#79576

FIELD SUMMARY REPORT

PHYTOREMEDIATION STUDY OF BURNING GROUND SPRING WETLAND

2000-2001

IT CORPORATION

AUGUST 2002

3658

Table of Contents

1.0	Introduction
1.1	Site Contamination
1.2	Objectives
2.0	Literature Review
3.0	Methods
3.1	Field Sampling
3.2	Laboratory Uptake Experiments
3.3	Analytical Procedures
4.0	Results and Discussion
4.1	Description of Burning Ground Spring Natural Wetland
4.2	Behavior of RDX in the Wetland
4.3	RDX Uptake Experiments Using Nimulus and Torreyochloa
5.0	Conclusions and Recommendations
6.0	References

List of Tables

Table 1 HE and Metabolites in Water Samples from September 2000
Table 2 HE and Metabolites in Water Samples from April 2001
Table 3 HE and Metabolites in Water Samples from June 2001
Table 4 HE and Metabolites in Water Samples from October 2001
Table 5 RDX Concentration in Quarterly Surface Water Samples
Table 6 Redox Measurements at Burning Ground Spring Wetland
Table 7 Plant Species Tested for RDX and Metabolites
Table 8 Concentration of RDX in Laboratory Plants

List of Figures

Figure 1 Map of Burning Ground Spring wetiand sample locations
Figure 2 Reactors used to test RDX uptake by Mimulus
Figure 3 Reactors used to test RDX uptake by Torreyochloa
Figure 4 Water flow through Burning Ground Spring wetland
Figure 5 Channelized flow at Burning Ground Spring wetland
Figure 6 RDX concentration in Burning Ground Spring wetland over time
Figure 7 Decrease in mass of RDX over time due to uptake by Mimulus
Figure 8 Decrease in mass of RDX over time due to uptake by Torreyochloa

1.0 INTRODUCTION

This report presents the results of a phytoremediation pilot study conducted in support of the PRS 16-021(c)-99 Corrective Measure Study (CMS). The phytoremediation study is a component of the PRS 16-021(c)-99 CMS and is one of several Bench-and-Pilot studies that have been or will be conducted to address various site contaminant concerns.

A RCRA facility assessment (RFA) and two phases of a RCRA facility investigation (RFI) have been conducted on PRS 16-021(c) (LANL 1990, LANL 1996, and LANL 1998). The results of these investigations have demonstrated the need for corrective action at this site. The Building 16-260 outfall drainage channel is known to have contained a large volume of highly contaminated material and is thought to be the principal source of contamination impacting the springs and associated alluvial system in Cañon de Valle.

1.1 Site Contamination

Contaminants known to be present in the 260 drainage area include barium, cyclonite (RDX), 2,4,6-trinitrotoluene (TNT), 1,3,5,7-tetranitro-1,3,5,7-tetrazacyclooctane (HMX), dinitrotoluene (DNT), amino-DNT, 1,3,5-trinitrobenzene (TNB), acetone, chloromethane, dichloroethane, isopropyltoluene, tetrachloroethene, trichloroethene, anthracene, bis(2-ethylhexyl)phthalate, butylbenzylphthalate, copper, cadmium, chromium, cobalt, lead, nickel, silver, vanadium, uranium, and zinc. High explosives (HE), barium, and low levels of other contaminants have also been measured in Cañon de Valle waters, the R-25 regional aquifer monitoring well, nearby shallow (approximately 17 to 80 ft deep) boreholes, and TA-16 springs. The TA-16 springs include: Martin Spring, SWSC spring, and Burning Ground spring.

Chemical analyses of the TA-16 springs were compared to levels observed from springs issuing from Tschicoma Dacite and the Tshirege member of the Bandelier tuff outside of Los Alamos National Laboratory (LANL) boundaries (LANL, 1998). The TA-16 springs showed concentrations of several major cations and anions greater than the surrounding spring levels (LANL, 1998). Low levels of VOCs have been detected at all three springs. These VOC detects were sporadic, occurring primarily during the quarterly sampling round of June 1997.

Time-series analysis of the springs data suggests extreme variability in the concentration of constituents (up to a factor of 20 in RDX concentration at Martin Spring). Similarities in element variability and flow rate changes over time indicate that SWSC Spring and Burning Ground Spring are hydrogeologically similar, but that Martin Spring probably represents a different hydrogeological system (LANL, 1998).

Human health and ecological screening assessments performed as part of the Phase II RFI identified barium, bis(2-ethylhexyl)phthalate, HMX, RDX, and 2,4,6-TNT as the contaminants of potential concern (LANL 1998). RDX due to toxicity and HMX due to high concentrations present the most significant potential risks to human health and the environment.

1.2 Objectives

High explosives have been measured in the waters of the three springs at TA-16. These contaminants pose a potential risk to human health and the environment. Different corrective measures are being explored to reduce the contaminants in the springs. At Martin Spring, a pair of stormwater filters have been installed. The first unit contains granular activated charcoal (GAC) to treat high explosives and the second unit contains ion exchange resin to treat barium. This treatment system requires regular maintenance and media regeneration to ensure proper function.

As an alternate to a constructed treatment system, treatment by natural means is also being explored. High explosives have been shown to degrade in constructed wetlands (Sikora et al., 1998). It is likely that natural wetlands are also capable of reducing explosives contamination. At Burning Ground spring there is a 200 m^2 wetland area between the spring outlet and the confluence with the main Cañon de Valle channel. This wetland is the focus of a study to investigate the potential for natural attenuation of RDX. Concentrations of the parent compounds and primary metabolites were monitored at several locations within the wetland. The study also examined the ability of two dominant plant species to take up RDX. This investigation will help understand the processes occurring in the wetlands and their effect on HE degradation in the Cañon de Valle system. Specific objectives were to:

- Monitor levels of RDX and TNT across the Burning Ground Spring wetland and determine if a significant reduction in parent compound concentration by wetland plants and microbes could be detected.
- Monitor concentrations of primary metabolic breakdown products (MNX, TNX, 2ADNT, and 4ADNT) to help determine if degradation of RDX and TNT is occurring in the wetlands.
- Observe seasonal trends in HE concentration and wetland performance.
- Conduct laboratory studies with selected wetland plant species present at the Burning Ground Spring site and determine if they are capable of taking up RDX.

The overall objective of the study was to assess the effectiveness of natural wetlands as a treatment technology for the HE contaminated waters present in Cañon de Valle.

2.0 LITERATURE REVIEW

Phytoremediation of explosives by wetland plants has been examined in laboratory studies and field demonstrations. Several wetland plant species are known to uptake of TNT and RDX. These plant species include: sago pondweed (*Potamogetonpectinatus L.*), water stargrass (*Heteranthera dubia*), elodea (*Elodea canadensis*), parrotfeather (*Myriophyllum aquaticum*) reed canary grass (*Phalaris arundinacea L.*), wool grass (*Scirpus cyperinus*), and sweetflag (*Acorus calamus L.*) (Best et at, 1999). Wetland plants have also been shown to transform TNT to 2-amino-4,6-dinitrotoluene (2ADNT) and 4-amino-2,6-dinitrotoluene (4ADNT) (Best et al., 1997). Recent studies with 14C-labeled RDX indicate that reed canary grass can convert RDX to formaldehyde (Just and Schnoor, 2000).

Natural wetlands often contain both aerobic and anaerobic sediment zones (Mitsch and Gosselink, 1993). As a result, both oxidative and reductive bacterial degradation may occur in addition to plant uptake. Anaerobically, TNT is converted to 2-amino-4,6-dinitrotoluene (2ADNT) and 4-amino-2,6-dinitrotoluene (4ADNT), and sometimes to the diamino compounds 2,4-diamino-6-nitrotoluene (24DANT) and 2,6-diamino-4- nitrotoluene (24DANT). Aerobic TNT biodegradation can result in 2,6-dinitrotoluene (26DNT), 2,4-dinitrotoluene (24DNT), 2-nitrotoluene (2NT), 4-nitrotoluene (4NT), and 3-nitrotoluene (3NT). Under anaerobic conditions, bacteria can sequentially reduce RDX to a mononitroso metabolite NINX (hexahydro-l-nitroso-3,5-dinitro-1,3,5-triazine) and the trinitroso metabolite TNX (hexahydro-1,3,5-trinitroso-1,3,5-triazine).

A field demonstration conducted at Milan Army Ammunition Plant (MAAP) showed that phytoremediation of high explosives using constructed wetlands is feasible (Sikora et al., 1998). Two different systems were tested: a lagoon system with surface flow and a gravel bed system with subsurface flow. Both systems were designed with an influent flow rate of 5 gallons per minute and a hydraulic retention time of 10 days. Groundwater contaminated with HE was pumped from wells to the system.

The lagoon system contained sago pondweed, water stargrass, elodea, and parrotfeather in two cells arranged in series. This system was able to degrade TNT to metabolites 2ADNT, 4ADNT, and 24DANT. The TNT concentration was reduced by more than 85 percent under most conditions, while TNT by-product concentrations increased during the experiment. The removal efficiency of RDX in the lagoon system was much lower, only 25 percent. RDX is mainly degraded under anaerobic conditions, which were not prevalent in the lagoon cells. Known RDX metabolites hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) were not detected except in a single sample (Sikora et al., 1998).

The gravel bed system contained canary grass, wool grass, sweetflag, and parrotfeather in an anaerobic cell followed by an aerobic cell. The gravel-based system reduced TNT concentrations from 1,250 ppb to less than 2 ppb. Total nitroaromatic concentrations, including RDX and HMX, were reduced from 3,200 ppb to 50 ppb except during the winter months. TNT concentrations were reduced to the detection limit in 1.7 days during summer months and 3.3 days during winter months. RDX removal was slower, and complete removal occurred only during the summer. RDX was degraded to metabolites MNX and TNX. The decrease in removal efficiency during the winter was attributed to reduced microbial activity at lower temperatures. Less than two percent of the added explosives accumulated in the gravel, sediment, or plant tissues, indicating that microbes and/or plants degraded the explosives (Sikora et at., 1998).

