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Waterways Experiment Station

Plant Uptake of Explosives From Contaminated Soil and Irrigation Water at the Former Nebraska Ordnance Plant, Mead, Nebraska

by Richard A. Price, Judith C. Pennington, Steven L. Larson, WES
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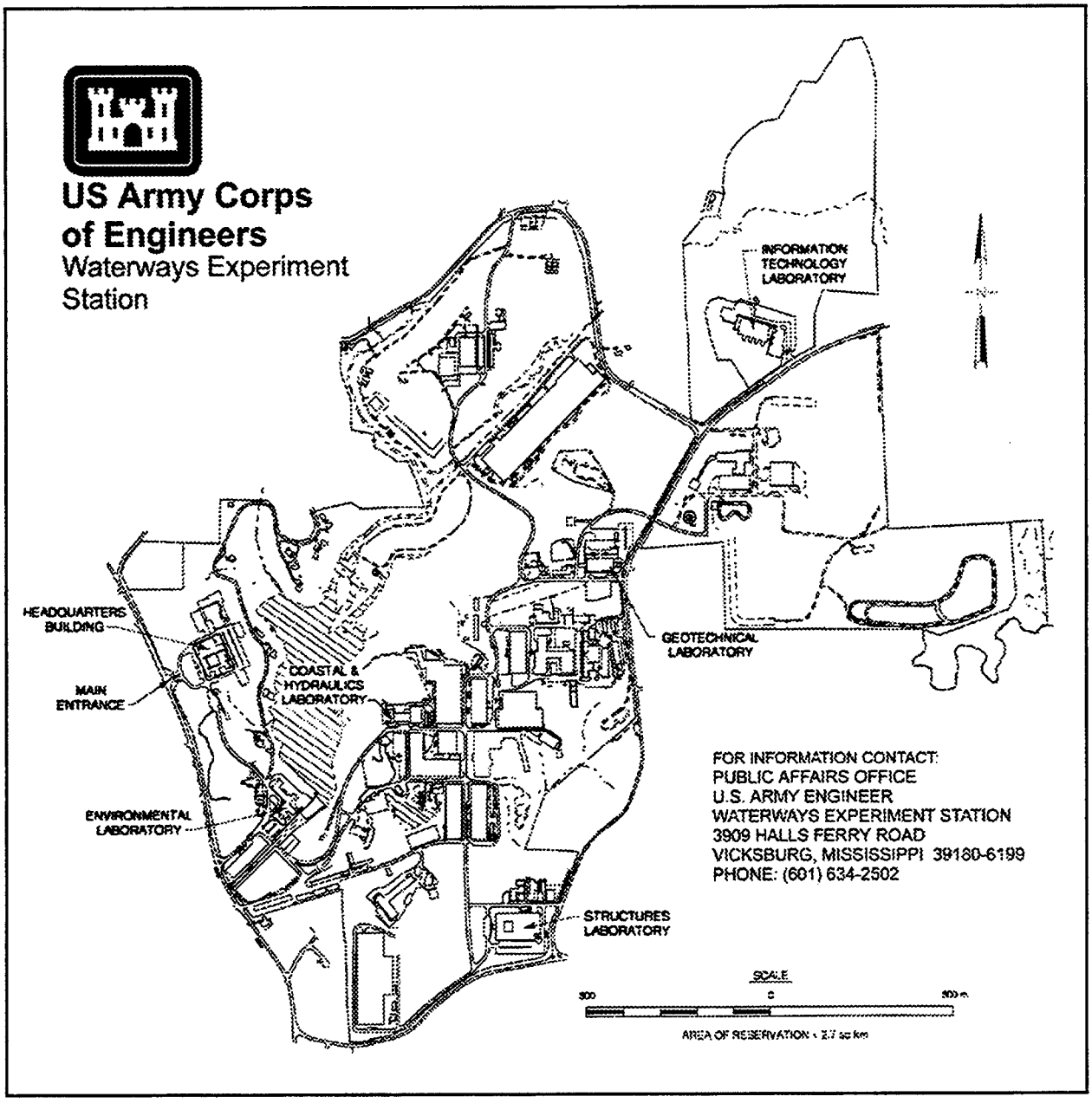

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Preface

The report herein was prepared for the U.S. Army Engineer District, Kansas City, Kansas City, MO, by the Environmental Laboratory (EL) of the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, in association with Mississippi College, Clinton, MS, and AScI Corporation, McLean, VA. The research was conducted in support of the former Nebraska Ordnance Plant Operable Unit 3 Remedial Investigation. The Principal Investigators were Dr. Judith C. Pennington, Ecosystem Processes and Effects Branch (EPEB), Environmental Processes and Effects Division (EPED), EL, WES, and Mr. Richard A. Price, Fate and Effects Branch (FEB), EPED. Analytical chemistry was performed by Dr. Steve Larson, Environmental Chemistry Branch, Environmental Engineering Division, EL. Project monitors were Mr. Garth Anderson, Mrs. Natalie Tillman, and Mr. Ed Louis, Kansas City District.

Authors of this report are Mr. Price, Dr. Pennington, and Dr. Larson, WES; Mr. David Neumann, Mississippi College; and Ms. Charolett Hayes, AscI Corporation. The report was reviewed by Drs. James M. Brannon, EL, and Elly Best, AScI Corporation. The study was conducted under the direct supervision of Dr. Richard E. Price, Chief, EPEB, and Dr. Bobby L. Folsom, Jr., Chief, FEB, and under the general supervision of Dr. Richard E. Price, Chief, EPED, and Dr. John Harrison, Director, EL.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

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1 Introduction

Background

The former Nebraska Ordnance Plant (NOP) is a Superfund site in Saunders County, Nebraska. Explosives were loaded, assembled, and packed into bombs, boosters, and shells at the site during World War II and the Korean Conflict. The ordnance were loaded with 2,4,6-trinitrotoluene (TNT), amatol (TNT and ammonium nitrate), tritonal (TNT and aluminum), and Composition B [TNT and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)]. Process wastewaters were discharged into sumps and bomb wash pits and their associated drainage ditch systems. In 1956 the NOP was placed on standby and declared excess in 1959. Currently, the property is owned by the University of Nebraska, the National Guard and Army Reserves, the Department of Commerce, and private individuals. Since explosives and volatile organic compounds were detected in soils and groundwater at the site, three operable units (OUs) were defined to address remediation, OU I, OU II, and OU III. This project falls under OU III, which includes possible waste disposal sites. Preliminary remediation goals (RGs) for the site are 2 μg RDX per liter in groundwater and 5.8 and 17.2 mg kg^{-1} for RDX and TNT in soils, respectively (Rust Environmental Infrastructure 1995).

Objectives

To prepare the Operable Unit III Risk Assessment addressing the potential hazards associated with future uses of the site, data describing plant uptake of explosives, especially RDX, were needed. Greenhouse studies were conducted using selected agronomic species, corn, tomato, lettuce, and radish, to measure plant uptake of explosives from contaminated soil and uptake of RDX from irrigation water. A reference plant was included in all greenhouse studies to develop a database from which future studies could predict uptake by growing the reference plant and extrapolating to the database for other crops. In addition to the greenhouse studies, a mass balance study with two species, tomato and radish, was conducted to determine the distribution of radiolabeled carbon from [^{14}C]RDX in each compartment of the test: soil, plant, and air. Since trichloroethene (TCE) was detected in 28 of 128 groundwater wells at the site

(Woodward-Clyde 1993), a soil-partitioning study was also conducted to determine the effects of TCE in irrigation water on bioavailability of RDX in soils.

Literature Review

The earliest work concerning plant uptake of explosives indicated toxicity of TNT wastes to duckweed (*Lemna perpusilla*), a tiny aquatic flowering plant (Schott and Worthley 1974). Authors tested several other explosives and defined the highest "no-effect" concentration range in mg L^{-1} for each compound tested as follows: TNT, 0.1 to 1.0; 2,4-dinitrotoluene, 0.1 to 0.5; 4-amino-2-nitrotoluene, 10 to 50; and 2-nitrotoluene, 10 to 100. Toxicity was determined by plant death or depression in growth. RDX was not examined. In another early work, depression in yields of ryegrass and orchardgrass by pink water, a wastewater containing approximately 140 mg TNT per liter of water was reported (Palazzo and Leggett 1983). Pink water resulted from washdown of explosives loading and packing operations. The same authors conducted a hydroponic study in which yellow nutsedge (*Cyperus esculentus)* was grown in TNT concentrations of 0, 5, 10, and 20 mg L^{-1} (Palazzo and Leggett 1986). Plant yields were affected beginning at 5 mg L^{-1}. Reduction in yields by TNT have also been reported in several other grasses (Kentucky bluegrass, chewings fescue, perennial ryegrass, and orchardgrass) and in legumes (red clover, alfalfa, and birdsfoot trefoil) (Palazzo, Bailey, and Graham 1988).

Uptake of TNT and two of its common transformation products, 4-amino-2,6-dinitrotoluene (4-ADNT) and 2-amino-4,6-dinitrotoluene (2-ADNT), from soils was first reported for yellow nutsedge (Pennington 1988a,b; Folsom et al. 1988). Yellow nutsedge took up TNT and 4-ADNT from soils, but not 2-ADNT (Pennington 1988b). The author concluded that plant uptake of TNT was limited due to interactions with the soil, especially with clay. Plant yields were dramatically affected by the type of soil contaminated. Yields were significantly reduced beginning at a soil concentration of 200 mg kg^{-1} when plants were grown in silt, but were unaffected when plants were grown in clay containing up to 400 mg TNT per kg (Folsom et al. 1988). Concentrations of TNT in plant tissues seemed to reach a maximum of 30 to 40 mg kg^{-1} that was independent of soil concentration of TNT. A small amount of 4-ADNT and of 2-ADNT was also found in plants.

Plant uptake of TNT by agronomic species, bush beans (*Phaseolus vulgaris*), wheat (*Triticum aestivum*), and blando brome *(Bromus mollis)*, was studied in hydroponic cultures (Cataldo et al. 1989). Plants were subjected to 10 mg TNT per L^{-1} of nutrient solution. Plants were analyzed after 1 and 7 days; therefore, fruiting structures were not formed. Results indicated concentrations of <0.35 mg TNT per kg fresh shoots and leaves and about 1.5 mg TNT per kg fresh roots except for bean roots, which contained 4.1 mg TNT per kg. Aminodinitro-toluenes were also detected and in greatest quantities in roots, up to 22 mg per kg fresh weight in brome. The same authors studied bush bean uptake of TNT from soils amended with 10 mg TNT per kg soil. Seeds accumulated <0.6 mg TNT per

kg during the 60-day test, while leaves, stems, pods and roots accumulated up to 8.98, 23.99, 0.59, and 104.04 mg kg^{-1}, respectively. Uptake was affected by soil properties, with greatest uptake from soils lowest in percent clay and organic matter.

A more recent study of effects of TNT and 4-ADNT on germination and early seedling development by tall fescue (*Festuca arundinacea*) indicated that germination decreased linearly as TNT concentration increased, but was not significantly affected by 4-ADNT at the same concentrations (Peterson et al. 1996). Concentrations <30 mg TNT per liter or 7.5 mg 4-ADNT per liter had little effect upon seedling growth and development. Use of tall fescue as a phytoremediation tool was suggested due to the high water use and extensive fibrous root systems of the species.

A survey of plant species at Joliet Army Ammunition Plant examined TNT concentrations in native vegetation. Results indicated no explosives in aboveground plant tissues. However, TNT, 2-ADNT, and 4-ADNT were found in some root samples of false boneset (*Kuhnia eupatorioides*), teasel (*Dipsacus sylvestris*), and bromegrass (*B. inermis*) (Schneider et al. 1994).

Results of several recent studies for development of plant species for phytoremediation of explosives in groundwater or surface water or in constructed wetlands are in preparation or in review (Best, Miller and Larson in ; Best, Miller, Zappi et al., in preparation; Best, Sprecher, Larson et al., in preparation; Hughes et al. 1997; and Thompson and Schnoor, in preparation). Thompson and Schnoor (in preparation) used poplar tree cuttings (*Populus deltoides x nigra)* to assess plant uptake of TNT and RDX. Mass balance results after 20 days exposure to ^{14}C-labeled TNT indicated greater uptake of radioactivity from hydroponic solution (86 percent of added radioactivity) than from amended soils (12 percent of added radioactivity). The activity was concentrated in the roots (57 percent and 10 percent for hydroponic and soil treatments, respectively) as opposed to leaves (19 and 0.7 percent, respectively) or stems (22 and 2 percent, respectively). After only 2 days of exposure to ^{14}C-labeled RDX in hydroponic cultures, significant activity was found in plant leaves (26 percent of added radioactivity). Uptake of RDX from soils was not studied.

In a series of phytoremediation studies by the Corps of Engineers for three Army ammunition plants, plant uptake of explosives by aquatic and wetland plants was explored. Results of a study using TNT-contaminated water from Volunteer Army Ammunition Plant in a flow-through system indicated that elodea *(Elodea canadensis),* coontail *(Ceratophyllum demersum),* and pondweed *(Potamogeton nodosus)* could not survive in the explosives-contaminated site water (Best, Miller, and Larson, in preparation). Narrow-leaved cattail *(Typha angustifolia)* survived. No TNT was found in cattail nor in dead plants, but 2ADNT and 4ADNT were detected in both; 24DNT was detected in dying plants only. In similar flow-through system studies performed with groundwater from Iowa Army Ammunition Plant, lethal concentrations for the following species were defined: coontail *(Ceratophyllum demersum),* pondweed *(Potamogeton nodosus),* and common arrowhead *(Sagittaria latifolia)* (Best, Miller, Zappi et

al., in preparation). The lethal concentration for TNT was 5 to 7 mg L^{-1}. For RDX, 5 to 6 mg L^{-1} were found to be toxic. Concentrations in plant tissues were not reported. In a study of groundwater at Milan Army Ammunition Plant, results of mass balance studies with ^{14}C-labeled TNT indicated 20 to 83 percent of added radioactivity recovered in plant material (Best, Sprecher, Larson et al., in preparation). Activity tended to be higher in emergent than in submersed plant species. Results using ^{14}C-labeled RDX showed greater uptake by submersed (18 to 57 percent) than by emergent species (19 to 29 percent), the opposite of TNT results.

Hughes et al. (1997) also investigated plant uptake of TNT by aquatic species, Eurasian watermilfoil (*Myriophyllum aquaticum)* and *Catharanthus roseus.* Results indicated transformation of TNT by plants to the monoamino transformation products, 2ADNT and 4ADNT. In mass balance studies, 22 to 33 percent of added radioactivity was recovered in plant extracts. Schneider et al. (1996) report accumulation of TNT, the aminodinitrotoluenes and dinitrotoluenes in plant roots of several agronomic species grown hydroponically and in soils. Species included bush beans (*Phaseolus vulgaris*), carrot (*Daucus carota),* radishes (*Raphanus sativus),* kale (*Brassica oleracea),* and lamb lettuce (*Valerianella locusta).*

Plant uptake data for RDX were extremely limited. Plant uptake of RDX by the agronomic species, bush beans *(Phaseolus vulgaris),* wheat (*Triticum aestivum),* and blando brome *(Bromus mollis),* has been studied in hydroponic cultures (Harvey et al. 1991). Plants were subjected to 1, 2.5, 5, and 10 mg RDX per liter of nutrient solution. Results indicated bioaccumulation of RDX in bush beans. After only 1 day of exposure to a hydroponic solution of 10 mg RDX per liter of solution, leaf tissue approached 20 mg kg^{-1}, and stem and root tissues contained about 10 mg kg^{-1}. After 7 days of exposure, foliar concentrations were 97 mg kg^{-1}, and stem and root tissue concentrations had changed little. Results of other hydroponic studies using corn, sorghum, and wheat showed that RDX was readily absorbed (Banwart and Hassett 1990). Concentrations of RDX in plant tops (in milligrams per kilogram) were approximately 10 to 15 times higher than the concentrations in the hydroponic solution (in milligrams per liter).

2 Site Characterization

Background

Soils

The NOP is located in Saunders County, Nebraska, near the town of Mead in an area referred to as Todd Valley. A description and distribution of soils in the area were determined from the Soil Survey of Saunders County, Nebraska (U.S. Department of Agriculture and University of Nebraska Conservation and Survey Division 1965). The soils in this area are of the Sharpsburg-Fillmore soil association, comprised of mostly Sharpsburg silty clay loam soil on well-drained sites. The Fillmore silty clay loam soils are primarily found in low, poorly drained areas. Butler silty clay loam soils typically form terraces along drainage areas. Some small areas of sandy Ortello soils are also present in the area. When drained, these silty clay soils are well suited to agriculture. Sharpsburg soils comprise most of the land area currently in row crop and pasture agricultural activity at the NOP site.

Site usage

In characterizing the NOP site, past and present land use activities were important in selecting areas from which to collect soils necessary to conduct laboratory tests. Soils contaminated with explosives occur predominantly near the actual former ordnance loading sites (Load Lines 1 through 4). However, "clean" (reference) soils of the same type, in this case the Sharpsburg soil, were also required. Most of the site is occupied by former manufacturing and storage facilities or current activities such as feedlots, pasture, row crops, etc. Any site close to facilities of the NOP or associated with agricultural chemicals or irrigation water were considered unlikely to contain "clean" soils. Except for a few scattered woodland sites, little evidence of historically undisturbed areas on the former NOP exists. Some of these wooded sites were used for collecting reference soil for the laboratory tests.

Methods and Materials

Soil sampling

Soils samples were collected from Load Line 2 Area A (LL2A) to screen for explosives concentrations and distribution (Figure 1). The LL2A is a 13.4- by 28-m fenced site on the east side of Load Line 2. The LL2A was selected as a candidate for explosives-contaminated soil due to its comparatively large area and previous estimates of both high TNT and high RDX concentrations (Rust Environment and Infrastructure 1995). Fifteen soil cores were collected to a depth of 30.5 cm according to the layout in Figure 2. The T3, C3, and B3 samples were collected from the center of the ditch. All other samples were collected from outside the ditch. Soils were also collected from two wooded areas to serve as potential reference soil sites (Figure 3). Five samples were collected from within an area measuring 84 m^2 from each wooded site. Cores were collected with a 6.35-cm-diam auger, placed into amber glass jars, and transported on ice to the U.S. Army Engineer Waterways Experiment Station (WES) for analysis of explosives.

Irrigation water sampling

Irrigation water wells were selected based on information provided by Woodward-Clyde and previous analysis (Woodward-Clyde 1993). Both contaminated and "clean" wells were selected. However, access to irrigation wells was dependent upon the working condition of the associated pumps and assistance from the University of Nebraska personnel. Four irrigation wells were sampled, IR13, IR15, IR16, and IR20 (Figure 4). After generous purging of the wells, samples were collected into 1-L amber glass jars and transported on ice to WES for analysis of explosives.

Results and Discussion

Soil analysis

Explosives concentrations were highly variable between individual samples collected from LL2A (Table1). Neither RDX nor TNT was restricted to the boundary of the ditch. Concentrations of RDX and TNT were highest for the T-3 sample, exceeding 4,000 and 3,000 mg kg^{-1}, respectively. Soil that would provide approximately 1:1, 2:1, and 10:1 ratios of RDX to TNT could be collected from three locations within LL2A. The remedial cleanup goal of 5.8 and 17.2 mg kg^{-1} of RDX and TNT, respectively, is approximately a 1:3 ratio. The northern reference site (REF1) had two samples with detectable concentrations of RDX and one sample with detectable concentrations of TNT. The southern reference site (REF2) had no detectable concentrations of explosive and was used as the "clean" reference soil.

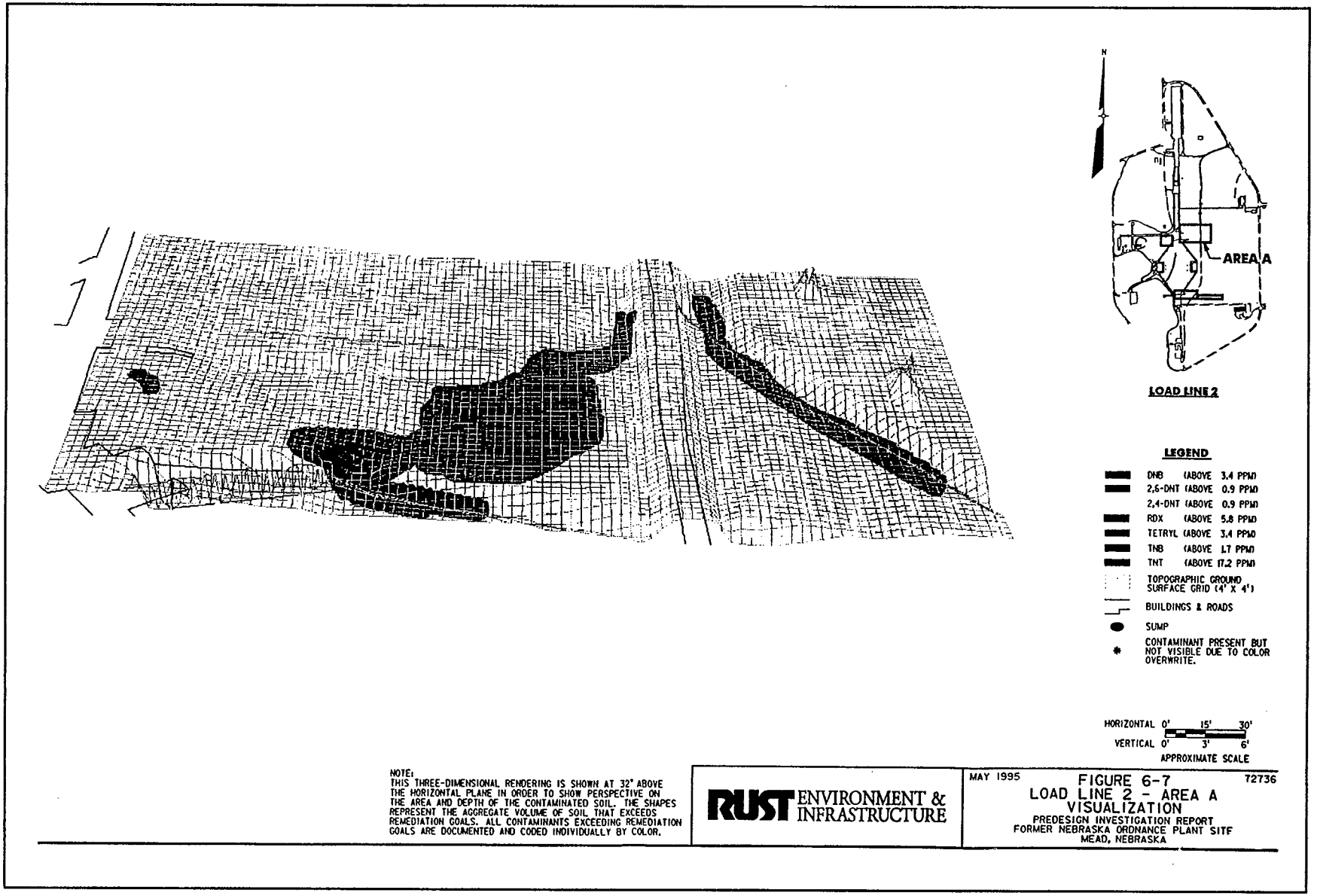

Figure 1. Location of Load Line 2 Area A (Rust Environment and Infrastructure)

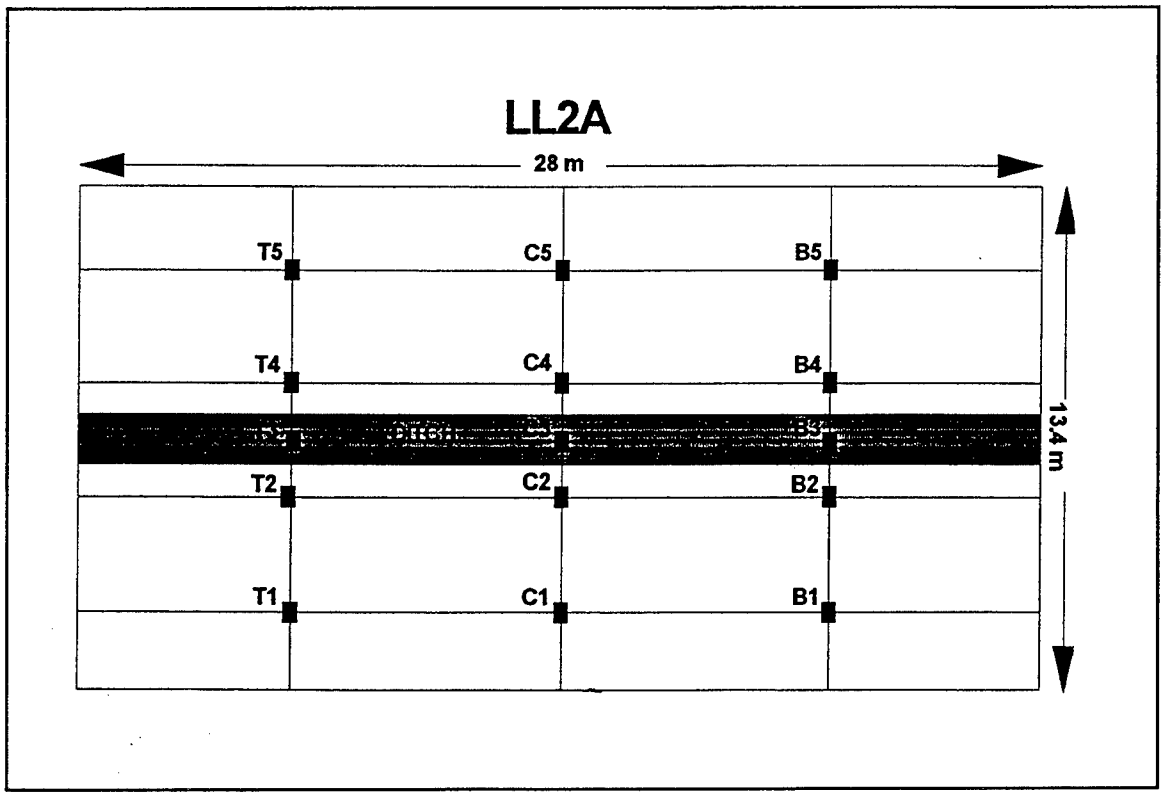

Figure 2. Sample locations in Load Line 2 Area A

Irrigation water analysis

The sample from IR13 had 0.3 μg L^{-1} and 3.75 μg L^{-1} HMX and RDX, respectively (Table 2). Explosive contaminants from the other three irrigation wells were below detection limits. Although 3.75 μg L^{-1} is above the 2.0 μg L^{-1} action level for RDX, higher levels were desired for the laboratory tests. After further consultation with Woodward-Clyde, two monitoring wells were selected (MW05 and MW48) as contaminated and clean irrigation water sources, respectively. The MW05 well data had previously shown RDX concentrations up to 98 μg L^{-1} (Woodward-Clyde 1993).

