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Technical Report EL-97-2
January 1997

US Army Corps of Engineers
Waterways Experiment Station

Screening of Aquatic and Wetland Plant Species for Phytoremediation of Explosives-Contaminated Groundwater from the Iowa Army Ammunition Plant

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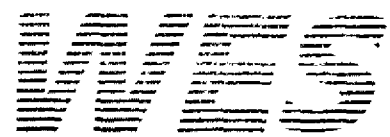

Prepared for U.S. Army Engineer District, Omaha

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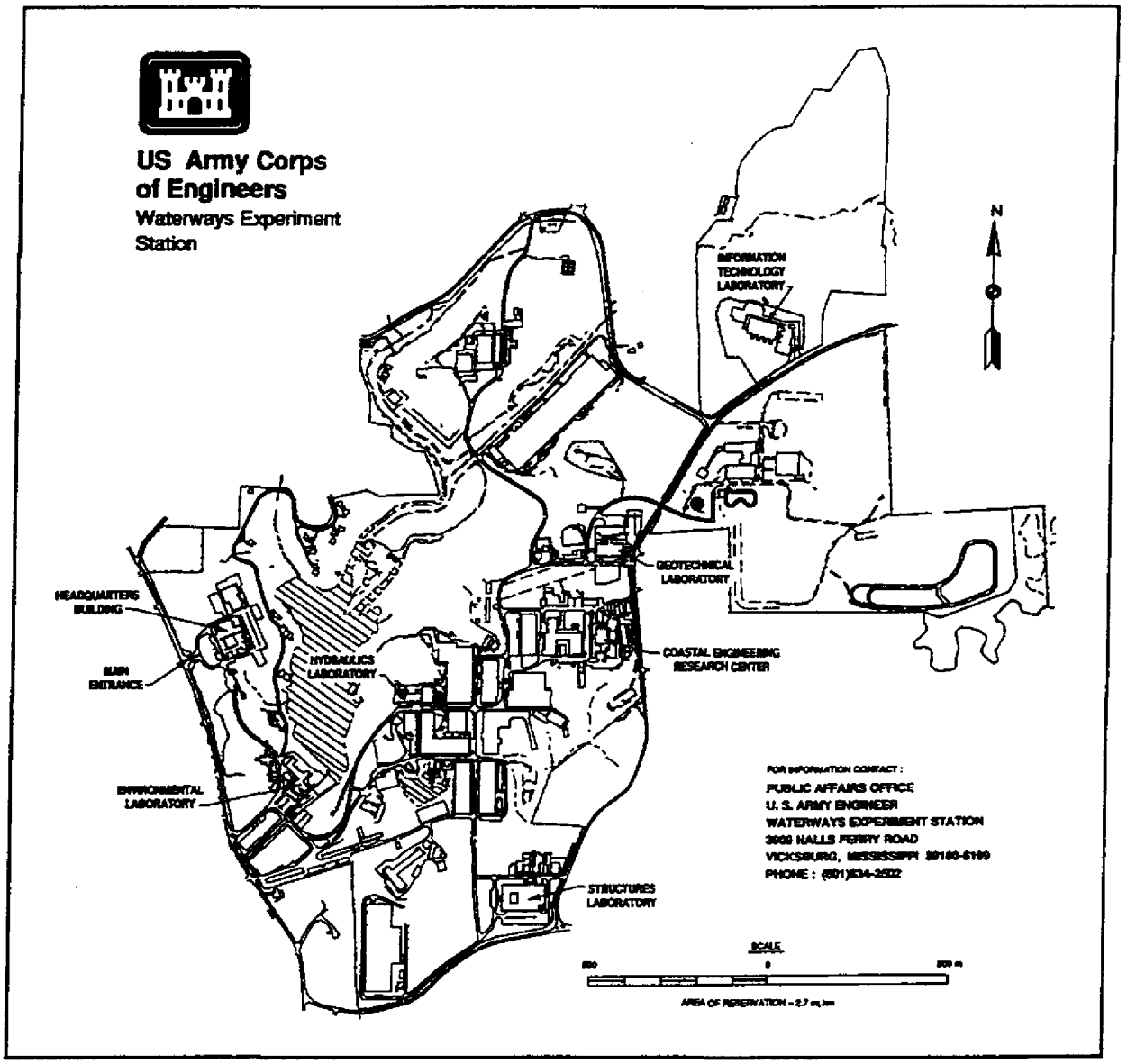
Final report

Prepared for U.S. Army Engineer District, Omaha
Omaha, NE 68102-4978



Waterways Experiment Station Cataloging-in-Publication Data

Screening of aquatic and wetland plant species for phytoremediation of explosives-contaminated groundwater from the Iowa Army Ammunition Plant / by Elly P.H. Best ... [et al.] ; prepared for U.S. Army Engineer District, Omaha.
72 p. : ill. ; 28 cm. — (Technical report ; EL-97-2)
Includes bibliographical references.

1. Groundwater — Pollution. 2. Explosives, Military — Environmental aspects. 3. Aquatic plants. 4. Wetland plants. 5. Iowa Army Ammunition Plant (U.S.) I. Best, Elly P.H. II. United States. Army. Corps of Engineers. Omaha District. III. U.S. Army Engineer Waterways Experiment Station. IV. Environmental Laboratory (U.S. Army Engineer Waterways Experiment Station) V. Series: Technical report (U.S. Army Engineer Waterways Experiment Station) ; EL-97-2.
TA7 W34 no.EL-97-2

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Preface

The work reported herein was conducted as part of the U.S. Army Engineer District, Omaha, "Optimization of Constructed Phytoremediation Systems for Treatment of Contaminated Groundwater at the Iowa Army Ammunition Plant" project involving the Omaha District as the lead agency, with the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, providing technical support. The project encompassed two treatability studies and supporting laboratory experiments. The Wetlands Phytoremediation Team was composed of engineers and scientists from both WES and the U.S. Environmental Protection Agency (USEPA) and the Uplands Phytoremediation Team of researchers from WES, USEPA, and the University of Iowa. The Design Assistance Team provided the expertise during the design activities and was composed of personnel from the Omaha District and both technology teams. Funding was provided by the Omaha District.

Principal Investigator for this study was Dr. Mark E. Zappi, Environmental Restoration Branch, Environmental Engineering Division (EED), Environmental Laboratory (EL) WES. The report was prepared by Dr. Elly P. H. Best, ASci Corporation, Vicksburg, MS, with contributions from Dr. Zappi; Drs. Herb L. Fredrickson and Susan L. Sprecher, Ecosystem Processes and Effects Branch, Environmental Processes and Effects Division (EPED), EL; and Mr. Jerry L. Miller, Environmental Resources Engineering Branch, EED.

Technical assistance was provided by Ms. Anne B. Stewart, ASci Corporation, and Mr. Robbie Godwin, EPED. Analysis of explosives and TNT degradation products in water was performed by Mr. Mike Ochman and Ms. Margaret Richmond, ASci Corporation. Analysis of explosives and degradation products in plants was performed by Dr. Steve L. Larson, Environmental Chemistry Branch (ECB), EL. Nutrients, metals, and ions in water were determined by the ECB. Various components in the sediments were determined by Ms. Susan Fox, ASci Corporation, and the ECB.

The financial support of the Strategic Environmental Research and Development Program is gratefully acknowledged. Dr. L. Carreira (USEPA, Athens) prepared the test protocol and performed the analyses for nitroreductase evaluation.

This study was conducted at WES under the general supervision of Dr. John W. Keeley, Director, EL; Mr. Norman R. Francingues, Chief, EED; and Dr. Richard E. Price, Acting Chief, EPED.

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

This report should be cited as follows:

Best, E. P. H., Zappi, M. E. Fredrickson, H. L., Sprecher, S. L. Larson, S. L., and Miller, J. L. (1997). "Screening of aquatic and wetland plant species for phytoremediation of explosives-contaminated groundwater from the Iowa Army Ammunition Plant," Technical Report EL-97-2, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

1 Introduction

Explosives and Phytoremediation

Munitions material such as 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and their combustion and decomposition products can enter the environment from production activities and field usage and disposal (Small and Rosenblatt 1974; Spanggord et al. 1983). The presence of these substances is of concern because of their potential toxicity and mutagenicity (Marvin-Sikkema and De Bont 1994).

The purposeful use of plants for cleanup of the environment has received relatively little attention despite the fact that plants, like microorganisms, play an important role in nature in sustaining and restoring environments. The capabilities of plants to absorb, accumulate, and metabolize, directly or indirectly, various organic substances suggest their use in the remediation of contaminated environments (phytoremediation).

In the aquatic environment, both TNT and RDX can disappear rapidly from water due to photolysis because they are sensitive to irradiance above 290 nm (ultraviolet (UV) and visible light). Adsorption to sediment is not significant. Biotransformation of TNT in water by microorganisms is potentially important, since rather rapid degradation rates have been found (Spanggord et al. 1980a). The most common initial degradation step of TNT was recently confirmed to be reduction of one or more nitro groups (products: 2-amino-dinitrotoluene (2ADNT), 4-amino-dinitrotoluene (4ADNT)) followed by irreversible binding of the products to organic matter (Rieger and Knackmuss 1995). Only under strict anaerobic conditions was mineralization to CO_2 demonstrated. Another aerobic, degradation pathway is described in which one or more nitro groups are removed (products: 2,4-dinitrotoluene (2,4DNT), 2,6-dinitrotoluene (2,6DNT), 2-nitrotoluene (2NT), 4-nitrotoluene (4NT), 3-nitrotoluene (3NT); Vorbeck et al. 1994). The biodegradation rate of RDX in water is slower than that of TNT. Biodegradation of RDX is proposed to proceed under anaerobic conditions via sequential reduction of the nitro groups to a point where destabilization and fragmentation of the ring occurs (McCormick, Cornell, and Kaplan 1981). Degradation products include hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine,

hexahydro-1,3-dinitroso-5-nitrohydrazine, 1,2-dimethylhydrazine, formaldehyde, and methanol (McCormick, Cornell, and Kaplan 1984).

Recently, it has been suggested that TNT disappears rapidly from water in the presence of several algae and submersed macrophytes, and that TNT derivatives with reduced nitro groups are early biodegradation products; no mineralization to CO_2 was demonstrated (Schnoor et al. 1995). This was largely confirmed by studies similar to that described in this report, evaluating submersed (Best et al., in preparation) and emergent macrophytes (Anonymous 1995). Degradation of TNT by freshwater sediments has been seen to originate largely from aquatic plant enzymes (Van Beelen and Burris 1995). Screening studies by Best et al. (in preparation) and Anonymous (1995) indicated slow disappearance of RDX in the presence of aquatic macrophytes where plant-stimulated activity of microorganisms inherent to the explosives-contaminated water may have contributed to removal.

Phytoremediation of Explosives-Contaminated Groundwater from the Iowa Army Ammunition Plant

The Iowa Army Ammunition Plant (IAAP) encompasses a 26-square mile (67-sq km) area in Burlington, IA (longitude 91° 20' W, latitude 40° 48' N), and has ongoing munition manufacturing activities. Explosives contamination has been detected at several locations. The U.S. Army Engineer District, Omaha, in conjunction with the U.S. Army Environmental Center has investigated various options for the removal and remediation of explosives contamination within both soil and groundwater matrices. Two sites, Lines 1 and 800, are scheduled for excavation of contaminated soils, and groundwater seeping into the excavation pit will be treated by constructed wetlands.

This report presents the results of a screening study to quantify the ability of 10 submersed and emergent macrophytes, adapted to lentic habitats in Iowa, to phytoremediate local explosives-contaminated groundwater. Species evaluated under hydroponic batch conditions were the submersed *Potamogeton nodosus* Poir. (American pondweed) and *Ceratophyllum demersum* L. (coontail) and the emergent *Alisma subcordatum* Raf. (water-plantain), *Sagittaria latifolia* Willd. (common arrowhead), *Carex vulpinoidea* Michx. (fox sedge), *Scirpus cyperinus* (L.) Kunth (wool-grass), *Eleocharis obtusa* (Willd) (blunt spikerush), *Phalaris arundinacea* L. (reed canary grass), and *Typha angustifolia* L. (narrowleaf cat-tail). Parrot-feather (*Myriophyllum aquaticum* (Vell.) Verdc.) was included in the evaluation to provide a comparison between the present study and other similar evaluations. The effects of native and heat-inactivated sediments on the explosives in the aqueous phase were also examined. The impact of a decrease in temperature from 25 to 10 °C on contaminant disappearance was assessed in three species.

2 Material and Methods

Plant Material

Species selection

The nine submersed and emergent species evaluated for ability to degrade explosives in IAAP groundwater were selected from 42 local native species shown to possess nitroreductase enzyme activity on the basis of U.S. Environmental Protection Agency (USEPA) immunoassay testing in the field at IAAP, August 1995 (Appendix A, Appendix B, and Best et al. 1996). Ecological traits important in field deployment were also considered, including perenniality, high biomass production, adaptability to water depth, and extensive root/rhizome systems. Attention was given to the ecological habitat range of the species, including their presence in wet meadow, marsh, and pond sites. The species chosen are listed in Table 1.

Source and acclimation of planting material

American pondweed was obtained from the Lewisville Aquatic Ecosystem Facility (LAERF), Lewisville, TX. Coontail was harvested from Brown Lake, Vicksburg, MS. Water-plantain and wool-grass were purchased from a commercial nursery, Green and Hagstrom, Fairview, TN. Common arrowhead, fox sedge, reed canary grass, and narrowleaf cat-tail were purchased from Southern Tier Consulting, West Clarkville, NY. Blunt spikerush was harvested from a small impoundment in Vicksburg, MS. Parrot-feather was obtained from USEPA-Athens, GA, in spring 1995; it was propagated further in outdoor ponds at LAERF.

Plants for evaluation were collected during the first 2 weeks of September 1995. Upon arrival, they were planted into N-amended Brown Lake sediment, submerged in a low-alkalinity solution (Smart and Barko 1985), and held in monocultures in a U.S. Army Engineer Waterways Experiment Station (WES) greenhouse.

Submersed plants were received as unrooted (coontail) or rooted (pondweed) apical shoots. Emergent plants were received as unrooted apical shoots

Table 1
Aquatic and Wetland Plant Species Used in Factorial Screening for Explosives Removal

Group	Family	Plant Species		Habitat
		Latin Name	Common Name	
Submersed				
Monocotyledons	Potamogetonaceae	*Potamogeton nodosus* Poir.	American pondweed (pondweed)	Pond
Dicotyledons	Ceratophyllaceae	*Ceratophyllum demersum* L.	Coontail	Pond
Emergent				
Monocotyledons	Alismataceae	*Alisma subcordatum* Raf.	Water-plantain	Pond, marsh
		Sagittaria latifolia Willd.	Common arrowhead (arrowhead)	Pond, marsh
	Cyperaceae	*Carex vulpinoidea* Michx.	Fox sedge	Marsh, wet meadow
		Scirpus cyperinus (L.) Kunth	Wool-grass	Marsh, wet meadow
		Eleocharis obtusa (Willd) Schultes in R. & S.	Blunt spikerush (spikerush)	Marsh, wet meadow
	Gramineae	*Phalaris arundinacea* L.	Reed canary grass	Wet meadow, upland
	Typhaceae	*Typha angustifolia* L.	Narrowleaf cat-tail (cat-tail)	Pond, marsh
Dicotyledons	Haloragaceae	*Myriophyllum aquaticum* (Vell.) Verdc[1]	Parrot-feather	Pond, marsh

Note: U.S. Army Engineer Waterways Experiment Station, September 1995. Common names used in the text in parentheses.
[1] Comparison species.

(parrot-feather); as whole plants (water-plantain, arrowhead, spikerush, and reed canary grass); and as rooted plants with shoots removed to a height of 10 cm (fox sedge and wool-grass). Rooting of apical shoots during the acclimation period was minimal. All culture solutions were aerated to enhance mixing and air/water carbon dioxide exchange.

Groundwater

Explosives-contaminated groundwater used for screening originated from Monitoring Wells G19 and G20 (see Appendix A). The water was transported to WES in two stainless steel 208-L drums at the beginning of September and stored in a cold room (5 °C). Both water types were mixed in equal quantities for use in the experiment. The chemical composition of this groundwater is given in Table 2. RDX was the primary contaminant with TNT and TNB also being of concern.

Table 2
Chemical Characteristics of the IAAP Groundwater Composite

Characteristic	Value
pH	7.5 ± 0.1
Macronutrients, micronutrients, mg L^{-1}	
Alkalinity	170 ± 1.4
Bicarbonate	170 ± 1.3
Carbonate	0.6 ± 0.2
Total dissolved solids	839 ± 58
Kjeldahl-N	1.6 ± 0.2
Nitrite/nitrate-nitrogen	0.15 ± 0.09
Ammonium-nitrogen	0.17 ± 0.01
Total-phosphorus	< 0.2
Phosphate-phosphorus	0.185 ± 0.020
Sulfate	56.35 ± 0.72
Calcium	121.16 ± 3.43
Iron	0.18 ± 0.04
Manganese	0.06 ± 0
Explosives, μg L^{-1}	
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	NA[1]
2,6-Diamino-,4-nitro-toluene (2,6DANT)	596 ± 36
2,4-Diamino-,6-nitrotoluene (2,4DANT)	23 ± 14
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	12,785 ± 1,744
1,3,5-Trinitro-benzene (TNB)	1,422 ± 88
1,4-Dinitro-benzene (1,4DNB)	--[2]
1,3-Dinitro-benzene (1,3DNB)	20 ± 4
Nitrobenzene (NB)	15 ± 3
2,4,6-Trinitrotoluene (TNT)	681 ± 70
2-Amino-dinitrotoluene (2ADNT)	60 ± 4
4-Amino-,2,6-dinitrotoluene (4ADNT)	41 ± 2
2,4,-Dinitrotoluene (2,4DNT)	--
2,6-Dinitrotoluene (2,6DNT)	6 ± 7
2-Nitrotoluene (2NT)	--
4-Nitrotoluene (4NT)	--
3-Nitrotoluene (3NT)	--

Note: Mean values of triplicates and standard deviations.
[1] Not analyzed.
[2] Below detection.

Sediment

Sediment used as a control in the experiment originated from Stump Lake, IAAP. Sediment was excavated at the beginning of September, transferred into polypropylene 19-L buckets, transported to WES, and stored in a cold room (5 °C). It was prepared for the experiment by decanting the water from each bucket and fully blending the remaining contents in a mechanical mixer. Dry weight was determined from a 34-g wet weight sample. The chemical composition of this sediment is given in Table 3. This sediment is low in organic matter (77 g kg DW^{-1}) and cation exchange capacity (CEC; 34 mgeq 100 g DW^{-1}), and it is expected that adsorption of explosives is low (Pennington and Patrick 1990; Pennington et al. 1995). A portion of this sediment was autoclaved (1 hr at 120 °C and 15 psi; 30 min at 120 °C and 15 psi) before used to inactivate soil organisms and enzymes. Autoclaved and unautoclaved sediment controls were included in the experiment.