3.0 METHODS

3.1 Field Sampling

Field samples at the Burning Ground Spring wetland were collected in September 2000, April 2001, June 2001, and October 2001. Sampling in January 2001 was cancelled because plants

were buried by snow. The four sample periods are representative of the high and low flow conditions as well as spring, summer, and fall seasonal conditions. Regionally on an annual basis since 1997 there has been less than average precipitation.

During each event, samples of surface water and sediment were collected at 22 locations within the wetland. Figure 1 shows a plan view of the sample locations. At each location soil and water samples were analyzed for high explosives. Water samples were collected as grab samples from flowing water. Sediment samples of the top six inches were collected at the same locations as water samples. In addition, dry soil samples were taken from the island and at two other locations outside the wetland. Two duplicate samples were collected, one at the spring outlet and one just before the confluence with the main channel.

There were additional samples collected from the spring outlet (influent) and from the point just above the confluence with the main channel (effluent). These samples were collected as part of an ongoing study and were analyzed for: alkalinity, perchlorate, anions, high explosives, high explosive breakdown products, metals (filtered and unfiltered), nitrogen isotope, low level tritium, and oxygen isotope.

Redox measurements were taken at four locations in the wetland: influent, SW-7, SW-12, effluent (Figure 1). Redox potential is the tendency of a medium to oxidize or reduce substances. Measurements were taken with a redox probe and portable mV meter. At each location, redox was recorded for three depths; one with the probe suspended in the surface water, one with the probe tip at the sediment surface and one with the probe approximately 2 inches below the sediment surface. Before and after each quarterly sampling trip, the probe calibration was verified using the recommended calibration solutions.

Three plant samples were collected at each of three locations (two within the wetland and one on the island) for a total of nine samples per trip. Sample locations are shown on Figure 1. Whole plants, including roots, were removed from the ground with a spade or shovel, rinsed to remove soil in the root zone, and frozen in Ziploc bags. Plants species collected at location P1 were smooth monkey flower (*Mimulus glabratus*), false manna grass (*Torreyochloapauci flora*) and an unidentified bryophyte. Plant species collected at location P2 were creeping bentgrass (*Agrostis palustris*), currant gooseberry (*Ribes montegenum*) and wild raspberry (*Rubus stigosus*). Plant species collected at location P3 were smooth monkey flower (*Mimulus glabratus*), false manna grass (*Torreyochloa paucijlora*) and white checkermallow (*Sidalcea candida*). Snow cover during the April 2001 sampling event prevented sampling of plants on the island (location P2) so only six plant samples were collected during this event.

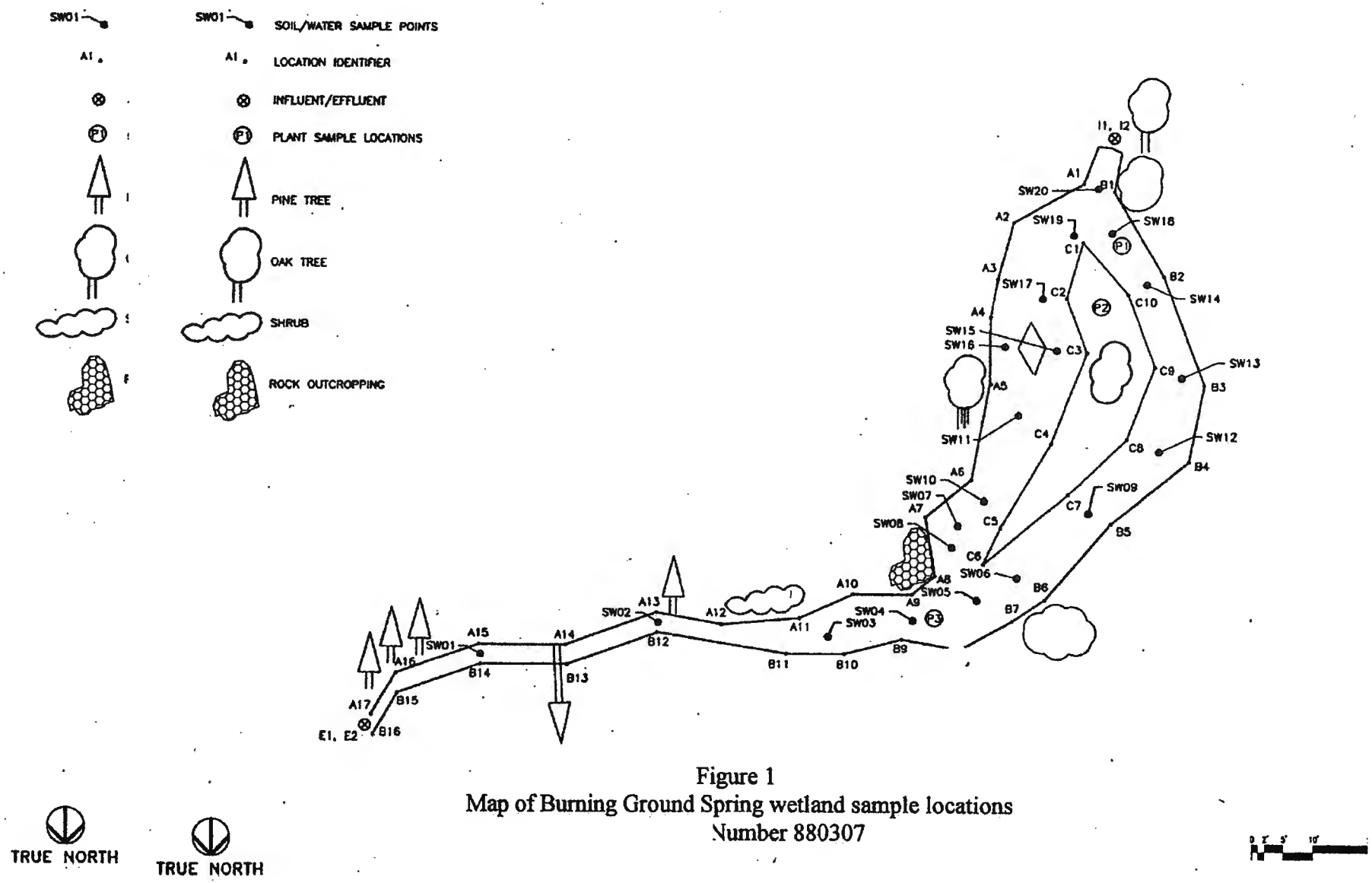


Figure 1
Map of Burning Ground Spring wetland sample locations
Number 880307

3.2 Laboratory Uptake Experiments

Laboratory tests were conducted with two of the dominant species from the site to check the ability to take up and/or degrade RDX. These tests were conducted under controlled environmental conditions. The plant species were exposed to different concentrations of RDX and both plant and media were monitored for RDX uptake or breakdown product.

Plants in the wetland were identified by Dorothy Hoard in October 2000, and two species of interest were selected for use in laboratory studies. The two main plants growing in the wetland stream channel are smooth monkey flower (*Mimulus glabratus*) and false manna grass (*Torreyochloa paucijlora*). A source of seeds/plants for these exact species could not be found, but two closely related species were ordered for laboratory uptake studies: *Mimulus guttatus* and *Torreyochloapallida.*

Mimulus plants were grown from seed in soil and the small plants were transferred to hydroponic solution. Torreyochloa plants were obtained as full grown bare-root plugs and were allowed to acclimate in hydroponic culture for several weeks before use in experiments. During uptake experiments, plants were grown hydroponically in glass Erlenmeyer flasks wrapped in tin foil (Figures 2 and 3). Triplicate plants were weighed and then dosed with Hoagland's solution containing 1 mg/L RDX. Triplicate control reactors were set up without plants to monitor non-plant losses such as sorption to glass or photolysis. Triplicate plants grown in undosed Hoagland's solution provided unexposed plant material for background analysis. Water use was monitored gravimetrically, and water samples were taken daily for High Performance Liquid Chromatography (HPLC) analysis. Hoagland's solution taken up by the plants was replaced as needed with undosed solution.

3.3 Analytical Procedures

Water, sediment, and plant samples were analyzed at the University of Tennessee for TNT, RDX, and metabolites including 2ADNT, 4ADNT, MNX, and TNX. Surface water and sediment samples were analyzed using EPA Method 8330: Nitroaromaties and Nitramines by HPLC analysis. This EPA method has also been successfully used to identify RDX metabolites hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-S-nitro-1,3,5-triazine (DNX), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) (Pennington et al., 1999). Plant tissue samples were analyzed using a modification of the EPA method (Larson et al., 1999). Plant tissues were homogenized then freeze-dried and extracted in acetonitrile. Samples were filtered through a florisil and alumina column prior to remove pigments prior to HPLC analysis. All water, soil, and plant extracts were run on a HPLC equipped with a Supelco LC-18 column.

