FIELD DEMONSTRATION OF IN-SITU BIOREMEDIATION OF RDX AND HMX USING MOLASSES

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By

Zachary Mahiohao Payne

Thesis Committee:

Roger Babcock, Chairperson
Chittarangan Ray
Tao Yan

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ABSTRACT

A nine-month, in-situ bioremediation study was conducted at Mākua Military Reservation on O‘ahu, Hawai‘i (USA) to evaluate the potential of molasses to enhance biodegradation of explosives (RDX and HMX) contaminated soil below the root zone. Molasses/water mixture (1:40 dilution) and clean water was applied to treatment plot and control plot, respectively in seven successive flood irrigation events. The change in concentration of explosives over time in lysimeter collected pore water samples was measured to determine treatment effectiveness. The mean concentrations of RDX from the two test plots differed very highly significant ($p < 0.001$) and degradation was most prevalent at depths from 5 to 13.5 ft. HMX was also degraded but only at a depth of 5 ft. The molasses/water mixture had a similar infiltration rate to that of water and was able to reach the deepest sensor (31 ft) within 5 days of application. Molasses was consumed within the top 13.5 feet of soil and greater molasses concentrations and/or more frequent flooding events may be required to treat deeper contamination. Use of the bioremediation method described herein could prevent explosives residuals from migrating to ground water and off-site.

Keywords: Explosives, In-situ remediation, Molasses, Irrigation, TOC

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
CALFX	combined-arms live-fire exercise training site
DNX	hexahydro-,3-dinitroso-5-nitro-1,3,5-triazine
DPA	demolition pit area
ft	feet
ft/d	feet per day
HMX	high-melting explosive
HPLC	high pressure liquid chromatography
LRL	laboratory reporting limit
mg/L	milligrams per liter
MMR	Mākua Military Reservation
MNX	hexahydro-1-nitroso-3,5-dinitro-,3,5triazine
NPOC	non-purgeable organic carbon
OB/OD	open burning/open detonation
PRG	Preliminary Remediation Goals
PVC	polyvinyl chloride
RDX	royal demolition explosive
TN	Total nitrogen
TNX	hexahydro1,3,5-trinitroso-1,3,5-triazine
TOC	Total organic carbon

ug/l	micrograms per liter
UXO	unexploded ordnance
VWC	volumetric water content

CHAPTER 1. INTRODUCTION

Contamination of soil and water with explosives residuals has been reported worldwide. Among 20 different chemicals used by the military, cyclic nitramine explosives hexahydro-1,3,5-trinitro-1,3,5-triazine (royal demolition explosive ($C_3H_6N_6O_6$) or RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (high-melting explosive ($C_4H_8N_8O_8$) or HMX) are two of the most powerful and commonly used explosives in conventional ammunitions (Spain et al., 2000). These compounds are released into the environment during testing, training, open burning/open detonation (OB/OD) and manufacturing (Hawari et al., 2005). Most high-order detonations deposit very little residues and the major deposition is due to low-order detonations (Hewitt et al., 2005). Live fire training ranges can also leave millimeter-sized particles of explosives even when rounds explode as intended (high-order) (Kalderis et al., 2011). Residues of these compounds are deposited generally as particles, fibers and slivers (Radtke et al., 2002; Walsh et al., 2002) which then dissolve slowly over time to continuously contaminate soil and ground water (Jenkins et al., 2006). There exist a number of sites having soil contamination remaining where waste disposal practices were discontinued 20 to 50 years ago (Craig et al., 1995). Weathering results in mass transfer of RDX into soil pore water (Phelan et al., 2003, Walsh et al., 2010). Both RDX and HMX migrate through subsurface soil and cause groundwater contamination due to solubility in water (50 mg/L and 5 mg/L for RDX and HMX at 25 °C, respectively) and weak binding affinity for soil (Hawari et al., 2005). RDX has been detected in the groundwater at military installations (Clausen et al., 2004; Lewis et al., 2009; Martel et al., 2009; Sunahara et al., 1999; Talmage et al., 1999). Compared to RDX, HMX is relatively less mobile because of its lower solubility and may accumulate on the ground surface adversely affecting training (ERDC/CRREL, 2007).

