10287

Environ Sci Pollut Res (2011) 18:908–917
DOI 10.1007/s11356-011-0449-9

RESEARCH ARTICLE

Concentration-dependent RDX uptake and remediation by crop plants

Diejun Chen · Z. Lewis Liu · Wanye Banwart

Received: 5 October 2010 / Accepted: 11 January 2011 / Published online: 28 January 2011
© Springer-Verlag (outside the USA) 2011

Abstract The potential RDX contamination of food chain from polluted soil is a significant concern in regards to both human health and environment. Using a hydroponic system and selected soils spiked with RDX, this study disclosed that four crop plant species maize (*Zea mays*), sorghum (*Sorghum sudanese*), wheat (*Triticum aestivum*), and soybean (*Glycine max*) were capable of RDX uptake with more in aerial parts than roots. The accumulation of RDX in the plant tissue is concentration-dependent up to 21 mg RDX/L solution or 100 mg RDX/kg soil but not proportionally at higher RDX levels from 220 to 903 mg/kg soil. While wheat plant tissue harbored the highest RDX concentration of 2,800 μg per gram dry biomass, maize was able to remove a maximum of 3,267 μg RDX from soil per pot by five 4-week plants at 100 mg/kg of soil. Although RDX is toxic to plants, maize, sorghum, and wheat showed reasonable growth in the presence of the chemical, whereas soybeans were more sensitive to RDX. Results of this study facilitate assessment of the potential invasion of food chain by RDX-contaminated soils.

Keywords Environmental contamination · Food chain safety · Phytoremediation · Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

Abbreviations

RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
TNT	Trinitrotoluene
HPLC	High performance liquid chromatography

Responsible editor: Elena Maestri

D. Chen · W. Banwart
University of Illinois,
Urbana, IL, USA

Z. L. Liu
National Center for Agricultural Utilization Research,
USDA-ARS,
1815 N University St,
Peoria, IL 61604, USA

Present Address:
D. Chen (✉)
National Center for Agricultural Utilization Research,
USDA-ARS,
1815 N University St,
Peoria, IL 61604, USA
e-mail: Diejun.Chen@ars.usda.gov

1 Background, aim, and scope

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) containing low levels of octahydro-1,3,5,7-tetrazocine is commonly used for production of explosives (Cataldo et al. 1987). It was heavily manufactured during the war time as well as in commercial applications such as coal mining (Etnier 1989). Waste water containing 2,4,6-trinitrotoluene (TNT) and RDX, two most commonly used military high explosives, has been directly discharged to lagoons during the manufacture, packaging, and decommissioning of outdated munitions (Freedman and Sutherland 1998; Atikovic et al. 2008). The toxic RDX is considered as an environmental hazard for soil and water supplies including contaminated areas that are leased for crop production or grazing (Gregory et al. 1991). The EPA standard for drinking water is less than 105 μg RDX/L (Etnier 1989). RDX and its degradation products pose a potential threat not only to food chain through plant uptake, but also can be further spread into terrestrial environments via polluted plants or animals.

The acute effects of RDX on survival, incidence of deformities, and behavior of larval zebrafish occurred at the high end of the most frequently reported concentrations of RDX in aquatic environments (Mukhi et al. 2005). RDX is degraded less rapidly than TNT from groundwater and emerging plants (Best et al. 1998). Although RDX did not obviously affect cricket survival, growth, and egg production, it did inhibit egg hatching (Zhang et al. 2006). RDX exerts its primary toxic effect in humans on the central nervous system and also involves gastrointestinal and renal effects (Etnier 1989). Exposure to the environmental toxicant RDX caused aberrant expression of many miRNAs related to various types of cancers, toxicant-metabolizing enzymes, and neurotoxicity (Zhang and Pan 2009).

Phytoremediation is defined as the use of green plants to remove, contain, or render harmless an environmental contaminant (Cunningham and Berti 1993). It is a growing technology that uses natural processes to break down, stabilize, or accumulate environmental pollutants (Pilon-Smits and Freeman 2006). It is essential to understand the fate of RDX in the plant and the potential of remediation. The hydroponic system has been used to study plant uptake of RDX (Banwart and Balbach 1991). A ^{14}C-labled RDX was found in its intact and bound forms in aquatic systems of parrot feather (*Myriophyllum aquaticum*) and in axenic hairy root cultures of *Vinca* (*Catharanthus roseus*) (Bhadra et al. 2001). The bioaccumulation of RDX in aerial tissues of hydroponic plants and the metabolism of RDX to polar metabolites were observed in bush been plants exposed to RDX (Harvey et al. 1991). Reed canary grass was used to degrade RDX and phytoremediation processes (Just and Schnoor 2004). The accumulation of RDX in aerial parts and metabolism of TNT in roots were observed for agronomic plants (Vila et al. 2007b). In this study, more than 80% of RDX taken up by plants was transported to aerial tissues while less than 25% of TNT taken up by plants was accumulated in aerial parts. It is believed that RDX concentrations in plants are decreased overtime, while those in worms are increased continuously in the RDX-contaminated soil (Best et al. 2006).

Although the RDX-contaminated soils have been an environmental concern for many years, knowledge of food chain contamination by RDX via plant uptake for main agricultural crops is limited. We are concerned that high levels of RDX could be accumulated in plant tissues and threaten food chain safety. The objectives of this study were to investigate yield of biomass for plants exposed to different levels of RDX in hydroponic solution and soil, to assess the differential uptake of RDX by four plant species, and to examine the recovery of added RDX from soil and plants at the termination of the experiment. Findings in this study will aid in risk assessment of crop plantation and food chain safety in RDX-contaminated areas.