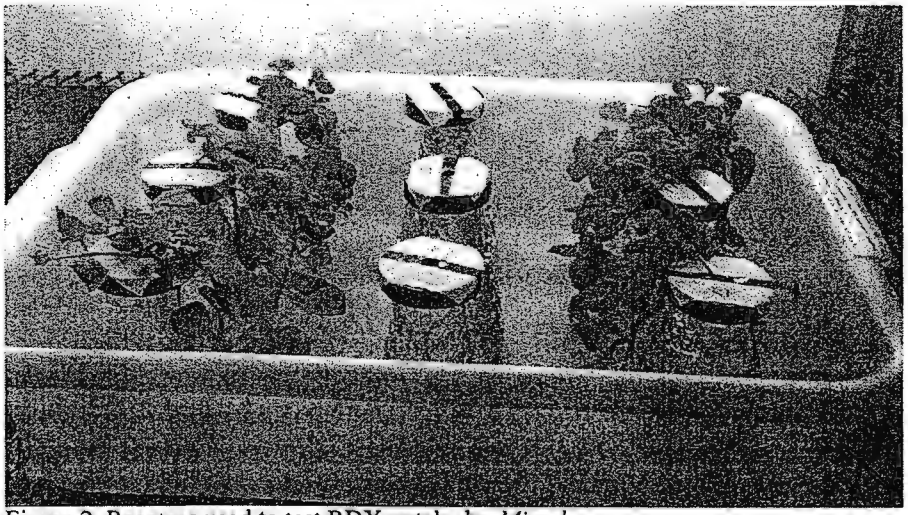
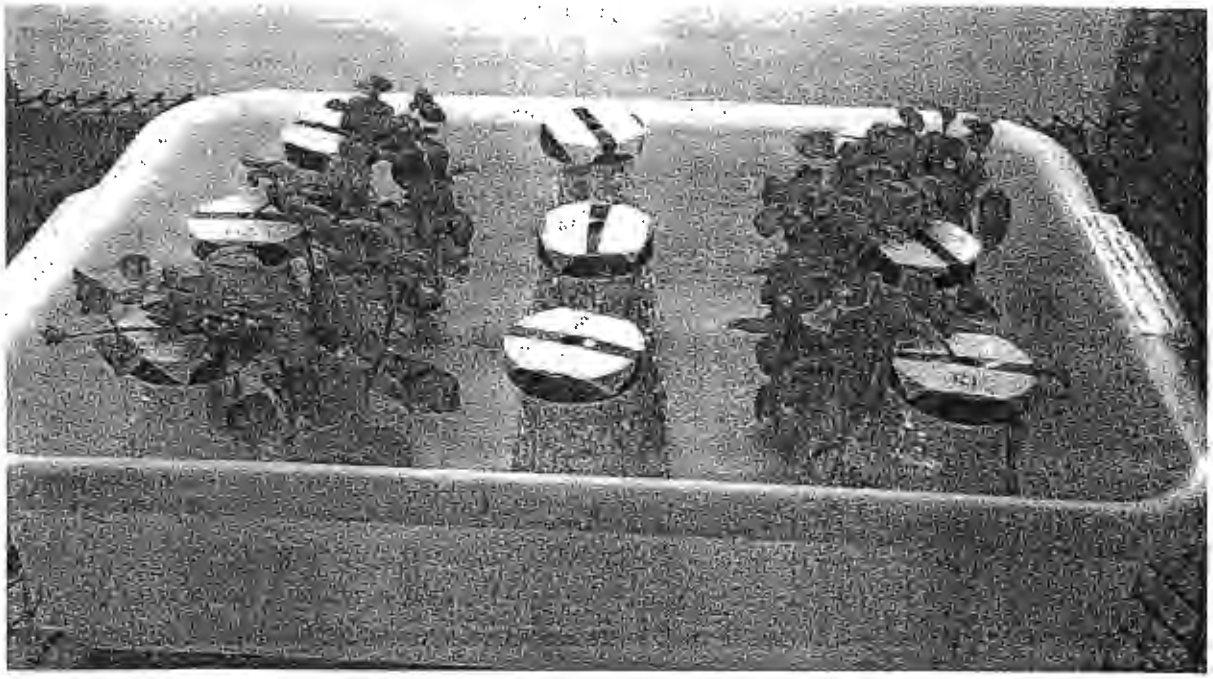


Figure 2 Reactors used to test RDX uptake by *Mimulus.*



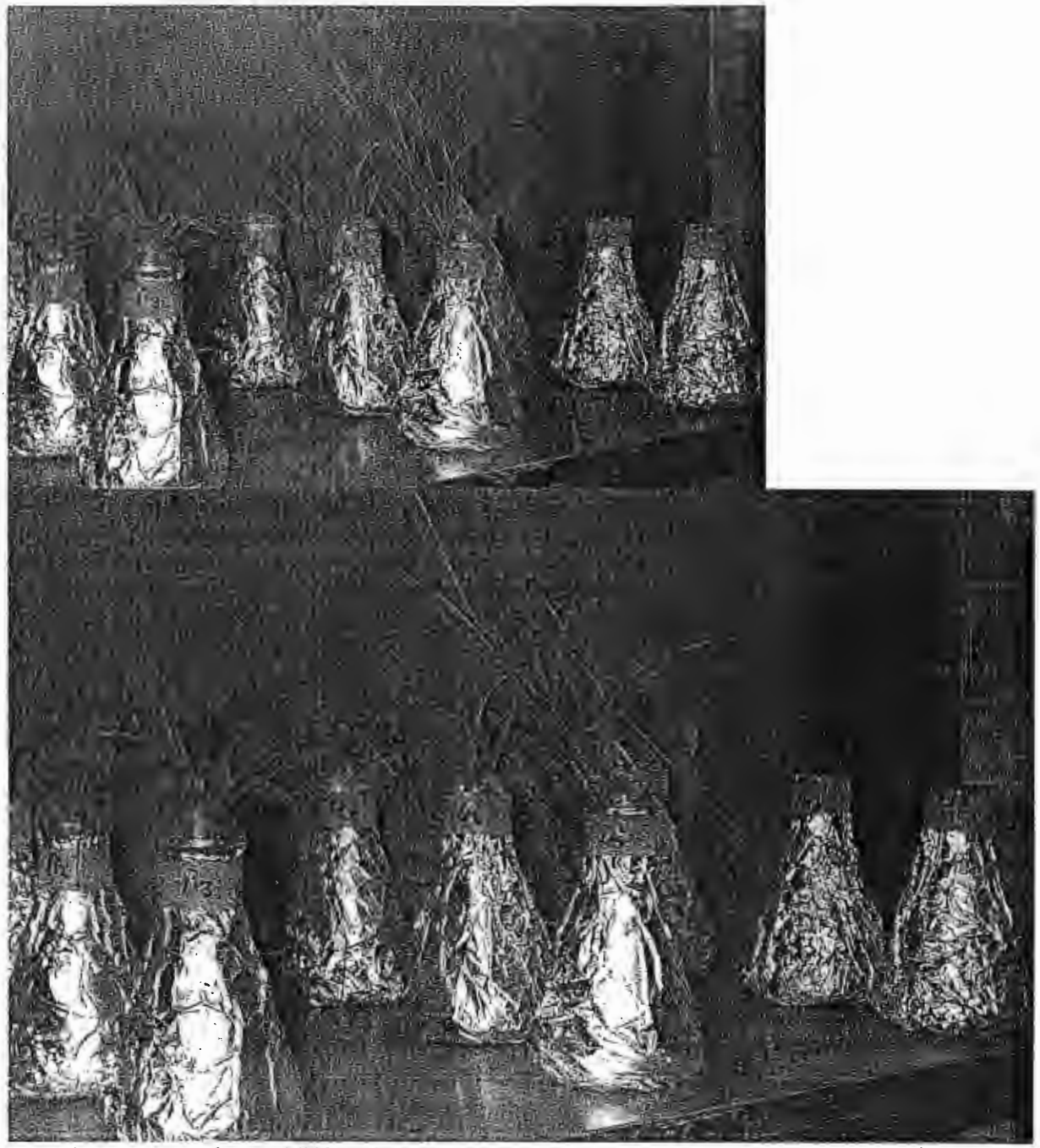


Figure 3: Reactors used to test RDX uptake by *Torreyocha*.

4.0 RESULTS AND DISCUSSION

4.1 Description of Burning Ground Spring Natural Wetland

The natural wetland examined in this report is apparently fed exclusively by water from Burning Ground spring. The spring is located in Cañon de Valle, and is thought to issue from perched water bearing zones in Bandelier Tuff in the vicinity of the TA-16-260 outfall. Water flows over a weir and into the wetland area. The area is densely vegetated with Mimulus and Torreyochloa during the growing season. The first one hundred feet of the wetland is wider and shallower; flow is divided around a central drier island. There are several areas where the water becomes pooled and stagnant. After the first hundred feet, the water becomes channelized and the flow velocity increases. This second half of the wetland contains more rocks and gravel, with fewer plants. Figures 4 and 5 illustrate the flow of water through the wetlands.

In October 2001, the flow rate at the beginning and end of the wetland was compared. The flow rate over the weir at the wetland influent was 0.5 L/s, whereas the effluent was 0.225 L/s. Thus, from the beginning to the end of the wetland the flow rate was reduced by about half. The flow was reduced again from the end of the wetland to the confluence with the Cañon de Valle channel, where the flow rate was measured as 0. 175 L/s. These flow rate reductions could be due to losses from evapotranspiration and/or infiltration within the wetland area.

Retention time in the wetland is likely to be an important factor in phytoremediation and/or microbial degradation of HE. Calculating the wetland volume and dividing by the flow rate over the weir estimated retention time in the wetland. The area of the wetland as mapped was approximately 195 m^2. A depth of five centimeters was assumed throughout the wetland based on water depths observed during field sampling activities, resulting in a volume of 10 m^3. Average annual flow rate is approximately 0.5 L/s (0.0157 cfs) (LANL, 1998). Based on these values, the approximate retention time in the wetland was six hours. Constructed wetlands to treat HE typically have retention times on the order of a week to ten days rather than a few hours. Altering the retention time may improve the effectiveness of phytoremediation and biodegradation.