The Mākua Military Reservation (MMR) on O‘ahu has been in operation since the 1940s as a combined-arms live-fire exercise training site (CALFX). The 4,249 acre MMR contains an 18 acre open burning/open detonation (OB/OD) area that was used for the disposal of ordnance from the 1960s through the early 1990s. Previous studies reported the presence of RDX and HMX in the OB/OD soils and vadose zone pore water at

concentrations above EPA Region 9 Preliminary Remediation Goals (PRGs). RDX was observed at concentrations up to 9 mg/kg (>5.5 mg/kg residential soils PRG) in soils (Daubel, 1985) and from 27 to 21,000 µg/l (average: 5,712 µg/l) in vadose zone shallow pore water. HMX was also observed in the vadose zone shallow pore water at concentrations from 3.8 to 2,700 µg/l (average: 1081 µg/l). These values are generally higher than the PRGs of 1,800 µg/l (GSLWES/Environet, 2006). These compounds are reported to be toxic to aquatic organisms (Sunahara et al., 1999), earthworms (Robidoux et al., 2001), mammals (Talmage et al., 1999) and human monocytes (Bruns-Nagel et al., 1999). RDX is classified as a possible human carcinogen class C (EPA, 1998) and may cause generalized seizures (Williams et al., 2011). HMX may be harmful to the central nervous system (CDC, 1997). The toxicity of these chemicals coupled with environmental persistence warrant cost effective and environmentally friendly remediation methods for the sustainable use of live fire training ranges.

Soil microorganisms normally cannot utilize explosives like HMX and RDX as a sole carbon and energy source, however, biodegradation has been successfully promoted by creating reducing conditions using a supplemental carbon source such as starch or molasses (Funk et al., 1993). RDX can be degraded by the two-electron reductive pathway (Nitroso route) and also by denitration (Crocker et al., 2006; Zhao et al., 2002). In the reductive pathway RDX is reduced to hexahydro-1-nitroso-3,5-dinitro-,3,5triazine (MNX) and then hexahydro-,3-dinitroso-5-nitro-1,3,5-triazine (DNX) and hexahydro1,3,5-trinitroso-1,3,5-triazine (TNX). The denitration pathway can be aerobic (Zhao et al., 2002) or anaerobic (Thompson et al., 2005). Similar to RDX degradation, HMX degradation can occur via reduction of nitro groups to form nitroso intermediates (Kalderis et al., 2011).

Previous work has demonstrated the effectiveness of molasses to facilitate complete bioremediation of RDX in the root zone of explosives contaminated Hawaiian soils (Lamichhane et al., 2012), however, no work had been done on contamination below the root zone. Unlike other tropical soils, Hawaiian soils exhibit a net positive charge, are acidic (pH 4.0–7.3), and have high iron and aluminum content. Because of the highly permeable substratum, there is an increased risk for contamination of groundwater if the

contaminants pass the carbon rich root zone (upper 30 cm) (Alavi et al., 2011). The main objective of this study was to evaluate whether molasses could be used to bioremediate RDX and HMX contamination in Hawaiian soils below the root zone (from 30 cm to 30 feet below ground surface). Another objective of the study was to assess the infiltration rate of molasses-fortified water.

CHAPTER 2. MATERIALS AND METHODS

A nine-month in-situ bioremediation study was conducted in MMR, O‘ahu, Hawai‘i (USA). Two test plots (a control plot and a treatment plot), 65 ft^2 in area, were constructed in a demolition pit area (DPA) at MMR. The DPA was formerly used to dispose of unexploded ordnance (UXO) and was known to contain elevated concentrations of RDX and HMX in surface soils (Environet, 2008). The triangular test plots consisted of 3 foot high water-tight walls to allow impounding of water up to 2 ft above the ground surface which would provide sufficient head for rapid infiltration. Three 30-ft deep boreholes were drilled in each test plot and nested lysimeters and moisture sensors installed. The field study included soil sample collection, periodic flood irrigation of the test plots and subsequent collection of pore water samples and soil moisture data. The control plot was irrigated with uncontaminated well water and the treatment plot received a 1:40 molasses mixture (1 part molasses to 40 parts uncontaminated well water). The 1:40 molasses concentration was chosen based upon the results of the previous greenhouse study which evaluated bioremediation/phytoremediation in the root zone (up to 30 cm depth) (Lamichhane et al., 2012). Each test plot received 500-gallons (1 gallon = 3.785 liter) of water during each flooding event and a total of seven flooding events were employed during the experimental period.

Two lysimeters (model 1920F1L24-B02M2, Soilmoisture Equipment Corp, Santa Barbara, CA, USA) and two moisture sensors (model CS616 L-60, Campbell Scientific, Inc., Logan, UT, USA) were installed in each borehole at depths from 5 to 30 ft to collect pore water and measure volumetric water content (VWC). Each lysimeter consisted of a polyvinyl chloride (PVC) body with a 2 bar (200 kilopascal) ceramic cup (diameter 1.9" and length 2.0") epoxy bonded to one end. Each moisture sensor was placed just above each lysimeter and bentonite clay plugs were installed above and below each lysimeter/moisture sensor pair. Two Jet Fill tensiometers (model 2725ARL, Soilmoisture Equipment Corp., Santa Barbara, CA) were installed at the near-surface (at 11 inch and 32 inch depth) of each test plot. Each tensiometer consisted of a long plastic tube with a

porous ceramic cup at the bottom, liquid reservoir at the top, and a vacuum gauge fixed below the reservoir.