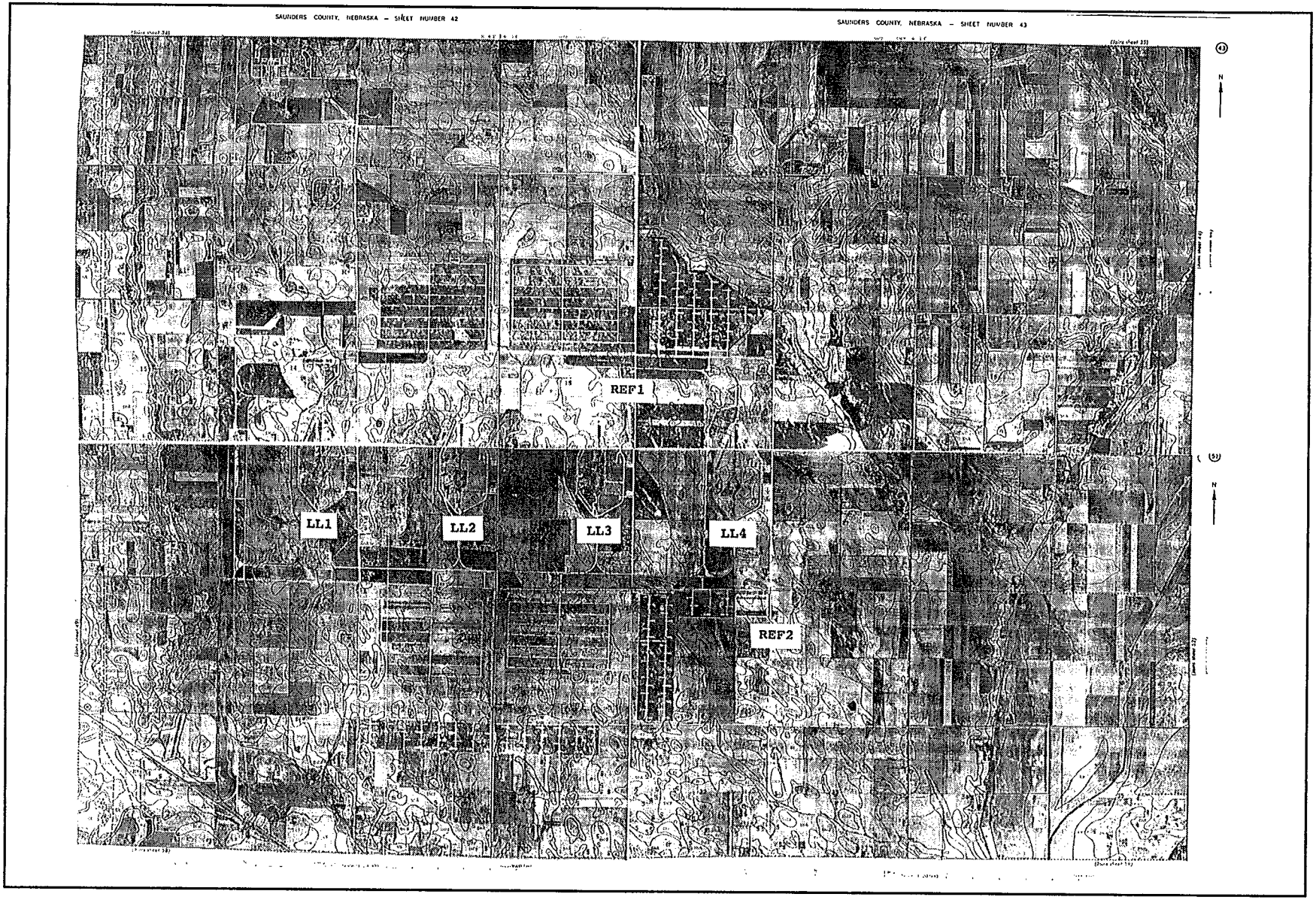

Figure 3. Reference soil locations

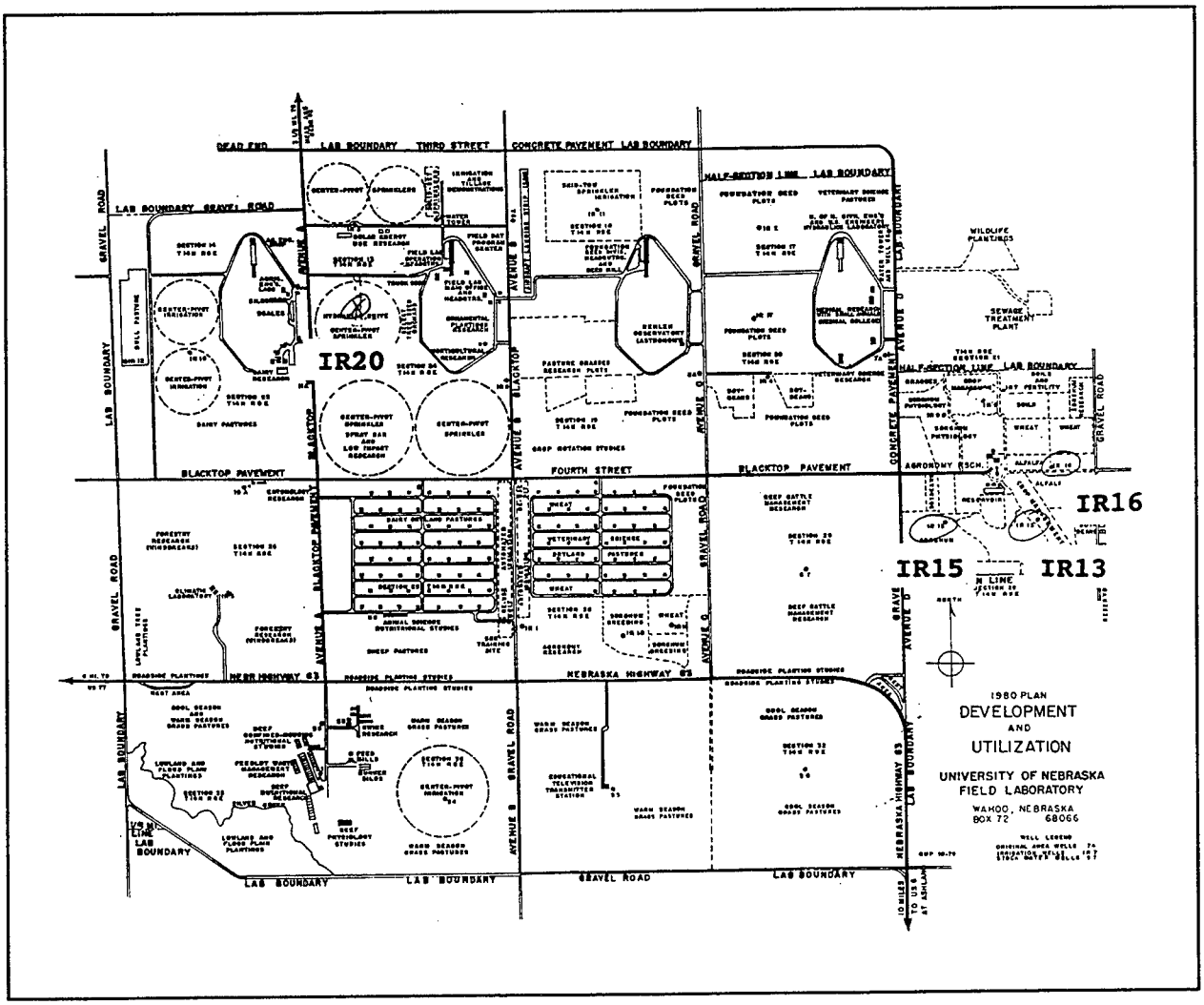

Figure 4. Irrigation well sample locations

Table 1
Soil Explosives Concentrations From Load Line Area A and Reference Soil Sites, mg kg^{-1}

LL2A Sample	HMX	RDX	TNB	DNB	Tetryl	TNT	4A-DNT	2A-DNT	2,6-DNT	2,4-DNT
T-1	0.210	0.346	<0.250	<0.250	<0.650	0.060	0.077	0.077	<0.260	<0.250
T-2	135	743	53.4	<2.5	<6.5	305	3.10	4.88	<0.260	1.42J
T-3	374	4,460	178	<2.50	<6.50	3,610	1.85J[1]	2.41J	<2.60	3.25
T-4	394	378	11.0	<2.50	<6.50	90.5	4.80	4.44	<2.60	<2.50
T-5	0.100J	1.08	<0.250	<0.250	<0.650	0.580	0.122J	0.087J	<0.260	<0.250
C-1	73.6	348	15.7	<2.50	<6.50	69.7	3.45	5.20	<2.60	0.892J
C-2	115	567	3.84	<2.50	<6.50	232	10.6	12.2	<2.60	1.10J
C-3	227	1,680	76.8	<2.50	<6.50	960	11.0	16.7	<2.60	2.18J
C-4	138	995	64.6	0.337J	<6.50	884	2.33J	3.03	<2.60	0.159J
C-5	0.250J	0.979J	0.678	<0.250	<0.650	1.47	0.148J	0.115J	<0.260	0.014J
B-1	17.6	209	149	1.66J	<6.50	2,690	5.35	8.66	<2.60	5.00
B-2	62.0	48.3	25.6	0.394J	<6.50	1,510	7.49	8.58	<2.60	1.94J
B-3	33.8	167	7.02	0.473J	<6.50	1,170	9.25	20.5	<2.60	3.61
B-4	58.9	251	4.40	<2.50	<6.50	12.5	2.31J	2.00J	<2.60	0.281J
B-5	0.693J	1.12	0.812	0.013J	<0.650	1.94	0.540	0.428	<0.260	0.056J
Reference Samples										
REF1-1	<2.20	0.059J	<0.250	<0.250	<0.650	<0.250	<0.250	<0.250	<0.260	<0.250
REF1-2	<2.20	<1.00	<0.250	<0.250	<0.650	<0.250	<0.250	<0.250	<0.260	<0.250
REF1-3	<2.20	<1.00	<0.250	<0.250	<0.650	<0.250	<0.250	<0.250	<0.260	<0.250
REF1-4	<2.20	<1.00	<0.250	<0.250	<0.650	<0.250	<0.250	<0.250	<0.260	<0.250
REF1-5	<2.20	0.053J	<0.250	<0.250	<0.650	0.015J	<0.250	<0.250	<0.260	<0.250
REF2-1	<2.20	<1.00	<0.250	<0.250	<0.650	<0.250	<0.250	<0.250	<0.260	<0.250
REF2-2	<2.20	<1.00	<0.250	<0.250	<0.650	<0.250	<0.250	<0.250	<0.260	<0.250
REF2-3	<2.20	<1.00	<0.250	<0.250	<0.650	<0.250	<0.250	<0.250	<0.260	<0.250
REF2-4	<2.20	<1.00	<0.250	<0.250	<0.650	<0.250	<0.250	<0.250	<0.260	<0.250
REF2-5	<2.20	<1.00	<0.250	<0.250	<0.650	<0.250	<0.250	<0.250	<0.260	<0.250

[1] J values are detected concentrations below method detection limits.

Table 2
Groundwater Explosives Concentrations From Irrigation Wells, µg L^{-1}

Irrigation Well No.	HMX	RDX	TNB	DNB	Tetryl	TNT	4A-DNT	2A-DNT	2,6-DNT	2,4-DNT
IR13	0.30	3.75	<0.20	<0.20	<0.50	<0.20	<0.20	<0.20	<0.20	<0.20
IR15	<0.20	<0.20	<0.20	<0.20	<0.50	<0.20	<0.20	<0.20	<0.20	<0.20
IR16	<0.20	<0.20	<0.20	<0.20	<0.50	<0.20	<0.20	<0.20	<0.20	<0.20
IR20	<0.20	<0.20	<0.20	<0.20	<0.50	<0.20	<0.20	<0.20	<0.20	<0.20

3 Collection of Soil and Irrigation Water

Methods and Materials

Collection of soils for greenhouse tests

Based on data resulting from the site characterization, contaminated soil was collected from three locations in Load Line 2 Area A. Two, six, and two drums were collected from areas B1, C2/C3, and T3, respectively (Figure 5). (These samples were subsequently designated NOPB, NOPC, and NOPT, respectively.) A backhoe was used to excavate the soil down to 0.3 m after first removing the majority of surface vegetation, where present (Figure 6). The soil was placed into new 208-L steel drums and transferred to a refrigerated truck. Twenty drums of uncontaminated soil were also collected from a reference site in the same manner (Figure 7).

Collection of groundwater for greenhouse tests

Based on data provided by Woodward-Clyde, groundwater was collected from one contaminated and one clean monitoring well, MW05 and MW48, respectively (Figure 8). Field support for groundwater collection was provided by Woodward-Clyde, including pumping equipment. Water was pumped from the well allowing for sufficient purging and optimization of flow prior to collection. Water was then transferred to 208-L closed-top drums. Five drums were collected from Well MW05, and 15 drums were collected from Well MW48. The drums were transferred to a refrigerated truck. Drums of water and soil were transported at 4 °C to WES.

Soil mixing and analysis

Upon reaching WES, the drums of soil from LL2A were segregated by location and then mixed in a soil lysimeter with a small rotary tiller (Figure 9).

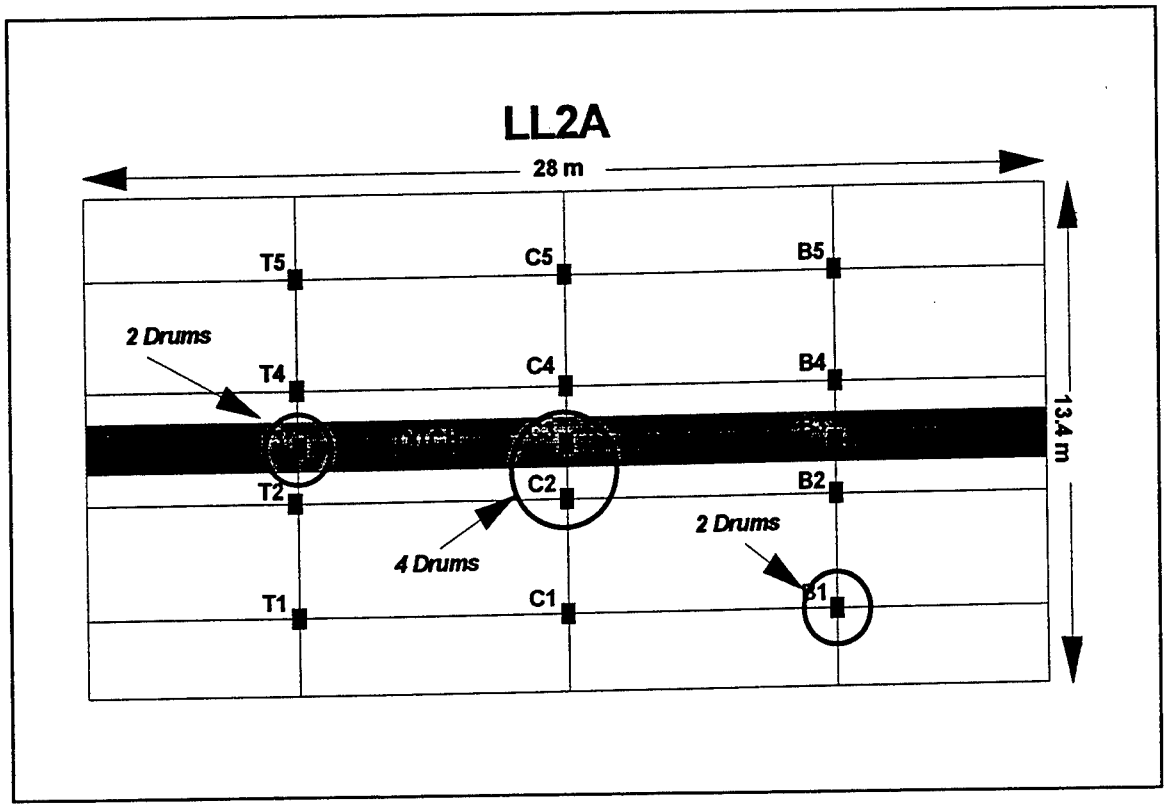

Figure 5. Locations of soil collection from LL2A for greenhouse tests

Figure 6. Collecting soil from LL2A with a backhoe

Figure 7. Collecting soil from reference site

Figure 8. Collecting groundwater from monitoring well (MW5-B)

Figure 9. Mixing soils from LL2A in a soil lysimeter

Due to the potential for photodegradation of TNT, light was kept at a minimum during the mixing process. The large volume of reference site soil required mixing on a concrete pad with a backhoe. After mixing each soil, three samples were collected for chemical analysis, and the soils were placed back into the drums until needed. Chemical analysis for explosives was conducted on all soils collected from the NOP site using EPA SW846 Method 8330 (U.S. Environmental Protection Agency (EPA) 1992). Soils from the NOP reference site were also analyzed for agricultural performance parameters: pH, phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), percent organic matter (OM), cation exchange capacity (CEC), and particle size distribution (percent sand, silt, and clay). Pettiet Agricultural Services (Leland, MS) performed analyses for the determination of pH, P, K, Mg, Ca, and CEC using the methods of Mehlich (1984) for P, K, Mg, and Ca. CEC was determined by summation of exchangeable bases (Ca, Mg, K) and total acidity. A determination of pH was accomplished by a glass electrode measure of a 1:2 soil to water mixture. OM was determined at WES by weight loss on ignition at 550 °C using Procedure No. 209E (American Public Health Association 1976). Particle size distribution was determined by the method of Day (1956) as modified by Patrick (1958).

Groundwater analysis

One 1-L sample was collected from drums 1, 8, and 15 of the MW5-B monitoring well and from drums 1, 3, and 5 of the 48-B monitoring well. The

samples were placed into amber jars and analyzed for explosives using the methods of EPA SW846 Method 8330 (EPA 1992).

Results and Discussion

Soil analysis

Concentration of RDX in the NOPB soil was significantly lower than concentrations at locations NOPC and NOPT, while concentrations of TNT at the three locations did not differ significantly (Table 3). Elevated levels of HMX and TNB were also found in LL2A soils. Due to the high concentrations of RDX and TNT, detection limits for the remaining compounds were high, resulting in no detectable concentrations except for 2-amino-4,6-dinitrotoluene (2A-DNT) in the NOPB and NOPC soils. The "J" indicates a value of a measurable peak that falls below the method detection limit (MDL) for that particular sample. Although previous core samples collected from the NOP reference site did not show any detectable concentrations of explosives, the NOPREF composite did contain trace concentrations of TNT. However, since trace levels of TNT were not expected to interfere significantly with plant uptake experiments, the soil was used as the reference soil. Phosphorus, potassium, and magnesium concentrations were considered more than adequate for plant growth, while calcium concentrations were considered low (Table 4).

Table 3
Mean (standard error) Explosives Concentrations of LL2A and Reference Soil Composites, mg kg^{-1}

Analyte	NOPB	NOPC	NOPT	NOPREF
RDX	283.33 (64.09)	1,810 (102.14)	2,683 (707.02)	<1.0 (0.0)
TNT	2,270 (1,010.36)	1,620 (208.17)	3,302 (96.10)	0.04 (0.01)
HMX	55.0 (9.45)	214 (10.58)	271 (66.88)	<2.2 (0.0)
TNB	84.67 (13.67)	151.33 (26.67)	128 (25.66)	<0.25 (0.0)
DNB	<25 (0.0)	<25 (0.0)	<25 (0.0)	<0.25 (0.0)
TETRYL	<65 (0.0)	<65 (0.0)	<65 (0.0)	<65 (0.0)
4A-DNT	<25 (0.0)	<25 (0.0)	<25 (0.0)	<0.25 (0.0)
2A-DNT	7.0 (2.75)	5.67 (1.36)	<25 (0.0)	<0.25 (0.0)
2,6-DNT	<26 (0.0)	<26 (0.0)	<26 (0.0)	<0.26 (0.0)
2,4-DNT	<25 (0.0)	<25 (0.0)	<25 (0.0)	<0.25 (0.0)

Table 4
Agricultural Analysis of NOP Reference Soil

pH	P mg kg^{-1}	K mg kg^{-1}	Mg mg kg^{-1}	Ca mg kg^{-1}	OM %	CEC Meq per 100 g	Sand %	Silt %	Clay %
5.65	429	1,339	720.5	2,505	5.14	28.5	31.5	46.6	21.9

Table 5
Mean Water Explosives Concentrations (standard error) From Monitoring Wells, µg L^{-1}

Monitoring Well No.	HMX	RDX	TNB	DNB	Tetryl	TNT	4A-DNT	2A-DNT	2,6-DNT	2,4-DNT
MW5-B	<0.20 (0.0)	0.25 (0.09)	<0.20 (0.0)	<0.20 (0.0)	<0.50 (0.0)	<0.20 (0.0)	<0.20 (0.0)	<0.20 (0.0)	<0.20 (0.0)	<0.20 (0.0)
48-B	<0.20 (0.0)	0.50 (0.21)	<0.20 (0.0)	<0.20 (0.0)	<0.50 (0.0)	<0.20 (0.0)	<0.20 (0.0)	<0.20 (0.0)	<0.20 (0.0)	<0.20 (0.0)

Analysis of groundwater

Groundwater from MW5-B (previously containing up to 98 µg RDX per liter) contained a mean of only 0.25 µg RDX per liter of RDX (Table 5). The "clean" well (48-B) contained a higher mean concentration of RDX (0.50 µg L^{-1}) than the contaminated well. These concentrations are below the 2-µg L^{-1} action level for the NOP groundwater and required the addition of RDX for plant uptake tests. Groundwater from these two wells could not be used for clean irrigation water in the plant uptake test, as the "clean" well did contain RDX. Therefore, reverse osmosis (RO) water was used for uncontaminated treatments.

4 Greenhouse Tests

Objectives

Phase 1: Plant uptake at remedial cleanup goals for soils

The objective of Phase 1 was to quantify the uptake of explosives into usable (edible) plant tissues when plants were grown in contaminated soil and/or irrigated with contaminated groundwater. Soil RDX and TNT concentrations were of the same concentrations as the remedial cleanup goals (RG) of 5.8 and 17.2 mg kg^{-1} of RDX and TNT, respectively. The concentration of RDX in irrigation water was 100 µg L^{-1}. The highest concentration of RDX detected in groundwater from the NOP site was 98 µg L^{-1} (Woodward-Clyde 1993) from BMW-005-082. Although the action level for RDX in groundwater at the NOP site is 2 µg L^{-1}, accumulation of enough RDX at this level to be detectable in plant tissues was considered very unlikely. If significant levels of RDX accumulated in plant tissues when plants are irrigated with groundwater containing 100 µg RDX per liter, then lower concentrations of RDX in groundwater could be addressed in Phase 2. Corn, tomato, radish, and lettuce represent field and garden crops likely to be grown in the NOP area. Yellow nutsedge (*Cyperus esculentus*) served as an index plant for explosives uptake in agricultural crops. Yellow nutsedge has been used successfully to predict heavy metal accumulations in agricultural plants (Folsom and Price 1989; Van Driel et al. 1983)) and has promise of predictive capabilities for explosives. A zero RDX concentration for soil and water was added as a control, and each experimental treatment was replicated five times. A description of treatment levels for Phase 1 is shown in Table 6.

Phase 2: Effects of explosives concentrations in soil and irrigation water on plant uptake

The objective in Phase 2 was to quantify the effects of concentrations of RDX and TNT in soils on plant uptake. Three agricultural crops, lettuce, tomato, and corn, and the reference plant, yellow nutsedge, were used in this experiment. Four soil RDX and TNT concentrations were tested including the 5.8- and 17.2-mg kg^{-1} soil concentration for RDX and TNT, respectively (Table 7). Two

Table 6
Phase 1 Soil and Irrigation Water Treatments

Label (treatment)	Soil RDX, mg kg^{-1}	Soil TNT, mg kg^{-1}	Water RDX, µg L^{-1}
S0W0 (clean soil and water)	0.00	0.00	0.00
S0W1 (clean soil, contaminated water)	0.00	0.00	100
S1W0 (RG soil, clean water)	5.80	17.2	0.00
S1W1 (RG soil, contaminated water)	5.80	17.2	100

Table 7
Phase 2 Soil and Irrigation Water Treatments

Label (treatment)	Soil RDX, mg kg^{-1}	Soil TNT, mg kg^{-1}	Water RDX, µg L^{-1}
S0W0 (clean soil and water	0	0	0
S1W0 (RG soil, clean water)	5.8	17.2	0
S2W0 (RG soil x 0.1, clean water)	0.58	1.72	0
S3W0 (RG soil x 10, clean water)	58	172	0
S4W0 (RG soil x 100, clean water)	580	1,720	0
S0W2 (clean soil, water RG x 250)	0	0	500
S0W3 (clean soil, water RG x 500)	0	0	1,000

RDX concentrations in irrigation water were tested. A zero concentration for soil and water was included as a control, and each treatment was replicated five times.