Table 3
Chemical Characteristics of Stump Lake Sediment

Parameter	Concentration	Unit
Nitrogen	2.590 ± 0.039	g kg DW^{-1}
Exchangeable ammonium-nitrogen	0.139 ± 0.001	g kg DW^{-1}
Phosphorus	0.518 ± 0.006	g kg DW^{-1}
Available phosphate-phosphorus	0.076 ± 0.007	g kg DW^{-1}
Calcium	13.47 ± 0.59	g kg DW^{-1}
Iron	22.00 ± 2.22	g kg DW^{-1}
Magnesium	3.80 ± 0.13	g kg DW^{-1}
Manganese	0.27 ± 0.00	g kg DW^{-1}
Sodium	9.97 ± 3.85	g kg DW^{-1}
Cation exchange capacity	34.00 ± 0.93	mgeq 100g DW^{-1}
Bulk density	0.74 ± 0.00	g DW mL^{-1}
Moisture	484.9 ± 0.07	g H_2O g FW^{-1}
Organic matter	76.8 ± 0.13	g kg DW^{-1}
Total organic carbon	11.67 ± 1.61	g kg DW^{-1}

Note: Mean values of triplicates and standard deviations; DW = dry weight; FW = fresh weight.

Experimental Design

Two synchronous experiments were carried out. One experiment investigated the effects of all plant species on explosives concentrations under incubation at 25 °C. Controls were groundwater without plants and sediment and groundwater without plants with autoclaved or unautoclaved sediment. This experiment was a randomized complete block design composed of three blocks, each containing 13 experimental units. A second experiment at 10 °C investigated the effects of three plant species, with controls being groundwater alone and groundwater with sediment. The latter experiment was composed of four blocks, each containing five experimental units. The overall total was 59 units.

Experimental Conditions

The screening was carried out over a 10-day incubation period, 19 to 29 September 1995. The 25 °C experiment was conducted in a large walk-in controlled environment growth chamber, the 10 °C experiment in two independent 92- by 83- by 204-cm controlled environment growth cabinets. Experimental units were 15- by 15- by 37.5-cm glass aquaria (reactors). After test materials were placed in them, they were filled with groundwater to a final depth of 15 cm, giving a uniform total test volume (rather than water volume) of 3.375 L. Submersed plants were incubated without mechanical support as approximately 15-cm apical shoots at a density of 9-g fresh weight (FW) L^{-1}, giving 30.4-g plant material per aquarium. Emergent plants, except parrot-feather, were incubated on top of stainless steel gauze mechanical supports, allowing only the root systems, crowns, and lower stems to contact the groundwater. Parrot-feather was incubated without mechanical support. As emergent aquatics were expected to have approximately half of their biomass above the water surface, about twice as much material was incubated than for the submersed species, 60.8 g FW. Most emergent plants used had either very small root systems (except fox sedge and reed canary grass) or grew from crowns from which it was difficult to separate individual plants. Therefore, an amount of plant material to fulfill the requirement of approximately 30.4 g FW in contact with groundwater was incubated in each aquarium. Parrot-feather was incubated at approximately 60 g FW per aquarium.

High-pressure sodium and metal halide lamps provided a full photosynthetic spectrum at 400 to 500 $\mu E\ m^{-2}\ s^{-1}$ at 22.5 cm above the water surface for the 25 °C experiment. Fluorescent, cool-white TL and TLC 26 lamps provided a large part of photosynthetic spectrum at 300 to 650 $\mu E\ m^{-2}\ s^{-1}$ at 22.5 cm above the water surface for the 10 °C experiment. Each block of experimental units was positioned within an area of similar irradiance. Autotimers provided a daylength of 14 hr. Temperature controls were set at a constant 25 °C in the growth chamber and 10 °C in both growth cabinets.

Culture solutions were not aerated in order to (a) mimic expected slow water flow under field conditions, and (b) produce the low oxygen (O_2) concentrations under which RDX removal was shown to be enhanced in a recent Tennessee Valley Authority (TVA) study (Unpublished communication).

Experimental Procedures and Sampling

At the beginning of the incubation period, groundwater was pumped from barrels into each aquarium, and predetermined weights of fresh plant material or sediment were added as required.

Water from each reactor was sampled at 1, 4, 12, 24, 48, 96, and 240 hr (10 days) for explosives analysis. Prior to sampling, contents of the reactor were mixed with a glass rod to collect a representative sample; 100 mL of water was then collected using a glass pipet and decanted into a glass bottle with a Teflon-lined cap. Water samples were refrigerated (5 °C) in the dark until further processing, usually within 24 hr of collection.

After the final water sampling, plant materials were removed and weighed. A dry:fresh weight (DW:FW) ratio was determined for each species by drying a weighed portion of material in a ventilated oven at 70 °C until constant weight was attained and reweighing. Relative growth rates were calculated by dividing the natural log (ln) transform of final plant DW by initial DW and dividing by the 10 days of incubation. Sediment was removed, weighed, placed in glass jars, and kept refrigerated until analysis. Oxygen concentration was measured within the reactors of one block of the 25 °C experiment using a YSI O_2 electrode.

Water remaining in three replicate reactors of the 25 °C experiment was mixed in equal volumes. Subsamples of these mixtures were placed in glass bottles, and the bottles were placed in a freezer (-20 °C) to await further analysis as follows: (a) 1 L for explosives (for comparison with ECB); (b) 100 mL for determinations of pH, alkalinity, bicarbonate, carbonate, total dissolved solids (TDS), and sulphate (SO_4); (c) 500 mL amended with 1 mL concentrated H_2SO_4 for determinations of Kjeldahl-N, NH_4-N, NO_2/NO_3-N, total-phosphorus (Total-P), ortho-phosphorus (PO_4-P), chemical oxygen demand (COD), and total organic carbon (TOC); (d) 100 mL amended with 1 mL concentrated HNO_3 for determinations of calcium (Ca), iron (Fe), magnesium (Mg), and manganese (Mn).

Chemical Analyses

Explosives in water

Solid phase extraction (SPE) was used for concentration of 100-mL water samples. Explosives were eluted in acetonitrile, evaporated almost to dryness

using N_2, redissolved in a 2-mL mixture of acetonitrile:water (50/50 vol/vol), and analyzed using high performance liquid chromatography (HPLC), using a method based on EPA Method 8330 (Jenkins et al. 1995). HPLC separations were performed on a Hewlett-Packard 1090 Series 2/M with ChemStation (Pascal Series) liquid chromatograph equipped with a diode array detector (Series 2), PV5 ternary solvent delivery system, thermostatically controlled column compartment, autosampler, auto-injector and reverse phase analytical C18 column (5 μm, 100- by 4.6-mm inner diam), and Octyl Desil Silane (ODS) guard column (5 μm, 20- by 4.0-mm inner diam). The column compartment was operated at 40 °C, and the flow rate of the mobile phase was 1.5 mL min^{-1}. The composition of the mobile phase was 68 percent 20 mM NH_4Cl and a 32-percent mixture of methanol and n-butanol (98:2, respectively). The compounds used for the calibrations are given in Appendix C.

Azoxy compounds were measured only in the 10-day water samples of one block of the 25 °C experiment due to the lengthy procedure required for their analysis.

Alkalinity, macronutrients, and ions in water

The pH was calibrated with known buffer solutions bracketing the pH of the samples (American Public Health Association (APHA) 1992). Alkalinity was determined colorimetrically as $CaCO_3$ (Method 310.2, USEPA 1979). Bicarbonate and carbonate were calculated using Method 4500-CO_2 D (APHA 1992). TDS was determined on samples filtered through glass fiber filters and dried at 180 °C (Method 160.1, USEPA 1979). Sulfate was determined colorimetrically (Method 375.2, USEPA 1979).

Kjeldahl-nitrogen (N) and total phosphorus (P) were measured colorimetrically in samples digested with sulfuric acid, potassium sulfate, and mercuric sulfate using a Lachat Quikchem AE Automatic Flow Injection Ion Analyzer (QuikChem Methods No. 10-107-06-2-D and No. 13-115-01-1-B, 1992). Ammonia-N was analyzed colorimetrically via the salicylate method using the Lachat System (QuikChem Method No. 12-107-06-2-A), and nitrate/nitrite-N was reduced over a cadmium column to nitrite-N and analyzed colorimetrically via the Lachat System (Quikchem Method No. 10-107-04-1-C). Phosphate-P was analyzed colorimetrically using the Lachat System ascorbic acid method (QuikChem Method No. 12-115-01-1-A).

COD was determined on a potassium chromate, sulfuric acid, mercuric sulfate digest using automated colorimetry (USEPA 1979 method 410.4). TOC was measured using nondispersive infrared spectrometry (Method 5310C, APHA).

The cation concentrations (Ca, Fe, Mg, and Mn) were determined after acidification with 1:1 hydrochloric acid to pH < 2 using Inductively Coupled Argon Plasma emission spectrometry (ICP; USEPA, SW-846 Method 6010).

Macronutrients, ions, CEC, bulk density, and organic matter in sediment

Total Kjeldahl-nitrogen (N) and phosphorus (P) were determined in soil digests using the same method as for water. Exchangeable ammonium was extracted from the soil with 1 M NaCl and filtered; the filtrate was analyzed colorimetrically for ammonia via the salicylate method using a Lachat System (QuikChem Method No. 12-107-06-2-A 1988). Available P was extracted using a dilute hydrochloric acid fluoride modified Bray extraction procedure and was analyzed colorimetrically via the ascorbic acid method using a Lachat System (QuikChem Method No. 12-115-01-1-A 1988). Metals (Fe, Mg, and Mn) were determined as follows: 1- to 2-g dry soil aliquots were digested in nitric acid/hydrogen peroxide at 95 °C followed by reflux with HCl (USEPA SW-846, Method 3050) and measured using ICP (USEPA SW-846, Method 6010). The CEC was determined in samples treated with sodium acetate followed by an isopropyl wash and back-extracted with ammonium acetate. The sodium concentration is then measured by ICP to obtain equivalents of cations.

Bulk density and moisture content were determined gravimetrically by drying a known quantity of fresh weight to constant dry weight at 105 °C (Allen et al. 1974). Concentrations of organic matter were determined by loss on ignition at 550 °C. TOC was measured using nondispersive infrared spectrometry (APHA, method 5310 D).

Explosives in plant material and sediment

Levels of explosives[1] and the metabolic/degradation products of TNT were determined in plants from the 25 °C experiment. Plant samples were quick-frozen in liquid N_2 and ground to a fine powder; 2 g FW portions were extracted in 10 ml acetonitrile by an 18-hr sonication in a water-cooled (5 °C) sonic bath. Samples were then centrifuged at 3,000 rpm for 5 min, and 5 mL of the extract supernatant was placed on a cleanup column prepared by layering 0.5 g of Florisil and 0.5 g of neutral alumina. The column was washed with another 5 mL of acetonitrile, and the resulting extract was diluted 1:1 with deionized water and analyzed by HPLC (EPA method 8330).[2] Sediments were analyzed similarly, without grinding.

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[1] Names of explosives and their abbreviations are given in Appendix E.

[2] Personal Communication, 1996, Steven L. Larson, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Data Analysis

Statistical summary and analysis of explosives concentrations in the groundwater were carried out on TNT and RDX separately, using STAT-GRAPHICS Plus (Version 7; Statistical Graphics Corporation, Bitstream, Inc., Cambridge, MA) to perform analysis of variance (ANOVA), regression analysis, and multiple range tests. Significance was tested at the 95-percent confidence level. Where log transformation was required of data on nitrobody concentrations that were below detection, the detection level was used in place of zero.

Data from HPLC analysis of all water samples were initially screened for outliers using a method based on Hotelling's T-square (Hotelling 1953); however, this excluded whole species or factors and was not informative. Subsequently, only those samples thought to have been incorrectly prepared for analysis or misinjected during HPLC were excluded. These amounted to 4 samples out of 419, or 0.9 percent.

The data points representing all sampling times were included in ANOVA comparisons among species and treatments, to identify those treatments with significantly lower concentrations over the entire incubation period.

3 Results and Discussion

TNT Concentration in Water: Effects of Plant Species and Temperature

TNT removal

At 25 °C, plant incubations achieved 94- to 100-percent TNT removal in 10 days while controls ranged from 62 to 85 percent (Figure 1; Table 4). Both submersed and three of the eight emergent species removed TNT to levels below the detection limit (of 0.1 $\mu g\ L^{-1}$) within the 10-day incubation period. This indicates that plants provide additional TNT sinks over those in sediment and groundwater alone. Increased removal may be attributed to increased adsorption sites on the plant mass, or enzymatic degradation of the TNT on and within the plant. Chemical analysis of the plant mass, discussed later, indicated that enzymatic degradation was the likely cause of TNT removal. It is important to note that the additional adsorption sites added by the plant mass are not additive upon the sediment sorption sites since the plant incubations were done without sediment.

Of the controls, groundwater alone removed the lowest percentage of TNT, 62 percent. This was probably due to photolysis and biodegradation by groundwater-inherent microorganisms. Volatilization is not a factor because TNT is not volatile. Both sediment incubations yielded approximately 20 percent more TNT removal than groundwater alone. Since it is assumed that autoclaving eliminates sediment-based microorganisms and most enzymatic activity, the removal achieved is probably due to adsorption and sediment nutrient-based stimulation of groundwater-inherent microorganisms. The actual mechanism cannot be determined from this study.

Significant differences among species and controls in TNT concentration in groundwater at 25 and at 10 °C over the whole incubation period are shown in Table 5 with reed canary grass, coontail, and pondweed ranking as the most active. At 10 °C, all three species were more effective than groundwater alone.

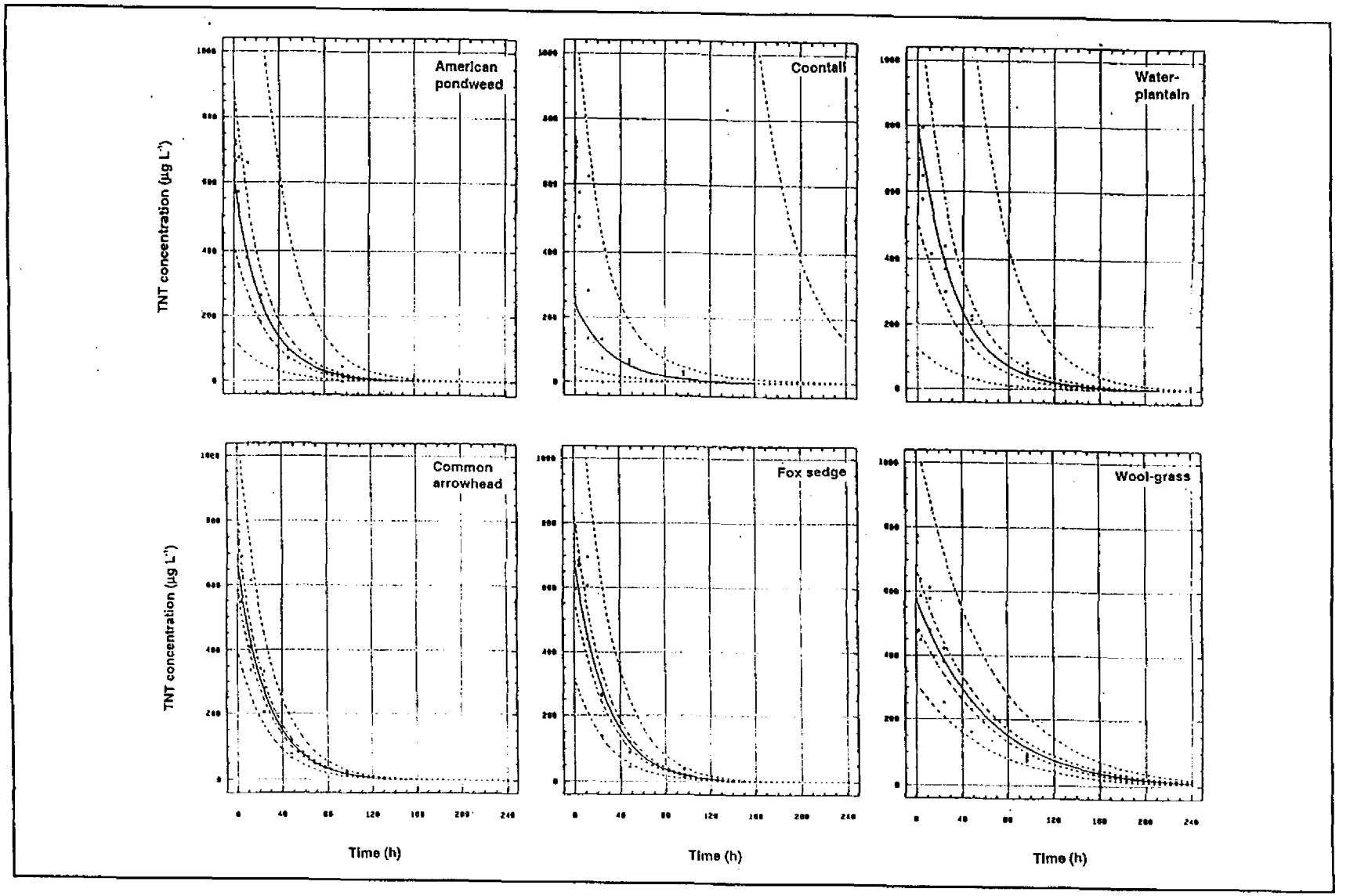

Figure 1. Changes in TNT concentration in groundwater with length of incubation at 25 °C with 1 of 10 aquatic plant species, alone, and with autoclaved and unautoclaved sediment (Drawn lines: fitted curves; interrupted lines, 95- and 90-percent confidence levels) (Sheet 1 of 3)

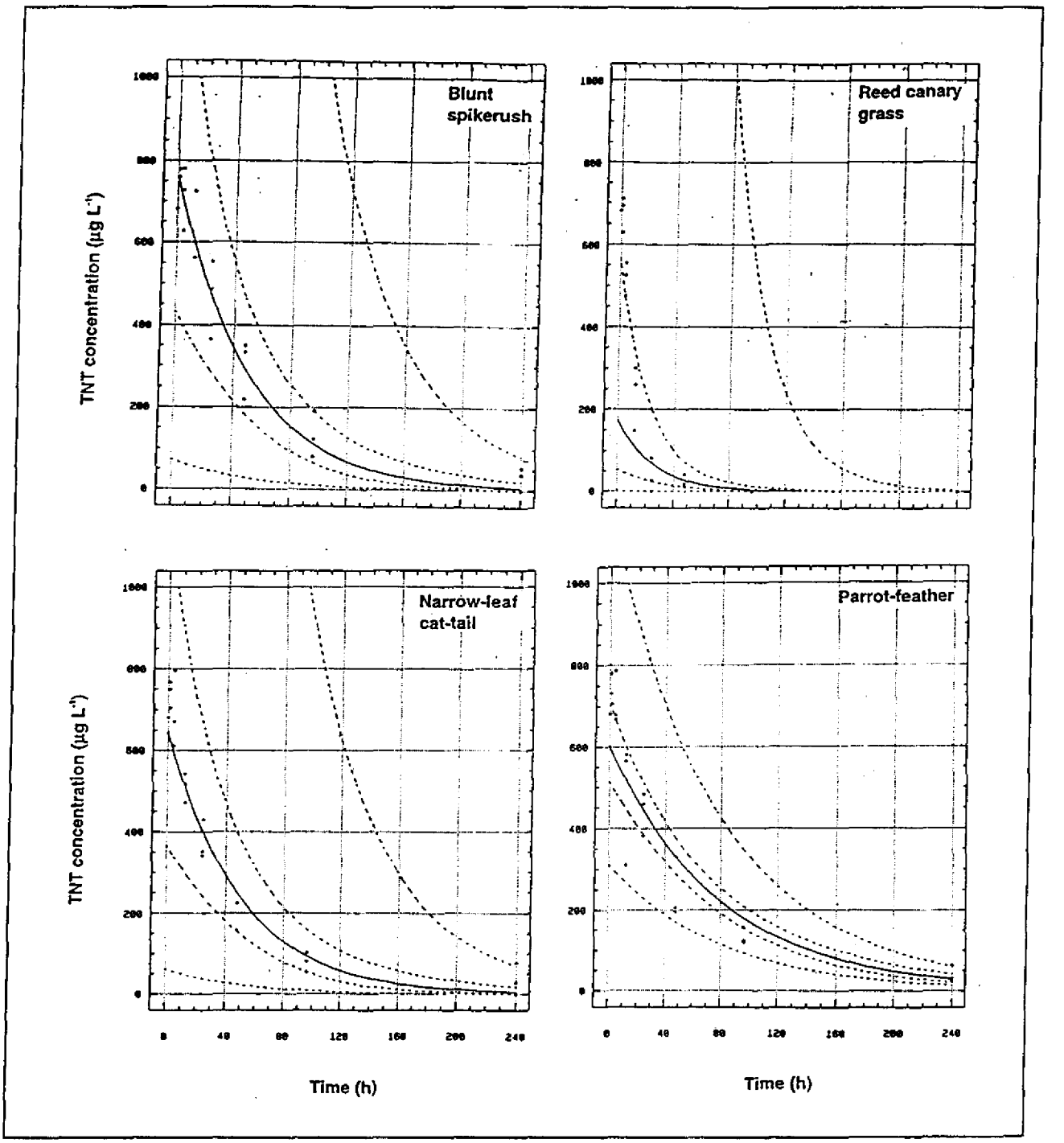

Figure 1. (Sheet 2 of 3)