2 Materials and methods

2.1 Chemicals

A purified RDX (96%) was supplied by the Joliet Army Ammunition Plant, Joliet, IL. HPLC-grade solvent and reagent-grade chemicals were purchased through Sigma (St. Louis, MO).

2.2 Plants

Crop genotypes were selected based on commonly used cultivars by Midwestern farmers. Thus, the results would be more applicable to practical field conditions where contaminated soils exist. Four widely used crop plant species were studied: maize (*Zea mays* cv. LH74-FR600), soybean (*Glycine max*), sorghum (*Sorghum sudanese* cv. SSG), and wheat (*Triticum aestivum* cv. Baker S-122) including both monocots and dicotyledonous. Soybean cultivars Williams 82 and Harper 87 were used for low and high levels of RDX treatments, respectively. Both were commonly used cultivars and the choice depended on availability at the time of the experiments.

2.3 Hydroponic experiments

Five-day-old seedlings were grown in a growth chamber and transplanted into a hydroponic assembly system. The hydroponic system consisted of a 10-L plastic tub with four openings on the lid. Each opening hosted one seedling of maize or soybean or two seedlings of sorghum or wheat supported by sponge materials. The hydroponic tubes were filled with a modified Hoagland nutrient solution (Below and Gentry 1987) spiked with RDX at 0, 6, 13, and 21 mg/L, respectively. The nutrient solutions were constantly aerated by slowly bubbling air from a small plastic tube. The experimental design consisted of six replications arranged in a completely randomized design. Plants were grown in the greenhouse with constant air temperatures of approximately 76°F and controlled light exposure. Plants were harvested 28 days after cultivation. Roots were rinsed with water and harvested separately from tops. Samples were dried in a forced-air oven at 25°C and ground into fine powder to pass a 20-mesh screen with a small Wiley Mill prior to RDX analysis.

2.4 Soil

Surface soil from 0 to 25 cm was collected containing no explosive residues near Group 61 of the Joliet Army Ammunition Plant, IL. It was identified as an Elliott silt loam (fine, illitic, mesic Aquic Agriudolls). The soil samples were analyzed to verify that the soil used did not contain any explosive residues. Soil samples were mixed

well, air-dried, and crushed with a soil grinder (Dynacrush 2, Dayton Electric MFG. Co., Chicago, IL). Soil samples were passed through a 4-mm sieve and stored in covered containers until use.

2.5 Soil treatment with RDX

Soils were treated with varied levels of a low concentration (0, 12.5, 25, 50, or 100 mg RDX/kg soil) or a high-concentration (0, 220, 494, or 903 mg RDX/kg soil). An appropriate amount of RDX was dissolved in 150 ml of acetonitrile in a glass flask. The RDX solution was gently sprayed on to the soil, while the soil was being continuously stirred in a motor driven stainless steel mixer (Intermatic, Dayton Electric MFG. Co., Chicago). An additional 50 ml of acetonitrile was applied rinsing the flask and added to the soil. After all the dissolved RDX was applied, the soil was thoroughly mixed continually for 90 min for each treatment. Controls (no RDX) also received 150 ml of ACN only.

2.6 Plants grown in RDX-treated soil

For the low levels of RDX treatments (0–100 mg RDX/kg), each 4.5″ plastic pot received 450 g of RDX-spiked soil. Soil in each pot received 125 ml of gentle watering prior to planting. Each pot was planted with ten seeds for maize and soybean, 14 for sorghum, and 20 for wheat. The seeds were then covered with an additional 50 g of the RDX-spiked soil to make up a total of 500 g soil in each pot. The surface soil of each pot was moistened with additional 30 ml of water. After emergence, seedlings were thinned to maintain five plants of maize and soybean, seven of sorghum, and ten of wheat in each pot. Each pot was watered daily with approximately 50 ml of water until harvest. Each plant pot received 50 ml solution every 5 days containing 10 g of a fertilizer (N–P_2O_5–K_2O at 20:20:20) per 1 gal of deionized water. Pots containing uncontaminated soil treated in the same manner served as controls. Three replications were carried out for each treatment. Plants were harvested 34 days after planting. Similar procedures were carried out for the high level RDX treatments (0–903 mg RDX/kg), except that seeds of each plant species were pre-germinated and seedlings transplanted. Seedlings at approximate 6 cm high pre-germinated in a growth chamber were transplanted and maintained in the RDX-spiked soils under the conditions mentioned above. Three plants were maintained in each pot for maize and soybean; five in each pot for sorghum and wheat. Plants were harvested 28 days after the planting.

2.7 RDX extraction from plant tissues

Plants were harvested by cutting the aerial portion just above the soil surface and placed in individual bags for each pot. Fresh weights were measured immediately at the harvest. Samples were dried in a forced-air oven at 26°C for 2 days, and dry mass was weighed. The dried plant samples were ground to powder. RDX was extracted from plant tissues using a modified procedure as previously described (Harvey et al. 1991; Chen 1993). Briefly, the ground samples were extracted using 10 ml of 0.1 N HCl with 0.2 g sample in a 25-ml Cortex tube with a Teflon-lined cap. The suspension was well mixed and went through a sonication treatment in tubes in a water bath for 15 h. Then a 2.0-ml aliquot was mixed with 2.0 ml of acetonitrile and allowed to stand for 15 min. The solution was filtered through a Nalgene 0.2 μm filter prior to HPLC analysis.