The control plot boreholes were labeled as C-1, C-2, and C-3 and the treatment plot boreholes were labeled as T-1, T-2, and T-3. Each borehole had two pore water collection points labeled A and B. C-1A and C-1B of the control plot were set at depths 5 ft and 13.5 ft, respectively. Other control plot lysimeters C-2A, C-2B, C-3A and C-3B were set at depths of 12 ft, 17 ft, 21.5 ft and 31 ft, respectively. Similarly, treatment plot bore hole water collection points T-1A, T-1B, T-2A, T-2B, T-3A and T-3B were set at depths of 5 ft, 13.5 ft, 21 ft, 31 ft, 11 ft and 19 ft, respectively. Figure S2 illustrates the layout of the test plots, bore hole locations and profiles, and depths of sensors and lysimeters (see Supporting Information).

2.1 Sample collection

Soil samples were collected in plastic bags during the drilling of each bore hole at approximately 18" (inch) intervals down to a depth of 20 ft below ground surface. Prior to each flood irrigation event, 0.5 bar of vacuum was placed on each lysimeter in order to sample percolating soil pore water. Several days after the flooding, the water sample collected in each lysimeter was pumped out for analysis. Sampling occurred after different durations based upon installation access restrictions as follows: event 1 – 15 days, event 2 – 7 days, event 3 – 2 days, event 4 – 10 days, event 5 – 5 days, event 6 – 3 days, and event 7 – 2 days. Lysimeter samples were collected in one liter glass amber bottles and then preserved on ice until analyzed. Moisture data were collected every 30 minutes from the 12 moisture sensors using a data logger and every 10 minutes from the four tensiometers.

2.2 Sample analyses

Soil samples were air dried, ground with a mortar and pestle, passed through a 30-mesh sieve, and then extracted with acetonitrile. The ultrasonic extraction method contained in EPA Method 8330 was employed for conducting soil extractions, modified by using 4 g soil samples and 20 ml acetonitrile. The detection limits for all energetics was 0.01 mg/kg in soil samples and 0.001 mg/l in liquid (pore water). Pore water samples from the

lysimeters were filtered through a 0.45 micron filter. Soil extracts and filtered lysimeter samples were analyzed for energetics (RDX and HMX), and their degradation products MNX, DNX, and TNX, using US EPA SW846 8330A method utilizing a CN reverse phase high pressure liquid chromatography (HPLC) column, with a C18 column for confirmation. HMX and RDX standard solutions were purchased from Chem Service, West Chester, PA (USA) whereas MNX, DNX, TNX standards were purchased from SRI International, Menlo Park, CA (USA). Total organic carbon (TOC) analyses were performed on a Shimadzu TOC-V instrument (Shimadzu Scientific Instruments, Columbia, MD, USA) using the non-purgeable organic carbon (NPOC) method. Total nitrogen (TN) was measured by the dry combustion method on the same Shimadzu instrument.

Statistical software SAS (SAS Institute Inc., Cary, USA) was used for all statistical analyses. The analysis of variance (ANOVA) GLM procedure was used to evaluate variations in the variables and difference in mean values of the variables in questions were tested at 5% significance ($p = 0.05$) level and the difference was considered significant, highly significant and very highly significant if p value was less than 0.05, 0.01 and 0.001, respectively.

CHAPTER 3. RESULTS AND DISCUSSION

3.1 Background soil concentrations of HMX and RDX

The presence of HMX and RDX in soil samples collected from all 6 boreholes (to a depth of 19.5 ft) revealed the existing (background) contamination before treatment and the potential of these chemicals to migrate downward and pollute ground water. Figure 1 shows the general trend of background concentration of HMX and RDX in subsurface soil samples for borehole C2. Figures S3-S7 shows the profiles for the other five bore holes (C1, C2, and T1-T3) (see Supporting Information).