Phase 3: Effects of soil properties on plant uptake

Variations in soil physical and chemical characteristics have been shown to affect the availability or mobility of explosive compounds into plants (Cataldo et al. 1989) and plant growth and yields (Folsom et al. 1988). The objective of Phase 3 was to quantify the effects of soil type on the uptake of explosives with emphasis on particle size and organic matter. Three soils were used in Phase 3: the NOP reference soil (moderate clay content and moderate organic matter) and two soils from the Vicksburg, MS, area (one high clay and high organic matter and one low clay and low organic matter). Each of these soils was mixed with contaminated soil from LL2A. To increase organic matter content, some treatments included amendments of composted cow manure (Table 8). Only two plants, lettuce and yellow nutsedge, were included in Phase 3.

Table 8
Phase 3 Soil Treatments

Label	Soil, Clay Content	Cow Manure, % by weight	RDX, mg kg^{-1}	TNT, mg kg^{-1}
S0FC	Clean, high clay soil	30	0.0	0.0
S0FS	Clean, low clay soil	30	0.0	0.0
S1UC	RG high clay	0	5.8	17.2
S1FC	RG high clay	30	5.8	17.2
S1US	RG low clay soil	0	5.8	17.2
S1FS	RG low clay soil	30	5.8	17.2
S1UN	RG medium clay (NOP)	0	5.8	17.2
S1FN	RG medium clay (NOP)	30	5.8	17.2

Methods and Materials

Soil preparation

Since the objective of this study was to simulate site conditions of the NOP, site soils were used, where available, for the greenhouse tests. Concentrations in the range of remedial cleanup goals (RG) of 5.8 and 17.2 mg kg^{-1}, for RDX and TNT, respectively, were used. To accomplish this, contaminated soils from LL2A were mixed with reference soil (NOPREF) and in Phase 3 with soils collected from Vicksburg, MS. Two soils from LL2A (NOPB and NOPC) were mixed in a soil lysimeter by a weight ratio of 2.5 to 1. This was done to create a contaminated soil with an RDX to TNT ratio of 1 to 3, or the same ratio as the 5.8 to 17.2 RG. NOP reference soil was weighed and placed into a small laboratory soil mixer (Figure 10). The mixed NOPB and NOPC (NOPBC) was then added to the NOP reference soil and mixed for 5 min. For the different crops, soil weight and pot size were adjusted to provide for optimum plant growth (Table 9). Once mixed, soils were placed into appropriately sized pots (Figure 11) (Folsom and Price 1989).

Preparation of irrigation water

Irrigation water was prepared by adding RDX in an RO water solution to a polyethylene tank containing 189 L of MW5-B groundwater while stirring with an electric stirrer. The tank was wrapped in black plastic sheeting to protect the water from light during storage in the greenhouse. Water for irrigation was collected from a valve in the bottom of the tank while stirring with an electric stirrer. Phase 1 irrigation water was prepared for a target concentration of 100 µg L^{-1}. For Phase 2 irrigation water, only the 1,000-ug L^{-1} concentration was prepared in the tank. Irrigation water for the 500-µg L^{-1} treatment was collected

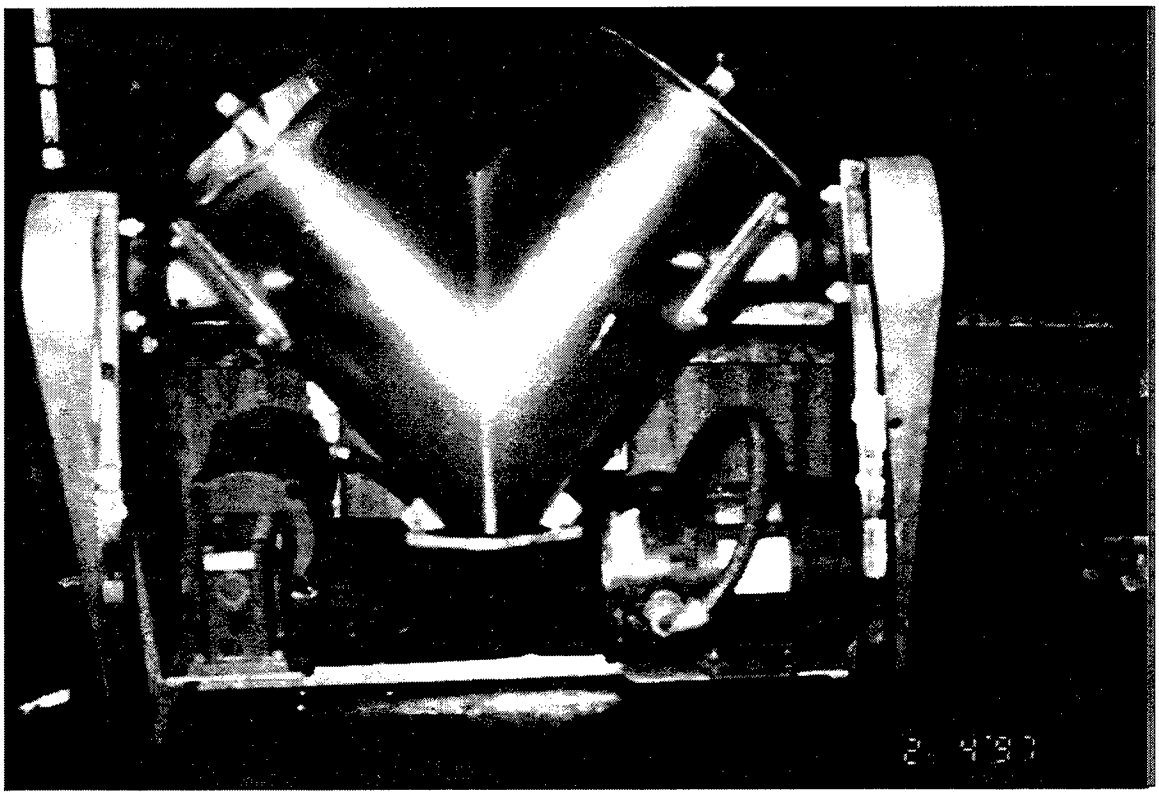

Figure 10. V-mixer used to mix LL2A soil with NOPREF soil

Table 9
Soil Weight and Pot Volume for Greenhouse Tests

Plant	Air Dry Soil Weight, kg	Pot Volume, L
Yellow nutsedge	4.5	7.6
Radish	6.0	11.4
Lettuce	6.0	11.4
Tomato	15.0	18.9
Corn	15.0	18.9

from the 1,000-μg L^{-1} tank and diluted by half with MW5-B groundwater as needed for irrigation. Since clean irrigation water was not collected from the NOP site, RO water was used for clean water irrigation and for supplemental water for all treatments. Total RDX loading for contaminated irrigation water is shown in Figure 12.

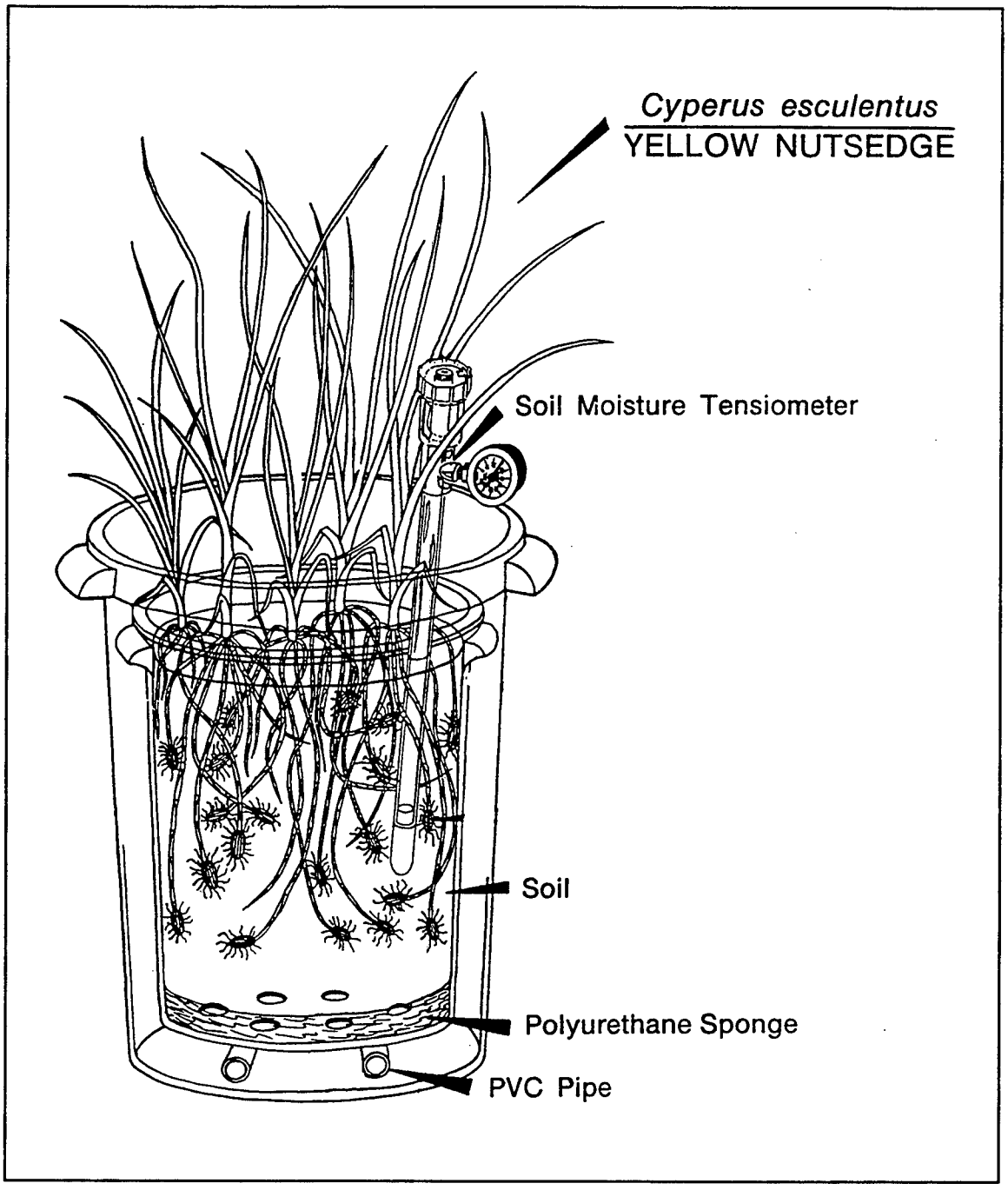

Figure 11. Schematic diagram of experimental unit for plant uptake test

Planting techniques

The pots were planted with seeds or seedlings of radish, lettuce, tomato, corn, and yellow nutsedge (Table 10). Variety, or cultivar, of each agronomic crop was those recommended by the University of Nebraska Cooperative Extension Service as suitable for use in Nebraska (Publication NF92-69, 1992). Seeds of

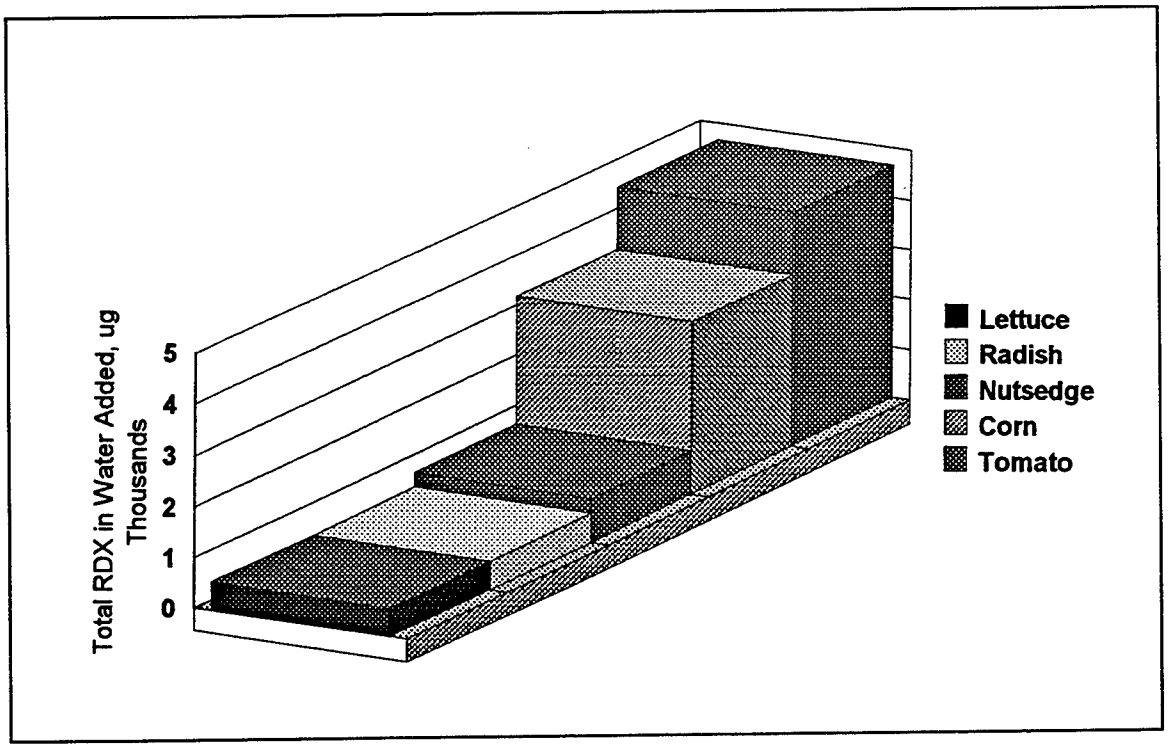

Figure 12. Total RDX loading from contaminated irrigation water, Phase I

Table 10
Plant Species and Planting Method

Common Name	Scientific Name	Variety/Cultivar	Planting Method
Radish	*Raphanus sativus*	White Icicle	Seedling
Lettuce	*Lactuca sativa*	Black Seeded Simpson	Seedling
Tomato	*Lycopersicon lycopersicum*	Early Girl hybrid	Seedling
Corn	*Zea maize*	Early Sunglow	Seed
Yellow nutsedge	*Cyperus esculentus*	NA	Seedling

Note: NA = Not applicable.

agronomic crops were obtained through BWI Companies, Inc. (Jackson, MS). Tubers of yellow nutsedge were obtained from Wildlife Industries, Inc. (Oshkosh, WI). Except for corn for which seeds were planted directly into test pots, plants were grown from seeds or tubers in the greenhouse to transplantable size. Lettuce, radish, and yellow nutsedge seedlings were transplanted three plants per test pot. After germination of the five corn seeds planted in each pot, seedlings were thinned to the two most vigorous plants. Tomatoes were transplanted one plant per pot. After planting, RO water was added to the soil surface and to the outer pot. Water was allowed to move through holes in the bottom of the inner

pot until the soil profile was completely moistened, and then water from the outer pot was removed.

Greenhouse Operation and Plant Growing Techniques

The five replicates of each treatment were randomly arranged in the greenhouse. Day length was maintained with an alternating pattern of high pressure sodium and high pressure multivapor halide lamps. The alternating lamps provide an even photosynthetic active radiation distribution pattern of 1,200 $\mu E\ m^{-2}s^{-1}$. The pots were placed at a height to allow maximum potential growth of each crop without heat damage from the light fixtures. Day length for the warm season crops (corn, tomato, and yellow nutsedge) was 16 hr and 12 hr for the cool season crops (lettuce and radish). Temperature was maintained for a summer environment of 32.2 °C (maximum) daytime and 21.1 °C (minimum) nighttime. Cool season crops, lettuce and radish, were subject to a 23.8 °C maximum day temperature and a 16.7 °C minimum night temperature. Relative humidity was maintained as close to 50 percent as possible. Soluble fertilizers, calcium nitrate ($CaNO_3$) and Miracle Grow, were added to ensure optimum plant growth. Foliar applied fungicides and insecticides were also used when necessary to control damaging insects and diseases. All treatment units received surface-applied irrigation water equivalent to $2.54 \times 10^5 l\ ha^{-1}$, or one acre-inch for each application. Each crop was irrigated up to three times weekly or less, depending upon water requirements of the crop. Moisture content of the soil was monitored using soil tensiometers to between 30 and 60 megapascals (Mpa) (field capacity is normally 30 Mpa). Any additional water requirements in excess of three weekly applications were supplemented with RO water by filling the outer container to the top of the inner container, allowing water movement through holes in the bottom of the inner container. Water was siphoned from the outer container when the tensiometer read less than 40 Mpa.

Plant Harvesting and Tissue Preparations

Forty-five days after planting, lettuce, radish, and yellow nutsedge were harvested in preparation for tissue analysis for explosives (Figures 13 through 15). Stainless steel scissors were used to cut the aboveground portion of lettuce and yellow nutsedge 5 cm above the soil surface. The tissue was weighed and washed in RO water to remove dust or soil particles. The aboveground as well as below-ground portion (root) of radish was harvested and weighed; however, only the edible root was analyzed. A small scrub brush was used to clean the radish root of soil to the extent that a home gardener would, noting that some soil particles may remain in pits and crevices of the root surface. Corn was harvested when the edible portions of the plants (kernels) were physically mature (76-80 days). (Figure 16 shows early growth). All of the aboveground portions of the corn plants were harvested after the kernels were removed from the plant and prepared for analysis. Corn kernels were removed from the cob to represent corn

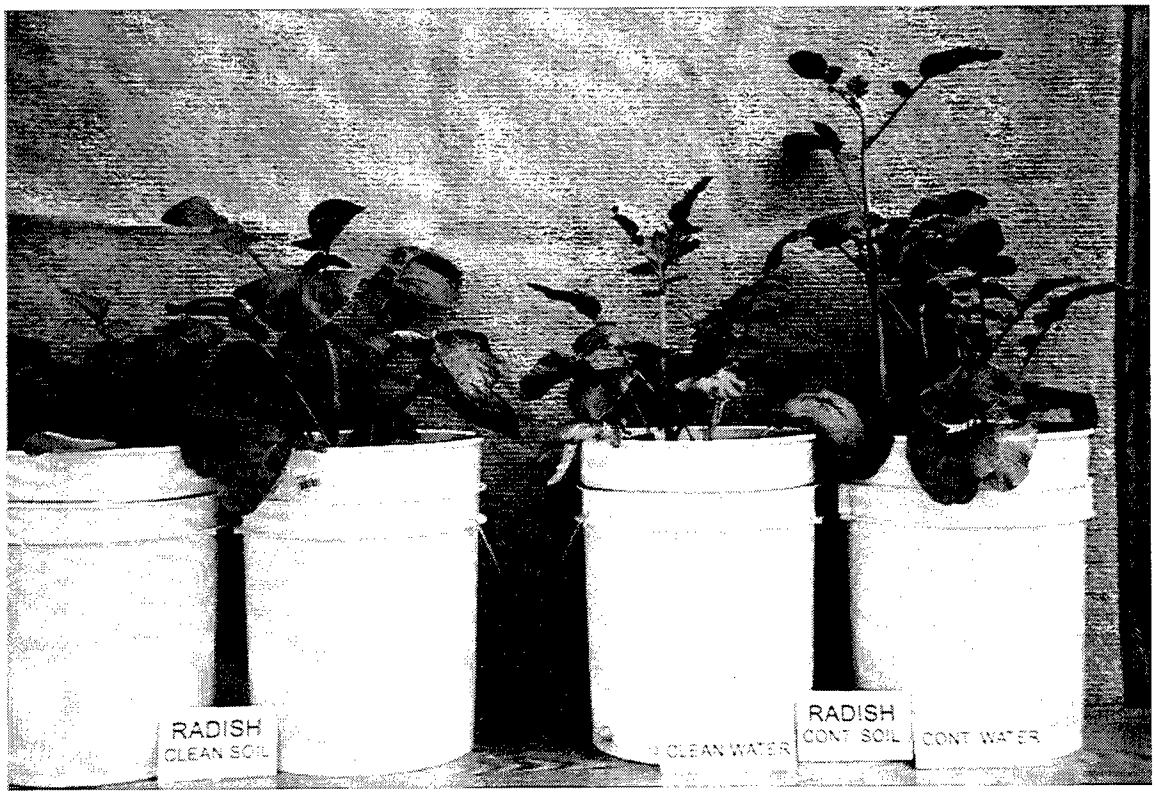

Figure 13. Radish plants in the four treatments

Figure 14. Lettuce plants in the four treatments

Figure 15. Yellow nutsedge in the four treatments

for human consumption. The cob was placed with the remainder of the plant (stalk, leaves, and shucks) to analyze as corn stover. Tomato fruit was harvested as it ripened, beginning as early as 50 days and ending on Day 85 (Figure 17). Both the fruit and vine of tomato plants were harvested and weighed; however, only the fruit was prepared for analysis of explosives. After collecting, weighing, and washing were completed, tissues was placed into plastic Ziploc bags and immediately frozen at -10 °C. In preparation for chemical extraction for explosives analysis, the tissues were ground in a green plant grinder then freeze-dried according to the methods in Appendix A. Subsamples were used to determine percent solids as freeze-dried weight. To determine explosives in plant tissues, modifications to Method 8330 for soils (EPA 1992) were used (Appendix A).

Statistical Analysis

All data were statistically evaluated using SAS software (SAS Institute, Inc., Cary, NC). Analysis of variance procedures were performed to determine significant differences between treatments, and the Waller-Duncan K-ratio test was performed to separate differences (Steel and Torrie 1980).

Figure 16. Corn plants in greenhouse at maturity

Figure 17. Tomatoes in greenhouse at maturity

Results and Discussion

Phase 1: Plant uptake at remedial cleanup goals for soils

The Phase 1 test was designed to address the effects of explosives in soil at the RG levels of 5.8 and 17.2 for RDX and TNT, respectively. Although the preplant RDX and TNT concentrations in the test soil were below the RG (Table 11), concentrations were within the range required and as close to the RG as can be expected when achieving concentration by mixing site soils. Four 190-L containers of site groundwater were spiked with RDX with a goal of achieving 100 $\mu g\ L^{-1}$. Extraction and analysis of the four containers showed the water to have a mean concentration of 134 ($\pm$11.18 standard deviation) $\mu g\ L^{-1}$. No other explosive compounds or their degradation products were detected. Since each crop has different water requirements and growth periods, the total amount of added irrigation water varied (Figure 12). Water requirements for the different crops were in the order of tomato > corn > nutsedge > lettuce = radish.

No significant differences in biomass due to treatment were evident except for corn ears and tomato vine (Table 12). Tomato vine weight was significantly higher in the contaminated soil receiving contaminated water than in other treatments. Corn ear weight was significantly higher in contaminated than in clean soil and when contaminated irrigation water was added to both clean and contaminated soil.

Table 11
Phase 1 Mean (standard error) Preplant Soil Explosives Concentrations, mg kg^{-1}

Treatment	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,4-DNT
S1W0	0.313 (0.263)	1.640 (1.252)	0.078 (0.038)	2.517 (3.059)	0.088 (0.049)	0.115 (0.051)	0.047 (0.011)

Table 12
Phase 1 Plant Biomass, g fresh weight mean (standard error)

Treatment	Radish Leaves	Radish Root	Lettuce Leaves	Nutsedge Leaves	Corn Stover	Corn Ears[1]	Tomato Fruit	Tomato Vine
S0W0	138.6 a (4.74)	48.8 ab (9.43)	185.4 a (10.64)	85.94 ab (3.74)	343.1 a (13.2)	44.83 c (10.58)	415.4 b (60.51)	528.8 b (38.23)
S0W1	144 a (9.51)	57 ab (11.17)	166.8 a (13.38)	97.98 a (5.74)	370.3 a (20.2)	81.8 b (14.87)	685 a (40.33)	566.8 b (20.35)
S1W0	137 a (7.58)	96.8 a (24.49)	191.8 a (6.19)	84.72 ab (4.02)	381.1 a (15.89)	105.21 b (7.16)	326.8 b (79.95)	533 b (28.82)
S1W1	152.8 a (9.55)	40.5 b (10.47)	188.2 a (9.5)	83.54 b (1.86)	375 a (12.47)	140.64 a (8.84)	361.4 b (122.71)	699.6 a (25.1)

Note: Means in a column with the same letter are not significantly different at the alpha = 0.05 level.
[1] Includes kernels and cob.

Contaminated irrigation water alone did not contribute to bioaccumulation of RDX in radish roots, tomato fruits, or corn kernels (Table 13). However, the leafy tissues of lettuce, yellow nutsedge, and corn stover accumulated detectable concentrations of RDX in one of five replicates for each crop. All crop tissues grown in contaminated soil accumulated RDX except for corn kernels. (Accumulations in tomato fruit were below method detection limit and were not significantly different from controls.) Accumulation of RDX significantly increased in lettuce grown in contaminated soil when contaminated water was also used for irrigation. For the contaminated soil and contaminated water treatments (S1W1), RDX uptake was in the order of yellow nutsedge > lettuce > radish root > corn stover > tomato fruit > corn kernel (no RDX detected).