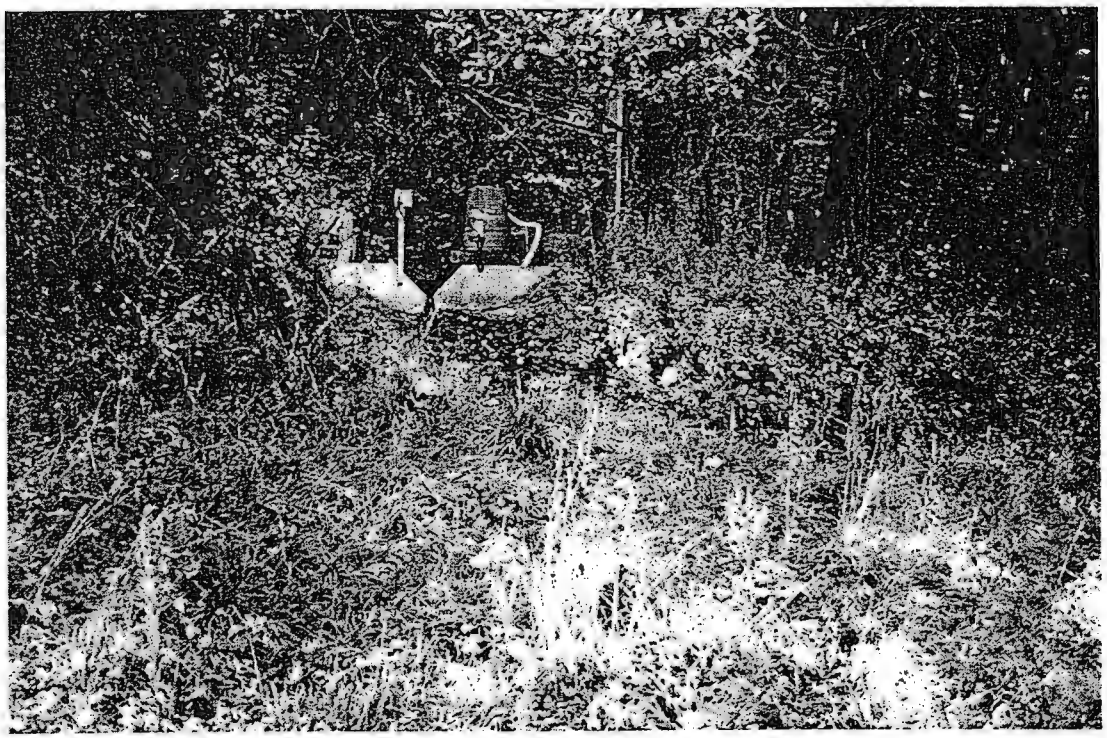


Figure 4a: Burning Ground Spring weir outlet. View looking up gradient.

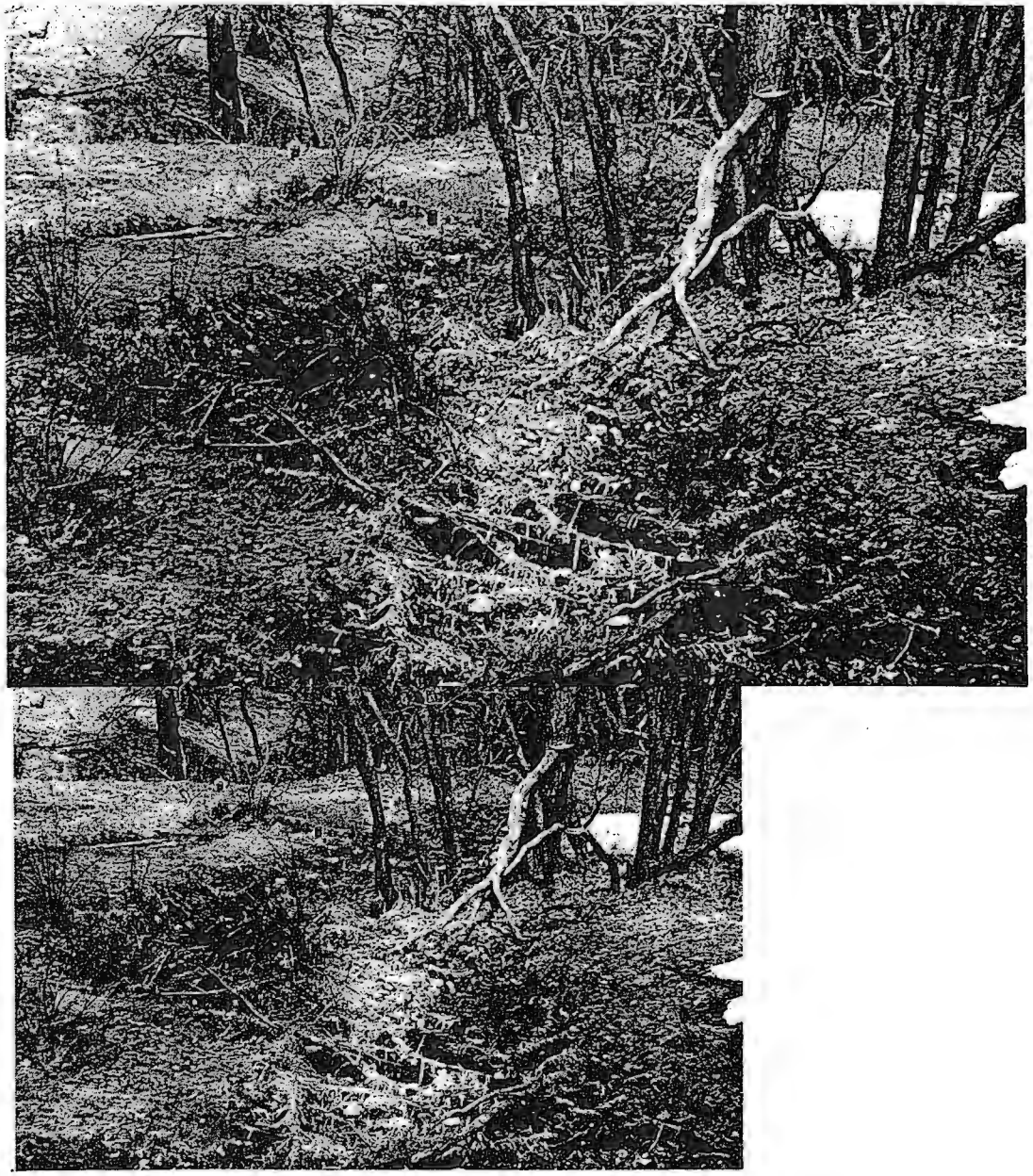


Figure 4b: Water flow through Burning Ground wetland area. View looking downgradient towards Cañon de Valle mainchannel

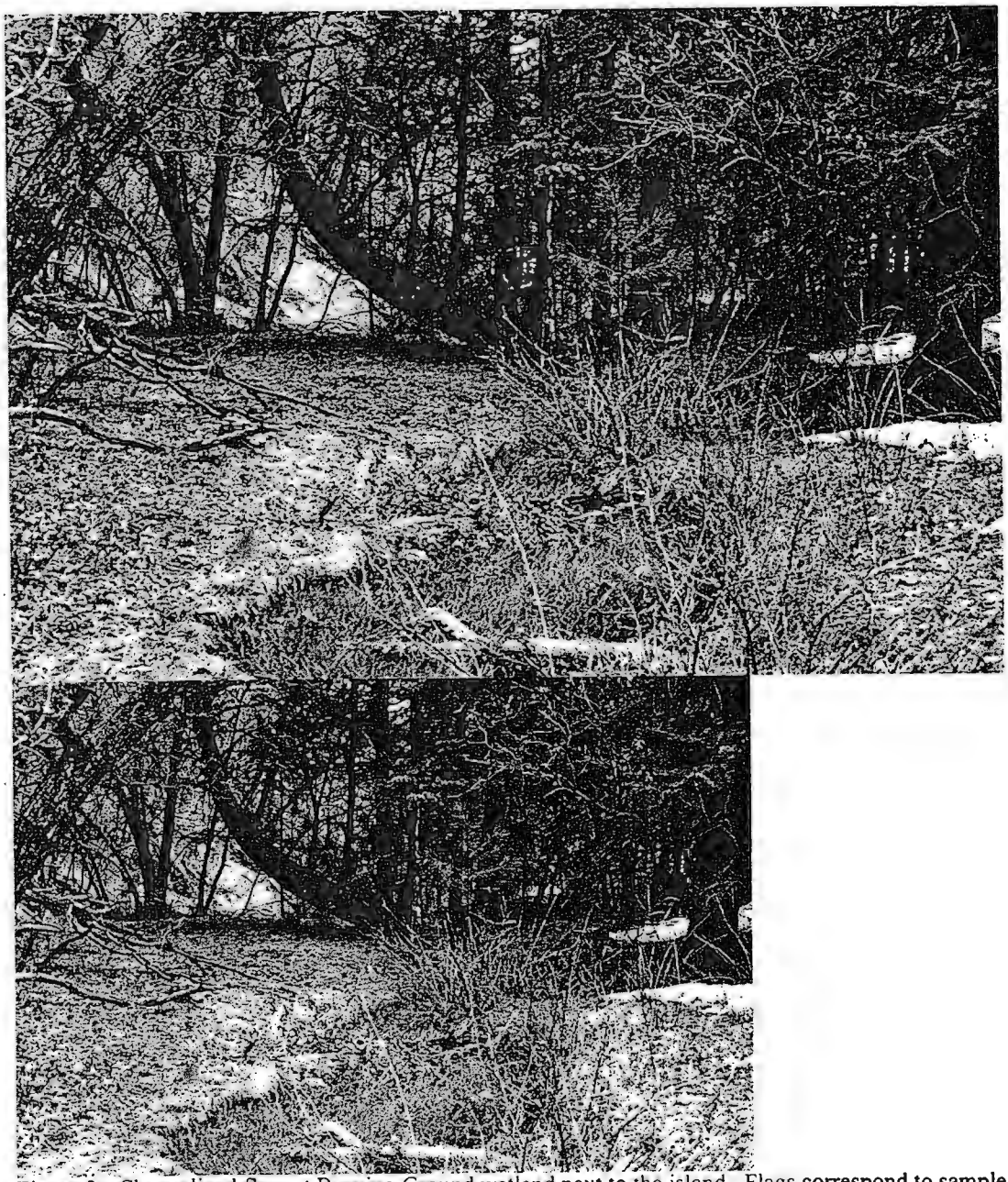


Figure 5a: Channelized flow at Burning Ground wetland next to the island. Flags correspond to sample locations.