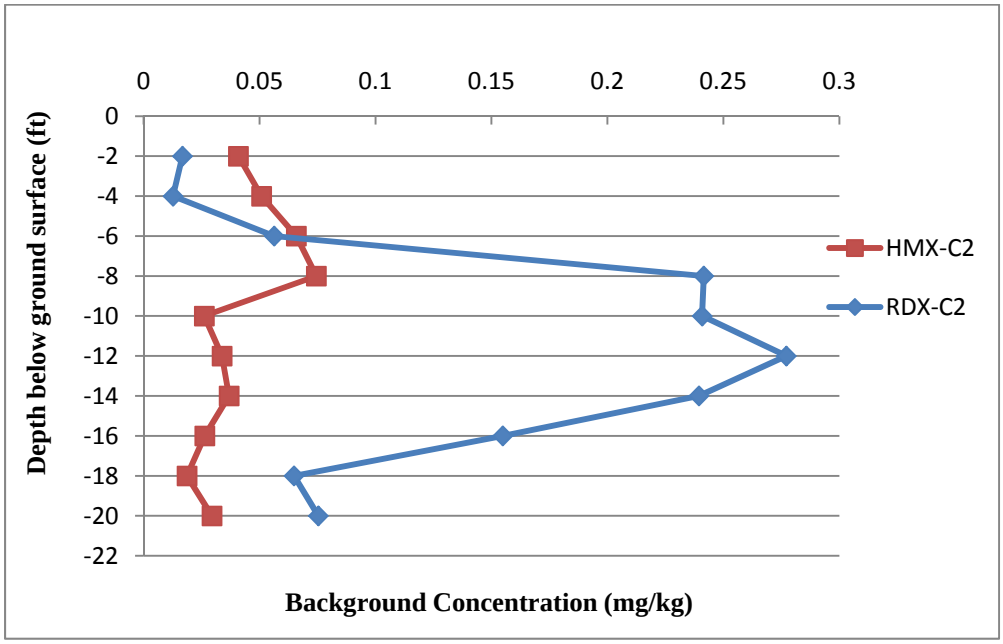

Figure 1: Distribution of HMX and RDX in subsurface soil samples (HMX and RDX profile of soil samples from bore hole 2 (C-2) of the control plot)

The background concentration of chemicals (HMX and RDX) in soil samples was not uniform: RDX concentration was low in near-surface soil samples whereas HMX concentrations tended to be largest within the upper ten feet. The highest concentrations of RDX occurred between depths of 5 to 15 ft. Two soil samples were also collected from greater depths (25 and 30 ft) in bore hole C-3. HMX was present at a concentration of 0.013 mg/kg at 25 ft but was not detected at 30 ft. The concentrations of RDX at depths

of 25 ft and 30 ft were 0.019 and 0.036 mg/kg, respectively. The background concentration of the degradation products MNX, DNX and TNX were below detection limits in all soil samples.

Using SAS, we checked whether the difference in concentrations within the control plot bore hole soil profiles (C-1, C-2, and C-3) and treatment plot soil profiles (T-1, T-2, and T-3) was statistically significant. The mean bore hole concentrations of HMX (0.046 mg/kg) and RDX (0.134 mg/kg) within the control plot were not significantly different (p = 0.22 and 0.63, respectively, for HMX and RDX). Though the concentration of HMX and RDX changed with depth (Figure 1), the change in concentration with depth was not significant for HMX (p = 0.28) but was highly significant for RDX (p = 0.007) within control plot soil samples. Similarly, the mean bore hole concentration of HMX was 0.053 mg/kg and RDX was 0.088 mg/kg in the treatment plot. The mean concentrations of HMX (p = 0.28) and RDX (p = 0.28) were not significantly different within treatment plot bore holes. The mean HMX concentration changed significantly with depth (p = 0.001), however, RDX did not (p = 0.179). We also checked if the concentration of chemicals in soil samples from the control and treatment plots differed statistically. The mean HMX concentration of 6 bore holes ranged from 0.041 mg/kg (bore hole 2) to 0.067 mg/kg (bore hole 4) and was not significantly different (p = 0.316). Similarly, the mean RDX concentration ranged from 0.06 mg/kg (bore hole 5) to 0.132 mg/kg (bore hole 2) and was not significantly different (p = 0.331). Overall, the background concentrations of RDX and HMX in the control and treatment plots are not statistically different.

3.2 HMX and RDX in pore water

A total of eighty four pore water samples (42 each from control and test plot) were tested for HMX and RDX and their degradation products (MNX, DNX and TNX). The concentration of HMX and RDX in pore water samples collected in lysimeters changed during different flooding events. HMX and RDX in the molasses treated plot behaved differently from the control plot. Though HMX degradation was not significant both with respect to depth (p = 0.20) and treatment (p = 0.45), much lower concentrations were observed in treatment plot samples than in control plot samples at a depth of 5 ft (Figure

2). At 5 ft depth, the HMX concentration of 0.009 mg/l in the control plot in the first event increased to 0.018 mg/l by the end of fourth event and remained relatively stable thereafter. We interpret this to mean that HMX particles in soil will constantly release HMX in solution over time. HMX was present in similar concentration at all other sampling points (below 5 ft) and was close to the detection limit. The initial HMX concentration of 0.016 mg/l at a depth of 5 ft at the treatment plot decreased over time (with subsequent flooding events) to below laboratory reporting limit (LRL) in contrast to control plot samples where an increase was observed. A similar trend, though statistically not significant at 5 % level, was also observed at a depth of 11 ft and 13 ft indicating enhanced biodegradation of HMX with molasses. Figure 2 below elucidates the effectiveness of molasses in biodegrading HMX in shallow depths. At a depth of 31 ft, HMX decreased to non-detect level in both plots during the third sampling event.