Phase 2: Effects of explosives concentration in soil and irrigation water on plant uptake

Preplant soil concentrations of RDX and TNT (Table 14) were very close to the target concentrations previously given (Table 7). Concentration of RDX in the irrigation water approached, but did not reach, the target concentrations of 500 and 1,000 µg L^{-1} (Table 15). Mean RDX concentrations were 406 and 812 µg L^{-1} for the 500- and 1,000-µg L^{-1} treatments, respectively. HMX was also detected in the spiked site water at 50 µg L^{-1}. Each batch showed little change in the concentration of RDX over time. Grant, Jenkins, and Golden (1993) studied

Table 13
Phase 1 Mean (standard error) Plant Tissue RDX Concentrations, mg kg^{-1}

Treatment	Radish	Lettuce	Nutsedge	Tomato	Corn Kernel	Corn Stover
S0W0	<1.6 b (0.0)	<1.6 c (0.0)	<1.6 b (0.0)	<1.6 a (0.0)	<1.6 a (0.0)	<1.6 b (0.0)
S0W1	<1.6 b (0.0)	0.82J[1] c (0.02)	0.96J[1] b (0.16)	<1.6 a (0.0)	<1.6 a (0.0)	0.94J[1] b (0.14)
S1W0	1.99 a (0.28)	9.62 b (1.19)	10.34 a (3.09)	0.61J a (0.15)	<1.6 a (0.0)	1.66 a (0.2)
S1W1	2.33 a (0.12)	13.64 a (1.64)	14.48 a (2.88)	0.49J a (0.18)	<1.6 a (0.0)	1.95 a (0.26)

Note: Means in a column with the same letter are not significantly different at the alpha = 0.05 level. [1] = Number of replicates with detectable RDX; J = All detected values below method detection limits (MDL).

Table 14
Phase 2 Mean (standard error) Preplant Soil Explosives Concentrations, mg kg^{-1}

Treatment	HMX	RDX	TNB	DNB	TNT	4A-DNT	2A-DNT	2,4-DNT
S2W0	0.255 (0.007)	0.673 (0.223)	0.085 (0.021)	<0.100 (0.00)	1.670 (0.212)	<0.100 (0.00)	<0.100 (0.00)	<0.100 (0.00)
S1W0	1.010 (0.127)	7.675 (0.177)	0.713 (0.004)	<0.100 (0.00)	17.05 (4.45)	0.603 (0.194)	0.570 (0.184)	0.118 (0.046)
S3W0	8.63 (2.93)	50.3 (6.93)	9.20 (2.26)	0.148 (0.032)	213 (91.9)	1.68 (2.31)	2.64 (0.403)	0.773 (0.131)
S4W0	93.3 (31.1)	667 (137.2)	80.25 (1.48)	1.105 (0.134)	1,700 (127.3)	<0.100 (0.00)	16.0 (2.97)	7.37 (0.205)

Table 15
Explosives Concentrations in Each High Concentration (1,000 µg L^{-1}) Batch Prior to Dilution for Greenhouse Irrigation Water, µg L^{-1}

Irrigation Water	RDX	HMX
Batch 1: Initial	834	45.4
Batch 1: Final	812	44.8
Batch 2: Initial	843	60.8
Batch 2: Final	812	56.7
Batch 3: Initial	803	75.8
Batch 3: Final	792	79.5

holding times for explosives and found RDX to be stable in groundwater samples held at 22 °C for up to 70 days. Irrigation water volumes varied for the different crops. RDX loadings from irrigation water are shown in Figure 18.

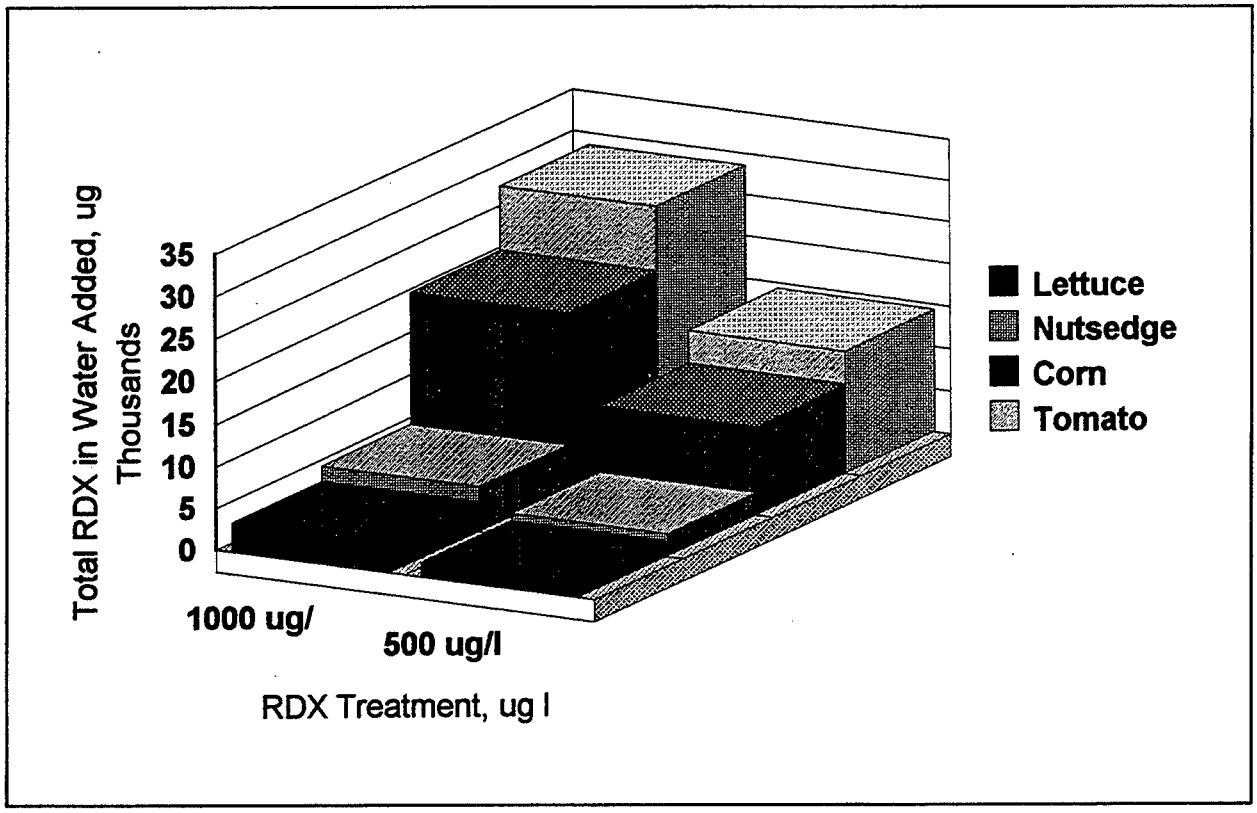

Figure 18. Total RDX loading from contaminated irrigation water, Phase 2

Yields of vegetative tissues generally increased as RDX concentration in the irrigation water increased from 0 to 1,000 μg L^{-1} (Table 16). Yields increased significantly in the 1,000-μg L^{-1} treatment (SOW3) compared with the control (S0W0) for lettuce, nutsedge, tomato vine, and corn stover. Lettuce, tomato fruit, and corn stover yields increased significantly compared with controls as RDX/TNT soil concentrations increased to the RG concentration levels (S1W0). The increase in yields may be an effect RDX or TNT has on soil-born diseases in some crops. A preliminary part of this study found blight on tomato was reduced with the addition of RDX and TNT to the NOP reference soil. Yields of yellow nutsedge and corn ears did not differ significantly as RDX/TNT concentrations increased from the control to the RG soil concentration (S1W0). Corn stover yields decreased as soil RDX/TNT concentrations increased. Yields were reduced for all plant tissues when RDX and TNT levels were increased to 58 and 172 μg kg^{-1}, respectively (S3W0 treatment). Tolerance to elevated concentrations of RDX and TNT in soils (S3W0) were in the order (most to least tolerant) of corn stover > tomato vine > nutsedge > corn ears > tomato fruit > lettuce. In the highest soil RDX and TNT treatment of 580 mg kg^{-1} RDX and 1,720 mg kg^{-1} TNT (S4W0), plant death occurred for all plants. However, plant survival may increase in soils contaminated with RDX in absence of TNT, or when TNT

Table 16
Phase 2 Plant Tissue Biomass, g fresh weight mean (standard error)

Treatment	Lettuce	Nutsedge	Tomato Vine	Tomato Fruit	Corn Ears[1]	Corn Stover
S0W0	55.12 cd (6.19)	68.72 b (5.67)	624.24 c (16.32)	76.7 a (28.4)	32.32 ab (4.99)	339.54 b (19.5)
S0W2	73.68 bc (16.03)	75.06 ab (3.10)	731.98 ab (18.60)	41.14 abc (12.12)	36.98 a (8.90)	352 b (6.85)
S0W3	126.64 ab (26.53)	83.78 a (3.87)	815.44 a (25.24)	67.46 ab (18.56)	43.84 a (8.35)	392.46 a (8.99)
S2W0	133.74 a (28.59)	66.44 b (4.05)	758.68 ab (32.41)	73.20 a (20.75)	32.5 ab (6.45)	301.48 c (11.39)
S1W0	160.5 a (29.41)	75.06 ab (3.91)	722.72 b (34.03)	22.56 bc (8.70)	19.48 bc (2.47)	284.26 c (7.43)
S3W0	2.02 de (0.54)	10.44 c (1.27)	19.62 d (2.87)	2.80 c[2] (0.0)	6.68 cd (1.97)	20.7 d (2.66)
S4W0	PD[3] e	PD d	PD d	PD c	PD d	PD d

Note: Means in a column with the same letter are not significantly different at the alpha = 0.05 level.
[1] Includes kernels and cob.
[2] Weight of one green tomato.
[3] PD = Plants died.

concentrations are reduced. Since this study evaluated the effects of both RDX and TNT in combination, the effects of RDX alone cannot be determined. TNT alone has been shown to decrease plant growth in some soil conditions. Skogerboe et al. (unpublished)[1] found plant growth limited to 50 percent at TNT concentrations of 300 mg kg^{-1}. Folsom et al. (1988) reported significant reductions in plant yields when TNT concentration in soil reached 200 mg kg^{-1} and plant death at 400 mg kg^{-1}. These tests were conducted in a soil low in clay contents. Yields decreased in TNT-contaminated soils as pH of the soil increased. However, plant yields were unaffected by TNT in clay soil containing TNT up to 400 mg kg^{-1}.

Contaminated irrigation water contributed to elevated levels of RDX uptake by lettuce, yellow nutsedge, and corn stover in both contaminated water treatments (S0W2 and S0W3) (Table 17). However, differences were not significant. Tomato fruit and corn kernels did not accumulate detectable concentrations of RDX. The data from Phases 1 and 2 are combined to illustrate the effects of RDX concentration in irrigation water on plant uptake of RDX (Figure 19). The concentration of RDX in these irrigation water treatments was roughly 65 to 400 times the RG concentration of 2 µg L^{-1} for site groundwater. Therefore, RDX concentrations in water near the RG concentration were not

[1] Skogerboe, J. G., Lee, C. R., Simmers, J. W., Brandon, D. L., and Karr, L. A. "Biotechnical slope stabilization and erosion control, SUBASE Bangor," U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS, and Naval Civil Engineering Laboratory, Port Hueneme, CA.

Table 17
Phase 2 Mean (standard error) Plant Tissue RDX Concentrations, mg kg^{-1}

Treatment	Lettuce	Nutsedge	Tomato Fruit	Corn Kernel	Corn Stover
S0W0	<1.6 (0.0) b	<1.6 (0.0) b	<1.6 (0.0) b	<0.8 (0.0) b	<0.8 (0.0) b
S0W2	11.14 (1.06) b	0.98J (0.05) b	<1.6 (0.0) b	<0.8 (0.0) b	0.74[2] (0.21) b
S0W3	21.32 (1.2) b	2.96 (0.27) b	<1.6 (0.0) b	<0.8 (0.0) b	1.78 (0.25) b
S2W0	7.90 (2.29) b	1.02[1] (0.22) b	<1.6 (0.0) b	<0.8 (0.0) b	<0.8 b
S1W0	154 (36.1) b	7.16 (5.67) b	5.32[4] (2.42) a	<0.8 (0.0) b	7.12 (0.45) b
S2W0	1,172 (157) a	62.46 (9.62) a	7.20[1][1] (0.0) a	6.14 (2.24)[2] a	55.82 (15.1) a
S4W0	PD[3]	PD	PD	PD	PD

Note: Means in a column with the same letter are not significantly different at the alpha = 0.05 level; J = All detected values below MDL; [1] = Number of replicates with detectable RDX; [4] = Number of replicates analyzed.
[1] Green tomato fruit.
[2] Kernel and cob.
[3] Plants died.

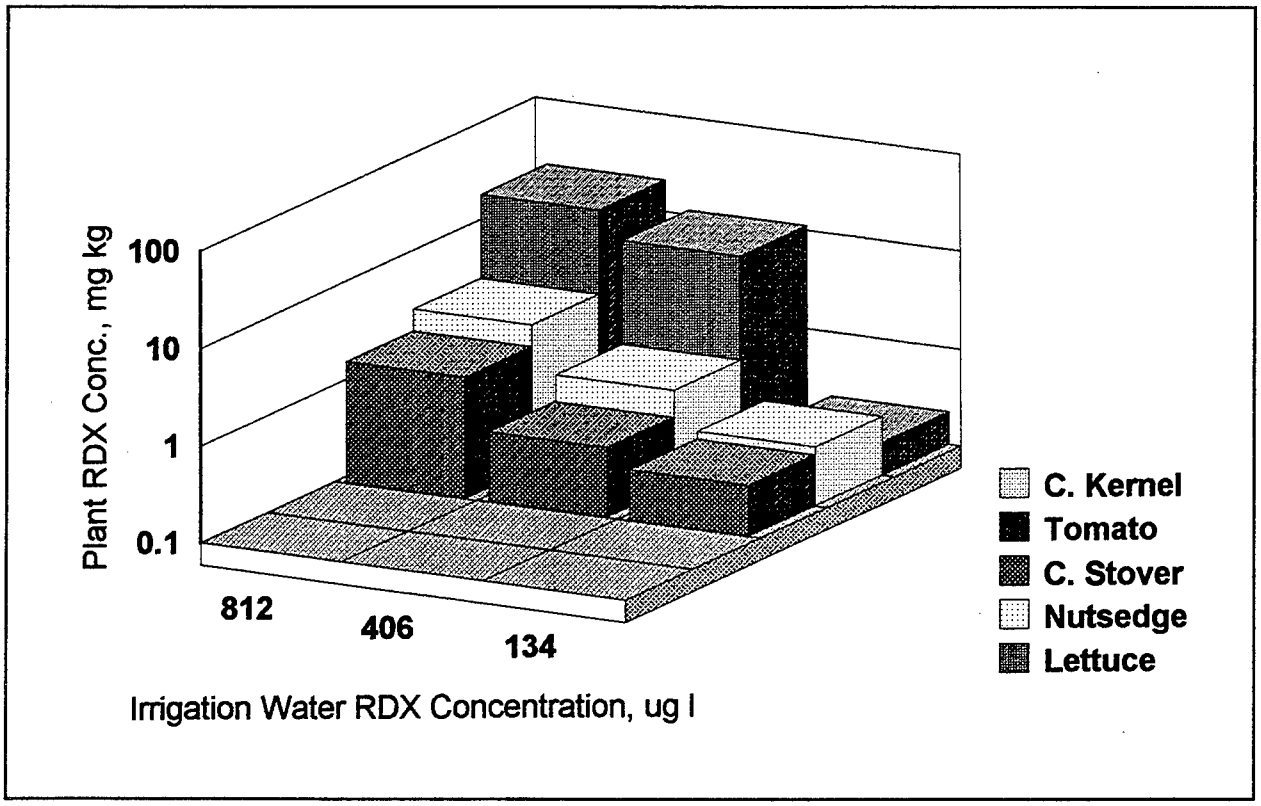

Figure 19. Effect of RDX concentration in irrigation water on plant uptake of RDX (from Phases 1 and 2)

expected to contribute to detectable RDX in plant tissues. (Current detection limits of RDX in plant tissues (milligrams per kilogram) are as follows: lettuce, nutsedge, and tomato fruit, 1.6; corn kernels and corn stover, 0.8.)

Increasing concentrations of RDX in soils contributed to plant uptake of RDX to the point of plant death (Table 17). Greatest uptake was by lettuce, yellow nutsedge, and corn stover. Corn kernels did not accumulate detectable concentrations of RDX until soil concentrations of RDX reached 58 mg kg^{-1}. Tomato fruit accumulated RDX at the 5.8- and 58-mg kg^{-1} soil level. Lettuce had the highest concentration of RDX, followed by yellow nutsedge, corn stover, tomato, and corn kernel. Other explosives compounds were also detectable in plant tissues from some treatments in Phase 2 (Table 18).

Table 18
Concentrations (mg kg^{-1}) of Analytes Other Than RDX in Plant Tissues of Phase 2 Experiments

Treatment	Crop	Replicate	HMX	TNT	2A-DNT	4A-DNT	MNX
S1W0	Lettuce	R1	3.76	<1.60	<1.60	<1.60	<1.60
S1W0	Lettuce	R2	4.32	<1.60	<1.60	<1.60	1.60
S1W0	Lettuce	R3	6.40	<1.60	<1.60	<1.60	3.68
S1W0	Lettuce	R4	6.00	<1.60	<1.60	<1.60	2.24
S1W0	Lettuce	R5	4.08	<1.60	<1.60	<1.60	1.60
S3W0	Lettuce	R1	37.2	<1.60	<1.60	<1.60	<1.60
S3W0	Lettuce	R2	34.4	<1.60	<1.60	<1.60	<1.60
S3W0	Lettuce	R3	49.1	<1.60	<1.60	<1.60	<1.60
S3W0	Lettuce	R4	42.2	<1.60	<1.60	<1.60	4.08
S3W0	Lettuce	R5	52.6	<1.60	<1.60	<1.60	2.48
S1W0	Nutsedge	R5	3.68	<1.60	<1.60	<1.60	<1.60
S3W0	Nutsedge	R1	5.04	<1.60	<1.60	<1.60	<1.60
S3W0	Nutsedge	R2	6.24	<1.60	<1.60	<1.60	<1.60
S3W0	Nutsedge	R3	5.60	<1.60	<1.60	<1.60	<1.60
S3W0	Nutsedge	R4	8.40	<1.60	<1.60	<1.60	<1.60
S3W0	Nutsedge	R5	4.96	<1.60	<1.60	<1.60	<1.60
S3W0	Corn kernel	R3	<0.80	<1.60	<0.80	<0.80	<0.80
S1W0	Corn stover	R4	<0.80	3.92	<0.80	<0.80	<0.80
S3W0	Corn stover	R2	4.24	<1.60	<0.80	<0.80	<0.80
S3W0	Corn stover	R3	3.44	1.44	<0.80	<0.80	3.98
S3W0	Corn stover	R4	4.80	<1.60	<0.80	<0.80	1.71
S3W0	Corn stover	R5	8.96	<1.60	<0.80	<0.80	4.72
S1W0	Tomato	R2	<1.60	<1.60	<1.60	<1.60	<1.60

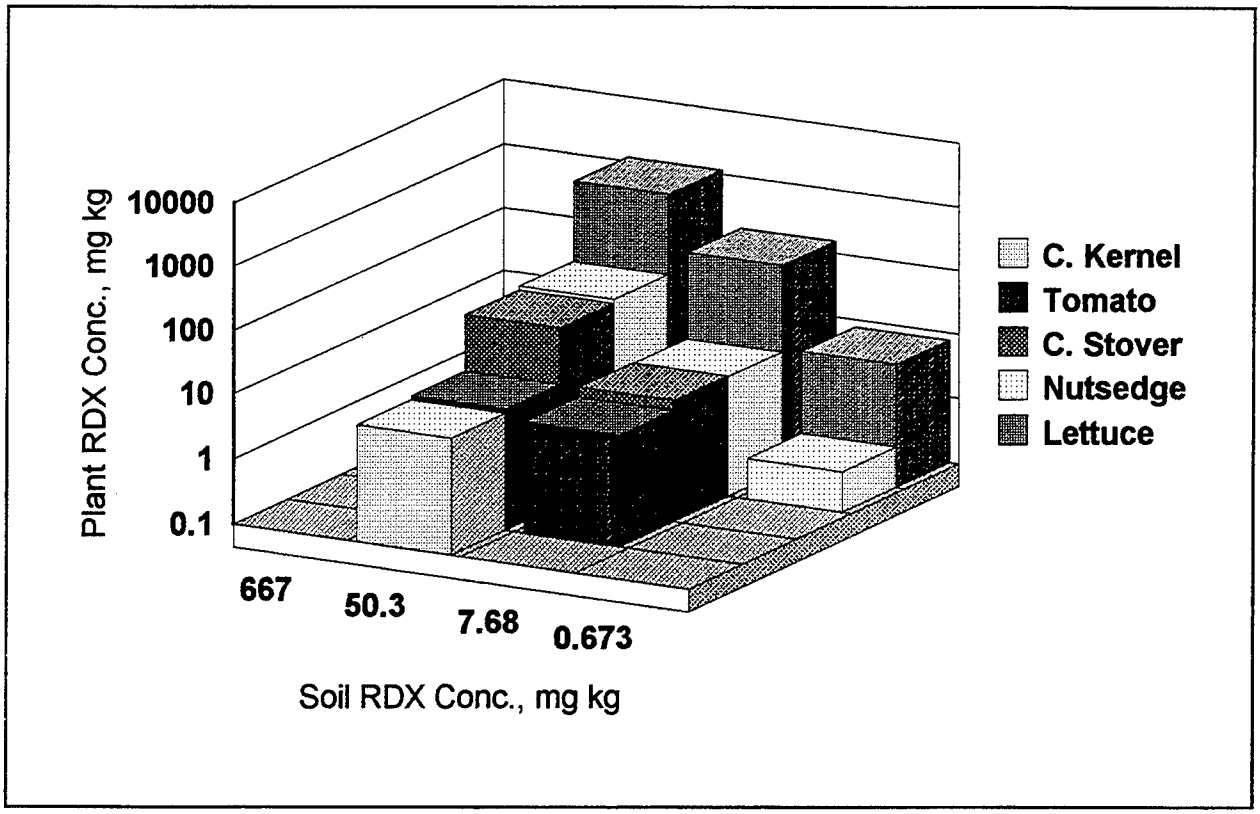

Figure 20. Effect of soil RDX concentration on plant RDX concentration

The accumulation of RDX by plant tissues (concentration, milligrams per kilogram) can be compared with soil RDX concentration (Figure 20). However, to determine the total uptake of RDX into plants, the total dry weight (g) of plant biomass (Table 19) is multiplied by the total RDX concentration. Total uptake is the total mass of RDX that has mobilized from the soil into plant tissues and can differ dramatically depending on the total biomass of the plant. Although the highest RDX concentrations in plant tissue occurred in the highest RDX soil

Table 19
Total Dry Weight (g) Biomass of Selected Plants

Treatment	Lettuce	Yellow Nutsedge	Corn Stover
S0W0	4.10	13.36	84.82
S0W2	5.22	15.73	91.1
S0W3	6.91	16.55	104.1
S2W0	14.68	13.23	78.57
S1W0	11.14	14.59	80.96
S3W0	0.237	1.82	4.98
S4W0	PD[1]	PD	PD

[1] Plant death.

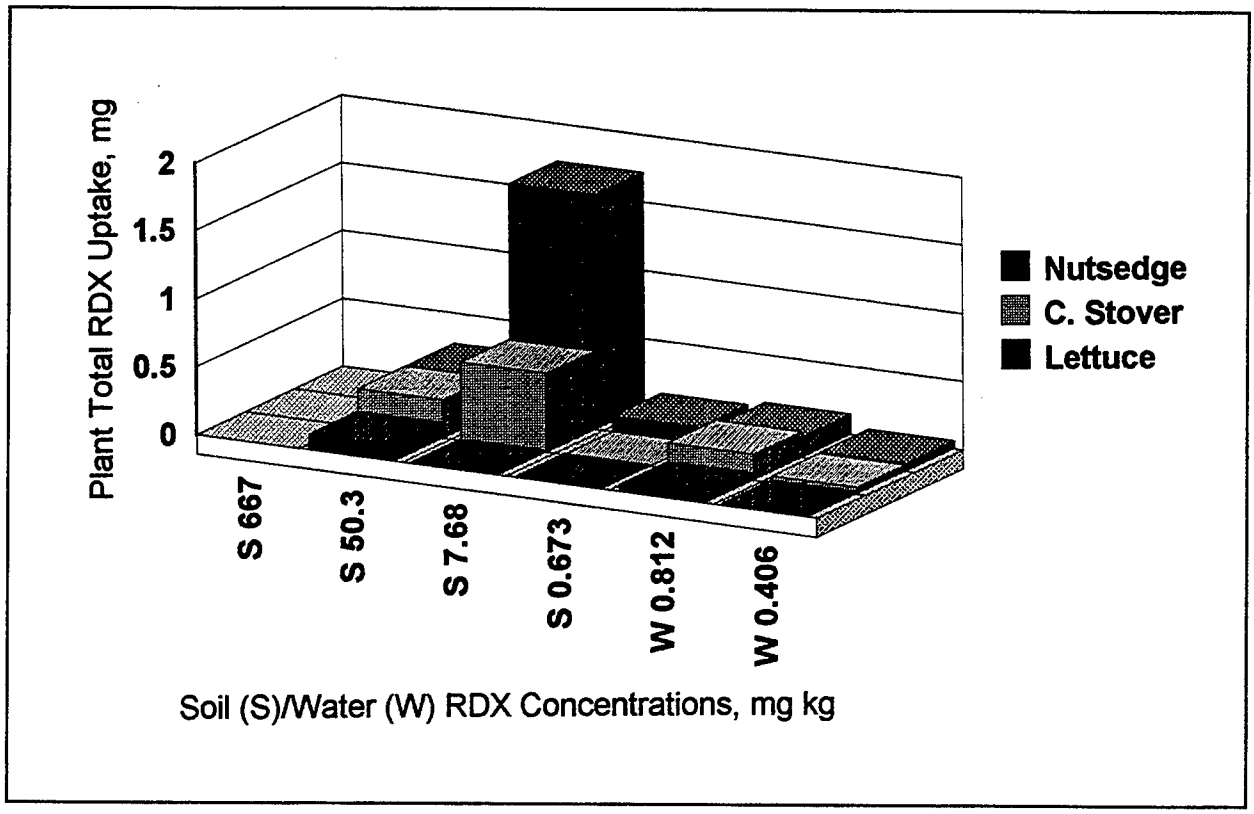

Figure 21. Effect of soil and water RDX concentration on total plant uptake of RDX

treatment (S3W0), the greatest total RDX uptake occurred when the soil RDX/TNT was at the RG concentration (S1W0), (Figure 21). These values may have important ramifications for human consumption of contaminant in crops.