2.8 RDX extraction from soil

Soil samples were taken from each pot at the time of planting and after harvest. At the planting, air-dried soil spiked with RDX were taken prior to the addition of water. Samples collected after harvest were free of visible root materials and passed through a 9-mesh screen prior to air drying. Soil samples were extracted following a USA-THAMA certified method (Chen 1993). Briefly, 2 g of the soil and 20 ml of acetonitrile were added to a 25-ml glass screw-cap test tube to allow the soil to disperse for 1 min using vortex and sonication for 18 h in a water bath modified based on a procedure described previously (Jenkins and Grant 1987). Following sonication, a 10-ml aliquot of sample was combined with 10 ml of 0.2% aqueous calcium chloride solution, mix well, and standing for 15 min. An aliquot of the supernatant was filtered through a 0.2-μm regenerated cellulose filter prior to analysis.

2.9 HPLC analysis

2.9.1 HPLC analysis for RDX in plant material

Analysis of RDX in plant tissues was performed using an HPLC system consisting of a Beckman 110 B pump and a 166 detector. A Micromeritics 728 autosampler was used to inject 50 μl of filtered plant extract onto a Supelco Pelliguard column (5 cm×4.6 mm, 40 μm pellicular CN-bonded phase) and a Supelco LC-CN analytical column (25 cm×4.6 mm, 5 μm). The mobile phase consisted of 16.5:13.5:70 (*v*/*v*/*v*) methanol/tetrahydrofuran/water at a flow rate of 1.0 ml/min. The RDX was detected at 254 nm, and peak areas were used to quantify explosive levels (Harvey et al. 1991, Chen 1993).

2.9.2 HPLC analysis for RDX in soil

Soil sample analysis was accomplished by injecting 50 μl of filtered extract onto a reverse phase C-18 guard column

(4.5 cm) and C-18 analytical (25 cm) column (Beckman 167 scanning detector module and a Beckman 406 analog interface module). The mobile phase consisted of 58/42 (*v*/*v*) methanol/water at a flow rate of 1.5 ml/min and RDX was detected at 254 nm.

2.10 Statistical analysis

Statistical differences were calculated using SAS software (Version 8, SAS Institute, Cary, NC, USA). A general linear model analysis of variance with Tukey's multiple comparison adjustment was used to determine significant differences between treatment combinations. Statistical significance was defined at $P<0.05$.

3 Results

3.1 Plant response to RDX

All tested plant species were able to maintain reasonable growth in the RDX-spiked hydroponic solutions at concentrations of 6 to 21 mg/L. However, visual stress or toxic symptoms were observed with a typical browning edge on leaves for maize, wheat, and sorghum. More severe symptoms of necrotic spots and premature leave dropping were observed on soybean plants. These symptoms were less notable at 6 mg/L level but more obvious at 21 mg/L levels for all four plant species. Maize plants appeared to be less sensitive to the RDX toxicity, especially at lower levels. The dry biomasses of four plants were decreased with the increase concentrations in hydroponic solution. However, the decreases were not significant different statistically, except for wheat at 21 mg/L compared with lower levels.

Similar to the plants grown in hydroponic solution, all plants grown in the RDX-spiked soils showed toxic symptoms at all levels for both low and high-concentration treatments. Typical symptoms were brown tips of leaves for sorghum and wheat, browning lesions for maize, and inter-veinal browning, necrotic spots on leaves, and defoliations for soybeans. Soybean plants exhibited the most severe symptoms of damage from the RDX-treated soils. Although there was a tendency of increased plant damage with increasing concentrations of RDX, all plants maintained reasonable growth for the duration of the experiments even at the highest levels of 903 m/kg soil.

In general, plant dry biomass appeared to be repressed by treatments with RDX in the hydroponic system. Dry biomasses from root and aerial parts decreased with the elevated concentrations of RDX in the hydroponic study for all plants (Table 1). Reduction of plant biomass by RDX was greater for root than the aerial portion for plants grown in hydroponic systems. Root dry weight was significantly reduced for maize, soybean, and wheat plants at 21 mg/L of RDX. In contrast, maize, soybean, and sorghum plants showed no significant biomass reduction in aerial parts. Wheat appeared to be affected considerably for reduced biomass production in both aerial parts and roots at 21 mg/L compared with a non-RDX-treated control. For plants grown in the soils at lower RDX concentrations from 12.5 to 100 mg/kg, the significant reduction of dry biomasses was only observed for wheat plant at 100 mg RDX/kg soil (Table 2). The reduction of biomass was not significant at the higher RDX concentrations from 220 to 903 mg/kg. It was unexpected that dry biomass of maize was increased significantly with the increased concentrations at high levels from 220 to 903 mg/kg RDX (Table 2).

3.2 Plant uptake of RDX

The uptake of RDX in both aerial parts and roots for four crop plant species was observed under the hydroponic system. Concentrations of RDX in plant for all four species were increased with the increased RDX concentrations in the solutions (Table 1). The RDX uptake and accumulation appeared to be higher in the aerial parts than in the roots as measured per unit of dry biomass (Table 1, Fig. 1a). RDX detected from plant tissues showed a RDX concentration-dependent accumulation from 6 to 21 mg/L (Fig. 1a). Among the four species, soybean displayed the lowest RDX uptake from the hydroponic solution.