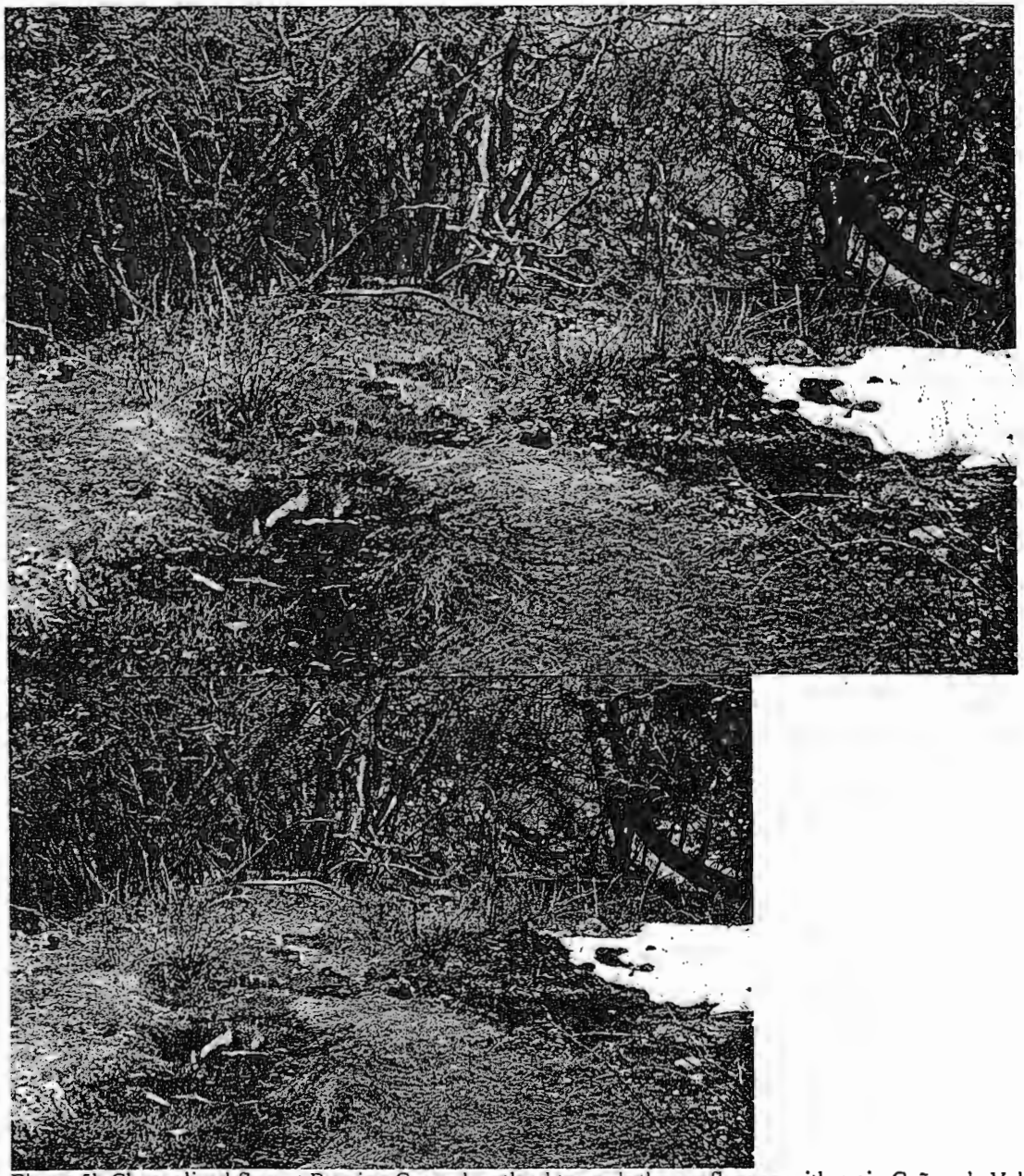


Figure 5b Channelized flow at Burning Ground wetland towards the confluence with main Cañon de Valle channel.

4.2 Behavior of RDX in the Wetlands

Surface water samples were collected and analyzed on a quarterly basis. HE concentrations for September 2000 are shown in Table 1. External laboratory analysis of duplicate samples collected at the influent and effluent locations generally agreed with HPLC screening results from the University of Tennessee. The external lab measured RDX concentration at the effluent of 19.0 ug/L, compared with 24.3 and 27.2 ug/L. Results for the influent from the external lab were 25.0 ug/L compared to 25.5 and 20.6 ug/L. The ADNT concentration of 0.53 ug/L reported by the external lab at the effluent was below the internal detection limit of 0.6 ug/L. There was not a noticeable change in concentration of RDX throughout the wetland. Concentrations were slightly lower at three locations: W-7, W-12 and W-16. Sample W-7 contained the RDX metabolites TNX and MNX. This water sample was also noted to have an anaerobic or rotted odor and more sediment than the other water samples, so it is possible that bacteria in the sediment were degrading RDX in the sample bottle while in transit. Sample temperature was not monitored while stored at LANL in coolers prior to shipping, and was noted to be 10-11 °C upon arrival at Knoxville, TN. This is slightly warmer than the desired 4 °C. However, this sample location (SW-07) also had a negative redox measurement of -324.3 mV, so anaerobic degradation was likely occurring in the wetland. Samples W-08 and W-12 contained low levels of MNX. These samples are both from locations where the wetland channel is wider and water is more likely stagnant.

Table 1: High explosive compounds and associated metabolite concentrations in water samples from September 2000.

Sample ID	RDX ug/L	MNX ug/L	TNX ug/L	TNT ug/L	ADNTs ug/L
Effluent*	19.0	NA	NA	U (2.5)	U (2.5)
SW-E1	27.2	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-E2	24.3	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-1	21.0	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-2	22.0	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-3	21.6	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-4	20.5	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-5	22.9	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-6	24.2	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-7	5.3	0.6	U (0.3)	U (0.3)	U (0.6)
SW-8	24.8	0.4	U (0.3)	U (0.3)	U (0.6)
SW-9	24.6	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-10	12.1	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-11	22.7	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-12	7.6	0.9	U (0.3)	U (0.3)	U (0.6)
SW-13	16.6	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-14	23.0	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-15	26.5	U (0.3)	U (0.3)	U (0.3)	U (0.6)

SW-16	9.8	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-17	23.0	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-18	26.7	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-19	23.7	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-20	24.9	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-I1	25.5	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-I2	20.6	U (0.3)	U (0.3)	U (0.3)	U (0.6)
Influent**	25.0	NA	NA	U (0.2)	0.53

*Samples analyzed by Severn Trent Laboratories, Inc.
**Samples analyzed by Recra LabNet
U = Undetected; approximate detection limit given in parentheses
NA = Not analyzed

Concentrations of HE in samples taken in April 2001 are shown in Table 2. Analysis of duplicate samples sent to an outside laboratory generally agreed with analytical results from the University of Tennessee lab. The concentration of RDX remained fairly constant, averaging about 25 ug/L. Metabolites MNX and TNX were detected in samples W-5, W- 7, W-8, W-9, W- I 0, W- 1 5, W-20, W-1 1, and W-12. Locations W-7, W-11, W-12, and W-13 had concentrations below 10ug/L. Locations W-7 and W- 12 also had lower RDX concentrations and elevated metabolite concentrations during the September 2000 sampling period. Anaerobic conditions at these and possibly other locations seem to promote degradation of RDX.

Table 2: High explosive compounds and associated metabolite concentrations in water samples from April 2001.

Sample ID	RDX ug/L	MNX ug/L	TNX ug/L	TNT ug/L	ADNTs ug/L
Effluent*	35.6	NA	NA	U (0.1)	0.43
SW-E1	28.5	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-E2	27.7	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-1	13.6	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-2	15.6	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-3	15.6	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-4	20.1	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-5	28.2	0.5	0.7	U (0.3)	U (0.6)
SW-6	21.8	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-7	4.1	0.4	U (0.3)	U (0.3)	U (0.6)
SW-8	25.6	0.6	1.4	U (0.3)	U (0.6)
SW-9	20.1	0.4	0.9	U (0.3)	U (0.6)
SW-10	17.0	U (0.3)	1.2	U (0.3)	0.7
SW-11	8.0	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-12	6.9	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-13	6.8	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-14	41.9	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-15	38.0	0.8	1.7	U (0.3)	U (0.6)
SW-16	40.4	U (0.3)	U (0.3)	U (0.3)	U (0.6)

SW-17	30.6	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-18	14.0	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-19	45.3	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-20	28.3	0.6	2.2	U (0.3)	U (0.6)
SW-I1	32.8	0.8	1.9	U (0.3)	U (0.6)
SW-I2	38.6	1.2	2.5	U (0.3)	1.0
Influent*	31.2	NA	NA	U (0.1)	0.38

*Samples analyzed by General Engineering Laboratory
U = Undetected; approximate detection limit given in parentheses
NA = Not analyzed

HE concentrations for June 2001 are shown in Table 3. Again, concentrations did not change from the beginning to the end of the wetland. The metabolite TNX was detected at very low levels at locations W- 12 and W- 16. HE concentrations for October 2001 are shown in Table 4. Concentrations were fairly constant throughout the wetland, and RDX or TNT metabolites were not detected in any samples.

Table 3: High explosive compounds and associated metabolite concentrations in water samples from June 2001.