Phase 3: Effects of soil properties on plant uptake

Unamended soils (S1UC, S1UN, and S1US) exhibited a wide range of clay content: 50.8-, 21.9-, and 9.9-percent clay, respectively (Table 20). Total organic matter of the same soils was 8.84, 5.14, and 3.20 percent, respectively. After the addition of composted cow manure, the amended soils (S1FC, S1FN, and S1FS) had a total organic matter content of 11.56, 11.04, and 9.30 percent, respectively, and particle size distribution changed slightly due to the bulking agents in the composted manure. Overall, the target RDX and TNT concentrations of 5.8 and 17.2 mg kg^{-1}, respectively, were achieved except in the amended and unamended NOP soil (S1UN and S1FN), where TNT concentrations were higher than the target concentration in the other soils (Table 21).

Growth of lettuce and yellow nutsedge increased as clay content of the soil increased or remained the same (Table 22). The addition of composted cow manure increased the growth of lettuce in the low-clay soils (S0FS and S1FS), while growth of yellow nutsedge benefited from the cow manure addition only in the uncontaminated high-clay soil (S0FC). This can be attributed to the natural differences in nutrient levels of the three soils and different nutrient requirements

Table 20
Organic Matter Content and Particle Size Distribution in Phase 3 Soils

Treatment	Organic Matter, %	Sand, %	Silt, %	Clay, %
S0FC: Clean High Clay + Manure	12.45	26.6	33.2	40.2
S0FS: Clean Low Clay + Manure	8.57	28.7	60.3	11.0
S1UC: RG High Clay	8.84	4.7	44.5	50.8
S1FC: RG High Clay + Manure	11.56	26.6	33.2	40.2
S1US: RG Low Clay	3.20	17.8	72.3	9.9
S1FS: RG Low Clay + Manure	9.30	28.7	60.3	11.0
S1UN: RG Medium Clay	5.14	31.5	46.6	21.9
S1FN: RG Medium Clay + Manue	11.04	30.6	42.2	27.2

Table 21
Phase 3 Mean (standard error) Preplant Soil Explosives Concentrations, mg kg^{-1}

Treatment	HMX	RDX	TNB	TNT	4A-DNT	2A-DNT	2,4-DNT
S1US	0.303 (0.286)	3.06 (3.303)	0.313 (0.237)	4.76 (1.73)	<0.10 (0.0)	0.086 (0.032)	<0.10 (0.0)
S1FS	1.12 (0.658)	5.78 (0.764)	0.425 (0.057)	2.10 (0.764)	0.323 (0.06)	0.420 (0.092)	<0.10 (0.0)
S1UC	0.23 (0.184)	1.58 (0.735)	0.165 (0.106)	2.31 (0.530)	<0.10 (0.0)	<0.10 (0.0)	<0.10 (0.0)
S1FC	0.70 (0.523)	2.48 (0.566)	0.125 (0.021)	1.58 (0.226)	0.240 (0.14)	0.235 (0.007)	<0.10 (0.0)
S1UN	0.605 (0.396)	4.90 (2.15)	0.753 (0.378)	39.6 (42.0)	0.16 (0.156)	0.438 (0.032)	0.055 (0.007)
S1FN	1.64 (1.58)	18.95 (231.84)	1.29 (1.45)	99.2 (138.2)	0.245 (0.276)	0.813 (0.421)	<0.10 (0.0)

of lettuce and yellow nutsedge. Uptake of RDX by lettuce and yellow nutsedge was significantly higher (318.4 and 405.2 mg kg^{-1}, respectively) when grown in the low-clay unfertilized soil than in the medium- and high-clay soils with or without fertilizer (Table 23). When composted cow manure was added to the low-clay soil, uptake was significantly reduced to 10.88 and 62.74 mg kg^{-1} for lettuce and yellow nutsedge, respectively. Amendments of composted cow manure had no significant effect on the uptake of RDX from the medium- and high-clay soils. The effects of clay content and organic matter (manure) are illustrated in Figures 22 and 23, respectively.

Table 22
Phase 3 Plant Tissue Biomass, g fresh weight mean (standard deviation)

Treatment	Lettuce	Yellow Nutsedge
S0US: Low Clay[1]	35.7 (3.98) e	46.44 (3.06) de
S0UC: High Clay[1]	258.58 (33.42) bc	135.96 (13.11) a
S0FS: Low Clay + Manure[1]	185.88 (29.74) d	25.04 (4.46) e
S0FC: High Clay + Manure[1]	321.48 (47.36) ab	78.9 (15.35) bc
S1US: Low Clay	71.68 (19.24) e	25.48 (6.21) e
S1FS: Low Clay + Manure	207.74 (16.65) cd	23.48 (3.85) e
S1UC: High Clay	340.78 (16.91) a	89.64 (12.02) b
S1FC: High Clay + Manure	315 (19.10) ab	74.42 (12.6) bc
S1UN: Medium Clay	259.98 (35.05) bc	42.95 (8.75) de
S1FN: Medium Clay + Manure	211.48 (11.24) cd	54.45 (12.71) cd

[1] Uncontaminated controls.

Table 23
Phase 3 Mean (standard deviation) Plant Tissue RDX Concentrations, mg kg^{-1} (All soil explosives concentrations were at the RG approximations (Table 21))

Treatment	Lettuce	Yellow Nutsedge
S1US (unfertilized, low clay)	405.2 (74.13) a	318.4 (49.28) a
S1FS (fertilized, low clay)	62.74 (20.42) b	10.88 (4.36) b
S1UC (unfertilized, high clay)	62.48 (16.02) b	70.22 (9.68) b
S1FC (fertilized, high clay)	117.96 (41.55) b	65.24 (10.89) b
S1UN (unfertilized, NOP mod. clay)	154.58 (51.92) b	NA
S1FC (fertilized, NOP mod. Clay)	117.75 (6.66) b	NA

Note: Means in a column with the same letter are not significantly different at the alpha = 0.05 level.

NA = Not analyzed.

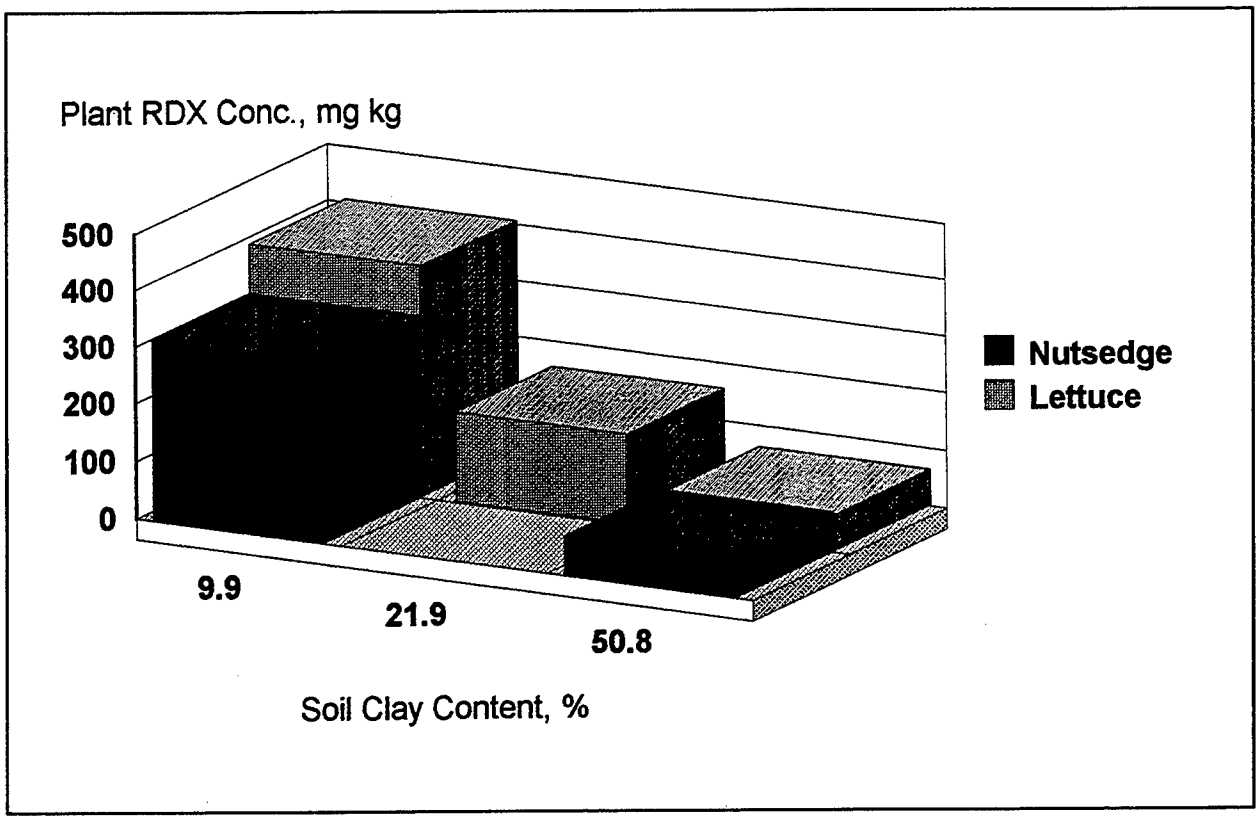

Figure 22. Effect of soil clay content on plant uptake of RDX

Yellow Nutsedge as an Index Plant for RDX Uptake by Agronomic Crops

As reported by van Driel et al. (1983), yellow nutsedge was used successfully to predict heavy metal accumulation by agronomic plants grown on contaminated sediments (Figure 24). Comparisons of RDX uptake by yellow nutsedge and all other plant tissues used in this study are shown in Figures 25 through 29. Lettuce uptake of RDX in this study was approximately 1.5 to 2 times higher than uptake by yellow nutsedge (Figure 25), while corn stover uptake of RDX was approximately 1.5 times lower than uptake by yellow nutsedge (Figure 26). Radish root uptake of RDX was 5 to 7 times less than uptake by yellow nutsedge (Figure 27). Tomatoes (Figure 28) and corn kernels (Figure 29) accumulated approximately 8 to 10 and 20 times less RDX than yellow nutsedge, respectively. The data at this point are insufficient to use yellow nutsedge as a tool for predicting RDX uptake by other agronomic crops, but results are promising. Additional studies of RDX uptake by plants will include yellow nutsedge and further contribute to a sufficient database. This database will serve as a tool to provide a rapid response and cost savings for questions concerning environmental or human risks and/or effective remedial cleanup/treatment of RDX-contaminated soil and water.

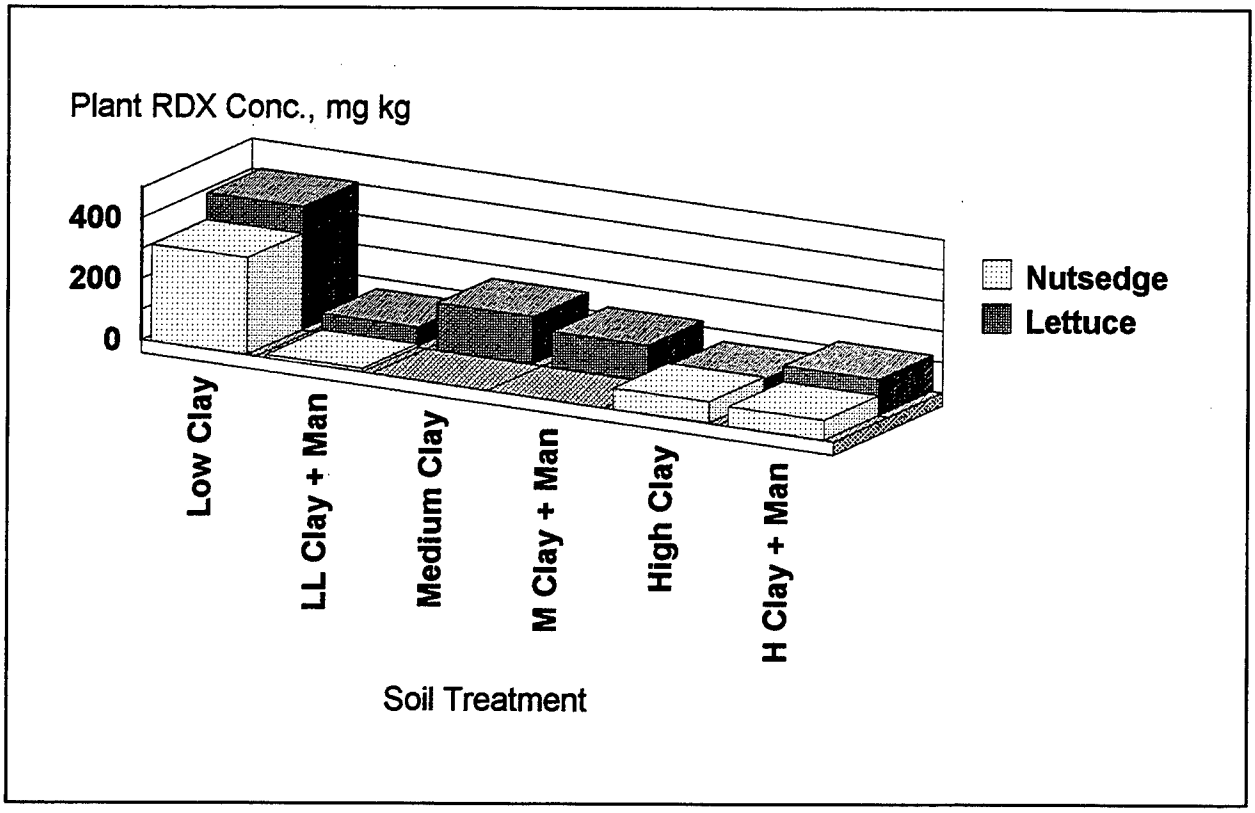

Figure 23. Effect of composted manure amendments to different soils on plant uptake of RDX

Conclusions

The results of this study demonstrate that leafy plant tissues accumulate RDX from both contaminated soils and irrigation water. Tomato fruit and corn kernels did not contain any detectable concentrations of RDX when irrigated with water containing up to 812 μg L^{-1} for the duration of one growing season. Since the RG level is 2 μg L^{-1} and previous groundwater data indicate concentrations below 100 μg L^{-1}, irrigation water will not likely contribute to short-term accumulation of RDX by tomato fruit and corn kernels. However, the long-term effects of contaminated irrigation water on loading of soils with RDX and subsequent accumulation by plants was not addressed. Leafy tissues of corn, lettuce, and yellow nutsedge accumulated increasingly higher concentrations of RDX as irrigation water RDX concentrations increased. When RDX in irrigation water was at the 100-μg L^{-1} level, only one of five replicates of corn, lettuce, and yellow nutsedge had detectable concentrations of RDX. Therefore, RDX concentrations near the RG level would not be expected to contribute to detectable accumulation of RDX by leafy tissues.

Leafy tissues of corn, lettuce, yellow nutsedge, radish roots, and tomato fruit accumulated significant levels of RDX when grown on NOP soil contaminated with RDX and TNT at the RG concentrations of 5.8 and 17.2 mg kg^{-1},

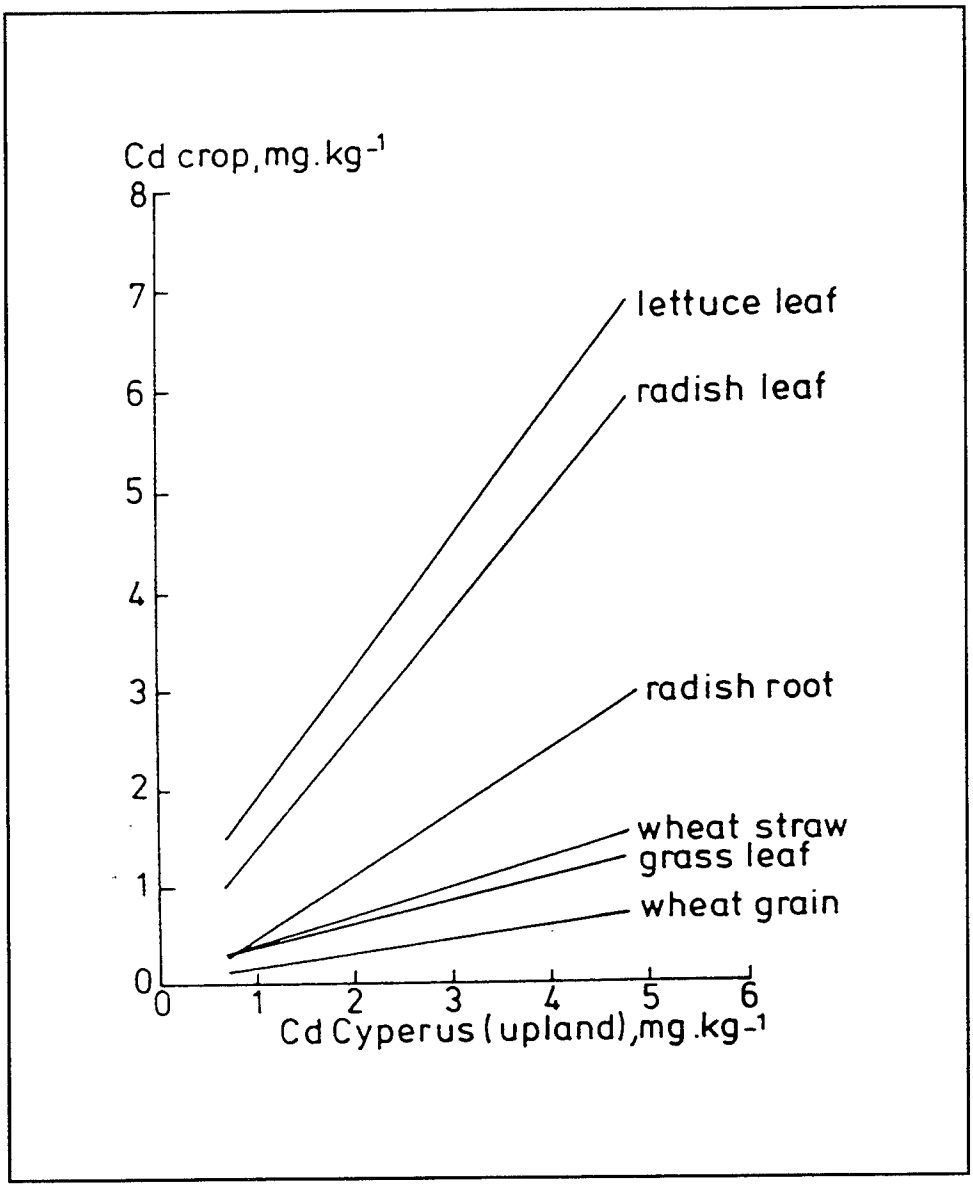

Figure 24. Yellow nutsedge used to predict cadmium uptake by agronomic crops

respectively. Increasing soil RDX and TNT concentrations up to 50.3 and 213 mg kg^{-1} reduced plant yields and significantly increased RDX uptake in lettuce, yellow nutsedge, and corn stover. Soil type and total organic matter content also affected the uptake of RDX by plants. Soils low in clay content contribute significantly to elevated levels of RDX in leafy tissues. Increasing the total organic matter with composted cow manure significantly reduced plant uptake for low-clay soils, but had little effect for soils with higher clay content.

Current data are insufficient at this time to use yellow nutsedge as a tool for predicting RDX uptake by agronomic crops. Additional studies will further develop the necessary database for comparing RDX uptake by yellow nutsedge and other plants, particularly agronomic plants. Once sufficient data are collected, future questions regarding RDX uptake by plants can be addressed

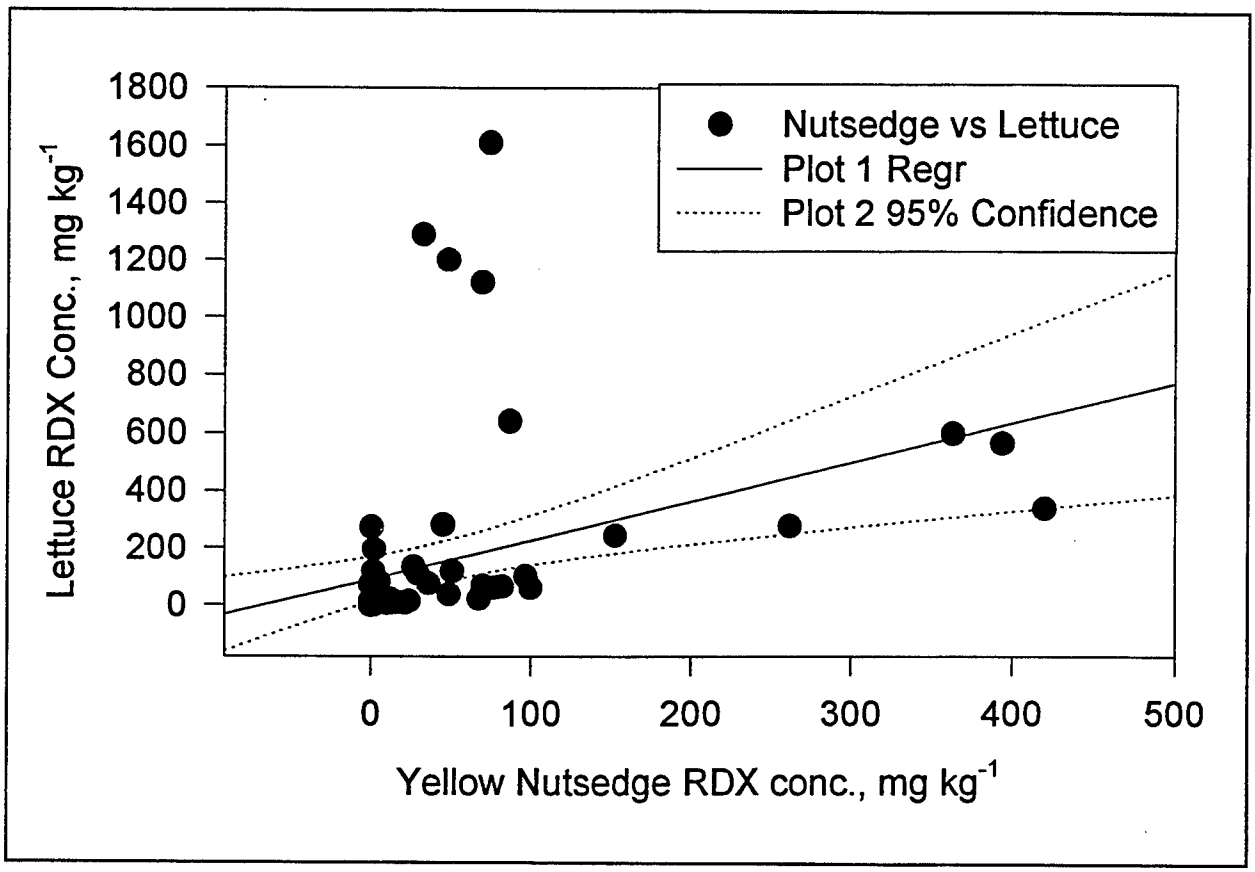

Figure 25. Comparison of RDX uptake by yellow nutsedge and lettuce

with the reference plant alone, eliminating the need for growing each plant in question.

Concentrations of RDX less than 100 μg L^{-1} in irrigation water are not expected to contribute to detectable RDX concentrations in plant tissues. However, this study demonstrated mobility of RDX into all plant tissues except corn kernels when soil RDX concentrations were above 58 mg kg^{-1}. These results suggest that human health hazards from ingestion of vegetables growing in soils contaminated at the RG be carefully evaluated and/or that the current RG be revised.