Similar to plants in hydroponics, RDX uptake and accumulation in plants grown in RDX-spiked soils showed concentration-dependent response and nearly linear relationships with escalating concentrations from 12.5 to 100 mg RDX/kg soil (Table 2, Fig. 1b). In general, RDX accumulation per unit of plant tissue was always higher than that in soil (Table 2). Wheat tissue contained the highest concentration of RDX (2800 μg/g) with a level approximately 28 times higher than the soil concentration (100 mg RDX/kg soil). In the soil with high levels of RDX (220 to 903 mg/kg), the RDX concentrations in wheat and sorghum displayed similar high levels of uptake, as observed in the low RDX level study (Table 2). The RDX concentrations in wheat and sorghum were higher than that in soil. However, the RDX accumulations per unit of plant biomass were not proportionally increased with higher concentrations from 220 to 903 mg/kg (Fig. 1c). The wheat plants only had about two times higher RDX in plant tissues than in the soil at RDX 903 mg/kg of soil. At RDX 903 mg/kg level, the RDX concentration in the plant was only 649 μg RDX/g for maize or 274 μg/g for soybean, which were both below the initial soil concentration of 903 mg RDX/kg. Overall, similar trends in RDX concentration in plants were observed for four

Table 1 RDX uptake and biomass production of four plant species grown in a hydroponic solution at varied RDX concentrations

Plant	RDX (mg/L)	Aerial parts			Roots		
		Dry mass (g)	RDX[a] (mg/kg)	Total RDX (μg)	Dry mass (g)	RDX[a] (mg/kg)	Total RDX (μg)
Maize	0	4.82 a[b]	0 a	0 a	1.47 a	0 a	0 a
	6	5.21 a	95 b	495 b	1.49 a	26 b	39 b
	13	5.08 a	171 c	869 c	0.99 ab	69 c	68 c
	21	3.78 a	300 d	1,134 c	0.79 b	156 d	123 d
Soybean	0	2.35 a	0 a	0 a	0.45 a	0 a	0 a
	6	2.26 a	76 b	172 b	0.37 ab	27 b	10 b
	13	1.89 a	138 c	261 c	0.28 b	57 c	16 bc
	21	1.77 a	168 c	297 c	0.25 b	80 d	20 c
Sorghum	0	1.79 a	0 a	0 a	0.76 a	0 a	0 a
	6	1.34 a	72 b	96 b	0.62 a	23 b	14 b
	13	1.60 a	196 c	314 c	0.58 a	66 c	38 c
	21	1.35 a	436 d	589 d	0.55 a	120 d	66 d
Wheat	0	1.46 a	0 a	0 a	0.37 a	0 a	0 a
	6	1.06 ab	65 b	69 b	0.39 a	18 b	7 b
	13	1.03 ab	239 c	246 c	0.29 ab	38 c	11 c
	21	0.89 b	408 d	363 d	0.20 b	85 d	17 c

[a] Values presented by a mean of six replications

[b] Means in a column with different letters are statistically significant at $P \leq 0.05$ for each plant species

plants in both studies with low and high levels of RDX. Wheat plants demonstrated the highest concentration of RDX accumulation followed by sorghum and maize, while the soybean plants were lower in the RDX uptake (Fig. 1b and c).

3.3 RDX removal from soil by plants

The total amount of RDX removed from hydroponics and soil per pot is a function of the RDX concentration in the plant tissue and the total dry biomass of a plant. For both hydroponics and the soil with lower level RDX, the total RDX removal by plants increased significantly with the increasing RDX concentrations for all species (Tables 1 and 2 and Fig.1). The highest amount of total RDX removal was observed at 3,267 μg by five maize plants followed by 2,743 μg by ten wheat plants at 100 mg/kg level. Soybean plants showed the least removal with 1,141 μg by five plants. However, the amount of RDX removal was not significantly increased as concentrations increased from 220 to 903 mg/kg. In the soil with 220 mg RDX/kg, total RDX removal was 1,515 and 1,150 μg for maize and wheat, respectively. At 494 mg RDX/kg soil, the highest removal of 1,772 μg/pot was achieved by maize plants, followed by sorghum with 1,336 and 1,025 μg/pot by wheat plant (Table 2). At the highest spiked level 903 mg/kg soil, the total amount of RDX removed was similar with that at the 494 mg RDX/kg level. Over all, there were no significant differences among the spiked levels for the high level treatments from 220 to 903 mg RDX/kg soil. While plants were able to uptake and accumulate RDX in tissue, a significant amount of intact RDX remained in the soil as detected by HPLC analysis (Figs. 2 and 3). As expected, the RDX detected in soil were proportionally increased with the increasing RDX spiked in the soil. However, a considerable amount of RDX was not recovered either from plant tissues, nor the soils except for the treatment of 903 mg/kg (Figs. 2 and 3). At the highest RDX-treated soil (903 mg/kg), only 3% to 8% of RDX was not recovered.

4 Discussion

Using RDX-spiked hydroponic solution and soils, we demonstrated that crop plants maize, sorghum, soybean, and wheat are capable of uptaking and accumulating RDX in a concentration-dependent fashion at concentrations below 100 mg/kg. Wheat plant tissues displayed the highest accumulation of intact RDX at 2,828 mg g^{-1} (d.w.). An individual 4-week-old maize plant was able to remove 653 μg RDX from soil. These results facilitate assessment of potential phytoremediation of the toxic explosives and contamination of food chain by the RDX soils.