Sample ID	RDX ug/L	MNX ug/L	TNX ug/L	TNT ug/L	ADNTs ug/L
Effluent*	**	NA	NA	**	**
SW-E1	18.3	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-E2	18.8	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-1	34.5	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-2	38.4	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-3	18.6	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-4	22.6	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-5	19.9	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-6	17.8	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-7	18.9	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-8	36.8	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-9	24.4	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-10	23.4	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-11	25.0	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-12	46.2	U (0.3)	0.4	U (0.3)	U (0.6)
SW-13	36.9	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-14	17.9	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-15	17.3	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-16	49.4	U (0.3)	0.4	U (0.3)	U (0.6)
SW-17	27.7	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-18	38.4	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-19	16.2	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-20	23.9	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-I1	31.5	U (0.3)	U (0.3)	U (0.3)	U (0.6)

SW-I2	21.3	U (0.3)	U (0.3)	U (0.3)	U (0.6)
Influent*	**	NA	NA	**	**

* Samples analyzed by outside laboratory
** Data unavailable
U = Undetected; approximate detection limit given in parentheses
NA = Not analyzed Data not available yet

Table 4: High explosive compounds and associated metabolite concentrations in water samples from October 2001.

Sample ID	RDX ug/L	MNX ug/L	TNX ug/L	TNT ug/L	ADNTs ug/L
Effluent*	**	NA	NA	**	**
SW-E1	31.0	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-E2	16.7	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-1	21.5	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-2	19.9	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-3	11.6	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-4	14.9	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-5	15.9	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-6	18.8	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-7	16.8	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-8	10.6	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-9	20.3	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-10	19.9	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-11	18.0	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-12	20.3	U (0.3)	0.4	U (0.3)	U (0.6)
SW-13	17.1	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-14	11.7	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-15	34.9	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-16	9.6	U (0.3)	0.4	U (0.3)	U (0.6)
SW-17	13.1	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-18	25.1	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-19	12.9	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-20	28.3	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-I1	15.1	U (0.3)	U (0.3)	U (0.3)	U (0.6)
SW-I2	23.0	U (0.3)	U (0.3)	U (0.3)	U (0.6)
Influent*	**	NA	NA	**	**

* Samples analyzed by outside laboratory
** Data unavailable
U = Undetected; approximate detection limit given in parentheses
NA = Not analyzed

RDX concentrations as a function of distance from the weir are summarized in Table 5 and plotted in Figure 6. Degradation of HE in the Burning Ground Spring wetland is more likely to occur in the stagnant areas. A significant change in RDX concentration is not evident from the

influent to the effluent of the wetland; although, concentrations in the first hundred feet from the weir varied more than those in the second hundred feet. This may be the result of the first portion's of the wetlands shallower and stagnant conditions, unlike the second portion's narrower channel with faster flowing water. This variation noted in RDX concentrations from the first 100 ft of the wetland could be due to small stagnant pockets of anaerobic activity.

Table 5: RDX concentration in Burning Ground spring surface water samples September 2000-October 2001.

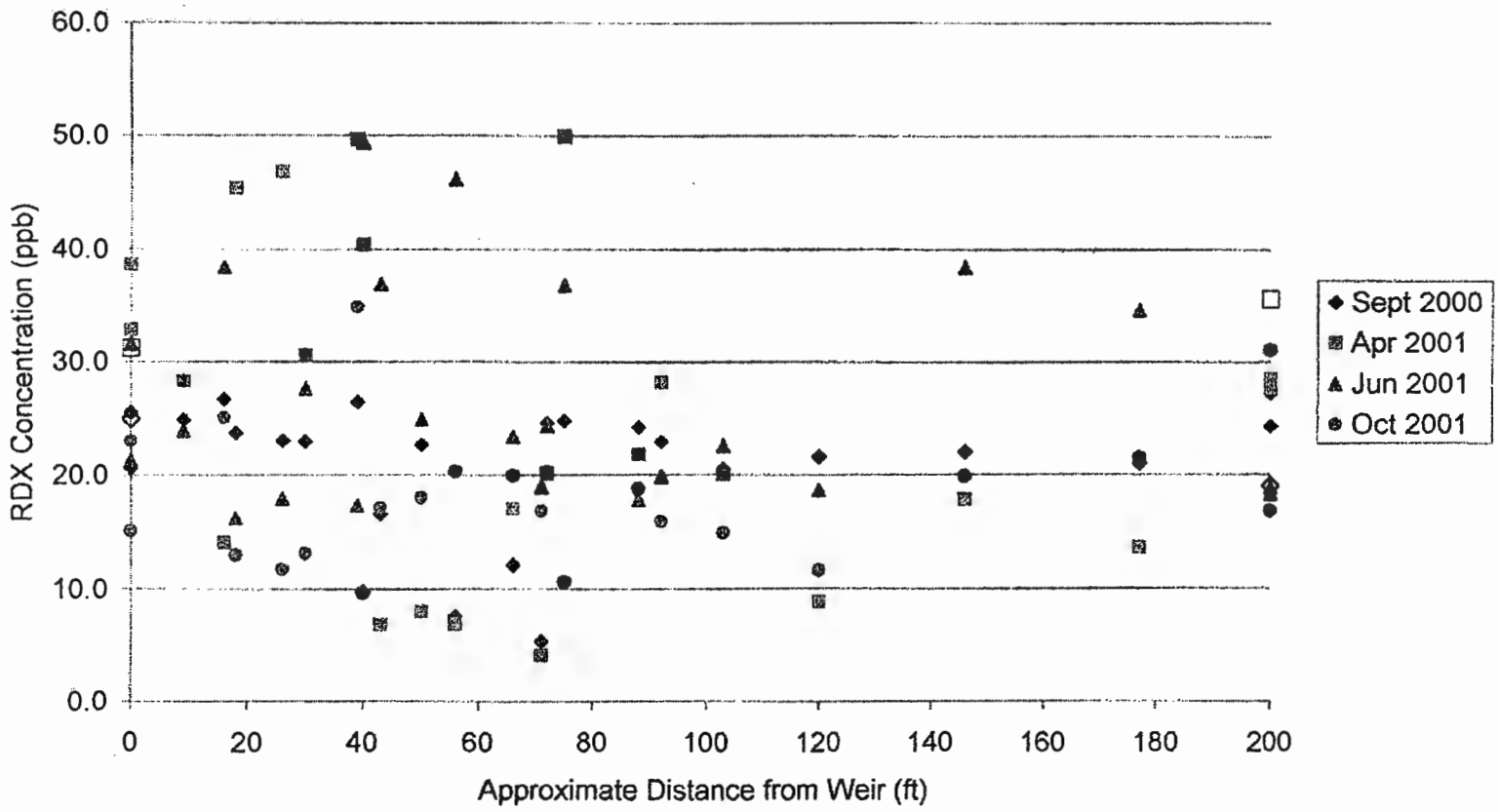
Sample ID	Distance from spring outlet (ft)	Sep 2000 RDX ug/L	Apr 2001 RDX ug/L	Jun 2001 RDX ug/L	Oct 2001 RDX ug/L
Effluent*	200	19.0	35.6	**	**
SW-E1	200	27.2	28.5	18.3	31.0
SW-E2	200	24.3	27.7	18.8	16.7
SW-1	177	21.0	13.6	34.5	21.5
SW-2	146	22.0	17.8	38.4	19.9
SW-3	120	21.6	15.6	18.6	11.6
SW-4	108	20.5	20.1	22.6	14.9
SW-5	92	22.9	28.2	19.9	15.9
SW-6	88	24.2	21.8	17.8	18.8
SW-7	71	5.3	4.1	18.9	16.8
SW-8	75	24.8	25.6	36.8	10.6
SW-9	72	24.6	20.1	24.4	20.3
SW-10	66	12.1	17.0	23.4	19.9
SW-11	50	22.7	8.0	25.0	18.0
SW-12	56	7.6	6.9	46.2	20.3
SW-13	43	16.6	6.8	36.9	17.1
SW-14	26	23.0	41.9	17.9	11.7
SW-15	39	26.5	38.0	17.3	34.9
SW-16	40	9.8	40.4	49.4	9.6
SW-17	30	23.0	30.6	27.4	13.1
SW-18	16	26.7	14.0	38.4	25.1
SW-19	18	23.7	45.3	16.2	12.9
SW-20	9	24.9	28.3	23.9	28.3
SW-I1	0	25.5	32.8	31.5	15.1
SW-I2	0	20.6	38.6	21.3	23.0
Influent*	0	25.0	31.2	**	**

*Samples analyzed by external laboratory

** Data not available yet

Figure 6

RDX concentration in Burning Ground Spring wetland



Sediment and soil samples were collected at each sampling event. Results of the September, 2000 analysis indicated that concentrations of HE in all sediment and soil samples were below the detection limit of 0.2 mg/kg. While this detection limit is comparable to the estimated quantitation limit given for soil in EPA Method 8330, it is only sensitive in the ppm range, rather that the ppb range achievable with water samples. Attempts to use larger sediment samples and concentrate the acetonitrile extract did not improve the detection limit significantly. Sediment samples from April, 2001 were analyzed and all were once again below the detection limit. Sediment samples from the remaining sampling events were collected and frozen but were not extracted or analyzed.

Redox potential measurements for all sampling periods are shown in Table 6. Redox values varied between locations and over time. However, the general trend observed was that redox measurements became increasingly negative with depth, indicating a transition from oxidized aerobic conditions to reduced anaerobic conditions. Wetlands usually have a 0.5-1 in. oxidized layer of sediment, while deeper sediments are more highly reduced (Mitsch and Gosselink, 1993).

Table 6: Redox measurements at Burning Ground spring sample locations September 2000 to October 2001.