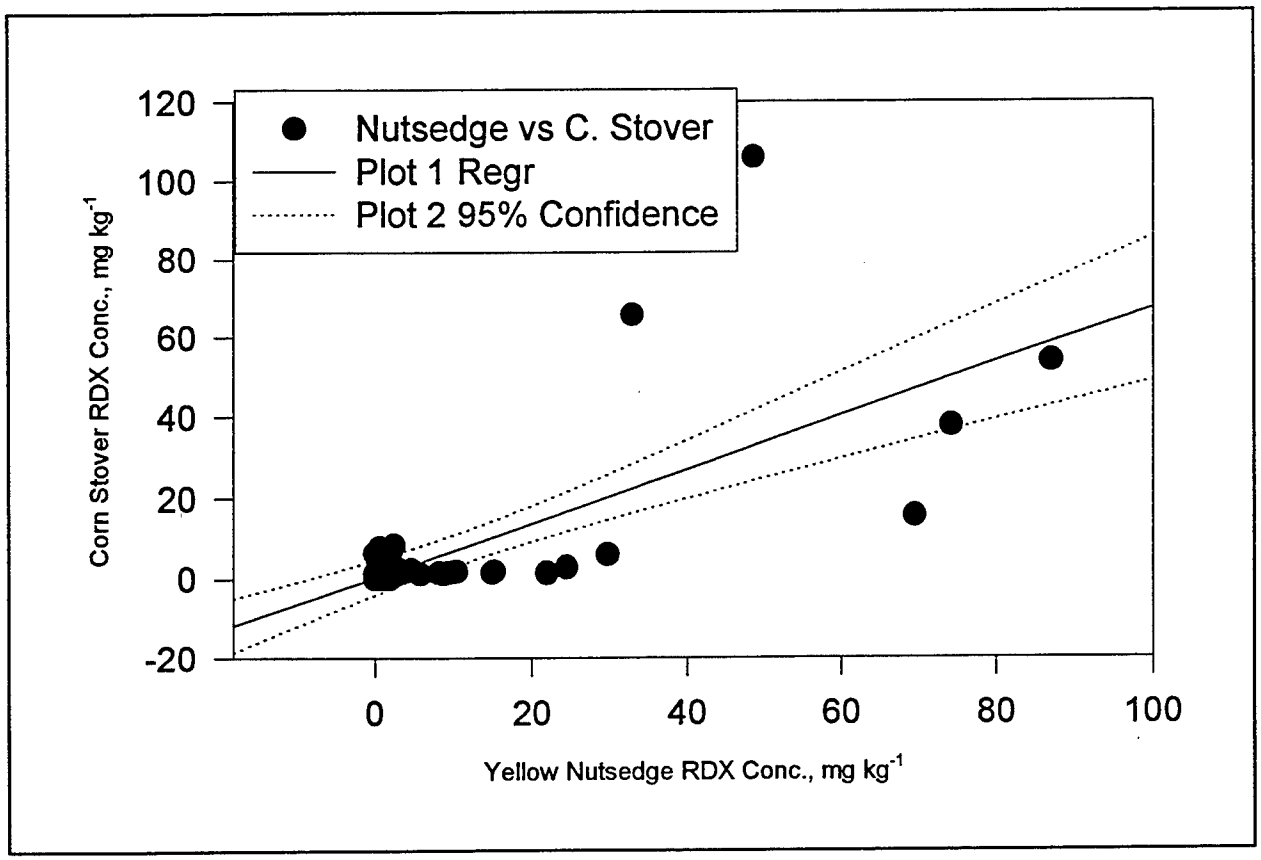

Figure 26. Comparison of RDX uptake by yellow nutsedge and corn stover

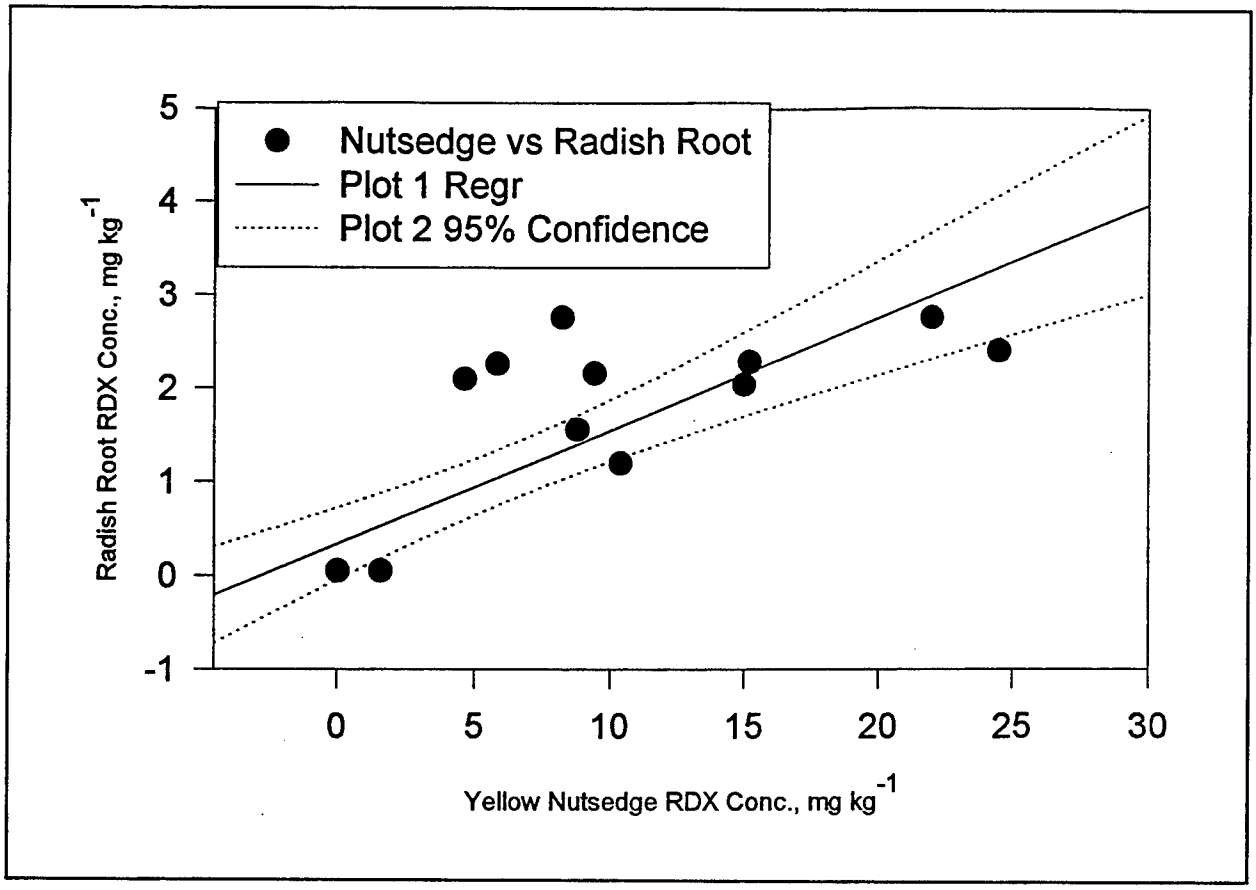

Figure 27. Comparison of RDX uptake by yellow nutsedge and radish root

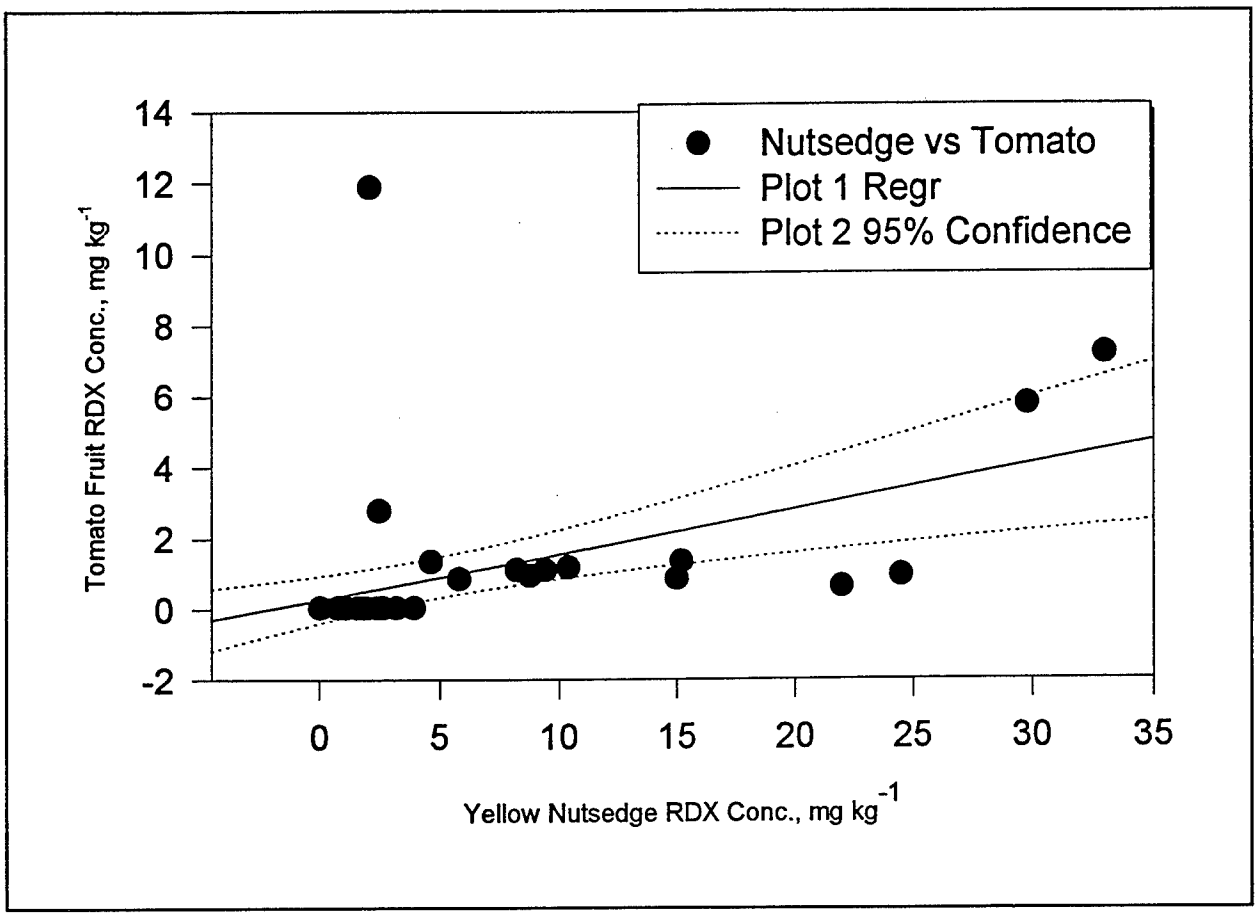

Figure 28. Comparison of RDX uptake by yellow nutsedge and tomato fruit

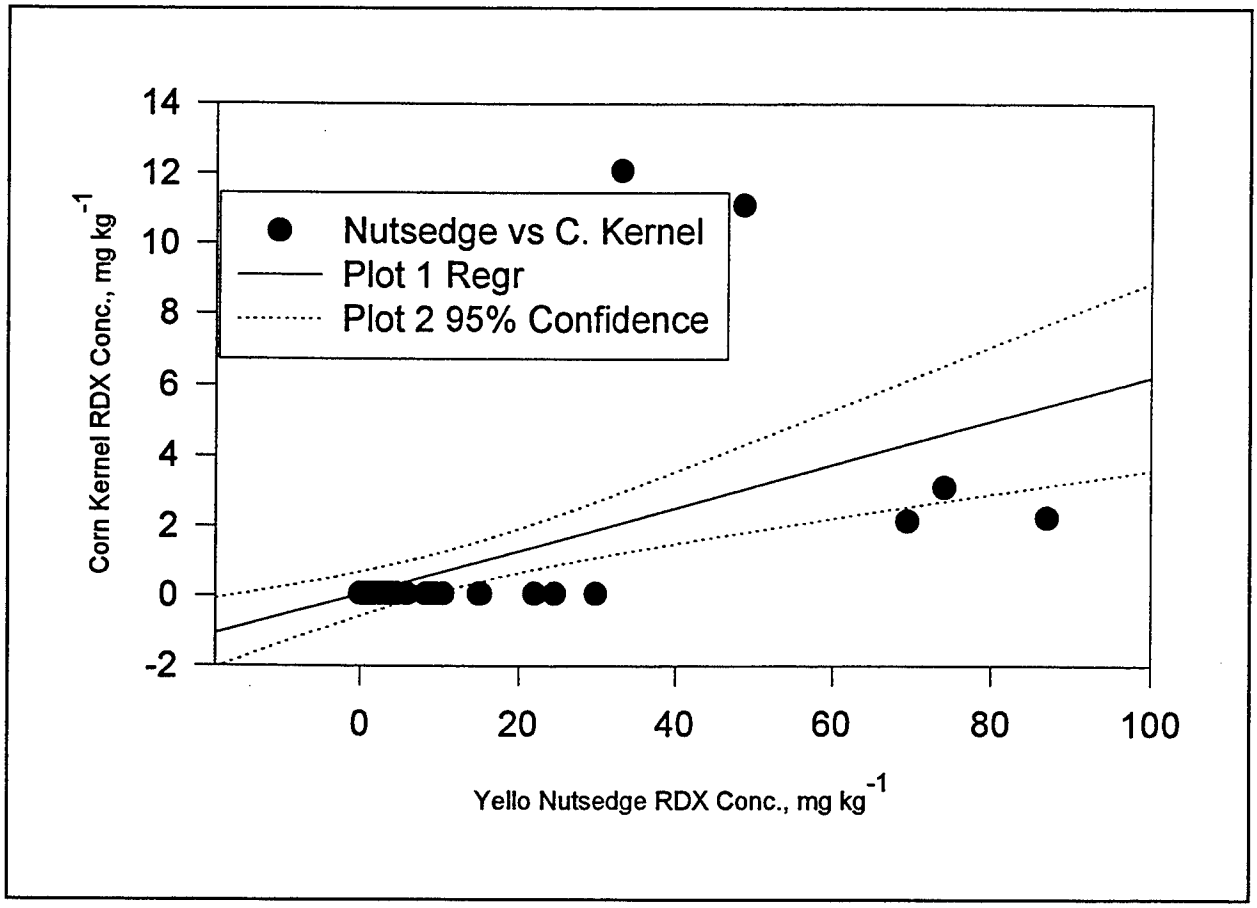

Figure 29. Comparison of RDX uptake by yellow nutsedge and corn kernels

5 Mass Balance

Experimental Design

Three of the plant species studied in the greenhouse experiments (tomato, radish, and lettuce) were subjected to mass balance studies using ^{14}C labeled RDX. Use of radiolabeled RDX in irrigation water or in soil allowed determination of mass balance of the radiolabeled carbon in all compartments of the test, i.e., soil, plants, and volatiles including carbon dioxide. The experimental design consisted of two treatments for each plant species: one treatment with contaminated soil and clean irrigation water, and the other with contaminated irrigation water and clean soil. Each treatment was replicated three times. Each replicate (chamber) contained one pot with two to three plants. One control (in two replicates) consisted of plants growing in clean soil and receiving clean irrigation water. This control was included to verify that all conditions for healthy plant growth were met in the execution of the experiment. Two additional controls (one replicate of each) received no plants. One of these contained contaminated soil and received clean irrigation water; the other contained clean soil and received contaminated irrigation water. These controls provided quantitative data on the fate of RDX independently of the plants.

Materials and Methods

Chambers

Rectangular growth chambers were constructed of 0.635-cm (0.25-in.) Plexiglas. The tomato chambers were 71.12 cm (28 in.) high by 60.96 cm (24 in.) wide by 60.96 cm long. The radish chambers were 44.45 cm high (17.5 in.) by 40.64 cm (16 in.) wide by 40.64 cm long. Each chamber was equipped with an air inlet, air outlet, and a port through which plants could be watered. Air was taken into the chambers by pulling a vacuum on the system at 10-12 mm Hg. The air inlet was fitted with a check value that would automatically shut off air flow in the event of a power failure. This safety precaution ensured that no radioactivity was lost to the room due to positive back pressure. Air exiting the system passed through a trap of activated charcoal to capture any volatile organic compounds and then a 1-L trap of 5N potassium hydroxide (KOH) to collect CO_2

Plants received a 14-hr photoperiod from a light bank in the walk-in environmental room. Light at the upper surface of the plant was approximately 500 $\mu Em^{-2}s^{-1}$ for tomatoes and 300 $\mu Em^{-2}s^{-1}$ for radish. Temperature in the chambers averaged 23 °C.

Soil amendment

For each replicate of the contaminated soil treatments of tomato and radish, 15 and 6 kg, respectively, of clean soil from the Nebraska Ordnance Plant site was spread in a thin layer and misted with a methanol solution to achieve 5.7 µg of cold RDX and 0.1 µg of radiolabeled RDX (specific activity of 688,816 dpm/µg) per g of soil. No TNT was added. After drying for 24 hr, amended soil was homogenized in a V-shaped mixer for 10 min to ensure a homogeneous distribution of the RDX. Amended soil was transferred to 18.9-L (5-gal) and 9.5-L (2.50-gal) plastic pails for tomato and radish tests, respectively. Each pail had drain holes in the bottom and was contained in a saucer. One pail was placed into each chamber after planting.

Irrigation water amendment

The RDX-contaminated water was prepared by adding 100 µg radiolabeled RDX per millileter of distilled, deionized water. Plants were watered as needed with this solution. Tomatoes and their control without plants received 1,100 µg total RDX. Radish plants and their control without plants received 350 µg total of RDX.

Plants

Plants were started in peat moss from seeds and transferred to growth chambers at 2 and 3 weeks for radish and tomato, respectively. Tomatoes (Burpee Tumbler Hybrid Catalog No. 15418AK Lot No. 9, Burpee, Warminster, PA) were transplanted in triplicate, and two were culled after survival of one was ensured (1 week). Four radish plants (White Icicle Lot 4301204, B.W.I., Texarkana, TX) were transferred to each replicate; none were culled. Four lettuce plants (Black Seeded Simpson Lot No. 4213410, Water Seed International, Inc., El Centro, CA) were planted in each reactor, and one was culled after plants became established (1 week).

Sampling and analysis

The KOH traps were changed every 7 days, and a 1-ml subsample was counted by liquid scintillation in Ultima Gold Liquid Scintillation Cocktail (Packard Instruments, Meriden, CT) using a liquid scintillation counter (LS) (Tricarb 2500 TR Liquid Scintillation Counter, Packard Instruments, Meriden, CT). All other sampling was conducted at the end of the test period,

approximately 11 weeks for tomato and approximately 6 weeks for radish. At the end of the test period, charcoal traps for volatile organic compounds were extracted with 5-ml methanol in sealed containers, sonicated for 12 hr, and 1 ml of the extracts was counted by LS. Aboveground plant tissues and tomato fruits and roots of radish plants were harvested, weighed, and homogenized for analysis. Subsamples were subjected to complete combustion (Model 307 Sample Oxidizer, Packard Instruments, Meriden, CT) followed by LS counting of the radiolabeled CO_2 trapped in Carbo-Sorb and Premafluor Liquid Scintillation Cocktail. Subsamples were also freeze-dried and analyzed by high performance liquid chromatography (HPLC) (See Appendix A for method). Soils were thoroughly mixed and also subjected to combustion and LS counting as well as HPLC analysis.

Total plant yields of tomatoes, radish, and lettuce were compared across treatments using a one-way analysis of variance. Differences between means were separated using an all pairwise multiple comparison procedure, the Student-Newman-Keuls Method available in Sigma Stat (Jandel Corp., San Rafael, CA).

Result and Discussion

Plant yields

Total dry weights of plants indicate the yield in grams of plant material produced in each test. Comparisons between yields for controls receiving no contamination and treatments receiving RDX in soil or irrigation water indicate the general health of plants under test conditions and impacts of contamination on plant growth (Table 24). Results indicated no significant difference between the health of treatments and clean controls of tomato and lettuce. However, treatment with RDX in soil and in irrigation water significantly reduced plant biomass in radish. These results are consistent with results of Phases 1 and 2 greenhouse studies.

Tomatoes

Mass balance for tomatoes growing in contaminated soil was 56 percent, and with contaminated irrigation water 70 percent (Table 25). Therefore, treatments exhibited a relatively high error term. Conducting mass balance studies with plants as large as tomatoes is difficult due to the necessity of confining the plants. In these experiments, moisture from the plants condensed in significant quantities onto the walls of the chambers throughout the study period. Tests at the end of the experiment when the chambers were disassembled indicated significant radioactivity in this condensate. Obtaining an accurate measure of the total volume of the condensate was not possible, but the volume is estimated to be about 3 L per chamber. Therefore, the condensate may be a significant contributor to the experimental error. The source of this radioactivity may be $^{14}CO_2$ dissolved in the condensate.

Table 24
Plant Yields (g dry weight) in Radiolabeled RDX Mass Balance Studies (Standard deviation of the mean of three replicates (except where noted) is given in parentheses)

Treatment	Fruit		Foliage	Total[1]
	Ripe	Green		
Tomato				
Control[2]	5.90 (0.36)	5.89 (3.98)	36.91 (10.61)	48.70 A
Contaminated water	2.94 (0.93)	2.16 (0.80)	38.48 (5.27)	43.58 A
Contaminated soil	4.32 (3.35)	1.08 (0.49)	40.77 (7.89)	46.17 A
Radish				
Control[2]	1.36 (0.54)		16.71 (1.30)	18.07 A
Contaminated water	2.86 (0.62)		8.15 (1.26)	11.01 B
Contaminated soil	1.09 (0.78)		12.02 (1.50)	13.22 B
Lettuce				
Control[2]	na		9.45 (2.12)	9.45 (2.12) A
Contaminted water	na		9.25 (2.66)	9.25 (2.66) A
Contaminated soil	na		9.23 (0.65)	9.23 (0.65) A

[1] Total values within plant species that are followed by the same letter are not significantly different. P = 0.657, 0.05, and 0.991 for tomato, radish, and lettuce, respectively.
[2] Values given for controls are means of two replicates.

Radiolabeled CO_2 recovered from contaminated soil treatments with (7.46 percent) and without (7.60 percent) plants were not significantly different (Table 25). However, treatments receiving contaminated water produced more $^{14}CO_2$ than treatments growing in contaminated soils. This difference may be explained by the microbiology of the tests. Microbial activity tends to be greater in the soil surface than at depths of even a few inches. Since all of the [^{14} C]RDX was applied to the surface soil in contaminated water treatments, these treatments were more susceptible to microbial mineralization. Furthermore, the contaminated water control without any plants significantly exceeded the contaminated water treatments with plants in $^{14}CO_2$ production. The loss of $^{14}CO_2$ in the condensate produced by the growing plants may explain this difference. These results also demonstrate that the [^{14}C]RDX is mineralized to $^{14}CO_2$ in the soil independently of the plants.

Mobilization of RDX or other radiolabeled degradation products of RDX into tomato fruit was less than 1 percent in both treatments. More radioactivity was observed in the plant foliage than in the fruit in both treatments. Foliage from each treatment contained about 7 percent of the total radioactivity originally present as [^{14}C]RDX.

Table 25
Percent Recoveries of Radioactivity From Various Compartments of Mass Balance Tests (Standard deviations of means of three replicates (except for single replicate controls without plants and two replicates for uncontaminated controls) are given in parentheses)

Treatment	Compartments				Total
	Soil	Foliage	Fruit	CO_2	
Tomato					
Uncontaminated controls	<0.002	<0.020	<0.002	<0.002	<d.l.
Contaminated water; No plants	77.47	na	na	33.39	110.86
Contaminated soil; No plants	88.79	na	na	7.60	96.39
Contaminated water	50.87 (4.27)	6.73 (0.95)	0.53 (0.06)	11.85 (2.40)	69.98
Contaminated soil	41.50 (1.68)	6.98 (3.16)	0.34 (0.22)	7.46 (0.56)	56.28
Radish					
Uncontaminated controls	<0.002	<0.020	<0.002	<0.002	<d.l.
Contaminated water; No plants	82.89	na	na	6.97	89.86
Contaminated soil; No plants	86.56	na	na	3.76	90.32
Contamianted water	69.73 (9.88)	6.22 (1.97)	1.46 (0.28)	6.48 (0.83)	83.89
Contaminated soil	67.57 (6.83)	14.40 (2.94)	0.52 (0.15)	3.88 (0.33)	85.58
Lettuce					
Uncontaminated controls	<0.002	<0.020	<0.002	<0.002	<d.l.
Contaminated water; No plants	73.22 (11.28)	na	na	12.83	86.05
Contamianted soil; No plants	97.06 (6.20)	na	na	2.91	99.97
Contaminated water	72.85 (7.97)	9.11 (0.81)	na	3.54 (0.21)	85.50
Contaminated soil	75.78 (8.46)	15.72 (2.13)	na	4.23 (0.86)	95.73

Note: d.1. indicates detectin limit; na indicates not applicable; <values are detection limits.

Results of HPLC analyses of the various test compartments (Table 26) indicate bioaccumulation of RDX in tomato fruit and foliage of contaminated soil treatments. Concentrations exceed the concentration of RDX detected in the soil (2.12 mg kg^{-1} by 3.5 and 7 times in fruit and foliage, respectively). Bioaccumulation was not observed in plants receiving contaminated irrigation water. These results are consistent with results of greenhouse studies.

The nitroso transformation product of RDX, MNX, was detected in soil from the RDX-contaminated soil treatment (0.140 mg kg^{-1}). The TNX was detected in soil from the RDX-contaminated control without plants (0.107 mg kg^{-1}). Both of these values are only slightly above the detection limit (0.100 mg kg^{-1}). No other

Table 26
Results of HPLC Analyses of Mass Balance Compartments (Standard deviations are given in parentheses. Analyte concentrations are in mg kg^{-1} dry weight)

Compartment	Treatment	Analyte[1]		
		RDX	MNX	TNX
Tomato				
Soil	Uncontaminated controls	<1.00	<1.00	<1.00
	Contaminated water; No plants	0.025J	<0.100	<0.100
	Contaminated Soil; No plants	3.39	0.140	<0.100
	Contaminated water	0.438 (0.03)	<0.100	<0.100
	Contaminated soil	2.12 (0.17)	<0.100	0.107 (0.02)
Foliage	Uncontaminated controls	<1.60	<1.60	<1.60
	Contaminated water	<1.60	<1.60	<1.60
	Contaminated soil	15.13 (3.20)	<1.60	<1.60
Fruit	Uncontaminated controls	<1.60	<1.60	<1.60
	Contaminated water	<1.60	<1.60	<1.60
	Contaminated soil	7.50 (1.77)	<1.60	<1.60
Radish				
Soil	Uncontaminated controls	<0.100	<0.100	<0.100
	Contaminated water; No plants	0.043J	<0.100	<0.100
	Contaminated soil; No plants	4.59	<0.100	<0.100
	Contaminated water	0.041J	<0.100	<0.100
	Contaminated soil	3.43 (0.25)	0.199 (0.01)	<0.100
Foliage	Uncontaminated controls	<1.60	<1.60	<1.60
	Contaminated water	<1.60	<1.60	<1.60
	Contaminated soil	159.0 (35.7)	<1.60	<1.60
Roots	Uncontaminated controls	<1.60	<1.60	<1.60
	Contaminated water	<1.60	<1.60	<1.60
	Contaminated soil	12.69 (5.56)	<1.60	<1.60

Note: Values followed by J are below statistically valid detection limits, but are quantifiable on chromatograms. RDX is hexahydro-1,3,5-trinitro-1,3,5-triazine; MNX is hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine; and TNX is hexahydro-1,3,5-trinitroso-1,3,5-triazine.