Our results showed that RDX is toxic to crop plant especially for soybeans. Similar phytotoxic symptom

Table 2 RDX uptake and biomass production of four plant species grown on RDX-spiked soils at varied RDX concentrations

Plant	RDX in soil (mg/kg)	Dry mass (g)	RDX In plant (μg/g d.m.)	RDX uptake by plant (μg/pot)[a]	RDX accumulation (μg/plant)
Low levels					
Maize (5/pot)	0	2.30 b[b]	0 d	0 d	0
	12.5	3.07 a	120 d	368 d	74
	25.0	2.91 ab	300 c	873 c	175
	50.0	2.87 ab	802 b	2,302 b	460
	100.0	2.70 ab	1,210 a	3,267 a	653
Soybean (5/pot)	0	2.82 a	0 e	0 e	0
	12.5	2.64 a	104 d	275 d	55
	25.0	2.76 a	181 c	500 c	100
	50.0	2.48 a	314 b	779 b	156
	100.0	2.32 a	492 a	1,141 a	228
Sorghum (7/pot)	0	1.31 a	0 d	0 c	0
	12.5	1.48 a	94 cd	139 c	20
	25.0	1.52 a	314 c	477 c	68
	50.0	1.22 a	1,052 b	1,283 b	183
	100.0	1.58 a	1,414 a	2,234 a	319
Wheat (10/pot)	0	1.17 ab	0 e	0 d	0
	12.5	1.25 ab	290 d	363 d	36
	25.0	1.23 a	888 c	1,092 c	109
	50.0	1.14 ab	1,723 b	1,964 b	196
	100.0	0.97 b	2,828 a	2,743 a	274
High levels					
Maize (3/pot)	0	1.50 a	0	0 a	0
	220	2.18 b	695	1,515 b	505
	494	2.86 c	602	1,722 b	574
	903	2.73 c	649	1,772 b	591
Soybean (3/pot)	0	0.84 a	0.00	0 a	0
	220	1.09 a	322	351 a	117
	494	0.76 a	358	272 a	91
	903	0.88 a	274	241 a	80
Sorghum (5/pot)	0	1.43 a	0	0 a	0
	220	1.01 a	1,133	1,144 b	229
	494	1.37 a	975	1,336 b	267
	903	1.00 a	1,218	1,218 b	244
Wheat (5/pot)	0	0.74 a	0	0 a	0
	220	0.72 a	1,597	1,150 b	230
	494	0.61 a	1,680	1,025 b	205
	903	0.64 a	1,915	1,226 b	245

[a] Values presented by a mean of six replications

[b] Means in a column with different letters are statistically significant at $P \leq 0.05$ for each plant species

caused by RDX accumulation was also observed for rice (Vila et al. 2007a). The symptoms on soybeans observed in our study are similar with those caused by triazine herbicide such as atrazine. RDX is a triazine compound which may account for the toxic symptoms of soybeans. Although RDX is phytotoxic, all plant species tested in this study were able to maintain reasonable growth for the duration of the experiments and no significant biomass reduction was observed except for wheat. Similar results were observed for perennial ryegrass *Lolium perenne* (Best et al. 2006). On the other hand, it is interesting to note that maize plant showed a significant higher biomass production at 494 and

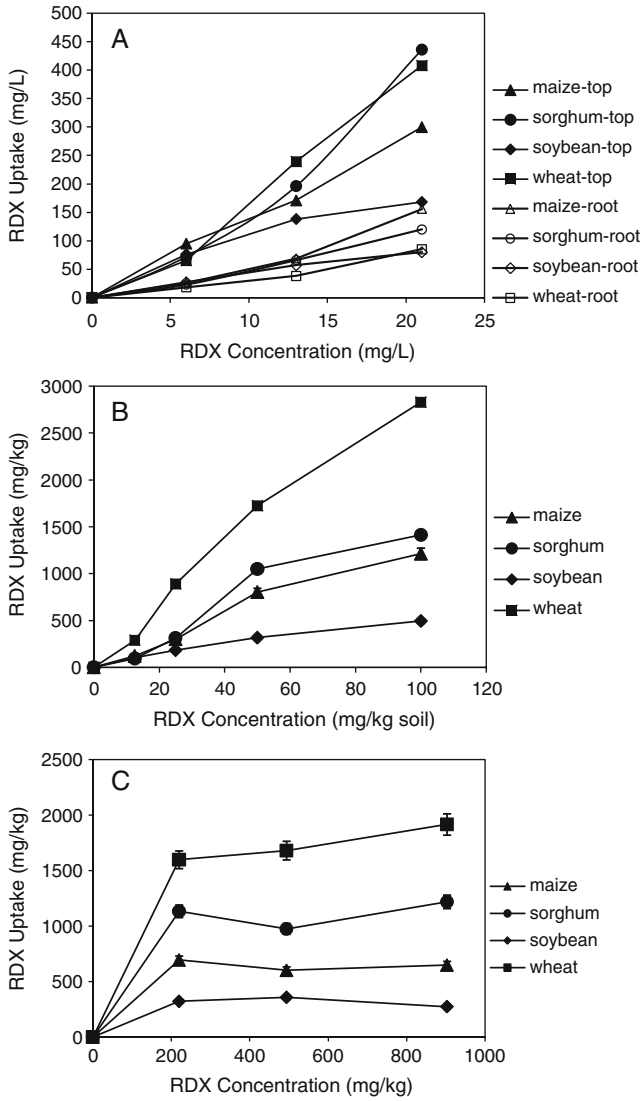

Fig. 1 **a** Plant uptake of RDX. RDX concentrations in dry biomass of plant tissues 28 days after exposure to the explosives in a hydroponic system for aerial portion/tops (*solid symbol*) and root (*open symbol*) for maize (*triangle*), sorghum (*circle*), soybean (*diamond*), and wheat (*squire*). **b** RDX concentrations in dry tissues of aerial portion for plants grown on RDX-spiked soils 34 days after exposure to the explosives at varied levels of low dose (12.5, 25, 50, and 100 mg/kg) and **c** high dose (220, 494, and 903 mg/kg) of RDX for maize (*solid triangle*), sorghum (*solid circle*), soybean (*solid diamond*), and wheat (*solid squire*) as measured by three replications

903 mg RDX/kg levels. The increased biomass of alfalfa (*Medicago sativa*) plants was also observed when grown in soil containing RDX (Best et al. 2006).