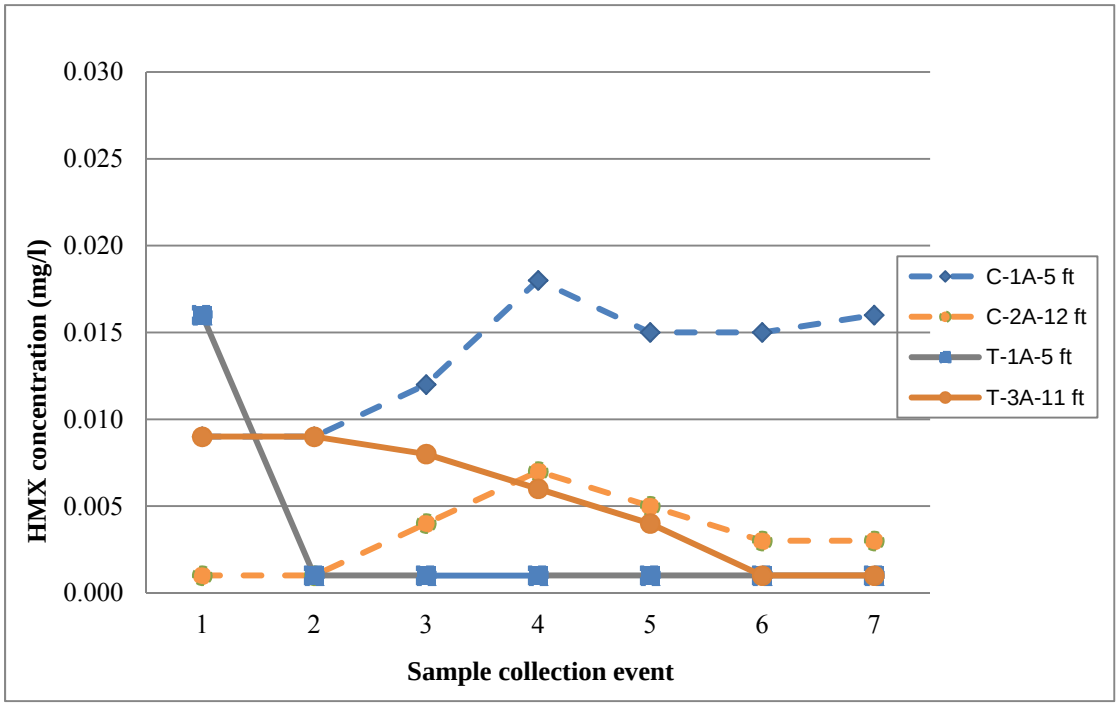

Figure 2: HMX concentration change in shallow depth (5 - 12 ft) pore water samples from control plot samples (C-1A-5 ft and C-2A-12 ft) and molasses treated plot (T-1A-5 ft and T-3A-11 ft).

The RDX concentrations in pore water samples are shown in Figure 3. The treatment plot RDX concentrations generally decrease during the study while they increase in the

control plot. The increasing concentrations of RDX in the control plot indicate dissolution of RDX in near-surface soils and leaching downward. If the decreases in RDX in the treatment plot were due to washout, then the same should be observed in the control plot. Instead, the decreasing concentrations of RDX in the treatment plot indicate molasses-enhanced biodegradation of the at-depth RDX and the RDX leaching from shallower depths. The difference in mean concentration of RDX with respect to both depth and treatment was very highly significant ($p < 0.001$).