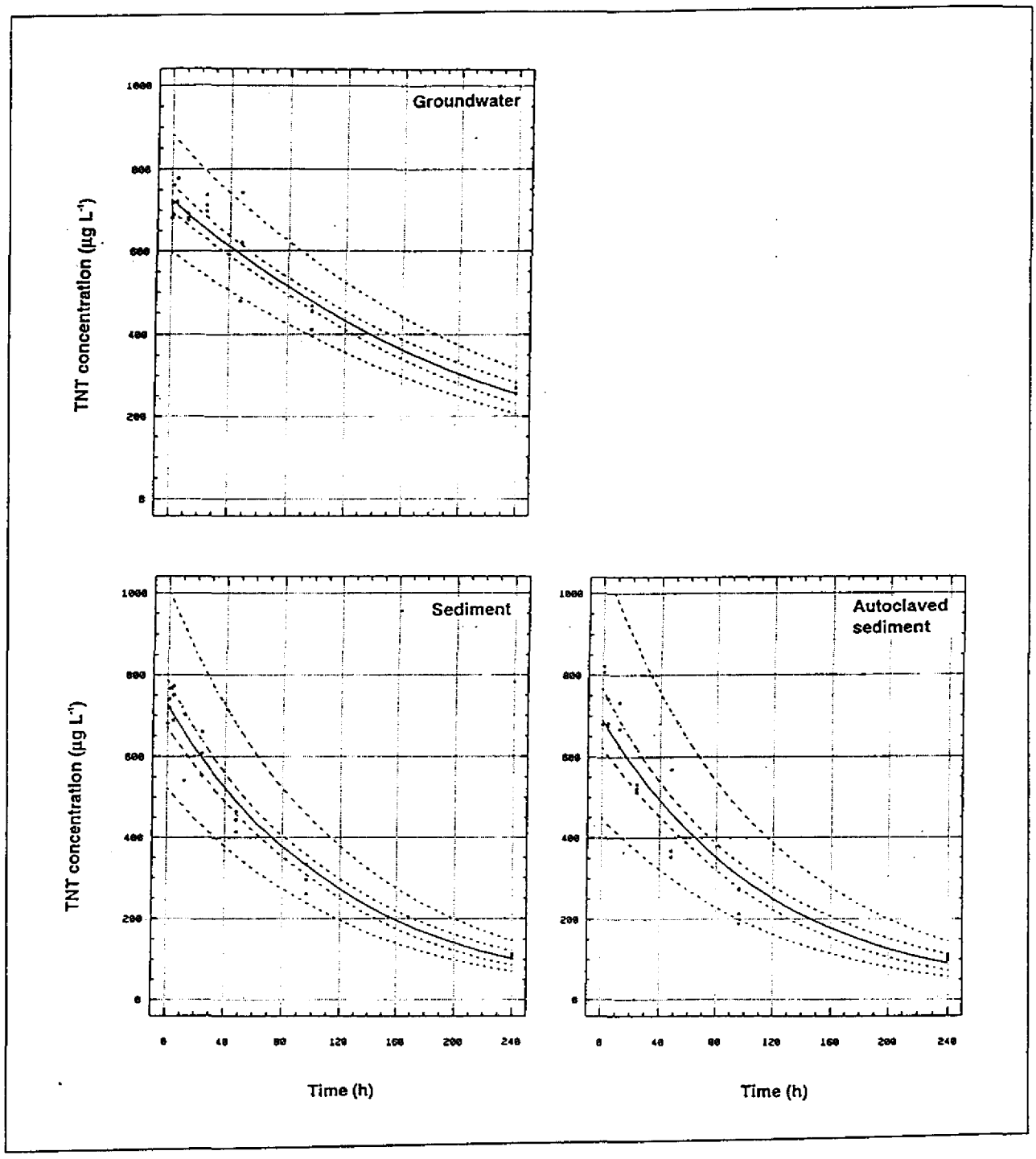

Figure 1. (Sheet 3 of 3)

Table 4
TNT Concentrations ($\mu g\ L^{-1}$) in Groundwater Over 10-Day Incubations With Plant Species and Controls at 25 °C

Treatment	Incubation Period, hr								Removal, %
	0	1	4	12	24	48	96	240	
Submersed									
Pondweed	682	703	573	474	230	86	22	--[1]	100
Coontail	682	708	515	247	69	63	27	--	100
Emergent									
Water-plantain	682	761	673	616	369	197	60	4	99
Arrowhead	682	764	626	453	272	86	17	--	100
Fox sedge	682	720	675	580	223	85	30	--	100
Wool-grass	682	666	558	557	352	216	82	13	98
Spikerush	682	760	712	629	469	301	133	33	95
Reed canary grass	682	677	519	236	36	25	--	--	100
Cat-tail	682	741	693	517	374	202	80	35	95
Parrot-feather	682	740	712	486	441	244	134	39	94
Controls									
Groundwater	682	723	740	685	715	614	444	261	62
Sediment	682	742	737	793	607	442	295	108	84
Autoclaved sediment	682	879	700	675	523	430	225	105	85

Note: Percent removal was based on concentrations at 10 days. Means of triplicates.
[1] Below detection.

Kinetics

To examine kinetic differences among treatments, two types of regression were performed. An exponential regression model, $Y = \exp(a + bX)$, where Y = concentration and X = time, with a negative slope, was used to describe TNT decrease over time. These regression statistics were used to calculate first order rate constants (slope of the regression; μg TNT L^{-1} hr^{-1}), half-life of TNT in hours, $t_{1/2} = -\ln 2/b$, and to extrapolate hydraulic retention time (HRT required to reach a TNT cleanup level of 2 $\mu g\ L^{-1}$. While half-life statistics are independent of concentration and intercept, this time to target concentration does involve these data; therefore, different species with the same half-life do not necessarily provide the same cleanup kinetic.

Table 5
Treatment Effects on TNT Concentration in Groundwater Over 10-Day Incubation

Treatment	LS Mean	Homogeneous Groups
25 °C Incubations		
Reed canary grass	271.91	a
Coontail	301.52	ab
Pondweed	348.63	bc
Arrowhead	362.52	cd
Fox sedge	374.28	cde
Wool-grass	390.74	cdef
Cat-tail	414.65	defg
Water-plantain	420.22	efgh
Parrot-feather	434.65	gh
Spikerush	464.76	h
Autoclaved sediment	527.42	i
Sediment	550.75	i
Groundwater	607.84	j
10 °C Incubations		
Reed canary grass	463.85	a
Spikerush	526.50	b
Parrot-feather	563.73	b
Sediment	625.45	c
Groundwater	682.54	d

Note: Multiple range analysis by treatment. ANOVA showed that treatment and time affected the TNT concentration significantly ($P < 0.001$; blocks as covariates).

Based on the first order rate constants (Table 6; Figure 1), reed canary grass had the most rapid TNT removal rate (0.038 $\mu g\ L^{-1}\ hr^{-1}$), followed closely by pondweed, arrowhead, and fox sedge (0.037 $\mu g\ L^{-1}\ hr^{-1}$), and by coontail (0.033 $\mu g\ L^{-1}\ hr^{-1}$). This ranking is consistent with the chemical removal results mentioned above indicating that these plant species performed best in removing the compounds analyzed during this study. Estimated HRTs for the plant species varied from 4.9 days for reed canary grass to 19.8 days for parrot-feather. The groundwater control required the longest HRT (61.4 days) for removing TNT of all systems evaluated. When rate constants were normalized for plant dry mass (K_n; data not shown), coontail, pondweed,

Table 6
Curve Fit Statistics for TNT Concentrations in Groundwater Over 10-Day Incubations With Plant Species and Controls

Treatment	Y = exp (a+bX)			Half Life, hr	Days to 2 $\mu g\ L^{-1}$	Initial Mass Incubated, g DW	
	Intercept a	Slope b	R^2			In Contact With Water	Total
25 °C Incubations							
Submersed							
Pondweed	6.407	-0.037	0.95	18.7	6.4	3.73 ± 0.39	3.73 ± 0.39
Coontail	5.493	-0.033	0.42	21.0	6.1	3.03 ± 0.19	3.03 ± 0.19
Emergent							
Water-plantain	6.678	-0.030	0.89	23.1	8.3	4.64 ± 0.06	12.34 ± 0.26
Arrowhead	6.493	-0.037	0.99	18.7	6.5	19.05 ± 0.54	22.34 ± 0.62
Fox sedge	6.536	-0.037	0.99	18.7	6.6	22.62 ± 0.62	28.99 ± 0.50
Wool-grass	6.360	-0.016	0.96	43.3	14.7	26.47 ± 4.36	43.15 ± 2.39
Spikerush	6.636	-0.020	0.68	34.6	12.4	20.74 ± 7.89	35.83 ± 3.95
Reed canary grass	5.166	-0.038	0.65	18.2	4.9	21.05 ± 2.49	36.83 ± 5.62
Cat-tail	6.470	-0.020	0.68	34.6	12.0	7.47 ± 0.97	16.26 ± 0.32
Parrot-feather	6.400	-0.012	0.92	57.7	19.8	1.83	5.51 ± 0.09
Controls							
Groundwater	6.591	-0.004	0.94	173.2	61.4	0	0
Sediment	6.587	-0.008	0.95	86.6	30.6	152.84 ± 0	152.84 ± 0
Autoclaved sediment	6.540	-0.008	0.92	86.6	30.5	152.82 ± 0	152.82 ± 0
10 °C Incubations							
Emergent							
Spikerush	6.535	-0.008	0.88	86.6	30.4	15.16 ± 0.89	16.65 ± 0.92
Reed canary grass	6.442	-0.011	0.88	63.0	21.7	14.82 ± 1.30	20.40 ± 3.44
Parrot-feather	6.536	-0.005	0.80	138.6	48.6	1.87	5.61 ± 0.08
Controls							
Groundwater	6.645	-0.003	0.65	231.0	82.6	0	0
Sediment	6.639	-0.005	0.85	138.6	49.5	152.80 ± 0	152.80 ± 0

Note: Data not normalized for plant dry weight. Mean mass and standard deviations (N = 3). Initial concentration TNT in groundwater: 681 $\mu g\ L^{-1}$.

parrot-feather, and wool-grass were most effective. An example of other calculation procedures is given in Appendix E.

Table 7 illustrates the impact on removal of the lower incubation temperature for the treatments evaluated. The plant systems were impacted more by the temperature decrease than were the controls. It is estimated that at 10 °C it will take reed canary grass four times longer to remove TNT to target treatment levels than at 25 °C (22 versus 5 days). It will take controls only one to two times longer. Although this extends cleanup time to 83 days with groundwater alone, the temperature effect is an important consideration when designing a wetland system expected to be operative year-round. It is expected to be even greater when plants are under natural low-temperature conditions when lower mass and metabolic activity occur.

Table 7
Impact of Temperature on TNT First Order Rate Constants and Hydraulic Residence Times to Meet Treatment Goal (2 $\mu g\ L^{-2}$)

Treatment	K_{25}, $\mu g\ L^{-2}.hr^{-1}$	K_{10}/K_{25}	HRT_{10}/HRT_{25}
Emergent			
Spikerush	-0.020	0.40	2.45
Reed canary grass	-0.038	0.29	4.43
Parrot-feather	-0.012	0.42	2.45
Controls			
Groundwater	-0.004	0.75	1.35
Sediment	-0.008	0.63	1.62

RDX Concentration in Water: Effects of Plant Species and Temperature

RDX removal

RDX removal was slower than that observed for TNT at 25 °C (Table 8, Figure 2). Significant differences among species and controls in RDX concentration over the whole incubation period are shown in Table 9. Only treatment with reed canary grass was significantly more effective than water alone at 25 °C. At 10 °C, reed canary grass and spikerush were more effective. Most plants achieved no more removal of RDX than did controls. In three of the incubations with emergents, an increase in RDX was seen over the incubation period. This anomaly may result from interference of some plant constituent that has a similar retention time when eluted from the HPLC column used, and would contribute to an explanation of the 10-day incubations evaluated; however, reed canary grass, fox sedge, arrowhead, and coontail

Table 8
RDX Concentrations ($\mu g\ L^{-1}$) in Groundwater Over 10-Day Incubations With Plant Species and Controls at 25 °C

Treatment	Incubation Period, hr								Removal, %
	0	1	4	12	24	48	96	240	
Submersed									
Pondweed	12,785	12,145	12,875	12,073	12,923	12,631	13,049	11,491	10
Coontail	12,785	13,453	12,217	12,373	14,933	12,552	12,473	11,243	12
Emergent									
Water-plantain	12,785	11,817	13,151	12,269	12,380	12,379	14,851	14,851	-16
Arrowhead	12,785	11,576	12,626	13,309	12,577	12,685	12,225	10,847	15
Fox sedge	12,785	11,979	12,378	13,376	12,815	12,131	11,916	9,467	26
Wool-grass	12,785	11,870	11,838	13,117	12,710	11,430	12,027	11,831	7
Spikerush	12,785	13,056	11,823	12,875	13,067	12,127	12,085	13,202	-3
Reed canary grass	12,785	13,381	11,690	11,430	12,574	12,260	10,829	9,365	27
Cat-tail	12,785	12,543	13,784	11,620	13,179	13,033	13,802	12,783	0
Parrot-feather	12,785	12,598	11,418	12,057	14,463	12,714	12,912	13,363	-5
Controls									
Groundwater	12,785	13,327	11,712	12,863	12,172	11,116	11,999	11,395	11
Sediment	12,785	12,693	12,019	11,305	12,360	12,137	11,817	10,047	21
Autoclaved sediment	12,785	12,599	13,295	12,133	12,569	11,367	11,294	9,578	25

Note: Percent removal was based on concentrations at 10 days. Means of triplicates.

achieved 27-, 26-, 15-, and 12-percent RDX removal, respectively. Failure by planted reactors to achieve at least the same level of removal as the groundwater alone may be due to decrease in photolysis caused by shading.

For the incubations with groundwater alone, photolysis and biotransformation by groundwater-inherent microorganisms apparently were minor mechanisms of RDX removal. Low photolysis is supported by the relatively long half-life of 46 days found in the present study compared with the half-life of 13 days for RDX in surface water in winter estimated by Spanggord et al. (1980b). The increased RDX removal in sediment incubations indicates that mechanisms for removal other than photolysis are active in sediment. Actual mechanisms are not known, but are speculated to be adsorption and sediment-nutrients-based stimulation of groundwater-inherent microorganisms.

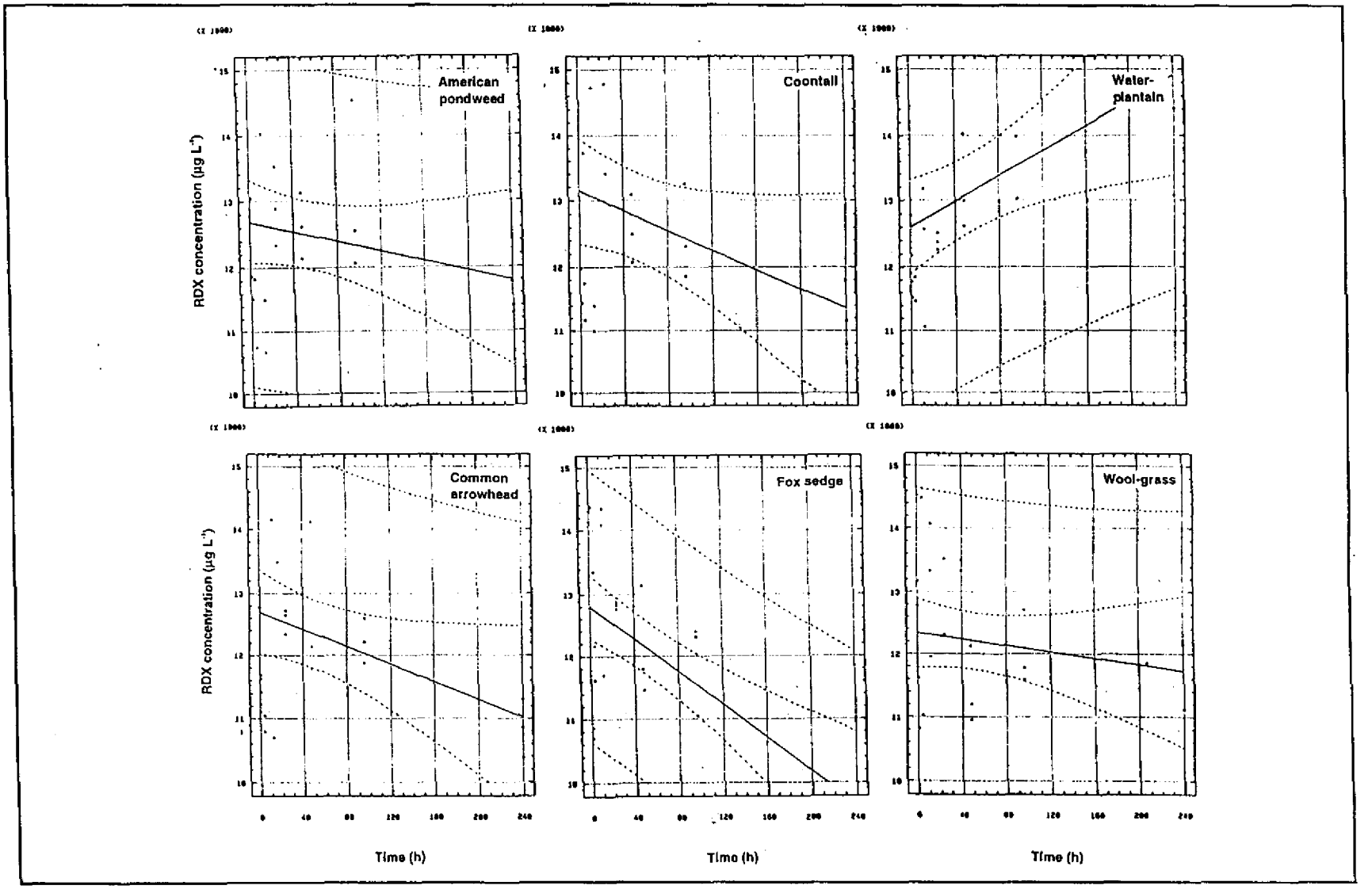

Figure 2. Changes in RDX concentration in groundwater with length of incubation at 25 °C with 1 of 10 aquatic plant species, alone, and with autoclaved and unautoclaved sediment (Drawn lines: fitted curves; interrupted lines, 95- and 90-percent confidence levels) (Sheet 1 of 3)