It is clear that the RDX uptake and accumulation in plant tissues are RDX concentration-dependent at relatively lower concentrations. The maximum RDX uptake by plant is 100 mg/kg as demonstrated by our study. At this level, the highest RDX accumulation reached as high as 2,828 μg/g (d.w.) for wheat plant. At higher RDX concentrations, such as 220 to 903 mg/kg, RDX accumulations in plant tissues were

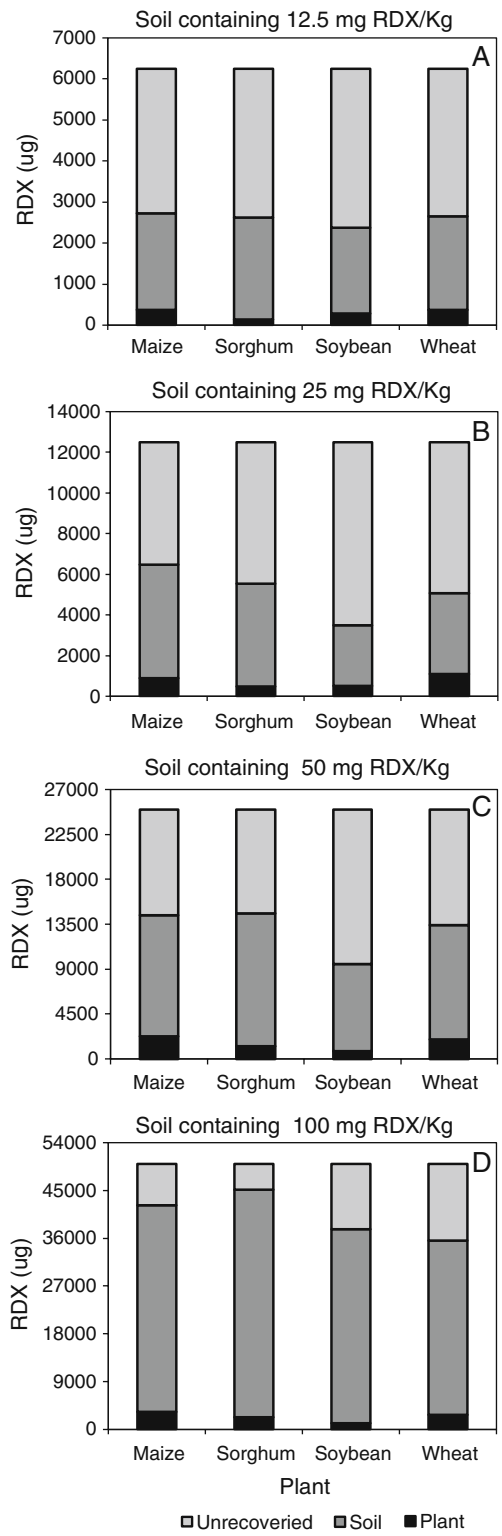

Fig. 2 RDX removal from low RDX-contaminated soil by plants. Total RDX amount detected in plant tissues (*black shaded*), remained in the soil (*gray shaded*), and undetected (*light gray shaded*) from soils spiked with RDX at concentrations of **a** 12.5, **b** 25, **c** 50, and **d** 100 mg/kg levels maintained in pots under greenhouse conditions

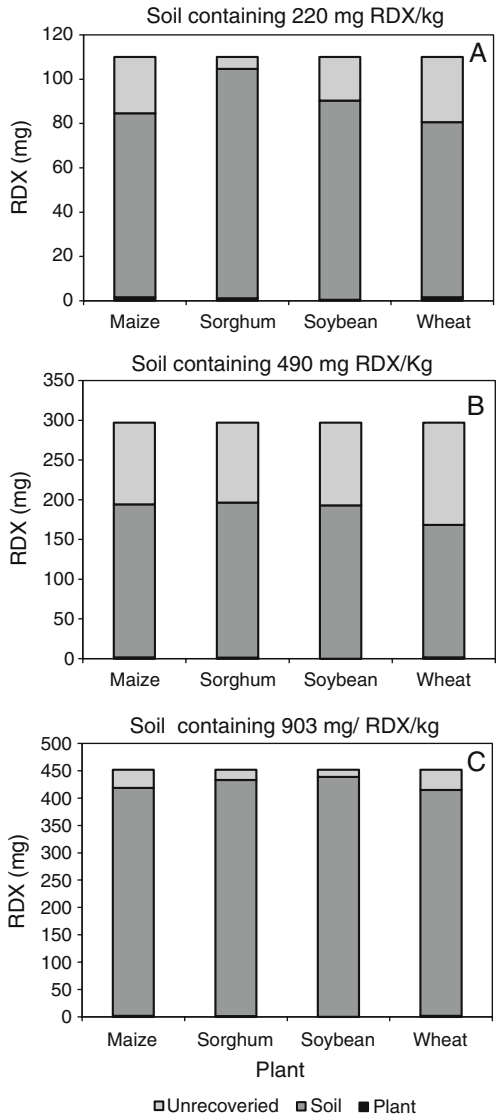

Fig. 3 RDX removal from high RDX-contaminated soil by plants. Total RDX amount detected in plant tissues (*black shaded*), remained in the soil (*gray shaded*), and undetected (*light gray shaded*) from soils spiked with RDX at concentrations of **a** 220, **b** 494, and **c** 903 mg/kg levels maintained in pots under greenhouse conditions

not proportionally increased but lower than that at 100 mg/kg. It is possible that the phytotoxicity of RDX at higher concentrations might reduce the rate of RDX uptake by plant. For example, the most sensitive soybean plants displayed the lowest RDX uptake and accumulation.