[1] Only the listed analytes were detected. Other analytes not detected, but for which analysis were performed, are given in Appendix A.

analytes except RDX were detected by HPLC in any compartment of the tomato study.

The most important conclusion illustrated by the tomato data is bioaccumulation of significant quantities of RDX in tomato fruit (7.5 mg kg^{-1} from soil containing 2.12 mg kg^{-1}). Mass balance results showed that uptake of RDX or any degradation products of RDX containing carbon into tomato fruit when plants were growing in contaminated soil or when plants were irrigated with contaminated water was small (less than 1 percent of added radioactivity) relative to the total RDX available. Most of the added radioactivity remained in the soil of both treatments. A significant amount of [^{14}C]RDX was mineralized in both treatments. Results for controls suggest that the mineralization occurs in the soil rather than in the plants.

Radish

Mass balance results for radish treatments and controls were relatively good. The average mass balance result for treatments receiving contaminated irrigation water was 84 percent; the control without plants was 90 percent. The average mass balance result for treatments growing in contaminated soils was 86 percent; the control without plants was 90 percent. The smaller plant biomass resulted in less condensation in radish tests than in tomato tests. This probably explains the greater recoveries in radish. An average of 6 percent of the radioactivity added to contaminated irrigation water was recovered in the radish leaf tissue; only 1.5 percent was recovered in the edible roots. None of this radioactivity was detected as RDX, MNX, or TNX in HPLC analyses (see below). This is likely due to the significant difference in detection limits of the two methods. When radishes were grown in contaminated soils, 14 percent of the added radioactivity was recovered in the leaf tissue, while only 0.5 percent was found in the roots.

Comparisons of treatments with controls containing no plants for both treatments suggest that mineralization was occurring in the soil rather than in the plant as was demonstrated for tomato. Treatments receiving contaminated irrigation water produced greater mineralization rates than treatments growing in contaminated soils. This result may be explained by the increased microbial degradation of the contaminant from water than from soil as explained for tomato.

Results of HPLC analyses of the various test compartments (Table 26) indicated bioaccumulation of RDX in radish root and foliage of contaminated soil treatments only. Concentrations in radish roots (12.69 mg kg^{-1}) were more than three times concentrations in the soil (3.43 mg kg^{-1}). Furthermore, concentrations in the foliage (159 mg kg^{-1}) greatly exceeded concentrations in the soil. Bioaccumulation was not observed using HPLC in plants receiving contaminated irrigation water.

The nitroso transformation product of RDX, MNX, was detected in the soil from the contaminated soil treatment (0.199 mg kg^{-1}). No other analytes except RDX were detected in any compartment of the radish study.

The most important conclusion illustrated by the radish data is bioaccumulation of significant quantities of RDX in edible radish roots (12.69 mg kg^{-1} from soil containing 3.43 mg kg^{-1}). However, radish foliage also accumulated RDX (159 mg kg^{-1}). Consumption of radish foliage as a salad green, although not typical, does happen. Mass balance results showed that most of the added radioactivity remained in the soil of both treatments. A significant amount of [^{14} C]RDX was mineralized in both treatments. Results for controls suggest that the mineralization occurs in the soil rather than in the plants, as was the case for tomato.

Lettuce

Mass balance for lettuce treatments was relatively good (Table 24). Results were comparable with recoveries found with radish foliage. As with radish, the smaller biomass resulted in less condensation than with tomato and, therefore, better recoveries. Plant uptake of radioactivity was also comparable with uptake by radish foilage. An average of 9.11 percent of the added radioactivity was recovered from lettuce receiving contaminated irrigation water, and 15.72 percent from lettuce growing in contaminated soil (Table 25). As with tomato and radish, mineralization of [^{14}C]RDX to $^{14}CO_2$ occurred. No HPLC analyses were conducted on these plants.

6 Effects of TCE on Solubility of RDX

Materials and Methods

To determine the effects of TCE concentration at a presumed postremediation concentration of 20 $\mu g\ L^{-1}$ on the aqueous phase concentration of RDX, batch partitioning tests were conducted in 40-ml Eagle Picher EPA vials (Eagle Picher, Miami, OK) without headspace. Three RDX concentrations, 0, 5, and 100 mg kg-1, and five TCE concentrations, 0, 5, 10, 15, and 20 $\mu g\ L^{-1}$, were selected for testing. Soils were clean plus contaminated site soils that were mixed to approximate the desired concentrations of RDX. Actual RDX concentrations achieved by mixing clean soil with soil containing 545 mg kg^{-1} RDX were 0, 3.73, and 101 mg kg^{-1}. Tests were conducted in three replicates for each treatment by placing soils, water, and appropriate concentrations of TCE into vials and shaking on a rotary tumbler for 2 hr. After partitioning, vials were centrifuged at 1,149 rcf for 10 min at 5 °C. TCE was analyzed in the solution phase according to EPA Method 8260 (EPA 1992). The solution phase was also analyzed for RDX by EPA Method 8330 (EPA 1992). Solution phase RDX concentrations for each TCE treatment were compared using a one-way analysis of variance available in Sigma Stat (Jandel Corp., San Rafael, CA).

TCE Results

When no RDX was present in the soils, the TCE partitioned in a linear fashion with the soil exhibiting a partition coefficient of 0.51 $\pm$ .02 (Table 27). TCE concentrations up to 20 $\mu g\ L^{-1}$ did not increase the solubility of RDX from soils (Table 28). These concentrations of TCE are probably too small to exert a significant effect upon partitioning of RDX. However, these concentrations of TCE represent what may be expected in site waters. When RDX was present in the tests, TCE concentrations were significantly reduced in the aqueous phase. These results suggest either an interaction between TCE and RDX or interference in the analysis of such low TCE concentrations when RDX concentrations are relatively high.

Table 27
Concentration of TCE in Solution Phase After Batch Partitioning, µg L^{-1} (Values represent means of three replicates with standard deviations given in parentheses)

Initial RDX Concentration (mg kg^{-1}) in Soil	Initial TCE Concentration (µg L^{-1}) in Treatment Solution				
	0	5	10	15	20
0	<0.005 (0.00)	3.29 (0.11)	6.52 (0.35)	9.92 (0.19)	13.5 (0.17)
5	<0.005 (0.00)	0.004J[1] ($5.8e^{-5}$)	0.009 ($2.1e^{-4}$)	0.013 ($5.8e^{-4}$)	0.017 ($5.8e^{-4}$)
100	<0.005 (0.00)	0.004J ($1.7e^{-4}$)	0.009 ($5.8e^{-4}$)	0.013 ($5.8e^{-4}$)	0.018 (0.00)

[1] Values followed by J are below the statistically valid detection limits, but are quantifiable on chromatograms.

Table 28
Concentration of RDX in Solution Phase After Batch Partitioning, mg kg^{-1} (Values represent means of three replicates with standard deviations given in parentheses)

Initial RDX Concentration (mg kg^{-1}) in Soil	Initial TCE Concentration (µg L^{-1}) in Treatment Solution				
	0	5	10	15	20
0	<0.020 (0.00)	<0.020 (0.00)	<0.020 (0.00)	<0.020 (0.00)	<0.020 (0.00)
5	0.612 A[1] (0.162)	0.505 A (0.026)	0.555 A (0.065)	0.544 A (0.041)	0.539 A (0.084)
100	10.460 B (1.08)	9.84 B (0.677)	9.85 B (0.636)	9.28 B (0.225)	9.21 B (0.492)

[1] Values followed by the same upper case letter across TCE concentrations are not significantly different at the P = 0.694 and P = 0.246 level for 5 and 100 mg kg^{-1} RDX treatments, respectively.

7 Conclusions

The results of this study demonstrated that at least some tissues of corn, lettuce, radish, tomato, and yellow nutsedge accumulated RDX from contaminated soil at the concentration of the remediation goal for NOP (5.8 mg kg^{-1}). Maximum plant concentrations were observed when the concentration of RDX in the soil was 58 mg kg^{-1}. Plants contained the following (mg kg^{-1}): tomato fruit, 7.2; corn kernel, 6.14; corn stover, 55.82; lettuce, 1,172; yellow nutsedge, 62.46. At soil concentrations of 580 mg kg^{-1}, the plants died; however, the concentration of TNT in the soil was high (1,720 mg kg^{-1}) and may be responsible for the lethal effects. None of the agronomic species accumulated RDX from contaminated irrigation water at the concentration of the remediation goal (2 µg L^{-1}). Neither tomato fruit nor corn kernels accumulated RDX when irrigation water contained up to 812-µg RDX per liter. However, long-term effects of contaminated irrigation water on loading of soils with RDX and subsequent accumulation by plants was not addressed. TNT was not detected in plants except in corn stover grown in contaminated soil.

Increasing soil RDX and TNT concentrations up to 50.3 and 213 mg kg^{-1}, respectively, reduced plant yields and increased RDX uptake in lettuce, corn stover, and yellow nutsedge. High organic matter content (composted cow manure amendment) and high soil clay content significantly reduced plant uptake.

Results of mass balance studies of tomato, radish, and lettuce, which were conducted with radiolabeled RDX ([^{14}C]RDX), indicated that most of the added radioactivity (typically about 75 percent) remained in the soil. However, significant amounts were accumulated in edible tissues. Accumulation of radioactivity in edible portions of plants grown in contaminated soil was as follows (percent of added radioactivity): tomato fruit, 0.34; radish root, 0.52; lettuce foliage, 15.72. Accumulation of radioactivity in edible portions of plants grown with contaminated irrigation water was as follows (percent of added radioactivity): tomato fruit, 0.53; radish root, 1.46; lettuce foliage, 9.11. When HPLC analyses were preformed on these tissues (lettuce was not analyzed), the following concentratins of RDX (mg kg^{-1} dry weight) were detected in plants grown in contaminated soil: tomato fruit, 7.50; radish root, 12.69. No contaminants were found in plants grown in contaminated irrigation water. Mineralization of [^{14}C]RDX to $^{14}C_2$ was significant (3 to more than 12 percent);

however, results for controls without plants suggest that the mineralization occurred in the soil rather than in the plants.

The data generated in this study form a significnat basis for development of yellow nutsedge as a reference plant for predicting bioaccumulation of explosives by plant species. Once this database is sufficiently expanded, bioaccumulation of untested species of interest can be anticipated without conducting laboratory growth experiments.

In summary, edible portions of plants except corn kernels bioaccumulated RDX in significant quantities from soils, but not from irrigation water, at the remediation goal concentrations. These results suggest that human health hazards from ingestion of vegetables growing in soils contaminated at the concentrations of remediation goals be carefully evaluated and/or that the level be revised.

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Appendix A
Analysis of Explosives in Plant Tissues: Modifications to EPA 846 Method 8330 for Soils

Background

This appendix addresses quantifying explosives and explosives degradation products in plant matrices. The extraction of the contaminants from the matrix required a different set of extraction techniques from those used for water and soil. Plants contain much higher organic content than soil or water and, as a result, chromatography of plant extracts is prone to interference. Sample cleanup is required to successfully analyze plant tissues using conventional high performance liquid chromatography techniques as described in EPA Method 8330 (U.S. Environmental Protection Agency (EPA) 1992; Jenkins 1989). Method 8330 for the analysis of explosives in water and soil samples was used as a base for the detection of nitroaromatic, nitramine, and their degradation products of explosives in plant samples (analytes listed in Table A1). Quantitation of these analytes required matrix-specific sample preparation, separation by reversed phase high performance liquid chromatography (HPLC), and ultraviolet detection.

Table A1
Target Analytes of EPA Standard Method 8330

Compound	Abbreviation	CAS Number
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4
1,3,5-Trinitrobenzene	TNB	99-35-4
1,3-Dinitrobenzene	DNB	99-65-0
Methyl-2,4,6-trinitrophenylnitramine	Tetryl	479-45-8
Nitrobenzene	NB	98-95-3
2,4,6-Trinitrotoluene	TNT	118-96-7
4-Amino-2,6-dinitrotoluene	4-A-DNT	1946-51-0
2-Amino-4,6-dinitrotoluene	2-A-DNT	355-72-78-2
2,4-Dinitrotoluene	2,4-DNT	121-14-2
2,6-Dinitrotoluene	2,6-DNT	606-20-2
2-Nitrotoluene	2-NT	88-72-2
3-Nitrotoluene	3-NT	99-08-1
4-Nitrotoluene	4-NT	99-99-0

Many interferences can be kept to a minimum through the use of rigorously clean reagents and sample processing equipment. Removal of the bulk of the polar, plant-based compounds present in the original extract is necessary, because of the lack of specificity inherent with ultraviolet (UV) detection. This is performed by a liquid chromatographic cleanup step using a specific stationary phase (alumina/florisil) with a specific mobile phase (pure acetonitrile). The presence of water in either the mobile or stationary phases of the cleanup system

will result in inadequate removal of the polar, plant-based compounds. During this procedure, reproducible losses of the more polar analytes can be expected.

Processing of sample material for removal of interferences inevitably results in some degree of analyte loss. For example, analyte recoveries are adversely affected by several factors: improper sample collection, extended sample storage time, exposure to electromagnetic and thermal radiation, as well as drying (EPA 1992; Beelen and Burris 1995; Comfort et al. 1995; Crawford 1993). Therefore, low recoveries are often tolerated to achieve reliable, reproducible analyses. Based on the results of a previous study of analysis of RDX in plant tissues (Larson, Escalon, and Parker 1997) in which inadequate removal of water from plant tissues was demonstrated to make determinations impossible, lyophilization was chosen. Lyophilization resulted in a reproducible loss in recovery that was offset by increased reliability and reproducibility of the analysis. This made possible the comparative experimental design used in this study.

The determination of explosives and explosives degradation products in complex matrices such as plant tissues requires careful attention to sample handling. Sample handing includes sample collection, storage, and transport; sample preparation; homogenization; drying; extraction; cleanup; and analysis. The determination of the concentrations of explosives and explosives degradation products is not made until the last step in the process. Therefore, consideration of the possible changes that can occur to analytes during the process is essential, so that analytical biases can be avoided or minimized. Recently, several researchers have examined the impact of freeze-drying on sample integrity and analyte recovery. Dao and Friedman (1996) recently published a comparison of gycoalkaloid content of fresh and freeze-dried potato leaves by reverse phase HPLC analysis. Because of the similarity of the molecular properties (polarity, reactivity, and molecular structure) of glycoalkaloids and explosives, this analytical problem is similar to that posed by sample preparation for determination of explosives in plant tissues. They concluded that freeze-drying was superior to analyzing fresh samples with recoveries between the two methods being similar and reproducibility greater for analyses of extracts from freeze-dried samples. A comparison study employing plant tissues containing RDX indicated that freeze-drying was superior to either fresh extraction or extraction following a nitrogen-drying procedure (Larson, Escalon, and Parker 1997). Zimmerman, Kramer, and Schnable (1996) published results concerning the use of lyophilization for improved handling of *Vicia faba* leaves prior to bioassay. Their results indicated nominal loss in activity during freeze-drying. Dewanji and Matai (1996) successfully used lyophilization as a sample preparation technique when evaluating leaf proteins in aquatic plants. Once again, a reproducible loss in recovery was offset by increased reliability and reproducibility.

In summary, the following advantages are gained by freeze-drying plant samples prior to analysis:

a. Removal of water further lyses cells, decreases particle size, and increases surface area for extraction of explosives.

b. Rate of drying is increased greatly over the recommended EPA Method 8330 practice of air-drying for soils, decreasing the time during which microbial alteration can occur.

c. Sample stays cold (0 °C) until it is completely dry, reducing thermal degradation and slowing microbial activity.

d. Freeze-drying stops enzyme-catalyzed, wound-induced, and moisture-dependent sample changes.

e. Freeze-drying produces a homogeneous sample for extraction.

f. Elimination of water allows a uniform extraction solvent (100-percent acetonitrile) to be used from sample to sample.

These advantages to maintaining sample integrity, sample handling, and extractability are generally thought to outweigh analyte loss during the drying process.

Materials and Methods

Instrumentation

The HPLC system consists of a 610 Fluid Unit pump, a 717 plus Autosampler, a 486 Tunable UV Absorbance detector monitored at 245 nm and Millennium 2.1 Chromatography Software. A Supelco LC-18 reverse phase HPLC column 25 cm by 4.6 mm (5 µm) was used as the primary column, and a Supelco LC-CN reverse phase HPLC column 25 cm by 4.6 mm (5 µm) was used as a confirmation column. The appropriate precolumn, Novapak C-18 or Novapak CN, was used.

Sonication extractions of plant material were performed using a temperature-controlled ultrasonic bath where the temperature did not exceed 30 °C. The solvents used in this method were acetonitrile, CH_3Cl (HPLC grade), methanol, CH_3OH (HPLC grade). The water used was organic-free reagent water (18 mega-ohm Milli-Q). The HPLC used was mobile phase (1:1 (v/v) methanol/reagent water).

Preparation of Plant Samples

Frozen plant samples were allowed to come to room temperature before a representative subsample was blotted with paper towels and weighed. The samples were cut into small (less than one centimeter) pieces and placed into a homogenizing chamber. Milli-Q water was added to just cover the top of the sample, and the mixture was homogenized using a sawtooth generator probe, beginning at 500 rpm and increasing in 2-min intervals to 2,500, 5,000, and 7,500 rpm.

After homogenizing, the sample was poured into a freeze-drier flask, covered with parafilm, and frozen (approximately 3-4 hr). The sample was freeze-dried until no water crystals were left (approximately 2 days).

After determining the freeze-dried weight, 0.25 g of freeze-dried sample was weighed into a 20-ml amber vial. A matrix spike was prepared by adding 0.100 ml of an acetonitrile solution containing 100 mg HMX, RDX, TNB, TNT, 4-A-DNT, and 2,4-DNT per liter to each vial. Acetonitrile (10.0 ml) was added volumetrically. The sample was mixed by vortex for 1 min and placed in a cooled (<30 °C) ultrasonic bath for 18 hr.

After sonication, the sample was centrifuged and allowed to sit for approximately 1 hr. Supernatant (5 ml) was transferred to a 20-ml vial. Filter columns were prepared by the following procedure:

a. Place a small piece of glass wool into a 14.60-cm glass disposable pipette.

b. Place 0.5 g of florisil into the pipette.

c. Place 0.5 g of alumina on top of florisil.

d. Rinse the filter column with 5 ml of acetonitrile. Discard the rinsate.

The supernatant (5 ml) was passed through, followed by an additional 5 ml of acetonitrile. The combined eluents were vortexed for 1 min. A 2-ml subsample of the supernatant was transferred along with 2 ml Milli-Q water to a 20-ml vial. The extract was filtered, the first 1 ml was discarded, and the remainder was retained in a 10-ml glass Teflon-capped vial for HPLC analysis.

Table A2
Plant Species and Quantities

Species	Fresh Weight, g
Yellow nutsedge	5.00
Lettuce	5.00
Radish	5.00
Corn Kernels	10.0
Corn Silage	10.0
Tomato	20.0

Method detection limits

Method detection limits (MDL) were determined for the plant species corn, corn silage, tomato, yellow nutsedge, and radish in an analogous manner to the MDL determination for soil analysis by EPA Method 8330. Frozen plant tissue

(100 g) was allowed to warm to room temperature. The tissue was cut into small pieces (approximately 1 cm in diameter) and homogenized as described above. The homogenized tissue was placed into a freeze-drying flask and stored in a freezer for at least 5 hr prior to lyophilization. The samples were freeze-dried for 48 hr and subsampled to determine dry weight. A 0.25-g sample of dried material was transferred to 20-ml amber vials. Acetonitrile (10 ml) spiked with explosive analytes at 0.125 mg kg^{-1} was added to the samples. The samples were extracted, cleaned up by liquid chromatography (alumina/florisil cleanup column), and analyzed as described above.

Results

Plant spiking and method detection limits

Figure A1 shows chromatograms acquired by spiking homogenized corn with purified explosives standards at 26.3 mg kg^{-1} fresh weight, 80 mg kg^{-1} dry weight. The elution order for the explosives in the standard mixture on the polar confirmation column, CN derivitized silica, was different from that observed on the C18 column. The use of two columns on which the same compounds exhibit large differences in retention factors served to confirm the peak identified on the primary column by ensuring the same compound is identified at the same concentration and distinctive retention time on the second column. (The likelihood of an unknown interfering peak matching retention times on a single column is quite high, but a significantly different molecular compound is not likely to have identical retention characteristics on two columns with dissimilar solid phases.)

For instrument calibration, purified reference standards of the analytes were used to prepare solutions with concentrations of 0.05, 0.1, 0.4, 1.0, and 4.0 mg L^{-1} (which corresponded to 0.4, 1.6, 4, 12, and 40 mg kg^{-1} fresh weight (assuming 90-percent water in plant tissues) or 4, 16, 40, 120, and 400 mg kg^{-1} dry weight) for instrument calibration. Stock sources were prepared from neat or crystalline stock explosives standards obtained from the Army Environmental Center at Aberdeen Proving Ground, MD. Excellent linearity was achieved over two orders of magnitude of concentration range. This allowed for quantitation of the explosive compounds. Retention times were stable throughout the two orders of magnitude in the calibrated concentration range.

Studies were performed in which the MDL and data reporting limits (LRL) were determined for the explosives in several of plant tissue types. Samples were prepared from unexposed reference plant material as described above and spiked immediately prior to extraction. Table A3 contains the results of seven replicate runs near the data reporting limit as well as the statistical interpretation of those results. Through the sample preparation and cleanup process, a concentration factor was introduced that depended on the mass of the plant tissue tested, the amount of water removed during sample preparation, and the volume of solvents used for cleanup and mobile phase matching. The USEPA SW846 (EPA 1992) method for determination of MDL and LRL was used to produce the results

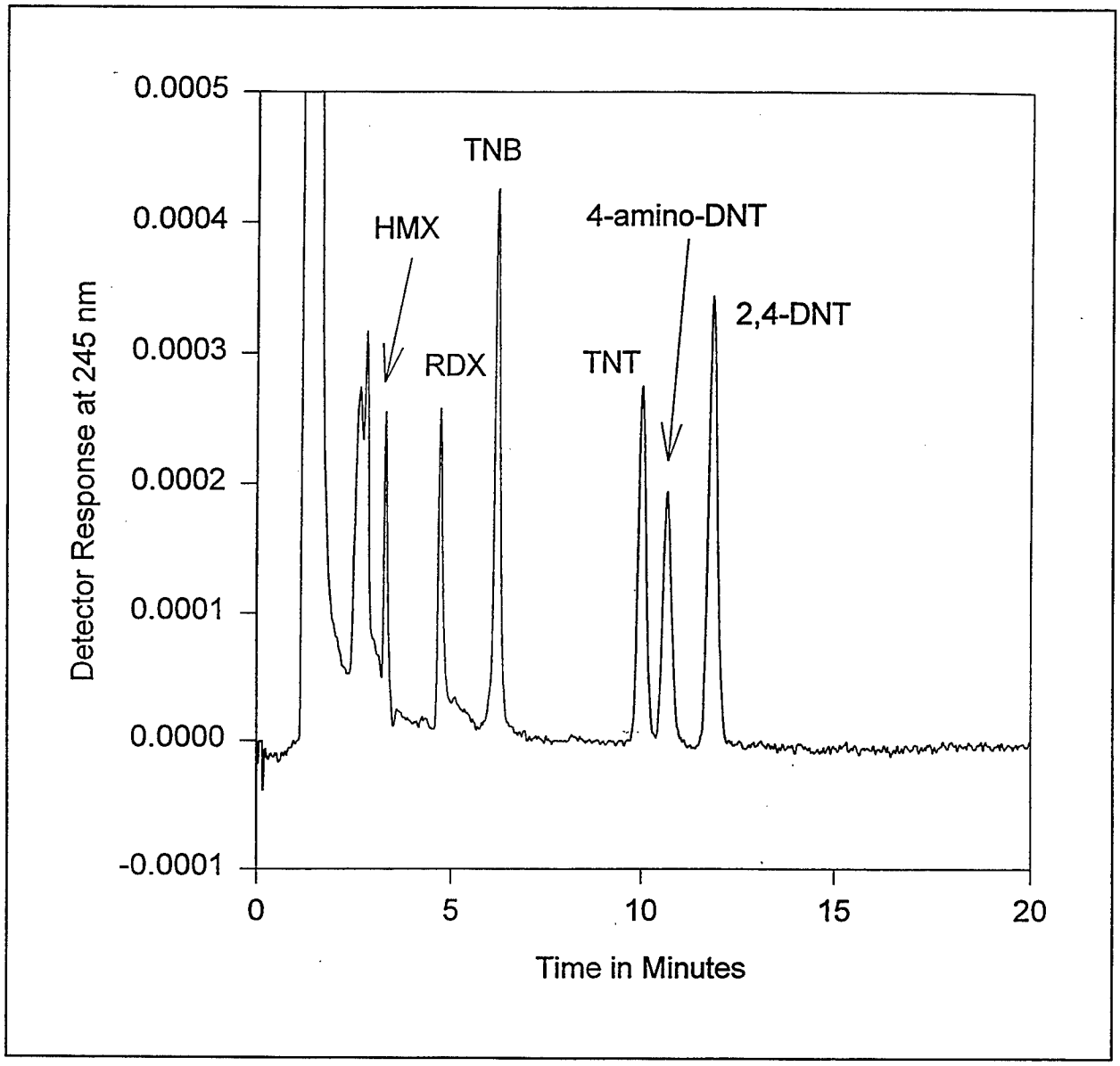

Figure A1. Corn extract spiked with explosives standards (at 1 ppm)

presented in Table A3. Table A4 provides a detailed description of MDL for RDX for various plant matrixes. As can be seen, large variations in detection limits were observed for different plant matrixes.