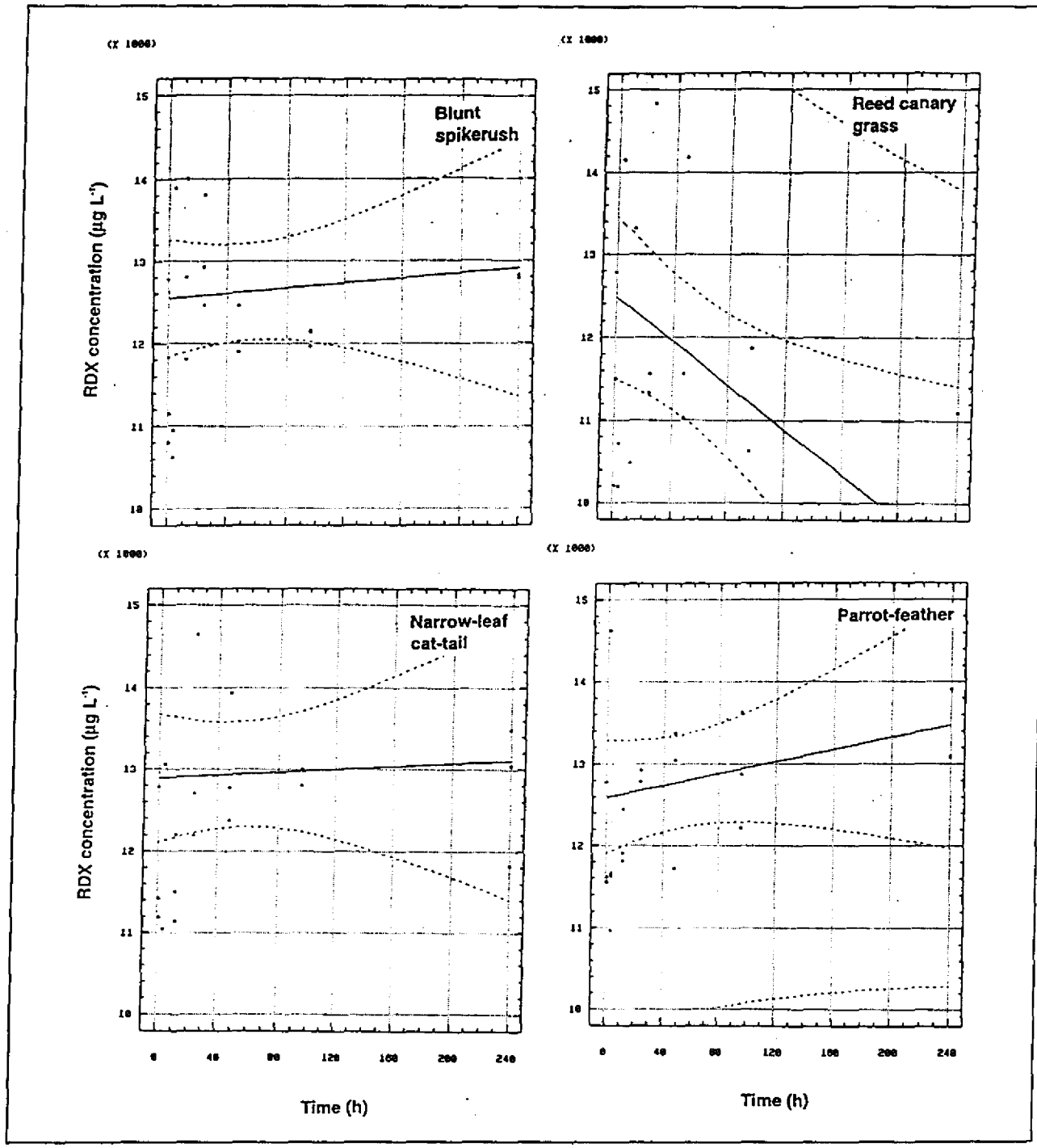

Figure 2. (Sheet 2 of 3)

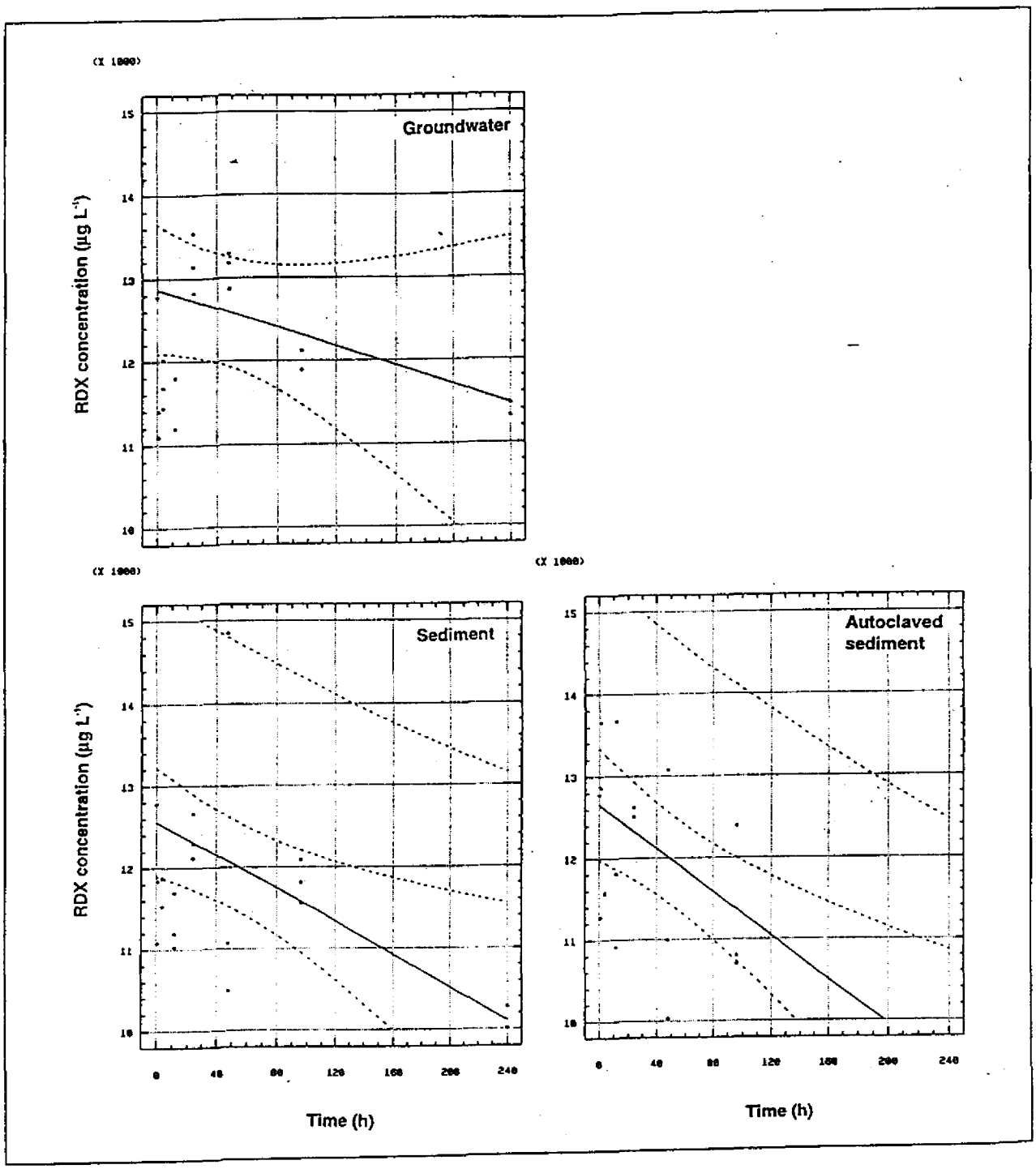

Figure 2. (Sheet 3 of 3)

Table 9
Treatment Effects on RDX Concentration in Groundwater Over 10-Day Incubation

Treatment	LS Mean	Homogeneous Groups
25 °C Incubations		
Reed canary grass	11,776.60	a
Autoclaved sediment	11,952.83	ab
Sediment	12,019.58	abc
Fox sedge	12,105.70	abcd
Wool-grass	12,200.94	abcde
Arrowhead	12,328.78	abcde
Pondweed	12,496.37	abcdef
Groundwater	12,578.76	bcdef
Spikerush	12,627.42	bcdef
Coontail	12,753.64	cdef
Parrot-feather	12,788.65	def
Cat-tail	12,941.22	ef
Water-plantain	13,110.22	f
10 °C Incubations		
Reed canary grass	11,715.19	a
Spikerush	12,199.56	b
Sediment	12,208.78	b
Groundwater	12,617.40	c
Parrot-feather	12,786.03	c

Note: Multiple range analysis by treatment. ANOVA showed that treatment affected the TNT concentration significantly (25 °C, $P = 0.008$; 10 °C, $P < 0.001$), as did time ($P < 0.001$; blocks as covariates).

Kinetics

A linear regression model, $Y = a + bX$, where Y = concentration, X = time, and b is negative over time, gave the best fit to change in RDX over time. These statistics were used to calculate zero order constants (slope of the regression; μg RDX L^{-1} hr^{-1}), half-life of RDX and HRT. However, fit was poor and incubations with four emergents had non-negative slopes (Table 10; Figure 2). Rate constants in sediment controls were higher than in groundwater alone.

Reed canary grass, fox sedge, and autoclaved sediment achieved the most rapid removal. Rate constants normalized for plant dry mass (K_n; data not shown) indicated that coontail, pondweed, reed canary grass, and fox sedge were the most effective species.

Table 10
Curve Fit Statistics for RDX Concentrations in Groundwater Over 10-Day Incubations With Plant Species and Controls

Treatment	Y = a+bX			Half Life, hr	Days to 2 $\mu g\ L^{-2}$	Initial Mass Incubated, g DW	
	Intercept a	Slope b	R^2			In Contact With Water	Total
25 °C Incubations							
Submersed							
Pondweed	12,694	-3.714	0.06	1,696	142	3.73±0.39	3.73±0.39
Coontail	13,150	-7.461	0.13	905	73	3.03±0.19	3.03±0.19
Emergent							
Water-plantain	12,597	9.654	0.24	--	--	4.64±0.06	12.34±0.26
Arrowhead	12,699	-6.985	0.15	902	75	19.05±0.54	22.34±0.62
Fox sedge	12,799	-13.053	0.51	490	40	22.62±0.62	28.99±0.50
Wool-grass	12,337	-2.562	0.03	2,320	200	26.47±4.36	43.15±2.39
Spikerush	12,546	1.532	0.01	--	--	20.74±7.89	35.83±3.95
Reed canary grass	12,485	-13.336	0.24	456	39	21.05±2.49	36.83±5.62
Cat-tail	12,894	0.889	0.01	--	--	7.47±0.97	16.26±0.32
Parrot-feather	12,569	3.635	0.04	--	--	1.83	5.51±0.09
Controls							
Groundwater	12,872	-5.836	0.07	1,110	91	0	0
Sediment	12,562	-10.211	0.29	604	51	152.84±0	152.84±0
Autoclaved sediment	12,657	-13.452	0.42	465	39	152.82±0	152.84±0
10 °C Incubations							
Emergent							
Spikerush	12,163	0.685	0.01	--	--	15.16±0.89	16.65±0.92
Reed canary grass	11,742	-0.516	0.01	10,366	947	14.82±1.30	20.40±3.44
Parrot-feather	12,569	4.315	0.19	--	--	1.87	5.61±0.08
Controls							
Groundwater	12,594	0.428	0.01	--	--	0	0
Sediment	12,451	-4.560	0.13	1,328	113	152.80±0	152.80±0

Note: Data not normalized for plant dry weight. Mean mass and standard deviations (N = 3). Initial concentration RDX in groundwater: 12,785 $\mu g\ L^{-1}$.

Decreased temperature appears to be more detrimental for RDX removal than for TNT (Tables 11 and 7). The predicted HRT for reed canary grass, the only plant to remove RDX to 2 $\mu g\ L^{-1}$ in the 10 °C incubations, increased 24 times at 10 °C. The same decrease in temperature only increased estimated HRT for TNT by approximately four times (Table 11).

Table 11
Impact of Temperature on RDX Zero Order Rate Constants and Hydraulic Residence Times To Meet Treatment Goal (2 $\mu g\ L^{-1}$)

Treatment	K_{25} ($\mu g\ L^{-1}.hr^{-1}$)	K_{10}/K_{25}	HRT_{10}/HRT_{25}
Emergent			
Spikerush	NR[1]	NR/NR	--
Reed canary grass	-13.336	0.04	24.28
Parrot-feather	NR	NR/NR	--
Controls			
Groundwater	-5.836	NR/-5.836	--
Sediment	-10.211	0.44	2.22

[1] NR = No removal of RDX observed.

At 10 °C, RDX removal was essentially eliminated except in the presence of sediment.

Concentration of TNB, Total ADNTs, Total DANTs, and Total Nitrobodies in Water: Effects of Plant Species and Temperature

TNB removal

TNB has long been recognized as a by-product of TNT photolysis and/or oxidation (Zappi 1995). The incubations with plants and sediment achieved over 90-percent TNB removal at 25 °C (Figure 3; Table 12). However, the planted reactors differed from controls in the trend of removal. The TNB concentrations remained high for a longer period in the controls than in the planted reactors' controls before decreasing. This indicates that plants are active in TNB removal.

The final extent of removal for groundwater alone was 38 percent. Since there was no difference between the autoclaved and nonautoclaved sediments, and there was limited TNB removal in the groundwater alone, nonbiological mechanisms (i.e., sorption or possibly cationic reduction) are suspected as being the primary source of TNB removal in the absence of plants.

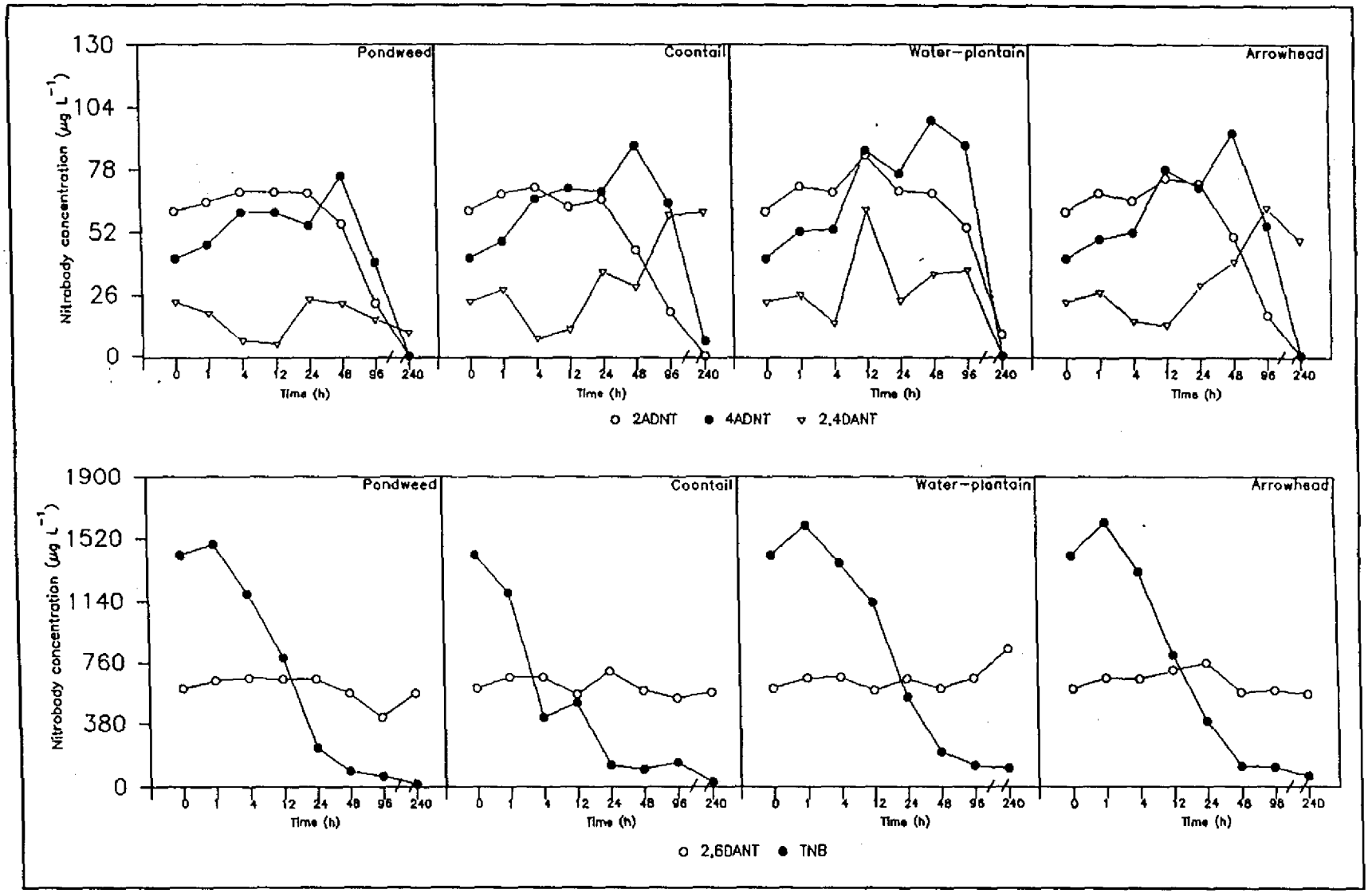

Figure 3. Changes in nitrobody concentration in groundwater with length of incubation at 25 °C with 1 of 10 aquatic plant species, alone, and with autoclaved and unautoclaved sediment (Mean values of triplicates) (Sheet 1 of 4)