Our hydroponic studies showed that more RDX are accumulated in the aerial parts than the roots. It has been observed that TNT and its metabolites remain predominantly in root tissues, while RDX and its metabolites are readily transported to leave tissues (Burken 2003; Brentner et al. 2010). The majority of RDX accumulation in aerial tissues was also observed for rice (Vila et al. 2007a).

As mentioned above, wheat plants displayed the highest RDX accumulation per unit dry weight. Sorghum also showed higher capacity and ranked as the second. Although the RDX concentrations in maize plant was not the highest, maize plants displayed the highest total RDX removal from soil due to its high biomass production. Maize plant removed 3,267 μg RDX per pot which accounted for 653 μg per one 4-week-old plant. RDX and TNT are important environmental contaminants and phytoremediation has been considered as cost-efficient abatement method (Rao et al. 2009).

The RDX recovery reported in our study is in its original form. About 95% of RDX detected from rice plant was also in its original form (Vila et al. 2007a). We also found that RDX concentrations per unit plant tissue were significantly higher than that of the initial RDX concentrations per unit of soil. It appeared that the RDX was transferred from the soil to the plant but not metabolized by the plants. Recent investigations indicated that RDX was resistant to microbial degradation and its structure was stable and persistent in soils (McCormick et al. 1981; Talmage et al. 1999). It appeared that a limited amount of RDX can actually be removed from soil. However, a new source of contaminated plant biomass is generated with relatively higher RDX concentrations per unit of biomass. RDX in a more concentrated form could be possibly spread out into a variety of different media by animals which consume plants containing RDX. Previous evidence showed that RDX accumulation in animals such as worms are constantly increasing (Best et al. 2006). Therefore, the food chain contamination is a potential threat if the RDX-contaminated animals and plants are consumed by human. Assessment of the food chain safety by RDX-contaminated soil and feasibility studies of such suggested phytoremediation methods are needed.

Although RDX was recovered from the plant tissue and extracted from soils, a significant amount of RDX was not recovered at most RDX concentration levels with the exception of the highest RDX treatment of 903 mg/kg (Figs. 2 and 3). The thermophilic bacteria play a dominant role in a small scale composting system (Emery and Faessler 1997), but the chance of microbial degradation was very limited under the normal plant growing temperature and conditions (McCormick et al. 1981; Talmage et al. 1999). Xenobiotic reductases and their respective bacterial strains have been reported to be able to transform RDX and a wide variety of explosive compounds, and *Pseudomonas fluorescens* I-C (XenB) degraded RDX faster than *Pseudomonas putida* II-B (XenA) under anaerobic conditions (Fuller et al. 2009). It was also demonstrated that RDX degradation did not occur under aerobic conditions alone and a complete degradation of RDX was achieved within 20 days using a consortium of bacteria from

wastewater activated sludge (Ibeanusi et al. 2009). It seems that the chance of degradation by the exposure to light and UV was limited since only a small portion of soil surface was affected. Inorganic and organic nitrogen sources were found to have a profound impact on RDX-biodegradation activity in cell cultures by *Rhodococcus* strain YH1 (Nejidat et al. 2008). Therefore, the nitrogen applied to soil possibly enhanced the degradation of RDX. In addition, soil has limited capability to bind RDX and the soil sorption of RDX varied with different soil types (Chen 1993). Adsorption coefficients indicate that RDX is only moderately adsorbed to soil and may leach to groundwater (Talmage et al. 1999). It is likely that the unrecovered RDX is leached by frequent watering during the growing season, particularly for the potted plants in the greenhouse. The efficiency of extraction procedures involving the particle size of soil and plant materials could also contribute to the incomplete recovery of RDX. In general, hydroponic system makes it easier for quantitative measurement compared with soil study. In future hydroponic studies, it would be useful to measure root architecture, growth rate, and levels of RDX uptake by different species over time with the same biomass for each species if possible.

5 Conclusion

Results of this study demonstrated that all four crop plants are able to uptake and accumulate RDX in plant tissues with the highest capacities of 2828 μg/g (d.w.) by wheat. RDX uptake and accumulation in plants are concentration-dependent from both hydroponic system and RDX-spiked soils. Maize was able to remove a maximum of 3,267 μg RDX/pot in its original form, the highest among the four crop plants, from soil by five 4-week-old plants.

