N5) to 94 mg/kg (A3). Zinc concentrations ranged from 38 mg/kg (A3) to 45 mg/kg (A4). Previous studies (see [18]) have determined EC50 values for copper to be 644 mg/L for earthworm survival tests, 47 and 55 mg/L for radish and cucumber, respectively, in seed germination tests, and 0.28 to 0.42 mg/L for 15-min Microtox. The EC50 values for zinc were 628 mg/L for earthworms, 53 and 61 mg/L for radish and cucumber, respectively, and 1.6 mg/L for 15-min Microtox. Microtox is much more sensitive to these metals than are the other tests. Therefore, toxicity of samples A3, A4, and N5 to Microtox tests may have been caused by elevated levels of heavy metals. Sample O1, moderately toxic to earthworm, had lead concentrations >900 mg/kg. Sample P1, highly toxic to all test organisms, had elevated zinc (139 mg/kg) and lead (900 mg/kg) concentrations. Sample E2 had high levels of RDX (>1,500 mg/kg) and HMX (>500 mg/kg), producing moderate (radish) to high (cucumber) phytotoxicity. Previous work has shown that RDX levels ≥100 mg/kg in soil caused significant biomass reduction in cucumber seedlings [19].

A more thorough investigation of the toxicity of these and other metals reported to cause contamination (e.g., chromium, cadmium) of soils in these areas [2] is needed to determine the full etiology of the toxicity. It is important to note that the scope of this study was to determine the extent of toxicity—in general, of explosives, degradation products, and compounds related to the production process—and not to identify all chemical modes of action responsible for soil toxicity. The study was performed to address data gaps that were evident after the remedial investigation. At this point in the risk assessment, it is up to the risk manager to decide whether further testing is needed or remediation is warranted.

Lowest-observable-effect concentration (LOEC) and no-observable-effect concentration (NOEC) of soil contaminants are useful for risk assessment and prioritization of areas for remediation at hazardous-waste sites [20]. The LOEC of TNT was 7 mg/kg and 19 mg/kg for Group 1 and Area L2, respectively. Lethal effects (survival <80%) and significant reductions in plant and earthworm growth ($p = 0.05$), and Microtox EC50s (<70%), occurred at and above these concentrations. The NOEC was not reported in this study because concentrations of TNT were not linear among sampling locations and would not accurately estimate actual no-effects

levels. It is important to emphasize that LOEC in this study was site specific and highly dependent upon soil characteristics and concentrations of other soil contaminants.

CONCLUSIONS

The TNT was the primary toxic explosive in soils at the sites examined in this study. Both TNT and TNB correlated positively with all bioassay end points. Biodegradation products of TNT other than TNB, as well as RDX and HMX, contributed little to soil toxicity. The LOEC of TNT in this study was 7 to 19 mg/kg. Areas of soil toxicity within Area L2 and Group 1 have been clearly defined. These data can be compared with results from studies of other components of the ecosystem and used by the risk manager to select sites for remediation or for additional analyses. Although preliminary analyses determined that heavy metals may cause a toxic response in some of the bioassays, more extensive toxicity testing and chemical analyses are needed to establish a direct cause–effect relationship with heavy metals and soil toxicity.

This study presents a simple, relatively inexpensive methodology for assessing soil toxicity caused by TNT, RDX, their degradation products, and other contaminants (e.g., heavy metals) related to the production process. Multiple bioassay screening using different levels of biological organization, as implemented in this study, can more accurately determine toxic regions of site soils than can screening with tests at the same level. For example, the Microtox assay was least sensitive to explosives, but may be more sensitive to heavy metals. Bioassay analysis using site soils, together with carefully planned transect sampling, chemical analysis of contaminants, and knowledge of site soil characteristics by the risk assessor can aid the risk manager to define accurately the type and extent of remediation required. Consequently, time and money can be saved in the long run.

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