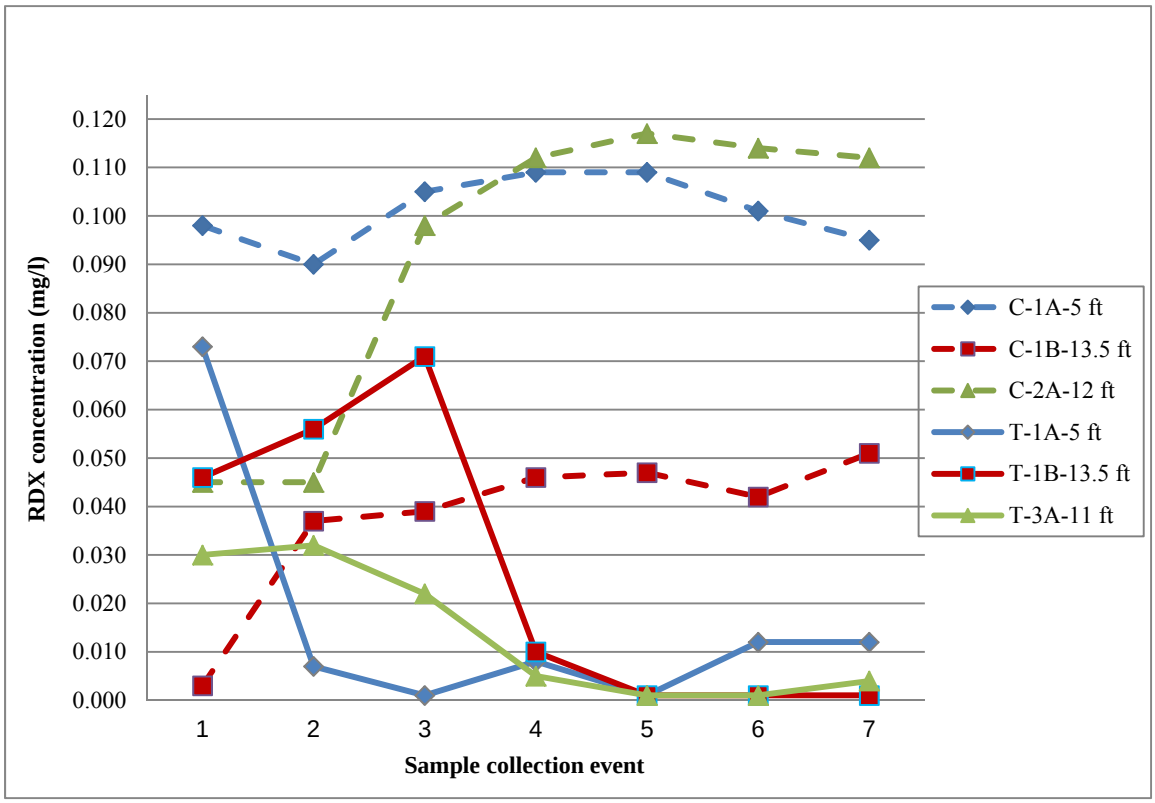

Figure 3: RDX concentration change in shallow depth pore water samples at control plot (C-1A-5 ft, C-2A-12 ft, and C-1B-13.5 ft) and molasses treated plot (T-1A-5 ft, T-1B-13.5 ft, and T-3A-11 ft).

The previous greenhouse treatability study carried out in the same Hawaiian soils found that molasses was effective in enhancing biodegradation of RDX within the root zone (~30 cm depth) but was not effective for HMX degradation (Lamichhane et al., 2012). The present study revealed that biodegradation of RDX can occur below the root zone down to a depth of approximately 13.5 feet if appropriate conditions can be created (addition of moisture and supplemental carbon source such as molasses). In addition,

contrary to the greenhouse treatability study, HMX in pore water samples was slowly biodegraded in the field plot study. This may be due to different environmental conditions in the root zone and in deeper pore water.

3.3 Degradation products of RDX

Degradation products MNX, DNX, and TNX were not detected in background soil samples but were detected in pore water samples and more frequently in samples from the molasses treated plot. Among the three, MNX had the highest frequency of occurrence (9 detections in treatment plot and 8 detections in control plot). The difference in mean concentrations of MNX were not statistically significant both with respect to treatment ($p = 0.068$) and depth ($p = 0.09$) at the 5% level. The degradation products DNX and TNX were not detected in control plot samples but were present in some treatment plot samples (2 DNX and 3 TNX detections out of total 42 samples). The presence and sequential depletion of MNX, DNX and TNX in pore water in the molasses plot (and their absence in soil prior to the experiment) indicates molasses enhanced biodegradation of RDX and HMX below the root zone.

3.4 Total organic carbon and total nitrogen

TOC concentrations were measured as a surrogate for molasses and values differed for the control and molasses treated plots. In the control plot, TOC concentrations were mostly less than 50 mg/l and were relatively stable (Figure 4) whereas much higher TOC concentrations were observed in several molasses treated plot samples (Figure 5). The difference in mean concentration of TOC with depth was highly significant ($p = 0.003$). The mean concentration of TOC differed very highly significantly ($p = 0.0006$) with treatment. Greater TOC concentrations at depths 5 to 13.5 ft in the molasses treated plot correlates with the depth of higher RDX degradation. This is further evidence that the presence of molasses is the cause of RDX biodegradation in pore water. Similar TOC concentrations observed in samples from both plots at greater depths (>17 ft) could be indicative of background conditions. In order to deliver molasses to depths greater than about 13.5 ft, it may be necessary to use higher molasses concentrations or greater frequency of flooding treatments.