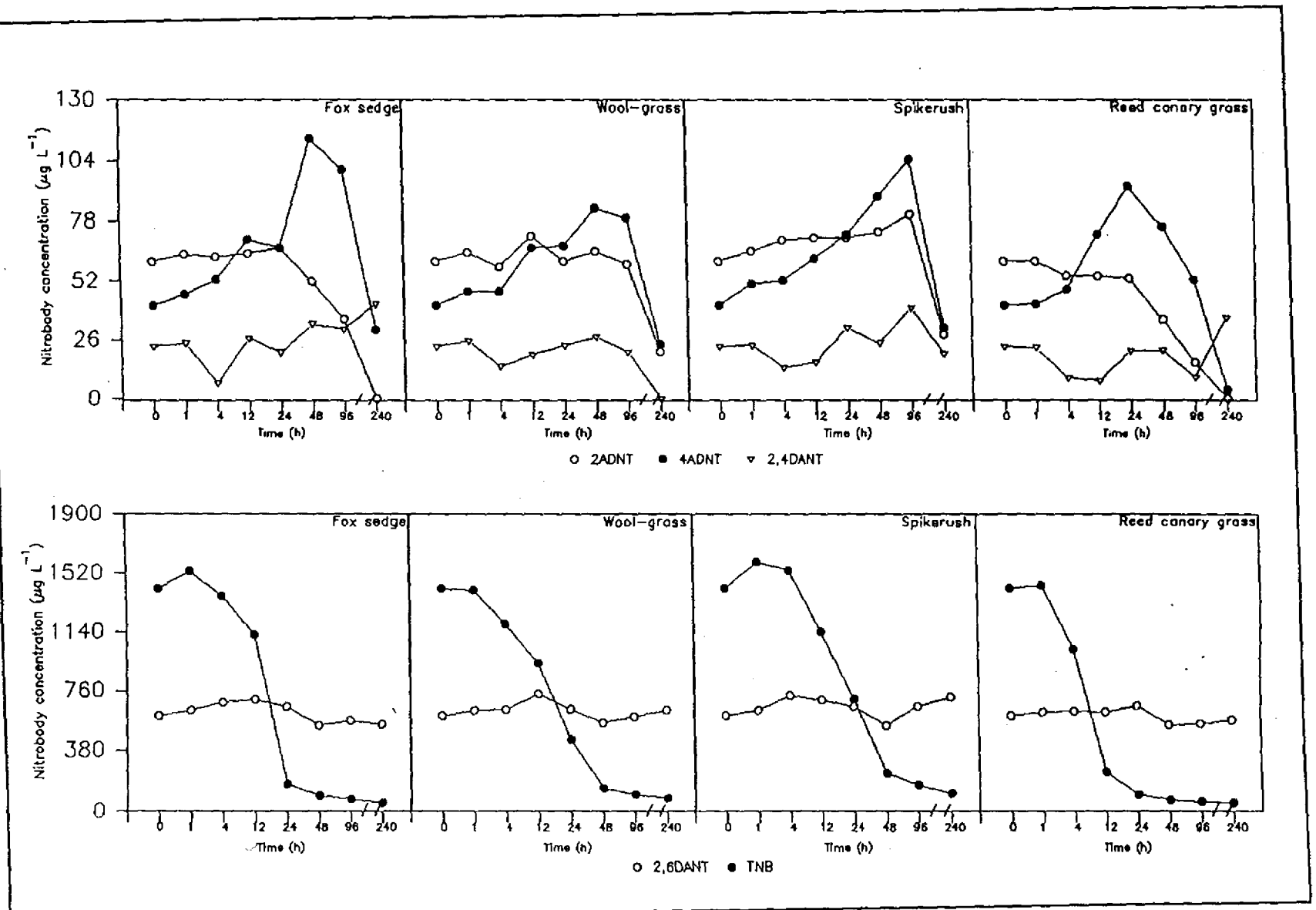

Figure 3. (Sheet 2 of 4)

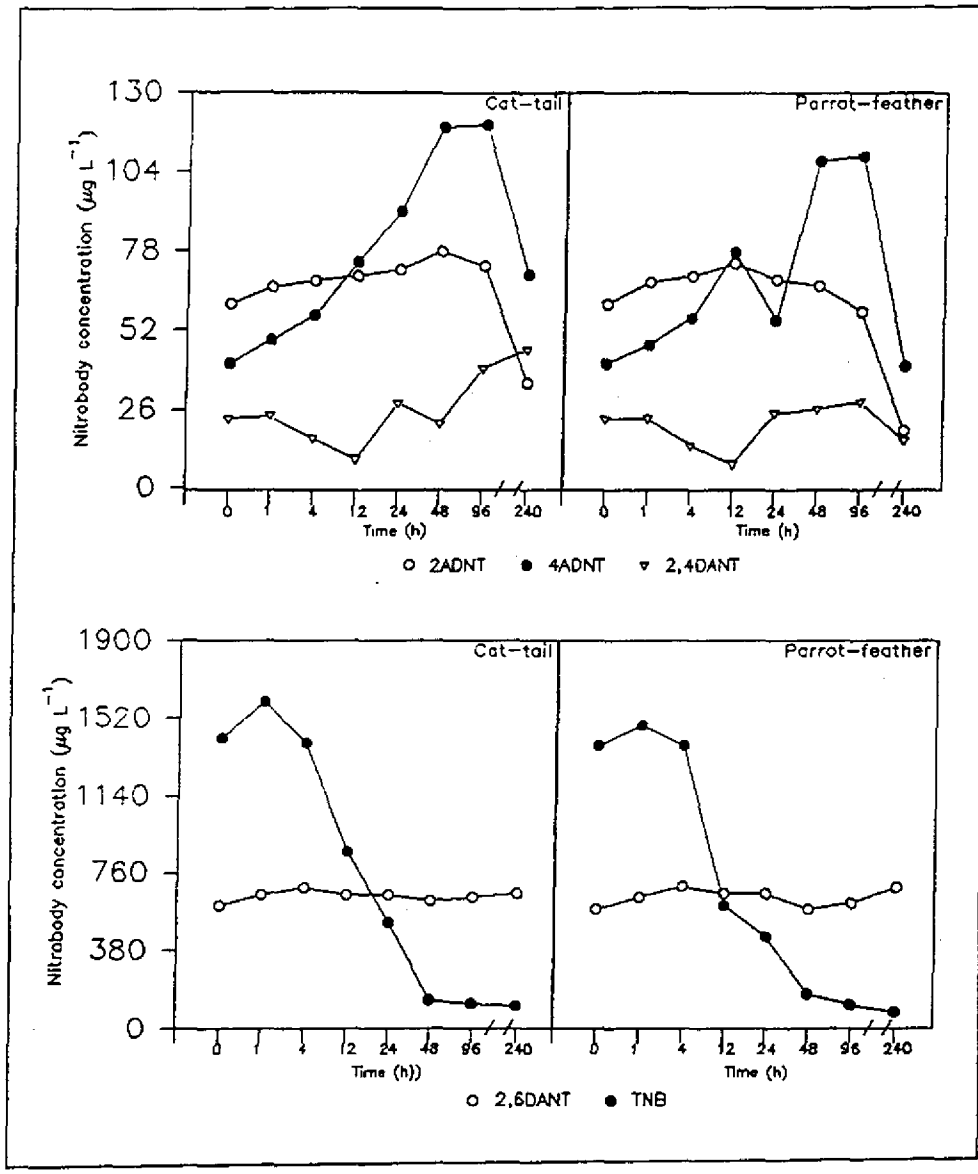

Figure 3. (Sheet 3 of 4)

Removal of total ADNTs

Aminodinitrotoluenes (ADNTs) are considered breakdown products of TNT by reduction of nitro-groups. They are commonly considered to be the first product of TNT reduction and the main mechanism proposed for the breakdown pathway of TNT within plants and microorganisms.

In most plant incubations, TADNTs (the sum of 2ADNT and 4ADNT) increased through 96 hr and then decreased below initial levels by 10 days in the 25 °C incubations (Figure 3; Table 13). Distinct differences among plant species were observed with two submersed and four of the emergent plants

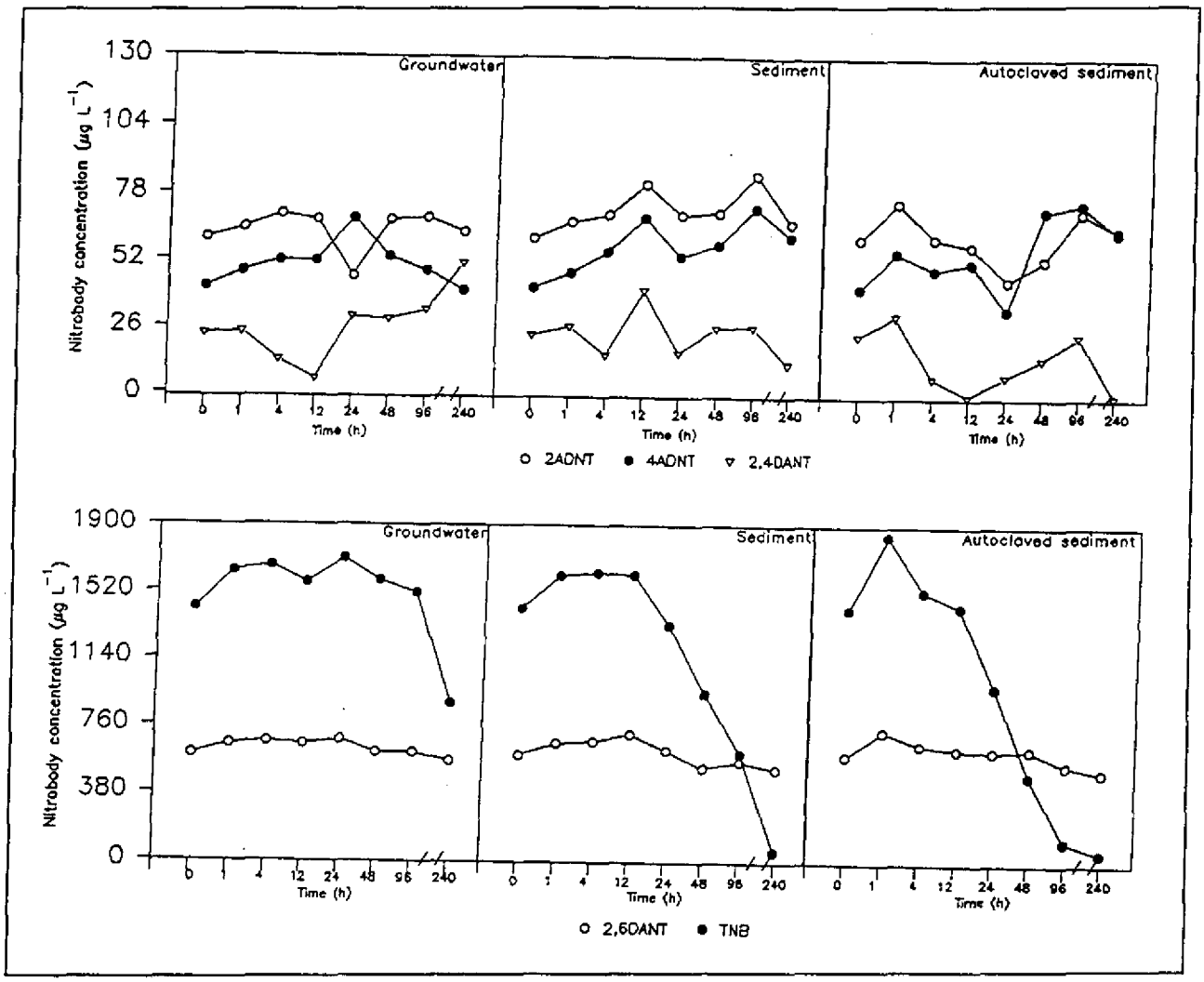

Figure 3. (Sheet 4 of 4)

having final removal percentages >70 percent. Cat-tail was the only plant that did not remove TADNTs, showing a 3-percent increase at 10 days. Pondweed and arrowhead were most effective removing TADNTs to levels below that of the HPLC detection limit within 10 days. Other plants that removed >90 percent TADNTs were coontail, reed canary grass, and water-plantain. Except for cat-tail, all emergents removed at least 40 percent of the TADNTs.

The groundwater alone incubation had no change in TADNTs. The sediment incubations both indicated an initial decrease in TADNTs and a net increase over time through the final sampling. This observation gives some evidence that the TNT removal observed in the sediment alone reactors is a reduction-based reaction and not completely that of adsorption. Based on the lack of an observed difference in performance between the autoclaved and

Table 12
TNB Concentrations ($\mu g\ L^{-1}$) in Groundwater Over 10-Day Incubations With Plant Species and Controls at 25 °C

Treatment	Incubation Period, hr								Removal, %
	0	1	4	12	24	48	96	240	
Submersed									
Pondweed	1,423	1,486	1,185	795	234	94	65	17	99
Coontail	1,423	1,193	424	509	127	105	147	33	98
Emergent									
Water-plantain	1,423	1,605	1,374	1,131	547	213	130	111	92
Arrowhead	1,423	1,618	1,323	807	401	120	115	64	96
Fox sedge	1,423	1,532	1,372	1,124	173	101	78	51	96
Wool-grass	1,423	1,410	1,190	938	455	149	108	82	94
Spikerush	1,423	1,588	1,536	1,137	703	240	165	111	92
Reed canary grass	1,423	1,439	519	236	36	25	--[1]	--	100
Cat-tail	1,423	1,604	1,399	860	515	142	122	111	92
Parrot-feather	1,423	1,521	1,419	615	460	175	122	82	94
Controls									
Groundwater	1,423	1,635	1,668	1,575	1,709	1,583	1,512	885	38
Sediment	1,423	1,615	1,633	1,617	1,333	941	608	62	96
Autoclaved sediment	1,423	1,848	1,529	1,445	984	489	125	62	96

Note: Percent removal was based on concentrations at 10 days. Means of triplicates.
[1] Below detection.

unautoclaved sediment systems, it is concluded that those reactions responsible for contaminant removal are not influenced by autoclaving (adsorption/iron reduction). A microbial population may have established itself over time in both sediment controls that ultimately began to reduce TNT into ADNTs, possibly feeding on the sediment detritus as a carbon source.

Removal of total DANTs

Diaminonitrotoluenes (2,6-diamino-4-nitrotoluene and 2,4-diamino-6-nitrotoluene; DANTs) are usually considered the second step in the reductive degradation of TNT. Planted reactors either produced little decrease or an increase in TDANTs in the 25 °C incubations, with water-plantain having the highest gain, 36 percent (Figure 3; Table 14). Plant species that did

Table 13
Total ADNT Concentrations ($\mu g\ L^{-1}$) in Groundwater Over 10-Day Incubations With Plant Species and Controls at 25 °C

Treatment	Incubation Period, hr								Removal, %
	0	1	4	12	24	48	96	240	
Submersed									
Pondweed	102	111	129	129	124	131	62	--[1]	100
Coontail	102	116	137	133	134	133	83	7	93
Emergent									
Water-plantain	102	124	122	170	146	167	142	9	91
Arrowhead	102	117	117	152	143	144	72	--	100
Fox sedge	102	110	115	135	134	166	136	31	70
Wool-grass	102	112	106	139	128	149	0139	45	56
Spikerush	102	116	122	133	144	162	186	60	41
Reed canary grass	102	102	102	127	146	111	69	4	96
Cat-tail	102	115	125	144	163	197	193	105	-3
Parrot-feather	102	115	126	152	124	174	167	60	41
Controls									
Groundwater	102	112	121	120	114	121	117	103	-1
Sediment	102	114	125	150	123	129	158	128	-26
Autoclaved sediment	102	130	110	110	78	125	145	128	-26

Note: Percent removal was based on concentrations at 10 days. Means of triplicates.
[1] Below detection.

decrease TDANTs were pondweed, fox sedge, and reed canary grass. Changes occurred largely in 2,6DANT, while 2,4DANT remained relatively constant.

The groundwater alone controls had no net change in TDANTs following a slight increase through 24 hr. Sediment incubations indicated a slight increase in TDANTs, followed by a net decrease of approximately 16 percent.

Removal of total nitrobodies

Table 15 lists the removal achieved at 10 days in the 25 °C incubations for all nitrobodies within the IAAP groundwater influent. Pondweed, coontail, arrowhead, and reed canary grass were the most effective removing over six chemicals at efficiencies > 80 percent. The groundwater alone incubations, in

Table 14
Total DANT Concentrations ($\mu g\ L^{-1}$) in Groundwater Over 10-Day Incubations With Plant Species and Controls at 25 °C

Treatment	Incubation Period, hr								Removal, %
	0	1	4	12	24	48	96	240	
Submersed									
Pondweed	619	666	670	663	685	594	436	582	6
Coontail	619	696	676	575	742	617	601	635	-3
Emergent									
Water-plantain	619	690	686	651	679	636	697	843	-36
Arrowhead	619	690	671	725	785	616	651	613	1
Fox sedge	619	659	692	733	677	571	600	590	5
Wool-grass	619	661	656	759	666	582	617	637	-3
Spikerush	619	659	740	716	692	565	696	733	-18
Reed canary grass	619	647	640	628	687	568	561	608	2
Cat-tail	619	678	705	664	681	645	679	702	-13
Parrot-feather	619	678	723	682	699	626	653	722	-17
Controls									
Groundwater	619	676	678	659	711	637	638	614	1
Sediment	619	687	690	755	644	551	590	529	15
Autoclaved sediment	619	768	666	638	632	655	572	511	17

Note: Percent removal was based on concentrations at 10 days. Means of triplicates.

which only photolysis and groundwater-inherent microorganism breakdown occurred, completely removed two compounds by over 80 percent (TDNTs and NB). The sediment incubations only differed for the TDNB removal, with 59 percent for unautoclaved versus 94 percent for autoclaved sediment.

The most difficult nitrobody compounds to remove were RDX, TADNTs, and TDANTs. RDX is regulated and is the primary contaminant at the IAAP site. The most effective species for RDX removal were fox sedge and reed canary grass. The two amino-reduced toluenes (ADNTs and DANTs), traditionally considered reductive degradation products of TNT, were most affected by pondweed, arrowhead, fox sedge, and reed canary grass. These same species were also most effective in TNT removal, achieving levels below analytical detection. This suggests that plant enzymes may be involved in reducing TNT to ADNTs and ADNTs to DANTs. Thus, DANTs build up over a 10-day period.

Table 15
Percent Removal of Nitrobodies Based on 10-Day Levels for the 25 °C Incubations

Treatment	Removal of Nitrobodies								No. Nitrobodies With >80% Removal
	TNT	RDX	TNB	TADNTs	TDANTs	TDNTs	TDNBs	NB	
Submersed									
Pondweed	100	10	99	100	6	100	100	100	6
Coontail	100	12	98	93	-3	100	100	100	6
Emergent									
Water-plantain	99	-16	92	91	-36	100	68	100	5
Arrowhead	100	15	96	100	1	100	100	100	6
Fox sedge	100	26	96	70	5	100	100	100	5
Wool-grass	98	7	94	56	-3	100	86	100	5
Spikerush	95	-3	92	41	-18	100	90	100	5
Reed canary grass	100	27	100	96	2	100	100	100	6
Cat-tail	95	0	92	-3	-13	100	85	100	5
Parrot-feather	94	-5	94	41	-17	100	100	100	5
Controls									
Groundwater	62	11	38	-1	1	100	-4	100	2
Sediment	84	21	96	-26	15	100	59	100	4
Autoclaved sediment	85	25	96	-26	17	100	94	100	5

Note: Initial nitrobody concentrations ($\mu g\ L^{-1}$) were TNT, 682; RDX, 12,785; TNB, 1,423; TADNTs, 102; TDANTs, 619; TDNTs, 7; TDNBs, 20; and NB, 15. Means of triplicates.

Table 16 presents the removal achieved for both the 25 °C and 10 °C incubations. Decreasing temperature resulted in slightly slower removal of TNT and TNB within the planted systems and had a far more dramatic impact on the removal of RDX and reduced nitroaromatics. Considerable differences in performance were observed in the control systems. The difference in lamps used in each respective growth chamber/cabinet may account for the difference in photolytic removal from the groundwater control, i.e., the lamps in the 25 °C growth chamber provided a better photonic flux than in the 10 °C growth cabinet.