Location	Probe Depth	Sep 2000 mV	Apr 2001 mV	Jun 2001 mV	Oct 2001 mV
Influent	surface water	264.0	-185.4	3.2	38.0
	sediment surface	234.0	-202.8	14.9	-422.9
	1 in below sediment surface	-389.0	-494.1	51.6	-395.5
SW-7	surface water	70.0	-130.3	-50.1	59.8
	sediment surface	-165.0	-139.8	-52.3	32.4
	1 in below sediment surface	-324.3	-408.7	-108.1	-382.5
SW-12	surface water	240.0	-32.5	0.7	99.4
	sediment surface	184.0	-46.0	9.1	25.1
	1 in below sediment surface	176.0	-306.8	-24.2	-358.2
Effluent	surface water	NM	-210.5	-14.1	38.0
	sediment surface	NM	-162.9	-40.3	-422.9
	1 in below sediment surface	NM	-34.7	-476.1	-395.5

NM = Not Measured

Concentrations of RDX in all plant tissue samples collected from the field are shown in Table 7. Concentrations of RDX, TNT, and all tested metabolites were below the detection limit of 0.2 mg/kg (200 ppb) -fresh weight (results not shown). The concentration of RDX in the water is approximately 30 ug/L or 30 ppb. If the plants are accumulating or bioconcentrating RDX then it must be at a lower concentration than the detection limit of 200 ppb. There is no direct evidence that RDX is being metabolized by the naturally occurring wetland plants. Therefore, the lack of detectable RDX and metabolites in plant tissues indicates that if the field plants are taking up RDX, the uptake does not result in accumulation or bioconcentration to detectable

levels. Thus, plant tissues will probably not pose a health risk to insects or animals feeding on plants in the wetland.

Table 7: Plant Species analyzed for RDX and metabolites.

Plant Tested	Location	Sep 2000 mg RDX/kg fresh wt	Apr 2001 mg RDX/kg fresh wt	Jun 2001 mg RDX/kg fresh wt	Oct 2001 mg RDX/kg fresh wt
Smooth monkey flower (*Mimulus glabratus*)	P1	U (0.2)	U (0.2)	U (0.2)	U (0.2)
False manna gras (*Torreyochloa paucifvlora*)	P1	U (0.2)	U (0.2)	U (0.2)	U (0.2)
Bryophyte (Unidentified)	P1	U (0.2)	U (0.2)	U (0.2)	U (0.2)
Creeping bentgrass (*Agrostis palustris*)	P2	U (0.2)	NS	U (0.2)	U (0.2)
Currant gooseberry (*Rives montegenum*)	P2	U (0.2)	NS	U (0.2)	U (0.2)
Wild raspberry (*Rubus stigosus*)	P2	U (0.2)	NS	U (0.2)	U (0.2)
Smooth monkey flower (*Mimulus glabratus*)	P3	U (0.2)	U (0.2)	U (0.2)	U (0.2)
False manna gras (*Torreyochloa paucifvlora*)	P3	U (0.2)	U (0.2)	U (0.2)	U (0.2)
White checkermallow (*Sidalcea candida*)	P3	U (0.2)	U (0.2)	U (0.2)	U (0.2)

* Detection limit 0.2 mg/kg in plant tissues
U = Undetected; approximate detection limit given in parentheses
NS = Not sampled

4.3 RDX Uptake Experiments Using Mimulus and Torreyochloa

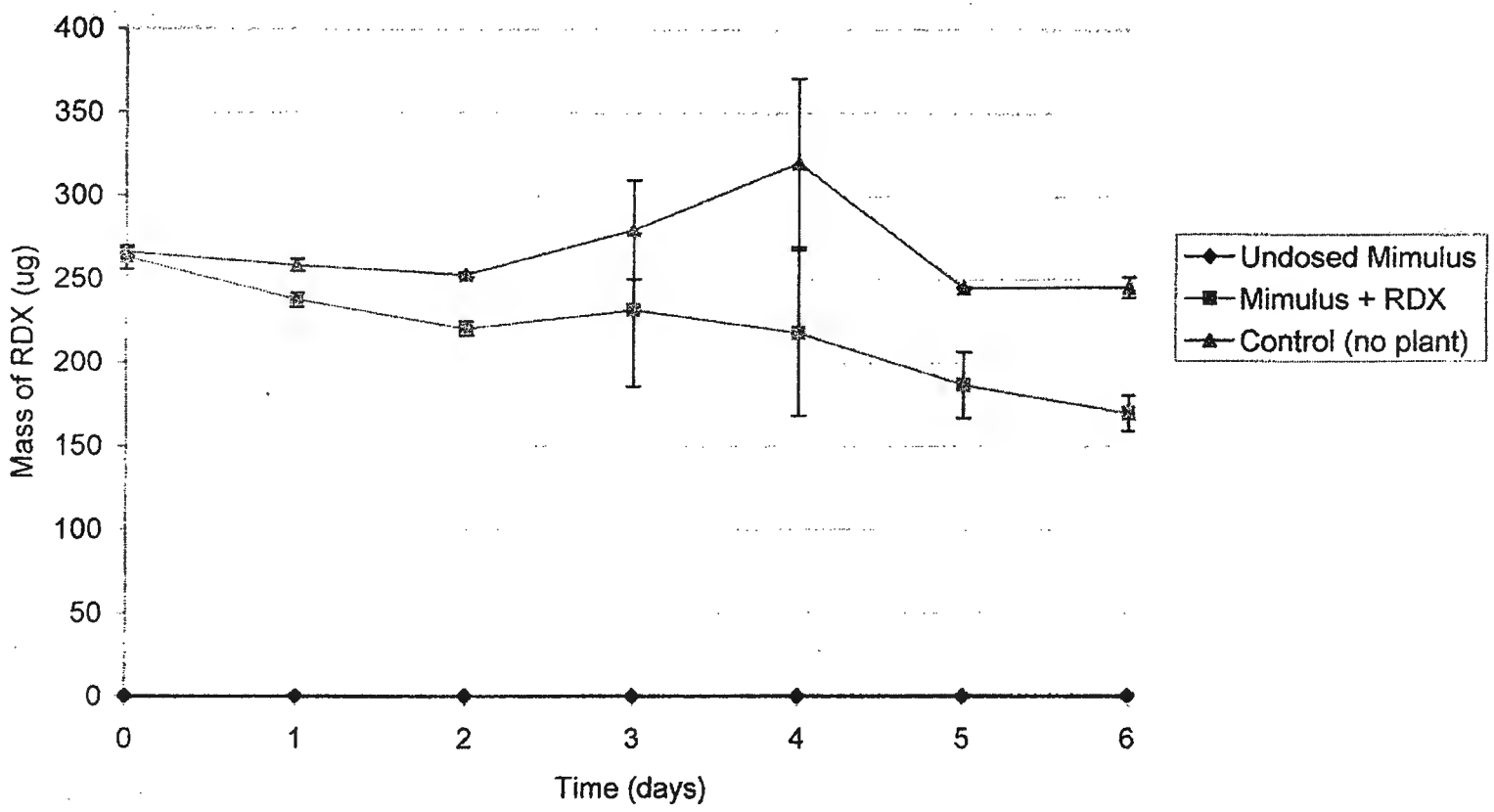
The mass of RDX in solution over time in reactors with and without Mimulus plants is shown in Figure 7. Losses from the control reactors averaged 8%. The control reactors had no plants present. A possible explanation for the 8% loss could be abiotic mechanisms such as sorption to the glass flasks or photodegradation. Mimulus plants are capable of taking up RDX from solution. Removal from the reactors containing Mimulus averaged 35% during the six-day period. Mimulus plant tissues were analyzed and found to contain an average of 8.7 mg RDX/kg fresh weight. Table 8 lists the RDX concentrations of the Mimulus and Torreyochloa plants. Based on this concentration, the total plant mass, and RDX removed, about 85 percent of the RDX removed from solution was recovered in plant tissues as RDX. Metabolites MNX and TNX were not detected above the limit of 0.2 mg/kg.

The mass of RDX in solution over time in reactors with and without Torreyochloa plants is shown in Figure 8. Losses from the control reactors averaged negative 4%, so loss could not be distinguished from normal sampling and analytical variation. Torreyochloa plants are capable of

taking up RDX from solution. RDX Removal from the reactors containing Torreyochloa averaged 19% over the six-day period, but the majority of removal appeared to occur during the

Figure 7

Decrease in mass of RDX due to uptake by *Mimulus*



first three days. Torreyochloa plant tissues were analyzed and found to contain an average of 3.9 mg RDX/kg fresh weight. Based on this concentration, the total plant mass, and RDX removed, about 58 percent of the RDX removed from solution was recovered in plant tissues as RDX. Metabolites MNX and TNX were not detected above the limit of 0.2 mg/kg.

Based on the average mass of RDX removed from solution and the amount of water used by the plants during the experiment, a hypothetical concentration of RDX in water taken up was calculated to be 0.76 mg/L compared with the initial solution concentration of 0.99 mg/L. Therefore, Mimulus plants do not take up RDX proportionately with water. Based on the average mass of RDX removed from solution and the amount of water used by the plants during the experiments a hypothetical concentration of RDX in water taken up was calculated to be 0.95 mg/L compared with the initial solution concentration of 0.94 mg/L. The concentration in solution and the theoretical concentration of water taken up by Torreyochloa was essentially the same. Therefore, Torreyochloa plants may take up RDX more efficiently than Mimulus. However, the short experiment time of six days may not reflect uptake efficiency over time. While Torreyochloa had a higher uptake concentration during this experiment, RDX removal from solution appears to have slowed and might not continue at that rate over a longer time period. Additionally, the final concentration in plant tissues was lower in Torreyochloa than in Mimulus. This could mean that Torreyochloa can degrade RDX more quickly than Mimulus. However, it is important to note that the Mimulus plants used in this experiment had a higher transpiration rate (average 2.2 mL/day/g-plant) than the Torreyochloa (average 1.1 mL/day/g-plant). Because transpiration is usually related to chemical uptake, Mimulus plants may be able to treat more water than Torreyochloa even though Torreyochloa may be more efficient in RDX uptake.