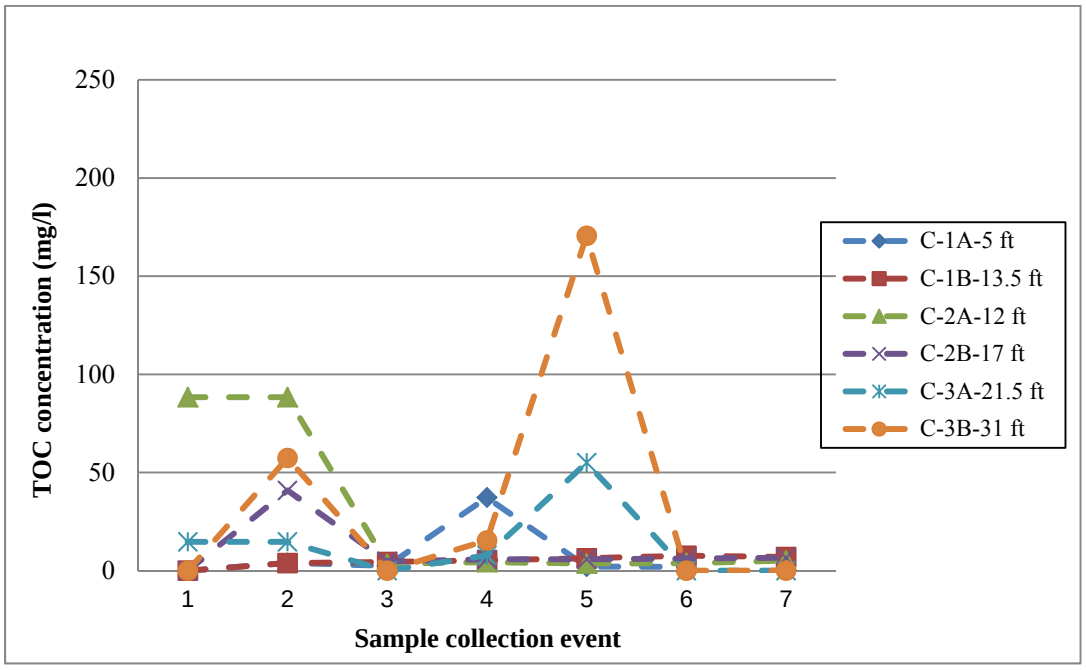

Figure 4: Change in TOC concentration with depth in pore water samples from the control plot in response to flooding events