Azoxy compounds in water

No azoxy compounds were detected in the 10-day samples incubated at 25 °C in any treatment.

Table 16
Comparison of the Percent Removal of Nitrobodies Based on 10-Day Levels for the 25 °C and 10 °C Incubations

Treatment	Removal of Nitrobodies								No. Nitrobodies With >80% Removal
	TNT	RDX	TNB	TADNTs	TDANTs	TDNTs	TDNBs	NB	
25 °C Incubations									
Emergent									
Spikerush	95	-3	92	41	-18	100	90	100	5
Reed canary grass	100	27	100	96	2	100	100	100	6
Parrot-feather	94	-5	94	41	-17	100	100	100	5
Controls									
Groundwater	62	11	38	-1	1	100	-4	100	2
Sediment	84	21	96	-26	15	100	59	100	4
Autoclaved sediment	85	25	96	-26	17	100	94	100	5
10 °C Incubations									
Emergents									
Spikerush	84	5	91	-18	NA	100	0	100	4
Reed canary grass	92	10	92	35	NA	100	9	100	4
Parrot-feather	71	-4	89	-2	NA	100	-11	34	2
Controls									
Groundwater	43	1	7	13	NA	9	-1	44	0
Sediment	67	11	68	-5	NA	-20	10	77	0

Note: Initial nitrobodies concentrations ($\mu g\ L^{-1}$) were TNT, 682; RDX, 12,785; TNB, 1,423; TADNTs, 102; TDANTs, 619; TDNTs, 7; TDNBs, 20; and NB, 15. Means of triplicates

Explosives and TNT Degradation Products in Plant Material and Sediments

Plant tissue of selected species from the groundwater incubations underwent HPLC analysis to assess the ultimate fate of explosives in contact with plants. Unexposed reference was also analyzed to assess whether natural chemical constituents coelute with targeted contaminants.

In incubated plants, TNT concentrations were usually near detection level (0.1 $\mu g\ L^{-1}$ in extract) and slightly higher in the aerial portions of water-plantain and arrowhead (Figure 4). TNT did not accumulate. RDX

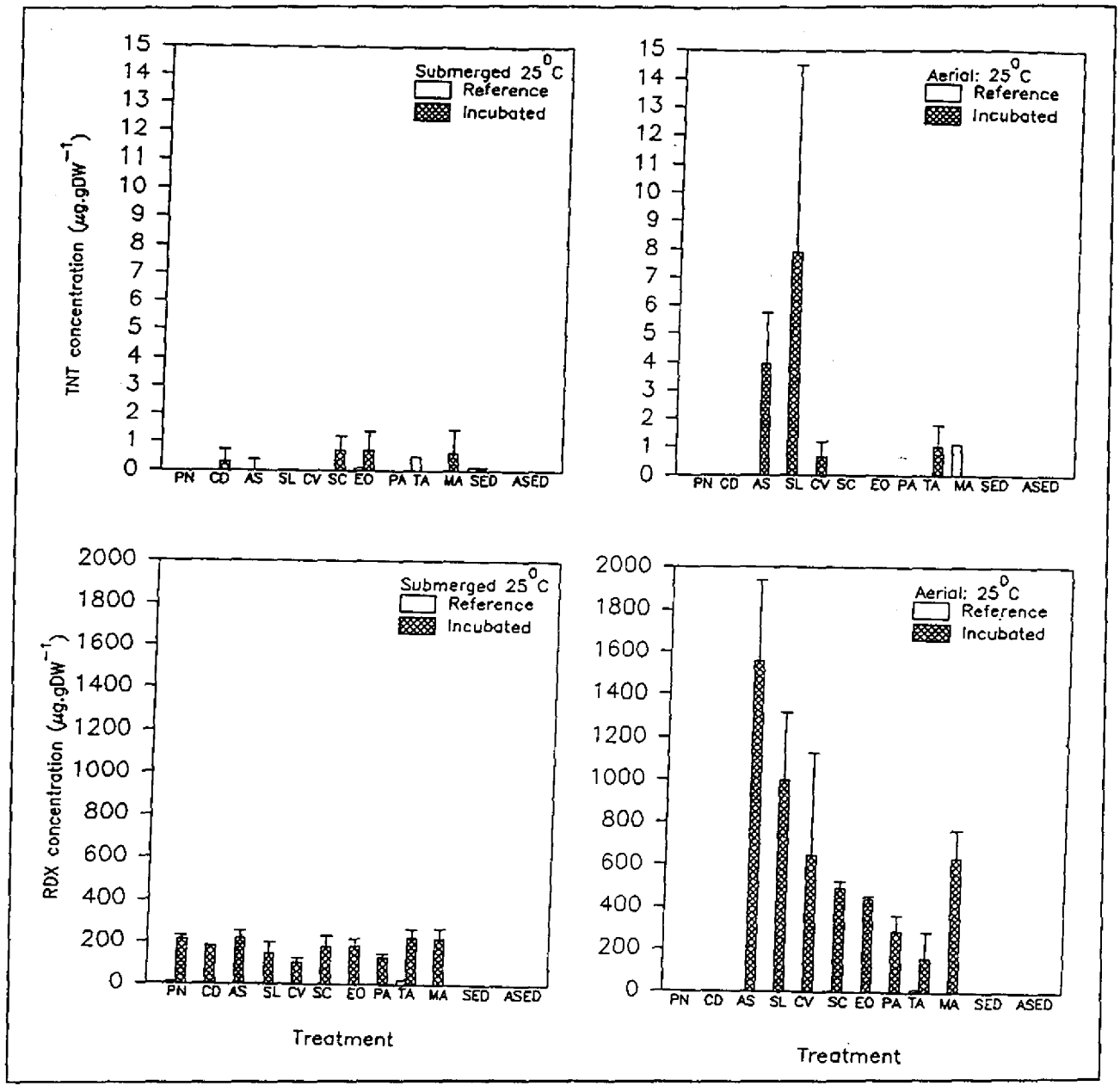

Figure 4. Concentrations of TNT and RDX in aerial and submersed portions of explosives-contacted plants and sediments (mean values of triplicates and standard deviations) and in reference plants (single values), 25 °C incubations (Abbreviations: PN, pondweed; CD, coontail; AS, water-plantain; SL, arrowhead; CV, fox sedge; SC, wool-grass; EO, spikerush; PA, reed canary grass; TA, cat-tail; MA, parrot-feather; SED, sediment; ASED, autoclaved sediment)

concentrations were substantial (to 200 μg g DW^{-1}) indicating translocation. The aerial portion of arrowhead, at 200,000 μg L^{-1} RDX, was 15 x higher than the final concentration in the incubation groundwater (13,000 μg L^{-1}). Apical transport of RDX has been reported for terrestrial plants by Cataldo,

Harvey, and Fellows 1990. Condensation and crystal formation on the outside of the plants is also feasible, since evapotranspiration was substantial (Figure 5). Reference plants appeared to have very little material that coeluted with RDX (Figure 4).

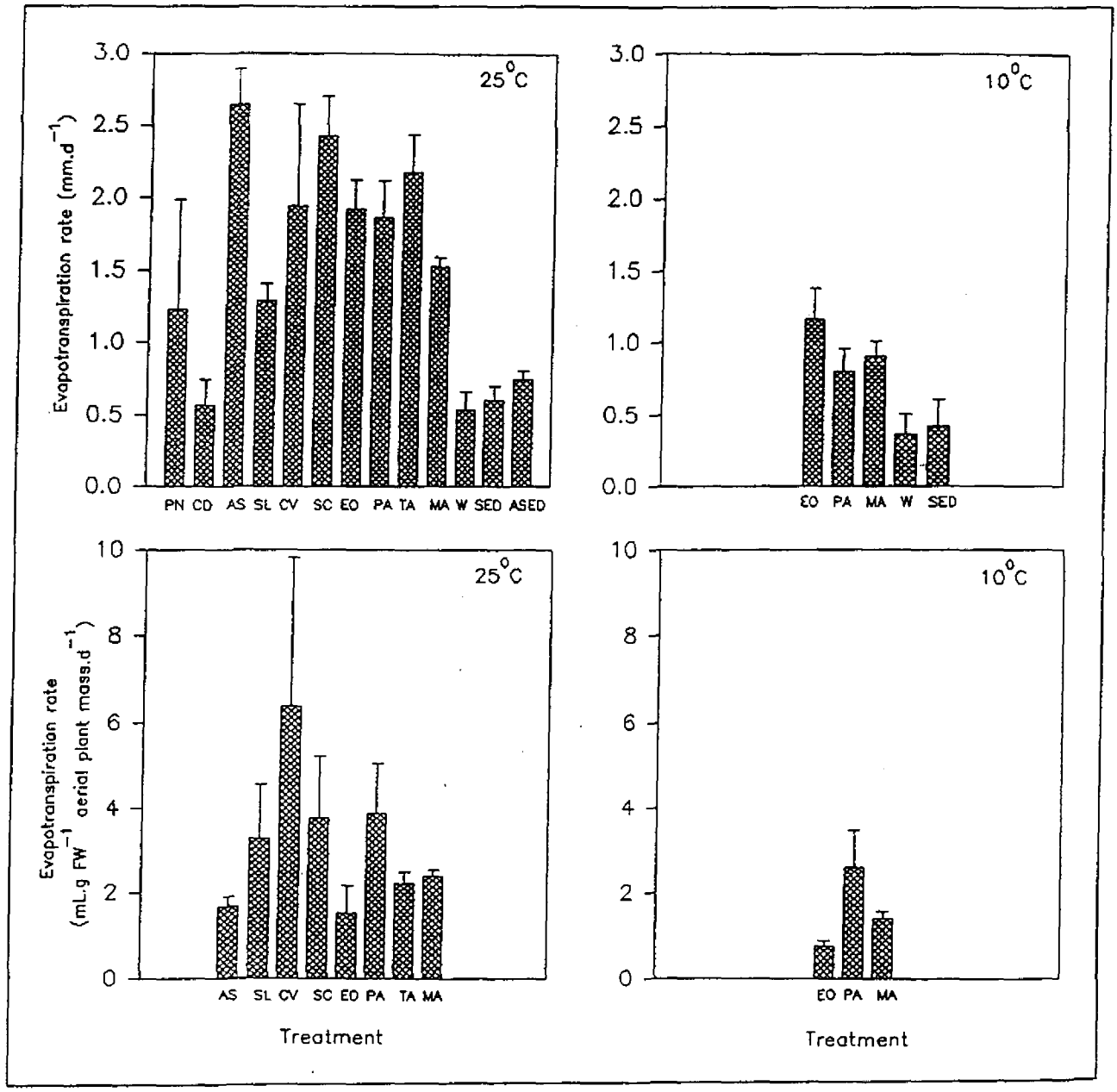

Figure 5. Evapotranspiration rates of groundwater following 10-day incubation (Mean values of triplicates and standard deviations) (Abbreviations: PN, pondweed; CD, coontail; AS, water-plantain; SL, arrowhead; CV, fox sedge; SC, wool-grass; EO, spikerush; PA, reed canary grass; TA, cat-tail; MA, parrot-feather; W, groundwater; SED, sediment; ASED, autoclaved sediment)

Concentrations of TNT-metabolites and TNT-photolytic products in plants were generally low (Table 17). Of note are (a) the occurrence of substantial 2ADNT and 4ADNT only in arrowhead, and 4ADNT in pondweed; in contrast, these metabolites were present in the groundwater initially and after incubation with most plant species and controls (Table 18); (b) the absence of 2,6DANT and 2,4DANT in all plant tissue, metabolites that had increased significantly in water incubated with some of the emergents; (c) the absence of products derived from TNT by removal of one or more nitro-groups in all plants and incubation water (only 2,4DNT shown in Table 17; other NTs were below detection); (d) the presence of TNB in most plant species, with higher concentrations in aerial than in submersed plant portions. In general, the aerial portions of arrowhead had the highest level of TNT metabolites.

Table 17
Concentrations of RDX and TNT, TNT-Metabolites, and TNT-Photolysis Products (μg g DW^{-1}) in Plant Tissue and Unautoclaved and Autoclaved Sediments After 10-Day Incubation

Plant/Sediment	In Contact With Water	RDX	TNT	TNT-Metabolites			TNT-Photolysis Products	
				2ADNT	4ADNT	24DNT	TNB	DNB
Submersed								
Pondweed	+	212	--[1]	--	51.9	--	0.9	1.6
Coontail	+	181	0.3	--	0.3	--	0.8	--
Emergent								
Water-plantain	-	1,557	3.9	2.6	2.7	--	1.5	2.1
	+	218	0.3	1.5	--	2.2	2.1	--
Arrowhead	-	998	7.9	18.5	11.3	2.4	11.2	0.5
	+	144	--	1.1	0.6	--	4.5	--
Fox sedge	-	645	0.7	0.8	1.2	0.2	2.9	3.7
	+	102	--	--	1.5	0.5	0.7	0.2
Wool-grass	-	492	--	1.3	--	--	0.6	1.6
	+	179	0.7	0.5	1.3	0.5	0.5	--
Spikerush	-	438	--	--	1.9	--	0.9	1.2
	+	182	0.7	1.5	--	--	1.0	--
Reed canary grass	-	287	--	0.3	1.3	--	4.0	0.1
	+	128	--	--	1.4	--	2.3	0.3
Cat-tail	-	159	1.0	--	--	--	0.8	--
	+	220	--	--	0.9	--	4.4	--
Parrot-feather	-	631	--	1.7	0.8	--	2.0	1.1
	+	217	0.6	3.5	0.5	0.8	6.0	0.8
Controls								
Sediment	+	0.2	0.1	--	--	--	--	--
Autoclaved sediment	+	0.3	--	0.2	--	--	0.1	--

Note: Means of triplicates.
[1] Below detection.

Table 18
Concentrations of RDX and TNT, TNT-Metabolites, and TNT-Photolysis Products ($\mu g\ L^{-1}$) in Groundwater After 10-Day Incubation

Treatment	RDX	TNT	TNT-Metabolites					TNT-Photolysis Products			
			2ADNT	4ADNT	26DANT	24DANT	26DNT	TNB	14DNB	13DNB	NB
Submersed											
Pondweed	11,491	--[1]	--	--	572	10	--	17	--	--	--
Coontail	11,243	--	--	7	574	60	--	33	--	--	--
Emergent											
Water-plantain	14,415	4	9	--	843	--	--	111	--	6	--
Arrowhead	10,847	--	--	--	564	49	--	64	--	--	--
Fox sedge	9,467	--	--	30	548	42	--	51	--	--	--
Wool-grass	11,831	13	21	24	636	--	--	82	--	3	--
Spikerush	13,202	32	28	31	713	20	--	111	--	2	--
Reed canary grass	9,365	--	--	4	573	35	--	51	--	--	--
Cat-tail	12,784	35	35	70	656	46	--	111	--	3	--
Parrot-feather	13,363	39	19	40	706	16	--	82	--	--	--
Controls											
Groundwater	11,394	261	63	40	562	51	--	885	8	13	--
Sediment	10,048	108	66	61	517	12	--	62	--	8	--
Autoclaved sediment	9,578	105	64	64	511	--	--	62	--	1	--
Initial groundwater	12,785	681	61	41	596	23	7	1,423	--	20	15

Note: Concentrations initial groundwater also given. Means of triplicates.
[1] Below detection.

The concentrations of explosives and TNT degradation products in the sediments were near detection level (Table 17), consistent with expected explosives adsorption based on low CEC and low organic-matter concentration (Pennington and Patrick 1990; Pennington et al. 1995).

Plant Health and Growth

Plant growth was minimal over 10-day incubation, probably due to the lateness of the growing season. Visual inspection during the incubations indicated that most plants recovered from transplant shock after 7 days. Coontail and arrowhead did not appear to recuperate in that period.

Water-plantain, wool-grass, cat-tail, and parrot-feather exhibited positive weight gains (Figure 6), while coontail and arrowhead exhibited the largest weight loss. Light levels were considered adequate for the submersed plants, being close to photosynthetic saturation (600-800 $\mu E\ m^{-2}\ s^{-1}$; Van, Haller, and Bowes 1976). To the emergent plants, however, light levels were

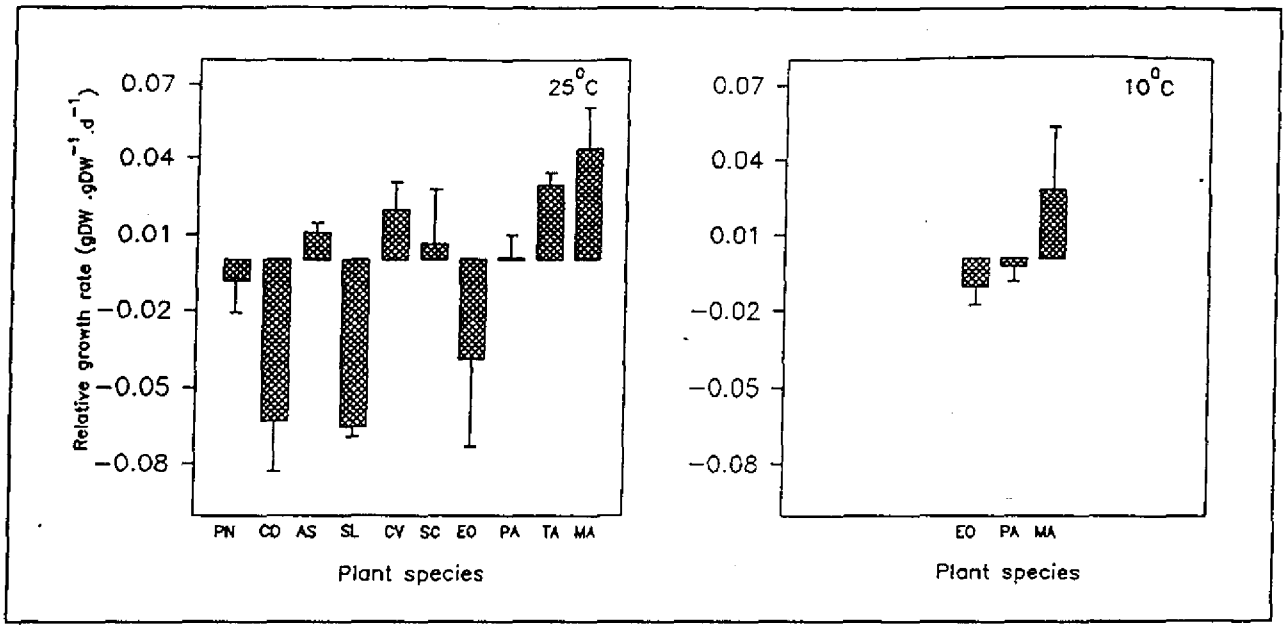

Figure 6. Relative growth rates over the 10-day incubation of plant species in explosives-contaminated groundwater (Mean values of triplicates and standard deviations) (Abbreviations: PN, pondweed; CD, coontail; AS, water-plantain; SL, arrowhead; CV, fox sedge; SC, wool-grass; EO, spikerush; PA, reed canary grass; TA, cat-tail; MA, parrot-feather)

approximately half of typical, but the amount supplied is considered adequate for maintaining activity.