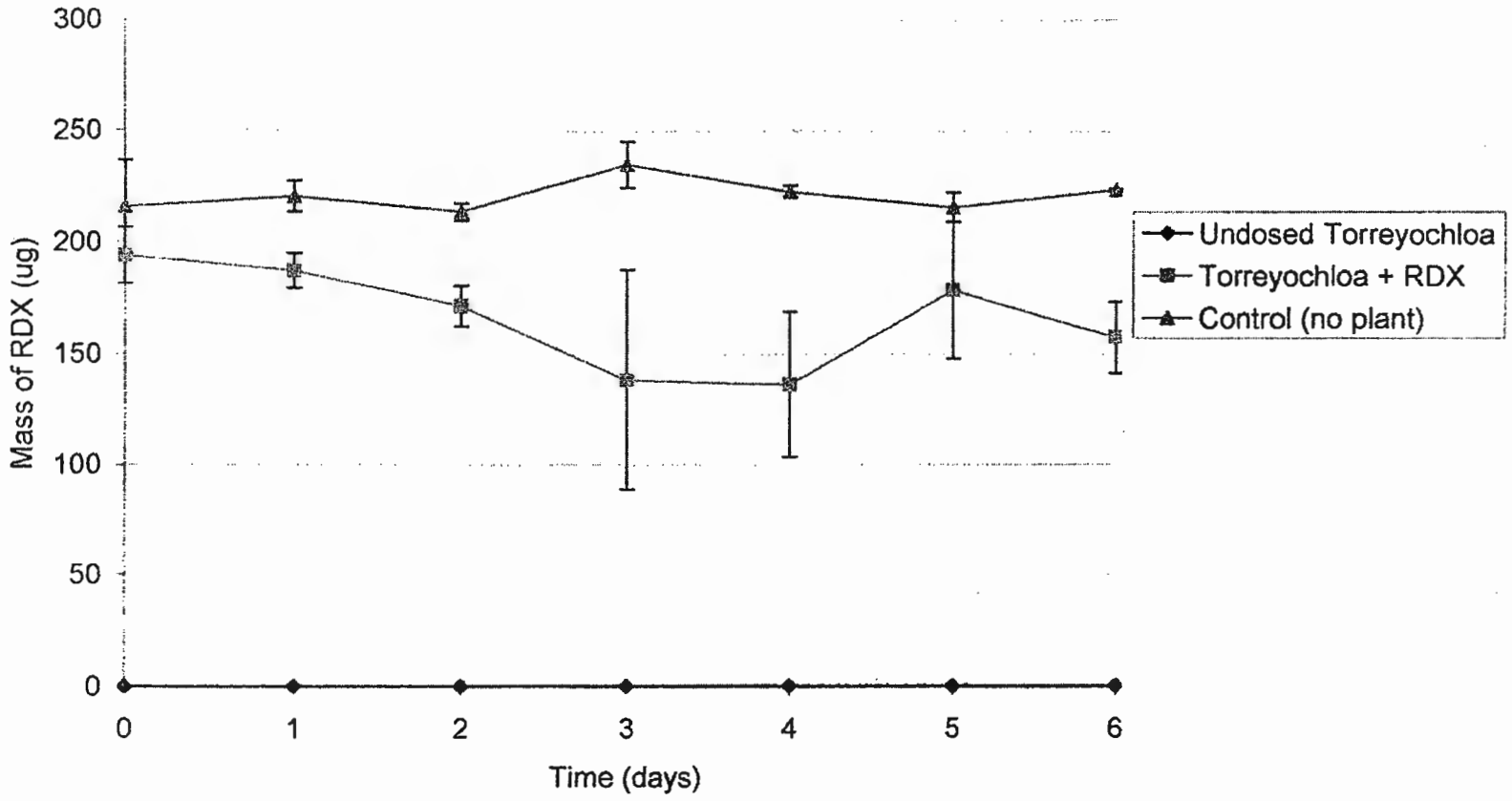
Table 8: Concentration of RDX in plants grown in the laboratory.

Plant Tested	Final Plant Mass (g)	RDX mg/kg fresh wt	MNX mg/kg fresh wt	TNX mg/kg fresh wt
Mimulus #1	10.0	9.2	U (0.2)	U (0.2)
Mimulus #2	8.6	7.9	U (0.2)	U (0.2)
Mimulus #3	9.2	8.3	U (0.2)	U (0.2)
Torreyochloa #1	5.0	3.8	U (0.2)	U (0.2)
Torreyochloa #2	5.9	4.5	U (0.2)	U (0.2)
Torreyochloa #3	7.0	3.5	U (0.2)	U (0.2)

U = Undetected; approximate detection limit given in parentheses

Figure 8

Decrease in mass of RDX due to uptake by *Torreyochloa*



5.0 CONCLUSIONS AND RECOMMENDATIONS

The results from the Burning Ground Spring wetland investigation indicate there is potential for RDX remediation within the wetlands. Under the current surface water flow pattern and retention time from the spring outlet to the confluence with Cañon de Valle there is no appreciable reduction in RDX concentrations. However within the wetland there are locations which show evidence for RDX biodegradation. This suggests that the wetland area could be modified to enhance the processes which encourage RDX biodegradation, i.e. increasing water residence time and providing more anaerobic environments. Key observations which support this are indicated below:

- RDX metabolites MNX and TNX were detected in several water samples, indicating that microbial degradation can occur in the wetlands. MNX and TNX detections were restricted to areas in the wetland where the water was slower moving and had lower redox potential. This observation is consistent with expectations for anaerobic biodegradation of RDX and the formation of the observed degradation intermediates. These observations further suggest that retention time and contact with anaerobic sediment are critical factors for successful RDX remediation.
- No strong seasonal variation was noted in the wetland concentration of RDX. Seasonal variation in the effectiveness of constructed wetlands has been noted at other sites and is thought to be related to temperature and its effect on microbial activity (Sikora, 1998). The water temperature in the Burning Ground spring wetland was fairly constant at 10-15 °C year round. Therefore, temperature related changes microbial activity are less likely to affect the RDX biodegradation at this site.
- Based on laboratory studies, naturally occurring Mimulus and Torreyochloa plants should be capable of taking up RDX. Both Mimulus and Torreyochloa took up RDX in the laboratory. Neither plant contained detectable concentrations of RDX when harvested from the wetland; however, this is probably due to the relatively high detection limit of RDX, MNX, and TNX in plant tissue compared to the low concentrations found in the wetland water. RDX uptake by native Mimulus and Torreyochloa is expected to occur during the growing season in the wetland provided the plants are in adequate contact with RDX impacted water.
- Under field conditions, retention time and direct contact with the plants may be inadequate for appreciable uptake of RDX. Similarly, microbial degradation is also likely to be limited by the retention time of water in the wetlands.
- Constructed wetlands typically have retention times of several days whereas Burning Ground Spring wetland has a retention time of only a few hours.
- In the present configuration of the wetlands the RDX concentration was not observed to decrease with passage through the wetland due to the short retention time.

The results of this evaluation indicate that RDX is not being appreciably biodegraded in the wetland, as it currently exists. The results also suggest that the wetland can be manipulated to greatly enhance the removal of RDX through the action of naturally occurring microorganisms and plants. Wetland renovations to improve RDX removal include altering water flow to increase

retention time and designing the water flow path so that the water moves slower and makes direct contact with sediment and plant roots for longer periods of time. These renovations may be accomplished without dramatically altering the natural appearance of the wetlands.

6.0 REFERENCES

Best, E.P.H., M.E. Sappi, H.L. Fredrickson, S.L. Sprecher, S.L. Larson, and M. Ochman. (1997) "Screening of Aquatic and Wetland Plant Species for Phytoremediation of Explosives-contaminated Groundwater from the Iowa Army Ammunition Plant." Annals of the New York Academy of Science 829: 179-194.

Best, E.P.H., S.L. Sprecher, S.L. Larson, H.L. Fredrickson, and D.F. Bader. (1999) "Environmental Behavior of Explosives in Groundwater from the Milan Army Ammunition Plant I in Aquatic and Wetland Plant Treatments: Uptake and Fate of TNT and RDX in Plants." Chemosphere 39: 2057-2072.

Just, C.L., and J.L. Schnoor. (2000) "A Preparation Technique for Analysis of Explosives in Plant Tissues." International Journal of Phytoremediation 2:255-267

LANL (Los Alamos National Laboratory), 1990. "RFA—initial site assessment," Los Alamos National Laboratory Report, Los Alamos, New Mexico.

LANL (Los Alamos National Laboratory), September 1996. "RFI Report for Potential Release Sites in TA-16: 16-003(k), 16-021(c), Field Unit 3," Los Alamos National Laboratory Report LA-UR-96-3191, Los Alamos, New Mexico.

LANL (Los Alamos National Laboratory). (1998) "Phase II RFI Report for Potential Release Site 16-021(c)," Los Alamos National Laboratory Report LA-UR-98-4101, Los Alamos, New Mexico.

Larson, S.L., R.P. Jones, L. Escalon, and D. Parker. (1999) "Classification of Explosives Transformation Products in Plant Tissues." Environmental Toxicology and Chemistry 18: 1270-1276.

Mitsch, W.J. and J.G. Gosselink. (1993) Wetlands. Van Nostrand Reinhold, New York.

Pennington, J.C., M. Zakikhani, D.W. Harrelson, and D.S. Allen. (1999) Monitored Natural Attenuation of Explosives in Groundwater: Cost and Performance. Technical Report EL-99-9, U.S. Army Corps of Engineers Waterways Experiment Station, Vicksburg, MS.

Sikora, F.J., R.A. Almond, L.L. Behrends, J.J. Hoagland, D.A. Kelly, W.D. Phillips, W.J. Rogers, R.K. Summers, F.C. Thornton, J.R. Trimm, and D.F. Bader. (1998) Demonstration Results of Phytoremediation of Explosives-Contaminated Groundwater Using Constructed Wetlands at the Milan Army Ammunition Plan. Milan, Tennessee. SFIM-AFC-ET-CR-97059. Tennessee Valley Authority, Muscle Shoals, AL.