References

Atikovic E, Suidan MT, Maloney SW (2008) Anaerobic treatment of army ammunition production wastewater containing perchlorate and RDX. Chemosphere 72:1643–1648

Banwart WL, Balbach HE (1991) HPLC separation of explosives and plant uptake of RDX and TNT. Final report to USATHAMA

Below FE, Gentry LE (1987) Effect of mixed N nutrition on nutrient accumulation, partitioning, and productivity of corn. Fertilizer Issues 4:79–85

Best E PII, Spreche SL, Larson S L, Fredrickson H L, Baderj D F (1998) Environmental behavior of explosives in groundwater from the milan army ammunition plant in aquatic and wetland plant treatments. Removal, mass balances and fate in groundwater of TNT and RDX. PII: SOO45-6535(98)00550-S

Best EPH, Geter KN, Tatem HE, Lane BK (2006) Effects, transfer, and fate of RDX from aged soil in plants and worms. Chemosphere 62:616–625

Bhadra R, Wayment DG, Williams RK, Barman SN, Stone MB, Hughes JB, Shanks JV (2001) Studies on plant-mediated fate of the explosives RDX and HMX. Chemosphere 44:1259–1264

Brentner LB, Mukherji ST, Walsh SA, Schnoor JL (2010) Localization of hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX) and 2, 4, 6-trinitrotoluene (TNT) in poplar and switchgrass plants using phosphor imager autoradiography. Environ Pollut 158:470–475

Burken JG (2003) Uptake and metabolism of organic compounds: green-liver model. In: McCutcheon SC, Schnoor JL (eds) Phytoremediation-transformation and control of contaminants. Wiley, Hoboken, New Jersey, pp 59–84

Cataldo D, Voris PV, Fellows R (1987) An evaluation of the environmental fate and behavior of munitions material(TNT, RDX) in soil and plant systems. Report. Pacific Northwest Laboratory. P.O.Box 999 Richland, Washington

Chen D (1993) Plant uptake and soil adsorption of RDX. University of Illinois. Thesis. University of Illinois, Urbana, IL

Cunningham SD, Berti WR (1993) Phytoremediation of contaminated soils: progress and promise. American Chemical Society, Division of Petroleum Chemistry, Preprints 38(2):265–268

Emery DD, Faessler PC (1997) First production-level bioremediation of explosives-contaminated soil in the United States. Ann NY Acad Sci 829:326–340

Etnier EL (1989) Water quality criteria for hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX). Regul Toxicol Pharmacol 9:147–157

Freedman DL, Sutherland KW (1998) Biodegradation of hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX) under nitrate-reducing conditions. Water Sci Technol 38:33–40

Fuller ME, McClay K, Hawari J, Paquet L, Malone TE, Fox BG, Steffan RJ (2009) Transformation of RDX and other energetic compounds by xenobiotic reductases XenA and XenB. Appl Microbiol Biotechnol 84:535–544

Gregory TP, Burton TD, Plaulson RL, Turley SD (1991) The acute and chronic toxicity of hexahydro-1, 3, 5-trinitro-1, 3, 5-traizine (RDX) to three freshwater invertebrates. Environ Toxicol Chem 10:1073–1081

Harvey SD, Fellows RJ, Cataldo DA, Bean RM (1991) Fate of the explosive hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX) in soil and bioaccumulation in bush bean hydroponic plants. Environ Toxicol Chem 10:845–855

Ibeanusi V, Jeilani Y, Houston S, Doss D, Coley B (2009) Sequential anaerobic–aerobic degradation of munitions waste. Biotechnol Lett 31:65–69

Jenkins TF, Grant CL (1987) Comparison of extraction techniques for munitions residues in soil. Anal Chem 59:1326–1331

Just CL, Schnoor JL (2004) Phytophotolysis of hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX) in leaves of reed canary grass. Environ Sci Technol 38:290–295

McCormick NG, Cornell JH, Kaplan AM (1981) Biodegradation of hexahydro-1, 3, 5-trinitri-, 3, 5-triazine. Appl Environ Microbiol 42:817–823

Mukhi S, Pan X, Cobb GP, Patiño R (2005) Toxicity of hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine to larval zebrafish (Danio rerio). Chemosphere 61:178–185

Nejidat A, Kafka L, Tekoah Y, Ronen Z (2008) Effect of organic and inorganic nitrogenous compounds on RDX degradation and cytochrome P-450 expression in Rhodococcus strain YH1. Biodegradation 19:313–320

Pilon-Smits EAH, Freeman JL (2006) Environmental cleanup using plants: biotechnological advances and ecological considerations. Front Ecol Environ 4:203–210

Rao MR, Halfhill MD, Abercrombie LG, Ranjan P, Abercrombie JM, Gouffon JS, Saxton M, Stewart CN Jr (2009) Phytoremediation

and phytosensing of chemical contaminants, RDX and TNT: identification of the required target genes. Funct Integr Genomics 9:537–547

Talmage SS, Opresko DM, Maxwell CJ, Welsh CJE, Cretella FM, Reno PH, Daniel FB (1999) Nitroaromatic munition compounds: environmental effects and screening values. Rev Environ Contam Toxicol 161:1–156

Vila M, Mehier S, Lorber-Pascal S, Laurent F (2007a) Phytotoxicity to and uptake of RDX by rice. Environmental Pollution 145:813–817

Vila M, Lorber-Pascal S, Laurent F (2007b) Fate of RDX and TNT in agronomic plants. Environ Pollut 148:148–154

Zhang B, Pan X (2009) RDX induces aberrant expression of MicroRNAs in mouse brain and liver. Environ Health Perspect 117:231–240

Zhang B, Freitag CM, Cañas JE, Cheng Q, Anderson TA (2006) Effects of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) metabolites on cricket (Acheta domesticus) survival and reproductive success. Environ Pollut 144:540–544

Supplied by the U.S. Department of Agriculture, National Center for Agricultural Utilization Research, Peoria, Illinois