An alkalinity of 1.84 mM at the beginning of the experiment is not expected to limit photosynthesis of submersed plants, but it decreased by 50-60 percent at the end of the 10-day period, and the latter level was in the range indicated to cause carbon limitation for growth of submersed plants (Van, Haller, and Bowes 1976). The pH of 7.5 to 9.0 favored the submersed plants like pondweed and coontail, as they prefer bicarbonate-carbon (Spence and Maberly 1985). Lack of water mixing may have limited carbon transport to diffusion alone and would have affected growth of the submersed plant species (Walker 1985). All emergent plants would have had access to carbon dioxide in air, where diffusion is far higher than in water. Oxygen levels in water usually ranged from >5 mg L^{-1} to saturation, being acceptable for aquatic plants (Figure 7).

Nitrogen and phosphorus nutrient levels were sufficient throughout the incubation. A major increase in nitrogen was observed, presumably caused by leaching of these substances from the plant biomass into the water, or their production via degradation of plant mass by microorganisms within the reactors. However, groundwater alone also had a major increase in NO_3-N (Table 19).

The increase in iron levels within the reactors may be indicative of plant mass degradation/decay.

In summary, slightly less than adequate conditions, some experimental and many system-oriented, were the likely cause for some of the lack of plant growth observed.

No data on toxicity of RDX to aquatic plants are available to date. Growth inhibition by the explosives concentration cannot be ruled out, since RDX concentration was in the range of 2 to 15 ppm, known in TNT as being toxic for aquatic plants and algae (Schott and Worthley 1974; Smock, Stoneburner, and Clark 1976). Toxicity of the IAAP water is suggested by the slow removal rates of parrot-feather in the present study compared with that documented in a similar study (Best et al., in preparation). However, differences in parrot-feather strain may have contributed to the contrasting reactions.

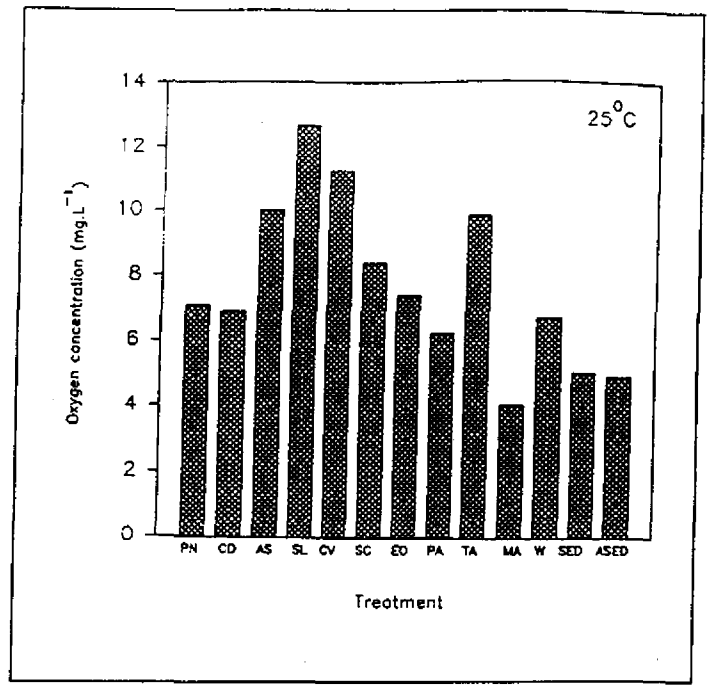

Figure 7. Oxygen concentrations in treated groundwater following 10-day incubation at 25 °C (Single values) (Abbreviations: PN, pondweed; CD, coontail; AS, water-plantain; SL, arrowhead; CV, fox sedge; SC, wool-grass; EO, spikerush; PA, reed canary grass; TA, cat-tail; MA, parrot-feather; W, groundwater; SED, sediment; ASED, autoclaved sediment)

General Discussion

Disappearance rates of explosives from groundwater

Disappearance rates seen here for TNT and RDX from groundwater, with or without plants, are rather low relative to literature values. Realistic comparison is difficult, however, because of nonuniformity among studies and calculation procedures (Appendix D). Similar submersed plant screenings at WES show TNT removal in 4 to 5 days in most species (Anonymous 1995; Best et al. 1996; this study). The longer half-lives of both TNT and RDX found in the present study are attributed to low photolysis with lack of UV light. If the contribution of the plants to the disappearance of explosives per se is linked directly to plant growth, this influence is expected to be low at the end of the growth season in autumn and far higher in spring and summer. However, this contribution can also be linked indirectly to plants, e.g., to the

Table 19
Chemical Characteristics of Initial Groundwater and After 10-Day Incubation

Treatment	pH	Alkalinity mg L^{-1}	COD mg L^{-1}	TDS mg L^{-1}	Kj-N mg L^{-1}	NO_2/NO_3-N mg L^{-1}	NH_4-N mg L^{-1}	Total-P mg L^{-1}	PO_4-P mg L^{-1}	SO_4 mg L^{-1}	Ca mg L^{-1}	Fe mg L^{-1}
Initial												
Groundwater	7.5	170	--	839	1.6	0.15	0.17	<0.2	0.185	56.35	121.36	0.18
After 10-Day Incubation												
Submersed												
Pondweed	8.8	56	63.4	774	3.2	71.6	0.19	0.28	<0.01	60.6	60.0	0.18
Coontail	8.0	78	75.1	758	1.9	73.8	0.35	0.39	<0.01	62.7	66.6	1.44
Emergent												
Water-plantain	8.1	263	113.0	966	2.9	80.5	0.33	0.62	0.33	91.9	152.0	1.39
Arrowhead	9.0	90	143.0	628	3.5	56.5	0.23	0.59	0.05	47.3	54.6	2.31
Fox sedge	8.1	295	156.0	516	5.7	19.6	0.29	0.46	0.01	47.2	72.4	1.40
Wool-grass	7.8	<5	148.0	978	3.1	89.1	1.18	1.13	0.69	66.7	149.0	9.96
Spikerush	8.0	<5	120.0	1,044	2.8	90.6	0.24	0.40	0.38	69.5	165.0	2.51
Reed canary grass	7.8	<5	148.0	486	9.8	<0.5	0.28	0.88	0.09	61.5	72.9	1.41
Cat-tail	8.3	111	94.2	878	1.8	93.8	0.27	0.43	<0.01	60.5	101.0	3.09
Parrot-feather	7.5	230	148.0	1,040	4.1	82.7	0.74	0.63	0.29	76.9	139.0	0.36
Controls												
Groundwater	8.3	128	41.6	910	<0.1	86.6	0.55	<0.2	0.09	62.3	103.0	0.20
Sediment	7.7	107	152.0	664	3.4	57.9	0.99	0.82	0.42	101.0	87.1	7.65
Autoclaved sediment	8.3	240	115.0	816	5.7	50.7	6.28	0.38	0.16	88.4	113.0	1.40

Note: Alkalinity expressed as mg $CaCO_3$ L^{-1}. Initial: mean values three determinations; after 10-day incubation: values single determination mixed sample of three blocks.

microorganism activity-stimulating effects of leachates. In case of the latter, greatest effects are expected either during the growth season when soluble carbohydrates are leached (about 10 percent of net primary production), or in autumn and winter when phenols are exuded to enter the environment during plant senescence and decomposition (Fletcher, Donnelly, and Hegde 1995).

Biotransformation

The generally negligible TNT-metabolite concentrations in plant material versus those in the incubation water suggest that either most TNT degradation occurs in the plants early in the incubation period, and is followed by TNT metabolite leaching from the plants to the water, or that most TNT is degraded outside the plants by microorganisms in the water but is stimulated by plant leachates. A combination of both mechanisms cannot be excluded. Only reductive pathways appeared to be active, and no evidence for the presence of a pathway involving the removal of one or more nitro groups from TNT was detected. The presence of reductive pathways of TNT and absence of nitro group removal from TNT in plants agree with studies on terrestrial plants (Palazzo and Legett 1986; Pennington 1988; Cataldo et al. 1989; Harvey et al. 1990).

The lack of significant differences in RDX decrease rates between plant species and groundwater suggests that breakdown by microorganisms was the dominant mechanism, probably stimulated by plant leachates. Translocation and accumulation of RDX into aerial plant portions agree with studies on terrestrial plants (Cataldo et al. 1989; Harvey et al. 1991). Since no RDX metabolites were screened, the degradation route of this compound cannot be assessed.

The RDX disappearance rate in the present study was not correlated with oxygen concentration, as found in a similar study involving submersed plants only (Best et al., in preparation).

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Appendix A
Site Reconnaissance Visit to Iowa Army Ammunition Plant, Burlington IA, 31 July-2 August 1995

The site reconnaissance visit is summarized below. A full report has been given elsewhere (Best et al. 1996).[1]

Goals of the Visit

The Iowa Army Ammunition Plant (IAAP) site visit was undertaken to allow participants in the U.S. Army Engineer District, Omaha, project "Optimization of Constructed Phytoremediation Systems for Treatment of Contaminated Groundwater at the Iowa Army Ammunition Plant" to accomplish the following:

a. Determine the taxonomy of local communities of aquatic and wetland plants.

b. Assess the nitroreductase activity of local plants using U.S. Environmental Protection Agency (USEPA)-developed Enzyme-Linked Sorbent Assay (ELISA).

c. Perform a visual assessment of site conditions.

d. Collect groundwater and sediment samples for physical, biological, and chemical characterization.

e. Collect site-specific historical data on groundwater quality and hydrodynamics.

[1] References cited in this appendix are located at the end of main text.

f. Meet with the Omaha District on planned excavation activities to help define pit configurations and locations.

Participants

Participants from cooperating agencies were Dr. Susan L. Sprecher of the U.S. Army Engineer Waterways Experiment Station (WES); Drs. L. Carreira (Dyn Corporation) and Rex Kerstetter (Furman University) representing USEPA-Athens; Messrs. Don Moses and Randy Sellers of the Omaha District; Dr. Diana Horton and Mr. Phillip Thompson of the University of Iowa. Mr. Rodger Allson, Mr. Joe Haffner, and Ms. Lori Landeche (Mason and Hanger-Silas Mason Co., Inc.) represented the IAAP; Mr. Derek Romitti represented the U.S. Army Environmental Center; Mr. Cyril Onewokae and Ms. Rebecca Goetzke represented Headquarters (Rock Island).

Local Communities of Aquatic and Wetland Plants

The IAAP reflects the status of the State as a whole in that the majority of its natural wetlands are riparian systems. A general list of common wetland herbaceous species found in Iowa (Table A1) indicates that sedges and bulrushes predominate at these sites (Marburger 1992).

Nitroreductase Activity of Local Using USEPA-Developed ELISA

Dr. Laura Carreira assayed nitroreductase (NR) activity of a large number of plants onsite (Table A2). She also provided more detailed NR activity data for 19 plant species collected at IAAP, the local parrot-feather used by USEPA as an NR activity standard, and two IAAP Stump Lake sediment samples (Table A3). Several species did not have NR activity higher than that found in sediment (64 - 68 $\mu g.g^{-1}$ fresh weight). Eleven species showed NR activity greater than parrot-feather: *Alisma subcordatum*, *Aster novae-angliae*, *Carex normalis*, *Carex vulpinoidea*, *Elymus virginicus*, *Eupatorium perfoliatum*, *Geum canadense*, *Penthorum sedoides*, *Sagittaria*, *Scutellaria lateriflora*, and *Typha*. With some species, leaf and stem activity was higher than root activity (e.g., *Carex normalis*).

Aquatic and Wetland Plant Species Selection

Criteria. In selecting plants for detailed characterization of explosives transformation in local groundwater, the potential for explosives degradation was taken into account as well as pertinent ecological, phenological,

Table A1
Common Herbaceous Wetland Species in Iowa (from Marburger 1992)

Family		Species	
Latin Name	Common Name	Latin Name	Common Name
Alismataceae[1]	Water-plantain	*Sagittaria latifolia* L.	Common arrowhead, Broad-leaf arrowhead
Asteraceae[1]	Aster	*Aster novae-angliae*	New England aster
Ceratophyllaceae[1]	Hornwort	*Ceratophyllum demersum* L.	Hornwort, Coontail
Cyperaceae[1]	Sedge	*Eleocharis smallii*	Small's spike-rush
Cyperaceae	Sedge	*Scirpus fluviatilis*	River bulrush
Cyperaceae	Sedge	*Scirpus heterochaetus*	Slender bulrush
Cyperaceae	Sedge	*Scirpus validus*	Softstem bulrush
Lemnaceae[1]	Duckweed	*Lemna minor* L.	Lesser duckweed
Lemnaceae	Duckweed	*Spirodela polyrhiza* (L.) Schleiden	Giant duckweed
Najadaceae	Naiad	*Najas flexilis* (Willd.) R. & S.	Slender naiad
Nymphaeaceae	Waterlily	*Nuphar luteum* (L.) Sibth. & Small	Spatterdock, Yellow waterlily
Nymphaeaceae	Waterlily	*Nuphar variegatum*	Yellow cowlily
Poaceae	Grass	*Leersia oryzoides*	Rice cutgrass
Poaceae[1]	Grass	*Phalaris arundinacea* L.	Reed canary grass
Poaceae[1]	Grass	*Spartina pectinata* Link	Prairie cordgrass
Polygonaceae[1]	Smartweed	*Polygonum amphibium* var. *coccineum*	Water smartweed
Polygonaceae[1]	Smartweed	*Polygonum amphibium* var. *stipulaceum*	Water smartweed
Potamogetonaceae[1]	Pondweed	*Potamogeton pusillus* L.	Small pondweed
Sparganiaceae	Bur-reed	*Sparganium eurycarpum* Engelm.	Giant bur-reed P F
Typhaceae[1]	Cat-tail	*Typha x glauca*	Blue cat-tail

[1] Present at IAAP.

morphological, and other properties that contribute to initial planting, functioning, and maintenance of a completed wetland.

- *Explosives degradation.* High activity of the NR enzyme is currently the major physiological selection tool to delineate rapidly those plants with ability to remediate explosives. The number of species found at IAAP with this characteristic provides a range of plants from which to choose wetland components.

Table A2
Plants Assayed Using ELISA-Based Field Test Kit for Nitroreductase Enzyme: IAAP, 1 and 2 August 1995

Family	Species	Habitats	Common Name	Relative NR Activity
Alismataceae	*Alisma subcordatum*	D	Southern water-plantain	Leaf/stem/root - moderate
Alismataceae	*Sagittaria cf. cuneata*	M	Arrowhead, Wapato	Leaf/petiole - weak
Alismataceae	*Sagittaria graminea*	P	Arrowhead	
Apocynaceae	*Apocynum sibericum*	B	Dogbane	Leaf/root - strong rxn
Asclepiadaceae	*Asclepias incarnata*	B	Swamp milkweed	Leaf/root - moderate
Asteraceae	*Ambrosia artemisiifolia*	B	Common ragweed	Leaf/stem - no rxn
Asteraceae	*Aster novae-angliae*	B	New England aster	Strong
Asteraceae	*Eupatorium perfoliatum*	B	Boneset	Root/leaf - moderate
Asteraceae	*Silphium perfoliatum*	B	Cup-plant	Leaf/stem/flower - no rxn
Ceratophyllaceae	*Ceratophyllum demersum*	P	Hornwort, Coontail	Leaf/stem - moderate
Cyperaceae	*Carex cf. normalis*	W	Sedgeleaf	Root - moderate
Cyperaceae	*Carex vulpinoidea*	W	Fox sedge	Leaf/stem - moderate
Cyperaceae	*Cyperus cf. esculentus*	WB	Flat sedge, Nutsedge	Leaf/petiole - moderate
Cyperaceae	*Eleocharis ovata* [= *E. obtusa*]	DMW	Blunt spikerush	Leaf - weak
Cyperaceae	*Eleocharis smallii*	DMW	Spikerush	Leaf/petiole - moderate
Cyperaceae	*Scirpus atrovirens*	DW	Black bulrush	Leaf/stem/root - weak
Cyperaceae	*Scirpus cyperinus*	W	Wool-grass	Leaf/stem - moderate
Equisetaceae	*Equisetum arvense*	B	Common or field-horsetails	Leaf/stem - weak
Equisetaceae	*Equisetum hyemale*	DWB	Common scouring rush	Stem - weak
Juncaceae	*Juncus cf. tenuis*	MW	Path rush	Leaf/stem - moderate
Juncaceae	*Juncus torreyi*	MW	Rush	Leaf/stem - weak
Lamiaceae	*Prunella vulgaris* var. *lanceolata*	WB	Self-heal	Root/stem/flower - very weak
Lamiaceae	*Scutellaria lateriflora*	WB	Skullcap	Leaf/stem - high to moderate
Lamiaceae	*Teucrium canadense*	B	American germander	Leaf/stem - low
Lemnaceae	*Lemna minor*	MP	Lesser duckweed	Whole plant - moderate
Lythraceae	*Lythrum alatum*	WB	Winged loosestrife	Flwr/lf/stem/root - weak
Onagraceae	*Ludwigia alternifolia*	B	Rattlebox, Seedbox	
Poaceae	*Bromus inermis*	WB	Smooth brome grass	Leaf/stem - weak
Poaceae	*Elymus virginicus*	WB	Virginia wild rye	Leaf/stem - moderate
Poaceae	*Leersia virginica*	WB	Cut grass	Weak

Note: Habitats: D = Watered ditch; M = Shallow marsh; W = Wet meadow; P = Pond; B = Bank of marsh/stream; rxn = reaction.

(Continued)

Table A2 (Concluded)

Family	Species	Habitats	Common Name	Relative NR Activity
Polygonaceae	*Polygonum* sp.	MP	Smartweed	Leaf/stem - weak
Potamogetonaceae	*Potamogeton foliosus*	P	Leafy pondweed	Stem/leaf - weak
Potamogetonaceae	*Potamogeton nodosus*	P	American pondweed	Stem/leaf - weak
Potamogetonaceae	*Potamogeton pusillus*	DM	Small pondweed	Leaf/stem - weak
Rosaceae	*Agrimonia parviflora*	B	Southern agrimony	Leaf/stem - very weak
Rosaceae	*Geum canadense*	B	White avens	Leaf/stem - moderate
Salicaceae	*Salix exigua* [*S. interior*]	MB	Sandbar willow	Root/leaf/stem - very weak
Saxifragaceae	*Penthorum sedoides*	B	Ditch stonecrop	Root/leaf/stem/flower - strong
Typhaceae	*Typha cf. angustifolia*	MP	Narrow-leaf cattail	Root/stem - moderate
Ulmaceae	*Ulmus cf. rubra*	B	Slippery elm	Leaf/stem - weak
Urticaceae	*Pilea fontana*	B	Clear weed	Leaf/stem - weak
Verbenaceae	*Verbena urticifolia*	B	White vervain	Leaf/stem - weak
Vitaceae	*Vitis riparia*	B	Frost grape	Stems only - moderate

However, NR metabolism is probably only one of several physiological pathways that plants use to degrade and detoxify TNT and other explosives.

In submersed plants, the majority of groundwater transformation is expected to occur in foliage. In emergents, it is expected to be proportional to the biomass of roots and leaf crowns in contact with the water. Thus, priority was given to submersed aquatic plants and wetland species adapted to standing water, allowing direct contact with groundwater-borne contaminants.

- *Plant communities.* Certain plants are found associated in natural conditions, linked by similar requirements for hydrology, soil type, and climatological conditions. As discussed above, certain associations were noted during the IAAP trip. For example, the grouping of water-plantain and arrowhead with cat-tail and spike-rush in shallow water at the edge of the Marsh site and the combination of sedges, bulrushes, rushes, and grasses in the Wet Meadow area.