Figure A2 provides an example of a chromatogram showing the separation and determination of RDX on a C18 analytical column in several plant tissues that were exposed to RDX-contaminated irrigation water (0.100 mg kg^{-1}) and soil (5.8 mg kg^{-1}). Several peaks are present that are not attributed to explosives contamination. Samples that are known not to have been exposed to explosives can be used to help eliminate irrelevant peaks. No standard reference material is currently available for this purpose. Therefore, a representative blank matrix

Table A3
MDL Results by Tissue-Type Spiking After Freeze-Drying

Compound		Conc mg L^{-1}	MDL-1	MDL-2	MDL-3	MDL-4	MDL-5	MDL-6	MDL-7	Avg mg L^{-1}	Std Dev	MDL mg L^{-1}	% Rec	LRL mg L^{-1}
Corn Fruit														
C18	HMX	0.125	0.099	0.113	0.127	0.094	0.096	0.088	0.090	0.101	0.014	0.042	80.80	0.141
CN		0.125	0.087	0.096	0.117	0.087	0.084	0.084	0.086	0.092	0.012	0.036	73.26	0.119
C18	RDX	0.125	0.116	0.115	0.122	0.119	0.102	0.113	0.118	0.115	0.006	0.019	92.00	0.064
CN		0.125	0.119	0.117	0.124	0.121	0.112	0.126	0.118	0.120	0.005	0.014	95.66	0.047
C18	TNB	0.125	0.118	0.119	0.123	0.119	0.108	0.118	0.119	0.118	0.005	0.014	94.17	0.046
CN														
C18	4A-DNT													
CN		0.125	0.125	0.125	0.127	0.133	0.123	0.128	0.132	0.128	0.004	0.011	102.06	0.037
C18	24-DNT	0.125	0.125	0.122	0.127	0.123	0.121	0.121	0.111	0.121	0.005	0.015	97.14	0.051
CN		0.123	0.123	0.120	0.126	0.125	0.128	0.122	0.119	0.123	0.003	0.010	98.63	0.033
C18	TNT	0.120	0.120	0.119	0.124	0.117	0.107	0.114	0.118	0.117	0.005	0.016	93.60	0.054
CN		0.121	0.121	0.119	0.126	0.123	0.117	0.125	0.118	0.121	0.004	0.010	97.03	0.035
C18	26-DNT	0.087	0.087	0.085	0.086	0.082	0.090	0.087	0.085	0.086	0.002	0.007	68.80	0.024
CN		0.085	0.085	0.084	0.085	0.089	0.083	0.087	0.084	0.085	0.002	0.006	68.23	0.021
C18	NB	0.091	0.091	0.090	0.092	0.085	0.097	0.091	0.088	0.191	0.004	0.011	72.40	0.035
CN		0.094	0.094	0.091	0.092	0.089	0.096	0.091	0.093	0.092	0.002	0.007	73.83	0.023
C18	DNA	0.085	0.085	0.084	0.085	0.081	0.089	0.084	0.083	0.084	0.002	0.007	67.54	0.024
CN		0.088	0.088	0.085	0.086	0.086	0.009	0.009	0.087	0.064	0.038	0.114	51.36	0.379

(Sheet 1 of 6)

Table A3 (Continued)

Compound		Conc mg L^{-1}	MDL-1	MDL-2	MDL-3	MDL-4	MDL-5	MDL-6	MDL-7	Avg mg L^{-1}	Std Dev	MDL mg L^{-1}	% Rec	LRL mg L^{-1}
Corn Fruit (continued)														
C18	TETRYL	0.125	0.122	0.132	0.141	0.139	0.138	0.136	0.130	0.134	0.007	0.020	107.20	0.066
CN		0.125	0.133	0.129	0.113	0.119	0.142	0.141	0.135	0.130	0.011	0.033	104.23	0.109
Corn Silage														
C18	HMX	0.125	0.122	0.123	0.126	0.121	0.134	0.126	0.122	0.125	0.004	0.013	99.89	0.045
CN		0.125	0.106	0.100	0.102	0.105	0.106	0.100	0.100	0.103	0.003	0.009	82.17	0.029
C18	RDX	0.125	0.113	0.113	0.117	0.119	0.113	0.114	0.113	0.115	0.002	0.007	91.66	0.024
CN		0.125	0.119	0.119	0.126	0.123	0.123	0.119	0.125	0.122	0.003	0.009	97.60	0.030
C18	TNB	0.125	0.112	0.113	0.120	0.123	0.115	0.116	0.112	0.116	0.004	0.013	92.69	0.042
CN														
C18	4A-DNT													
CN		0.125	0.122	0.127	0.123	0.126	0.128	0.125	0.123	0.125	0.002	0.007	99.89	0.023
C18	24-DNT	0.125	0.114	0.114	0.121	0.115	0.122	0.116	0.114	0.117	0.003	0.010	93.26	0.035
CN		0.125	0.127	0.121	0.124	0.129	0.125	0.121	0.127	0.125	0.003	0.009	99.89	0.031
C18	TNT	0.125	0.110	0.111	0.118	0.108	0.111	0.114	0.114	0.112	0.003	0.010	89.83	0.033
CN		0.125	0.116	0.124	0.118	0.120	0.122	0.120	0.120	0.120	0.003	0.008	96.00	0.026
C18	26-DNT	0.125	0.085	0.083	0.101	0.085	0.084	0.095	0.084	0.088	0.007	0.021	70.51	0.070
CN		0.125	0.085	0.087	0.085	0.103	0.086	0.101	0.086	0.090	0.008	0.024	72.34	0.080
C18	NB	0.125	0.091	0.090	0.108	0.090	0.091	0.101	0.090	0.094	0.007	0.022	75.54	0.072
CN		0.125	0.091	0.093	0.092	0.111	0.093	0.110	0.096	0.098	0.009	0.026	78.40	0.087

Table A3 (Continued)

Compound		Conc mg L^{-1}	MDL-1	MDL-2	MDL-3	MDL-4	MDL-5	MDL-6	MDL-7	Avg mg L^{-1}	Std Dev	MDL mg L^{-1}	% Rec	LRL mg L^{-1}
Corn Silage (continued)														
C18	DNA	0.125	0.085	0.084	0.101	0.084	0.084	0.094	0.084	0.088	0.007	0.020	70.40	0.068
		0.125	0.085	0.087	0.105	0.087	0.088	0.103	0.088	0.092	0.008	0.025	73.49	0.084
	TETRYL	0.125	0.130	0.138	0.130	0.121	0.127	0.130	0.116	0.127	0.007	0.021	101.94	0.071
		0.125	0.122	0.130	0.126	0.125	0.124	0.119	0.133	0.126	0.005	0.014	100.46	0.047
Yellow Nutsedge														
C18	HMX	0.125	0.000	0.000	0.000	0.000	0.000	0.130	0.124	0.036	0.062	0.186	29.030	0.620
CN		0.125	0.000	0.000	0.000	0.000	0.000	0.114	0.132	0.035	0.060	0.181	28.11	0.602
C18	RDX	0.125	0.134	0.132	0.126	0.128	0.122	0.120	0.125	0.127	0.005	0.015	101.37	0.051
CN		0.125	0.127	0.125	0.125	0.125	0.117	0.121	0.125	0.124	0.003	0.010	98.86	0.034
C18	TNB	0.125	0.130	0.130	0.122	0.124	0.109	0.114	0.110	0.120	0.009	0.027	95.89	0.089
CN														
C18	4A-DNT													
CN		0.125	0.134	0.129	0.127	0.130	0.124	0.123	0.136	0.129	0.005	0.014	103.20	0.048
C18	24-DNT	0.125	0.135	0.135	0.124	0.127	0.112	0.121	0.116	0.124	0.009	0.026	99.43	0.088
CN		0.125	0.134	0.131	0.127	0.127	0.122	0.121	0.130	0.127	0.005	0.014	101.94	0.047
C18	TNT	0.125	0.125	0.125	0.116	0.113	0.117	0.116	0.109	0.117	0.006	0.018	93.83	0.059
CN		0.125	0.126	0.126	0.121	0.115	0.117	0.116	0.123	0.121	0.005	0.014	96.46	0.047
C18	26-DNT	0.125	0.108	0.107	0.110	0.114	0.112	0.104	0.108	0.109	0.003	0.010	87.20	0.033
CN		0.125	0.104	0.108	0.108	0.109	0.107	0.107	0.105	0.107	0.002	0.005	85.49	0.018

(Sheet 3 of 6)

Table A3 (Continued)

Compound		Conc mg L^{-1}	MDL-1	MDL-2	MDL-3	MDL-4	MDL-5	MDL-6	MDL-7	Avg mg L^{-1}	Std Dev	MDL mg L^{-1}	% Rec	LRL mg L^{-1}
Yellow Nutsedge (continued)														
C18	NB	0.125	0.107	0.108	0.113	0.113	0.111	0.106	0.109	0.110	0.003	0.008	87.66	0.028
CN		0.125	0.108	0.111	0.112	0.109	0.109	0.107	0.111	0.110	0.002	0.005	87.66	0.018
C18	DNA	0.125	0.105	0.107	0.111	0.110	0.109	0.105	0.107	0.108	0.002	0.007	86.17	0.024
CN		0.125	0.104	0.106	0.106	0.107	0.105	0.104	0.102	0.105	0.002	0.005	83.89	0.017
C18	TETRYL	0.125	0.067	0.033	0.038	0.040	0.044	0.045	0.045	0.045	0.011	0.032	35.66	0.108
CN		0.125	0.051	0.030	0.036	0.035	0.036	0.042	0.054	0.041	0.009	0.027	32.46	0.089
Lettuce														
C18	HMX	0.125	0.085	0.081	0.073	0.086	0.081	0.077	0.067	0.079	0.007	0.020	62.86	0.068
CN		0.125	0.079	0.062	0.066	0.061	0.063	0.064	0.057	0.065	0.007	0.021	51.66	0.069
C18	RDX	0.125	0.112	0.118	0.113	0.118	0.119	0.118	0.111	0.0146	0.003	0.010	92.46	0.034
CN		0.125	0.126	0.122	0.124	0.120	0.122	0.123	0.118	0.122	0.003	0.008	97.71	0.026
C18	TNB	0.125	0.109	0.117	0.118	0.115	0.117	0.118	0.111	0.115	0.004	0.011	92.00	0.036
CN														
C18	4A-DNT													
CN		0.125	0.122	0.124	0.126	0.125	0.126	0.126	0.123	0.125	0.002	0.005	99.66	0.016
C18	24-DNT	0.125	0.117	0.120	0.122	0.119	0.121	0.120	0.116	0.119	0.002	0.006	95.43	0.021
CN		0.125	0.125	0.126	0.130	0.126	0.124	0.131	0.122	0.126	0.003	0.010	101.03	0.032
C18	TNT	0.125	0.110	0.117	0.106	0.114	0.362	0.368	0.414	0.227	0.145	0.435	181.83	1.451
CN		0.125	0.114	0.119	0.110	0.117	0.352	0.351	0.393	0.222	0.135	0.403	177.83	1.346

(Sheet 4 of 6)

Table A3 (Continued)

Compound		Conc mg L^{-1}	MDL-1	MDL-2	MDL-3	MDL-4	MDL-5	MDL-6	MDL-7	Avg mg L^{-1}	Std Dev	MDL mg L^{-1}	% Rec	LRL mg L^{-1}
Lettuce (continued)														
C18	26-DNT	0.125	0.087	0.090	0.097	0.103	0.091	0.093	0.094	0.094	0.005	0.016	74.86	0.052
CN		0.125	0.090	0.090	0.092	0.089	0.090	0.088	0.092	0.090	0.001	0.004	72.11	0.015
C18	NB	0.125	0.091	0.096	0.103	0.091	0.094	0.090	0.097	0.095	0.005	0.014	75.66	0.046
CN		0.125	0.094	0.095	0.100	0.092	0.092	0.094	0.094	0.094	0.003	0.008	75.54	0.027
C18	DNA	0.125	0.089	0.092	0.100	0.090	0.093	0.088	0.087	0.091	0.004	0.013	73.03	0.044
CN		0.125	0.096	0.090	0.089	0.088	0.088	0.089	0.087	0.090	0.003	0.009	71.66	0.030
C18	TETRYL	0.125	0.0062	0.046	0.043	0.043	0.047	0.051	0.053	0.049	0.007	0.020	39.43	0.068
CN		0.125	0.040	0.067	0.057	0.042	0.060	0.060	0.063	0.056	0.010	0.031	44.46	0.104
Tomato														
C18	HMX	0.125	0.100	0.097	0.099	0.099	0.102	0.112	0.099	0.101	0.005	0.015	80.91	0.050
CN		0.125	0.090	0.089	0.093	0.094	0.095	0.104	0.092	0.094	0.005	0.015	75.09	0.049
C18	RDX	0.125	0.117	0.114	0.113	0.113	0.117	0.116	0.116	0.115	0.002	0.005	92.11	0.018
CN		0.125	0.125	0.123	0.120	0.125	0.125	0.125	0.123	0.124	0.002	0.006	98.97	0.019
C18	TNB	0.125	0.117	0.117	0.117	0.117	0.121	0.117	0.117	0.118	0.002	0.005	94.06	0.015
CN														
C18	4A-DNT													
CN		0.125	0.119	0.116	0.118	0.116	0.116	0.118	0.118	0.117	0.001	0.004	93.83	0.013
C18	TNT	0.125	0.124	0.122	0.117	0.117	0.125	0.120	0.120	0.121	0.003	0.009	96.57	0.031
CN		0.125	0.117	0.116	0.114	0.116	0.121	0.119	0.117	0.117	0.002	0.007	93.71	0.023

(Sheet 5 of 6)

Table A3 (Continued)

Compound		Conc mg L^{-1}	MDL-1	MDL-2	MDL-3	MDL-4	MDL-5	MDL-6	MDL-7	Avg mg L^{-1}	Std Dev	MDL mg L^{-1}	% Rec	LRL mg L^{-1}
Tomato (continued)														
C18	24-DNT	0.125	0.123	0.121	0.123	0.121	0.126	0.123	0.122	0.123	0.002	0.005	98.17	0.017
CN		0.125	0.120	0.118	0.118	0.118	0.120	0.120	0.118	0.119	0.001	0.003	95.09	0.011
C18	26-DNT	0.125	0.099	0.101	0.102	0.099	0.103	0.102	0.102	0.101	0.002	0.005	80.91	0.016
CN		0.125	0.103	0.101	0.102	0.100	0.102	0.100	0.102	0.101	0.001	0.003	81.14	0.011
C18	NB	0.125	0.101	0.103	0.101	0.104	0.104	0.101	0.107	0.103	0.002	0.006	82.34	0.021
CN		0.125	0.107	0.107	0.103	0.105	0.104	0.103	0.107	0.105	0.002	0.006	84.11	0.019
C18	DNA	0.125	0.096	0.098	0.096	0.099	0.098	0.096	0.101	0.098	0.002	0.006	78.17	0.019
CN		0.125	0.103	0.101	0.099	0.102	0.102	0.101	0.104	0.102	0.002	0.005	81.37	0.016
C18	TETRYL	0.125	0.115	0.148	0.125	0.150	0.117	0.176	0.161	0.142	0.023	0.070	113.37	0.233
CN		0.125	0.137	0.176	0.156	0.175	0.177	0.173	0.127	0.160	0.021	0.062	128.11	0.207

(Sheet 6 of 6)

Table A4
Method Detection Limits for RDX (mg kg^{-1}) Values Given Represent Mean of Seven Replicates Spiked at 0.125 mg kg^{-1} Prior to Extraction

	Yellow Nutsedge	Silage	Corn	Lettuce	Tomato
Mean	0.127	0.112	0.120	0.122	0.124
MDL-Injected	0.003	0.009	0.014	0.003	0.005
MDL- Fresh Wt.	0.049 (79.5% water)	0.165 (77.1% water)	0.380 (66.1% water)	0.012 (95.17% water)	0.027 (93.1% water)
MDL-Dry Wt.	0.24	0.72	1.12	0.24	0.40

should be used when possible. This was easily seen in the radish chromatogram (Figure A2). The peaks at 4.5, 5.8, and 7.7 min were observed in the exposed radish and in an unexposed radish sample that has undergone an identical sample preparation and cleanup. With this information, the analyst can easily discount these peaks as noninterfering, naturally occurring compounds inherent to the radish matrix. The examples supplied in Figure A2 contained high levels of explosives contamination. When the analyte concentration is considerably smaller, such interferences become more important.

To assess the effect that freeze-drying has on the recoveries of spiked explosives, a laboratory study was performed in which recoveries of explosive analytes from samples spiked before and after freeze-drying were measured. A literature review on the effect of sample drying prior to analysis of explosives was performed along with a review of a number of methods and the effect of sample handling practices on recovery. Table A5 provides a comparison of the effect on RDX recovery of spiking prior to and following lyophilization. Significant loss of the spiked analytes can be attributed to freeze-drying of the spiked tissue prior to extraction. However, these losses appear to be highly reproducible within specific tissue samples used. The losses vary greatly depending on the tissue type from 32.57-percent recovery in corn fruit to 61.26-percent recovery in tomato fruit.

Concentration ranges

The concentration range is dependent on the matrix in which the explosives, by-products of explosives manufacture, and explosives degradation products are measured. Standards spiked into homogenized corn samples can be detected in the concentration range between 0.01 and 5 mg L^{-1} as injected, 0.08 to 40 mg kg^{-1} fresh weight (assuming 90-percent water in plant tissue) and 0.8 to 400 mg kg^{-1} dry weight. The testable concentration range varies considerably with the matrix encountered. Generally, the cleaner the sample, the less background signal is detected at the detection wavelength, resulting in a lower detection limit.

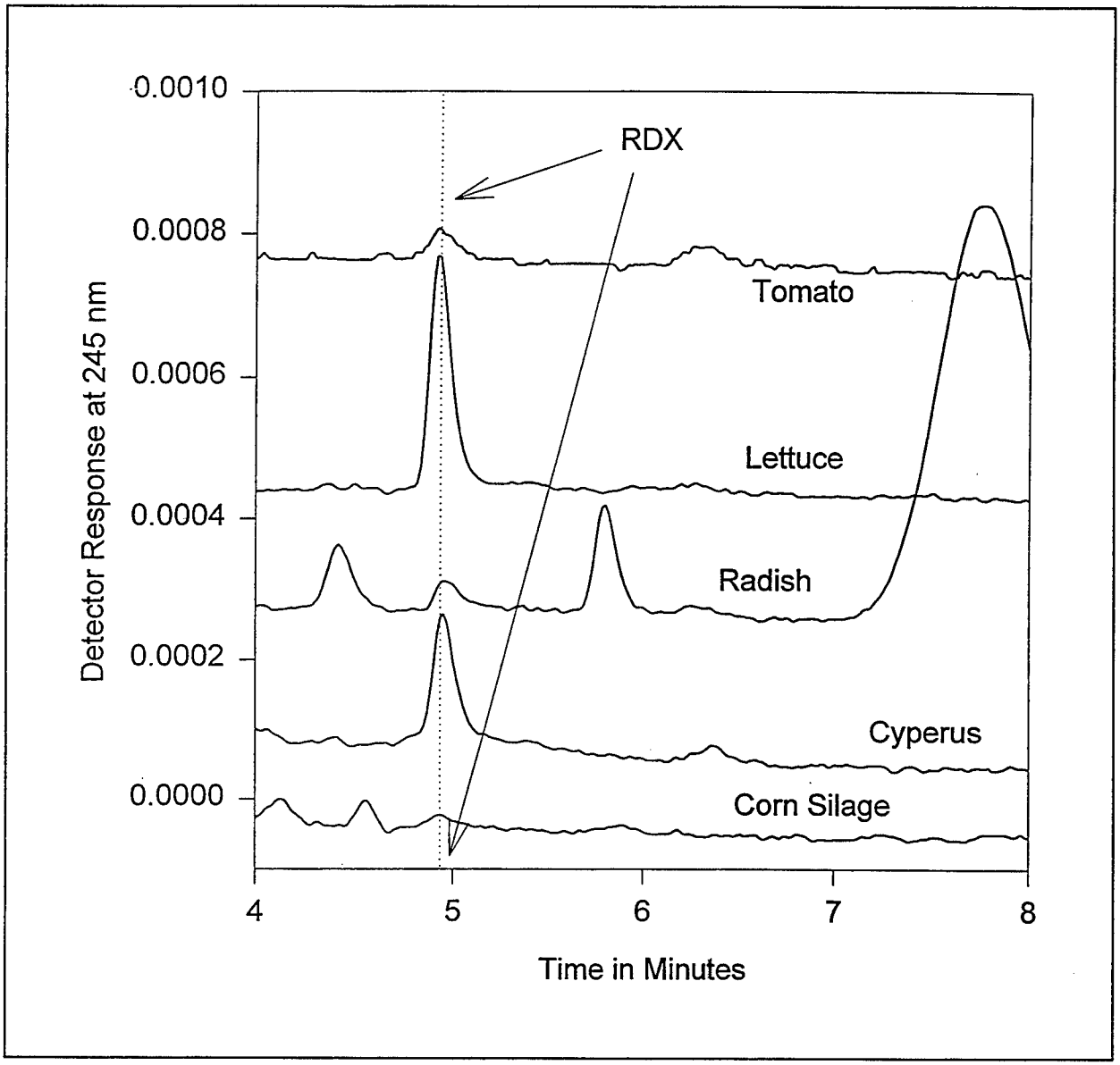

Figure A2. RDX detected in garden crops

Conclusions

A means of separation and quantitation of explosives in plant tissues has been developed. The plant tissues analyzed using this method in this study were yellow nutsedge (*Cyperus esculentus*), lettuce, radish, corn kernels, corn silage, and tomato fruit. Analyte recoveries were determined to range from 20 to 65 percent when examined through the entire method (including sample preparation, cleanup, and analysis). The method yields reproducible results for the tissues studied (i.e., corn fruit showed a 4-percent relative standard deviation over seven replicates). Dry weight detection limits for RDX were comparable

Table A5
RDX Recoveries with Prelyophilization and Postlyophilization Spiking (0.125 mg kg^{-1} Spike)

Tissue Type - Column Type	Conc mg L^{-1}	Post Avg mg L^{-1}	Pre Avg mg L^{-1}	Post Std Dev	Pre Std Dev	Post MDL mg L^{-1}	Pre MDL mg L^{-1}	Post % Rec	Pre % Rec	Post LRL mg L^{-1}	Pre LRL mg L^{-1}
Tomato-C18	0.125	0.115	0.066	0.002	0.005	0.015	0.005	92.11	52.91	0.018	0.048
Tomato-CN	0.125	0.124	0.077	0.002	0.006	0.018	0.006	98.97	61.26	0.019	0.059
Lettuce-C18	0.125	0.116	0.072	0.003	0.037	0.110	0.010	92.46	57.60	0.034	0.366
Lettuce-CN	0.125	0.122	0.058	0.003	0.003	0.008	0.008	97.71	46.63	0.026	0.027
Cyperus-C18	0.125	0.127	0.070	0.005	0.015	0.044	0.015	101.37	56.23	0.051	0.147
Cyperus-CN	0.125	0.124	0.069	0.003	0.015	0.045	0.010	98.86	54.97	0.034	0.152
Corn Fruit-C18	0.125	0.115	0.042	0.006	0.008	0.025	0.019	92.00	33.93	0.064	0.083
Corn Fruit-CN	0.125	0.120	0.041	0.005	0.007	0.020	0.014	95.66	32.57	0.047	0.066
Corn Silage-C18	0.125	0.115	0.060	0.002	0.026	0.079	0.007	91.66	48.23	0.024	0.264
Corn Silage-CN	0.125	0.122	0.064	0.003	0.025	0.076	0.009	97.60	50.86	0.060	0.255

with those from Method 8330 for explosives in soils: yellow nutsedge - 0.24 mg kg^{-1}, lettuce - 0.24 mg kg^{-1}, corn kernels - 1.12 mg kg^{-1}, corn silage - 0.72 mg kg^{-1}, and tomato fruit - 0.40 mg kg^{-1}.

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13. ABSTRACT ***(Maximum 200 words)***

Process wastes from ordnance loading (TNT, Amatol, Tritonal, and Composition B) have created groundwater and soil contamination at the Former Nebraska Ordnance Plant, Sauders County, Nebraska. Concern over hazards associated with future use of the site for vegetable gardening after remediation to low-level residual contamination prompted this study. The objective of the study was to quantify plant uptake of explosives, especially RDX, from contaminated soil or irrigation waters. Greenhouse studies were conducted using selected agronomic species, corn, tomato, lettuce, and radish, to measure plant uptake of explosives (RDX and TNT) from contaminated soil and uptake of RDX from irrigation water. A reference plant was included in all greenhouse studies to initiate development of a database from which future studies could predict uptake by growing the reference plant and extrapolating to the database for other crops. In addition to the greenhouse studies, a mass balance study with two species, tomato and radish, was conducted to determine the distribution of radiolabeled carbon from [^{14}C]RDX in each compartment of the test. At the preliminary remediation goal (RG) of 2 µg RDX per liter of groundwater, accumulation of RDX by plants was not detectable. However, all crops accumulated RDX from soils contaminated at the RG of 5.8 mg RDX per kilogram of soil and 17.2 mg TNT per kilogram of soil. All edible plant tissues accumulated RDX from soil except corn kernels. Bioaccumulation results from [^{14}C]RDX mass balance studies were consistent with results of greenhouse studies. These results suggest that human health hazards from ingestion of vegetables growing in soils contaminated at the RG be carefully evaluated and/or that the RG be revised.

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