- *Ecosystem/wildlife value.* Plants that provide varied habitat and food for highly desirable (e.g., ducks) or less desirable (e.g., muskrats) wetland wildlife species were considered as important in contributing to management goals at IAAP.

- *Life cycle.* Perennial plants usually contribute more aboveground and belowground biomass (living as well as detrital) to a plant community than annuals. In addition, they tend to be physiologically active later and earlier in the seasonal cycle. They also metabolize, although at

Table A3
USEPA-Athens Data on Nitroreductase Concentration of Sampled Plant Species

Species	Nitroreductase Concentration, μg g fresh weight^{-1}	
	Root	Shoot
Alisma subcordatum	184	239
Apocynum sibericum	129	46
Aster novae-angliae	225	260
Carex cf. normalis	95	146
Carex vulpinoidea	135	135
Ceratophyllum demersum[1]	--	87
Cyperus cf. esculentus	9	119
Eleocharis smallii	123	133
Elymus virginicus	106	218
Eupatorium perfoliatum	210	170
Geum canadense	181	109
Juncus cf. tenuis	38	21
Lemna minor[1]	--	83
Potamogeton nodosus	38	28
Penthorum sedoides	130	136
Sagittaria sp.	215	--
Scirpus cyperinus	25	79
Scutellaria lateriflora	200	126
Typha cf. angustifolia	148	198
Vitis riparia	31	42
Sediment from plant roots, Iowa APP	64	
Sediment from Stump Lake, Iowa APP	68	
Myriophyllum aquaticum[1]	--	135

[1] Whole plant concentration.

low levels, during winter, thereby maximizing the relative extent of plant activity throughout the year.

- *Biomass production.* Explosives transformation can be directly correlated to plant biomass. Therefore, highly productive species that are major components of their habitats were candidates for selection

because of their ability to rapidly maximize their relative growth densities.

- *Water depth adaption.* Aquatic and wetland plants may survive in a range of water depths, but most have optimal growth within a narrower regime. Species suitable for ponds, marshes, or wet meadows (Eggers and Reed 1987) will be needed to cover the range of water depths (approximately 1.5 m to periodically inundated shorelines) expected in the proposed constructed wetlands. The life form of the plant, whether emergent, floating leaved, submersed, or free-floating, determines its behavior in relation to hydrology. Therefore, a range of life forms were selected.

- *Root system.* A large perennial root system, with rhizomes or stolons that allow vegetative reproduction, contributes to rapid colonization and biomass increase while having a major role in reducing erosion by holding soil/sediment in place. The extent of water and soil remediation is expected to be correlated to density of the root system in emergent wetland plants.

- *Availability.* Constructed wetlands can be supplied with growing material from a range of commercial nurseries that deal in aquatic and wild plants or from plantings set up specifically to supply a project's needs. Commercial availability was balanced with the desire for locally adapted and locally acceptable material.

Species selection for batch tests. Nine local species were selected for further testing in this project; they are listed in Table A1. Additional IAAP wetland species may be recommended for phytoremediation planting schemes where needed to fulfill specific design and ecological management criteria.

Assessment of Site Conditions and Characterization of Groundwater and Sediment

Climate. The IAAP is located within an area with a midwestern climate of hot, humid summers and cold, wet winters. Summer and winter average temperatures are 23 °C and -3 °C, respectively. The growing season averages 183 days, with the freeze-free season from mid-April to mid-October. The Burlington area is wetter and warmer than most of Iowa. Average precipitation in this area is 1,032 mm, evenly distributed through the year. Snowmelt in spring and severe storms in summer can result in high runoff, surface erosion, and rapid increases in water levels of the IAAP's small impoundments and streams (National Oceanic and Atmospheric Administration 1979).[1]

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[1] Personal Communication, 1995, Joe Haffner, IAAP, Department of Commerce, Burlington, IA.

Sites and specific groundwater composition. Two sites are under consideration to host the future wetland, Line 800 and Line 1. Line 800 is a perched clay pan holding a dome of groundwater. Historic data on the groundwater (1981-1992) show maximum levels of TNT and RDX approaching 2,200 ppb and 14,000 ppb, respectively. It is estimated by the Omaha District that following site excavation and removal, only clean recharge from local precipitation would flow into the perched aquifer. The Omaha District has presented a plan to pump and recirculate water at this site to provide a steady hydrologic regime; the estimated water quality is 20 to 30 ppb TNT. No RDX estimates were presented. Line 1 will be excavated as a long, narrow trench with steep, upper slopes. The historical maximum explosive concentrations for the groundwater inflow into the proposed excavation pit (Brush Creek/groundwater) show that explosives levels vary with manufacturing-associated discharge. Weekly averages are 1,000 ppb TNT, 500 ppb RDX, and 100 ppb HMX.

Sediment. The sediment for the future wetland will be dredged from Stump Lake, a local natural lake. A 45-cm-thick layer will be used to contour the bottom of the excavation site. A chemical characterization is given in Table A3.

Selection and collection of test influent and sediments. Based on a review of the existing analytical data on the monitoring wells at the IAAP, it was determined that Monitoring Well No. G19 would provide a test influent closest to the estimated influent to the proposed wetland. This well had RDX and TNT concentrations of 8,400 ppb and 2,000 ppb, respectively.

During September 7 and 8, Mr. Jerry Miller and Mr. Sidney Ragsdale, WES, arrived at IAAP to collect approximately 9,000 L of groundwater from Well No. G19. Standard well sampling protocol, including three well volume purges, were used to collect the groundwater samples. A stainless steel submersible pump equipped with Teflon lines was used to pump the samples.

Upon pumping of Well G19 on 7 September, a pumping production rate of only 2.3 L per hour was obtained. Based on this observation, Dr. Mark Zappi, WES, and Mr. Ted Streckfuss, Omaha District, were contacted for instructions on whether to continue only pumping from G19 because the time requirements would be lengthy. Upon discussion, Dr. Zappi and Mr. Streckfuss agreed to collect samples from Well No. G20 also. This well had a much higher production yield than did Well No. G19. The intent was to composite the two samples using a 50/50 mixture. The targeted quantity of water was collected along with six buckets of Stump Lake sediments.

The groundwater samples were transported to WES in stainless steel 250-L drums under shaded conditions. The sediments were transported in plastic 20-L buckets under similar conditions. Upon receipt of the samples at WES, the groundwater and sediment samples were stored in a walk-in cooler set at 5 °C until required for experimentation.

Appendix B
Protocol for Enzyme-Linked Sorbent Assay (ELISA)-Based Nitroreductase Field Test, Iowa Army Ammunition Plant[1]

For the Iowa Army Ammunition Center site visit, 50 pretreated beads (USA/Scientific Plastics) were covalently bound with antibody by incubation in 1 mL 200 mM Na cyanoborohydrate containing 5 μL antibody produced against a sediment-derived nitroreductase (NR). All remaining immuno sites were blocked with a 5-percent nonfat milk solution.

In the field, 1 g of plant material was macerated in 1 mL 20-percent glycerol in 0.5 M KCI, using an automated Teflon microfuge-tube pestle. Plant extract (150 μL) was added to an NR antibody bead in a 1.5-mL screw-cap tube and mixed for 30 sec. The bead was then washed 5 $\times$ with 10 mM Tris, pH 8.

A second antibody conjugated to horseradish peroxidase (HRP), at a final dilution of 1:1000 in 100 mM PO_4, pH 7, was added to the bead and mixed for 30 sec, then washed as before.

To visualize the presence of the antigen (the NR in the plant extract), a substrate for HRP was added. This consisted of 3 μL of 30 percent H_2O_2 and 1 mg tetramethylbenzidine-HCI per 10 mL of 100 mM PO_4- citrate buffer, pH 6. This substrate buffer (150 L) was added to the bead in the tube, and enzyme activity was evaluated based on the development rate and the intensity of the blue color formed.

Forty-two plants were evaluated onsite using this method. Whole-plant specimens of 19 species that showed moderate to high activity NR were shipped to USEPA-Athens for further evaluation.

[1] Based on the protocol provided by Dr. Laura Carreira, Dyn Corporation, c/o USEPA-Athens.

Appendix C
Analytical Specifications, Calibration Compounds, and Method References

High Performance Liquid Chromatography Analysis of Explosives in Water

Samples (100 mL) were first concentrated using solid phase extraction (SPE) (Waters RDX cartridges, catnr 47220; SPE; Jenkins et al. 1995).[1] Subsequently, explosives were eluted from the cartridges using acetonitrile. The samples were evaporated to dryness using N_2, redissolved in a 2-mL mixture of acetonitrile:water (50/50 v/v), and subsequently analyzed using high performance liquid chromatography (HPLC).

HPLC separations were performed on a Hewlett-Packard 1090 Series 2/M with ChemStation (Pascal Series) liquid chromatograph equipped with a diode array detector (Series 2), PV5 ternary solvent delivery system, thermostatically controlled column compartment, autosampler, auto-injector and reverse phase analytical C18 column (5 μm, 100- by 4.6-mm inner diam) and ODS guard column (5 μm, 20- by 4.0-mm inner diam). The column compartment was operated at 40 °C, and the flow rate of the mobile phase was 1.5 mL min^{-1}. The composition of the mobile phase was 68 percent 20 mM NH_4Cl and a 32-percent mixture of methanol and n-butanol (98:2, respectively).

The compounds used for the calibrations were as follows:

a. RDX (obtained from NEN Research, Boston, MA).

b. 1,3-Dinitrobenzene; 2,4-Dinitrotoluene; 2,6-Dinitrotoluene; 5-Nitro-1,3-Dimethylbenzene (Aldrich Chemical Company, Milwaukee, WI).

[1] References cited in this appendix are located at the end of the main text.

c. 1,3,5-Trinitrobenzene; 2,4,6-Triaminotoluene; 2,4,6-Trinitrotoluene; 2-Nitrotoluene; 3-Nitrotoluene; 4-Nitrotoluene; Nitrobenzene (Chem Service Chemicals, West Chester, PA).

d. 2,4-Diamino-6-Nitrotoluene; 2,6-Diamino-4-Nitrotoluene; 2-Amino-4,6-Dinitrotoluene; 4-Amino-2,6-Dinitrotoluene; 4-Hydroxyamino-2,6-Dinitrotoluene, and the azoxy compounds: 4,4',6,6'-Tetranitro-2,2'-Azoxytoluene and 2,2',6,6'-Tetranitro-4,4'-Azoxytoluene (Dr. R. Spanggord, SRI International).

Appendix D: Comparison of Calculation Procedures for Explosives Decrease Rates in Groundwater

The equations describing the explosives decrease rates in the present report have been found by regression. Exponential regression fitted the TNT decrease rates best, and the equation describing this fitted curve is

$$Y = \exp(a + bX)$$

where

Y (μg L^{-1}) = explosives concentration at a given X

a = intercept, μg L^{-1}

b = curve slope, hr/μg L^{-1}

X = time, hr, at a given Y

This equation was used to calculate the time required to reach a cleanup level of 2 μg L^{-1} TNT. For example for pondweed, where the regression showed an intercept of 6.407 and a slope of -0.037, the time required was 154 hr, or 6.4 days (Table 6, main text). Using this equation to calculate the initial concentration of the groundwater yields a value of 3.162 hr, which is slightly higher than 0 hr. This difference originates from the data scatter early in the incubation period, possibly causing a small deviation of the fitted curve (Figure 1, main text).

Linear regression fitted the RDX decrease rates best, and the equation describing this fitted curve is

$$Y = a+bX$$

where

Y (μg L^{-1}) = explosives concentration at a given X

a = intercept, μg L^{-1}

b = curve slope, hr/μg L^{-1}

X = time, hr, at a given Y

This equation was used to calculate the time required to reach a cleanup level of 2 μg L^{-1} RDX. For example for pondweed, where the regression showed an intercept of 12,694 and a slope of -3.714, the time required was 3,417 hr, or 142 days (Table 10, main text). Using this equation to calculate the initial concentration of the groundwater yields a value of -24.5 hr, which is slightly lower than 0 hr. This difference originates from the substantial data scatter early in the incubation period, possibly causing a deviation of the fitted curve (Figure 2, main text).

If the explosives decrease rates depend directly on plant mass rather than on plant activity (but this is not yet known), normalizing the curve slope for weight of the plant parts in contact with the groundwater can be useful. Fresh weight is then the parameter to express plant mass in; dry weight:fresh weight ratios of submersed plants are usually 5-7 percent, in below-ground parts of emergent plants, 30-50 percent, and in aboveground parts, 10 percent (with root:shoot ratios varying per species and per season). If the explosives decrease rates depend directly on plant activity, however, normalizing the curve slopes for total plant mass, expressed in organic matter (dry weight), is more logical.

Another possibility to calculate explosives decrease rates over time is to model them following the first order kinetics equation

$$ln(C/C_0) = -KT$$

where

C = concentration at time T, day

C_0 = initial explosives concentration

K (day^{-1}) = rate constant determined as slope of $ln(C/C_0)$, mg L^{-1}, plotted against time

This equation generates K values in the present case, independent of plant biomass. However, the quantities of plant biomass incubated for the various species varied, and normalization of the K values for these biomass differences is desirable because then it becomes relatively easy to calculate wetland retention time for cleanup. If K and C_0 are known parameters and a target concentration of an explosive is given as C, the time required to decrease the concentration from C_0 to C is given by $T = 1n(C/C_0)/K$ (K having a negative sign). It is noted that the question remains whether this has to be on basis of total fresh weight, or dry weight, or on water-contacted fresh weight, or -dry weight. The first order kinetics calculation procedure was used to calculate the K value for pondweed. C_0 was 681 μg L^{-1} and C was 230 μg L^{-1} after 1 day of incubation (Table 4, main text). A K value of 1.085 day^{-1} was found at incubation of 3.73 g DW in a 3.375-L water volume, being synonymous with 62.2 g FW. L^{-1}. Using the thus-found K value to calculate the time required to reach the 2-μg L^{-1} explosives level yielded 5.4 days; the latter value is shorter than the 6.4 days found using the regression calculation procedure presented above.

An attempt to normalize the K values on plant biomass has been presented by Anonymous (1995).[1] It was assumed that the relationship between K and plant weight was simply as follows:

$$k = K/P$$

where

P = plant weight, g FW. L $water^{-1}$

k = a species-characteristic constant, L.g $FW^{-1}.day^{-1}$

Using this relationship, a k value of 0.059 L.g $FW^{-1}.day^{-1}$ was found for pondweed.

[1] References cited in this appendix are located at the end of the main text.

Appendix E
Abbreviations

2ADNT	2-amino-4,6-dinitrotoluene
4ADNT	4-amino-4,6-dinitrotoluene
2,4DANT	2,4-diamino-6-dinitrotoluene
2,6DANT	2,6-diamino-6-dinitrotoluene
DNB	dinitrobenzene
1,3DNB	1,3-dinitrobenzene
1,4DNB	1,4-dinitrobenzene
DNT	dinitrotoluene
2,4DNT	2,4-dinitrotoluene
2,6DNT	2,6-dinitrotoluene
NB	nitrobenzene
2NT	2-nitrotoluene
3NT	3-nitrotoluene
4NT	4-nitrotoluene
NT	nitrotoluene
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
TADNTs	total monoamino-dinitrotoluenes (= 2ADNT, 4ADNT)
TDANTs	total diamino-nitrotoluenes (= 2,4DANT, 2,6DANT)
TDNBs	total diamino-nitrotoluenes (= 1,4DNB, 1,3DNB)
TNB	trinitrobenzene
TNT	2,4,6-trinitrotoluene

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	January 1997	Final report

4. TITLE AND SUBTITLE
Screening of Aquatic and Wetland Plant Species for Phytoremediation of Explosives-Contaminated Groundwater From the Iowa Army Ammunition Plant

5. FUNDING NUMBERS

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
AScI Corporation, 3402 Wisconsin Avenue, Suite 5, Vicksburg, MS 39180-5378;
U.S. Army Engineer Waterways Experiment Station, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199

8. PERFORMING ORGANIZATION REPORT NUMBER
Technical Report EL-97-2

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Engineer District, Omaha
215 North 17th Street
Omaha, NE 68102-4978

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Available from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

A study was performed to quantify the ability of 10 submersed and emergent macrophytes to phytoremediate explosives-contaminated groundwater from the Iowa Army Ammunition Plant (IAAP). Species evaluated under hydroponic batch conditions were the submersed *Potamogeton nodosus* Por. (American pondweed) and *Ceratophyllum demersum* L.(coontail) and the emergent *Alisma subcordatum* Raf. (water-plantain), *Sagittaria latifolia* Willd. (common arrowhead), *Carex vulpinoidea* Michx. (fox sedge), *Scirpus cyperinus* (L.) Kunth (wool-grass), *Eleocharis obtusa* (Wiil.) (blunt spikerush), *Phalaris arundinacea* L. (reed canary grass), and *Typha angustifolia* L. (narrow-leaf cat-tail). Parrot-feather (*Myriophyllum aquaticum* (Vell.) Verdc.) was included in the evaluation to provide a comparison between the present study and other similar evaluations. The effects of sediments, native and heat-inactivated, on the explosives in the aqueous phase were also examined. The impact of a decrease in temperature from 25 to 10 °C on contaminant disappearance was assessed in three species and controls. TNT and RDX levels in the tested groundwater were 681 and 12,785 $\mu g\ L^{-1}$.

The results of this study indicate that the presence of plants did enhance TNT and TNB removal from IAAP groundwater. Most effective at 25 °C were reed canary grass, coontail, and pondweed. Groundwater and plant

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14. SUBJECT TERMS	15. NUMBER OF PAGES
See reverse.	72
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED		

13. (Concluded).

tissue analyses indicate that in the presence of the tested plants, TNT is degraded to reduced by-products and to other metabolites that were not analyzed. TNT removal was best modeled using first-order kinetics, with rate constants at 25 °C incubations ranging from 0.038 $\mu g\ L^{-1}\ hr^{-1}$ for reed canary grass to 0.012 $\mu g\ L^{-1}\ hr^{-1}$ for parrot-feather. These kinetics predict hydraulic retention times (HRTs) ranging from 4.9 days to 19.8 days to reach a TNT concentration of 2 $\mu g\ L^{-1}$. Decreasing incubation temperature to 10 °C affected reed canary grass more than parrot-feather, increasing estimated HRTs by factors of four and two, respectively.

The plant species tested showed a far lower potential for RDX removal from the IAAP groundwater. Most effective at 25 °C were reed canary grass and fox sedge. Analyses of plant material indicated the presence of RDX in underwater plant portions and in aerial plant portions, and RDX accumulation in the latter. RDX removal was best modeled using zero order kinetics, with rate constants for the 25 °C incubation ranging from 13.45 $\mu g\ L^{-1}\ hr^{-1}$ for reed canary grass to no removal in four species. Based on these kinetics, estimated HRTs to reach 2 $\mu g\ L^{-1}$ RDX increased from 39 days. Decreasing the temperature to 10 °C increased HRT 24-fold for reed canary grass.

By using a biomass-normalized K value, submersed plants are identified as having the highest explosives-removing activity (μg explosive $L^{-1}\ hr^{-1}\ g\ DW^{-1}$). However, biomass production of submersed plants is normally 5 to 10 times less than that of emergent plants per unit area; thus, in plant selection for wetland construction, both explosives-removal potential and biomass production are important determinants.

14. Subject Terms (Concluded).

Batch culture
Emergent aquatic plants
Explosives
Groundwater
Phytoremediation
RDX
Sediment
Submersed aquatic plants
TNT

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