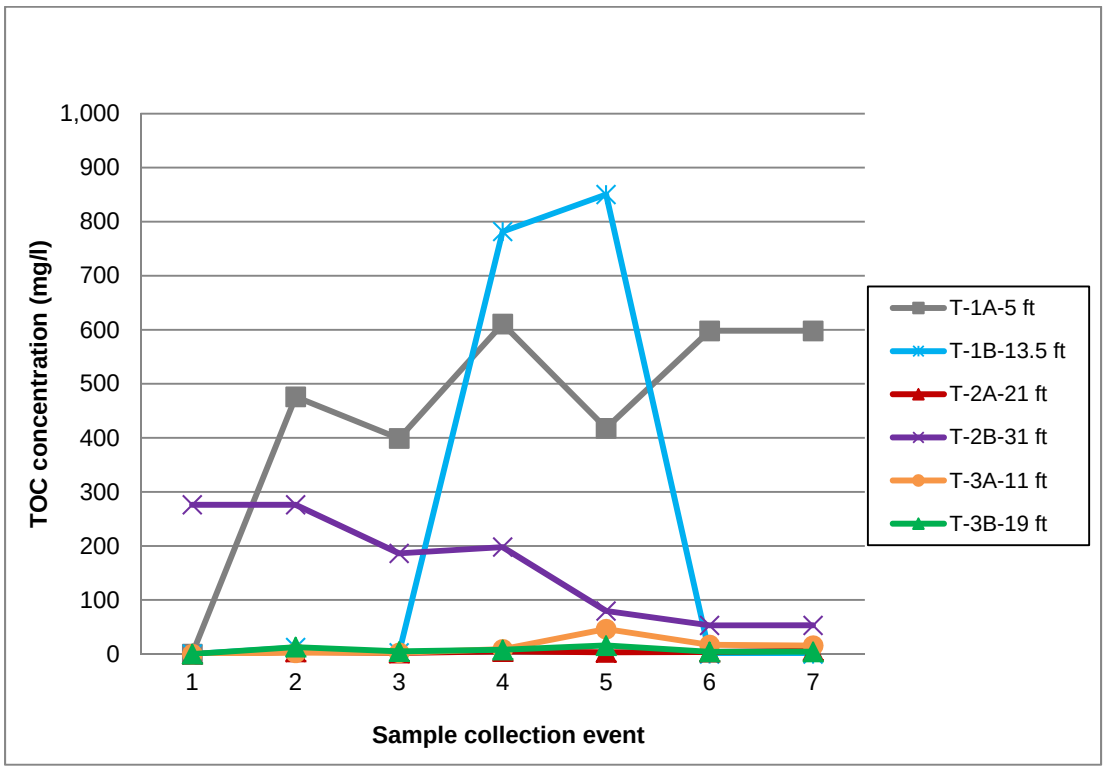

Figure 5: Change in TOC concentration with depth in pore water samples from the molasses treated plot in response to flooding events

Mean TN concentrations differed highly significantly ($p = 0.0004$) with treatment and also differed at 6% level ($p = 0.059$) with depth. TN concentrations were relatively stable at all sampling locations in the molasses plot with highest concentration in the zone of maximum explosive reduction (5-12 ft) likely due to liberation of N from degraded RDX. TN concentrations at similar depths in the control plot fluctuated and decreased slightly over time suggesting washout.

3.5 Infiltration rate measurement

VWC was recorded by 16 moisture sensors installed at different depths in bore holes of both test plots. The average infiltration rate was 4.12 ft/d (range: 0.65 - 5.17 ft/d) based on the VWC peak registered by the sensors corresponding to each flood irrigation event. An increase in VWC was registered within 24 hours after the flooding events at the shallow moisture sensors (e.g. C-1 in the control plot and T-1 in the molasses treated plot both at 5 ft depth), whereas it took about five days for the deep sensors (e.g., C-3 and T-2 at 31 ft depth) to register the VWC peak. Plots of change in VWC over time were generated for all moisture sensors. In general, infiltration rates increased with depth (weakly correlated, $r^2 = 0.39$). The VWC timing of peaks recorded by the moisture sensors at 31 ft depth (one each in control and test plots) show that the infiltration rate of water and the water molasses mixture is similar (Figures 6 and 7). It was also possible to determine the infiltration rate of precipitation that occurred during the experiment (average 0.65 ft/day). These data indicate that flood irrigation can be used to significantly increase infiltration rate in order to successfully rapidly deliver molasses deep into the unsaturated zone at MMR to enhance biodegradation.

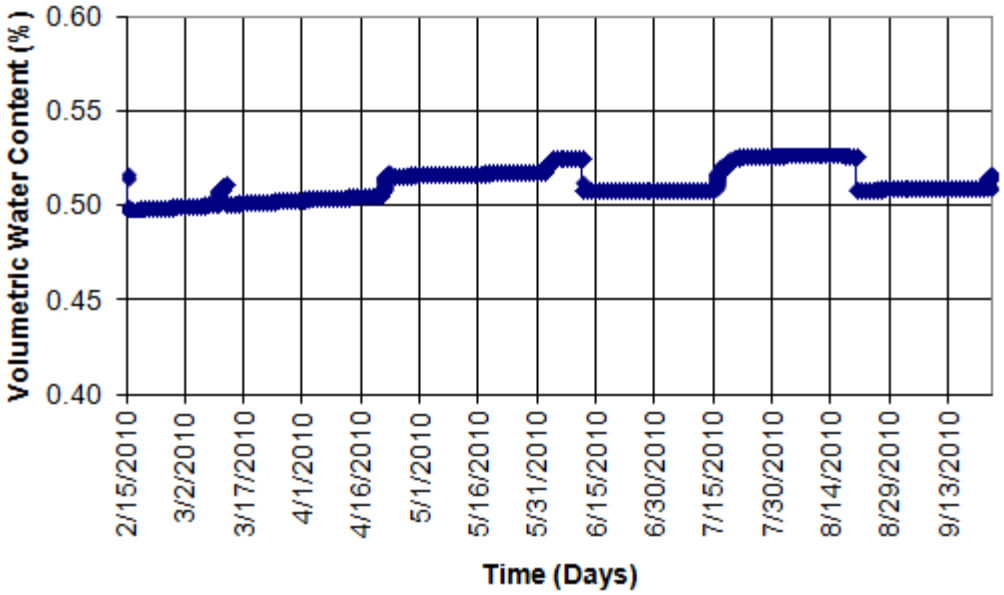

Figure 6: Fluctuations in moisture content recorded by the moisture sensor (C-3B) in the control plot at 31 ft depth

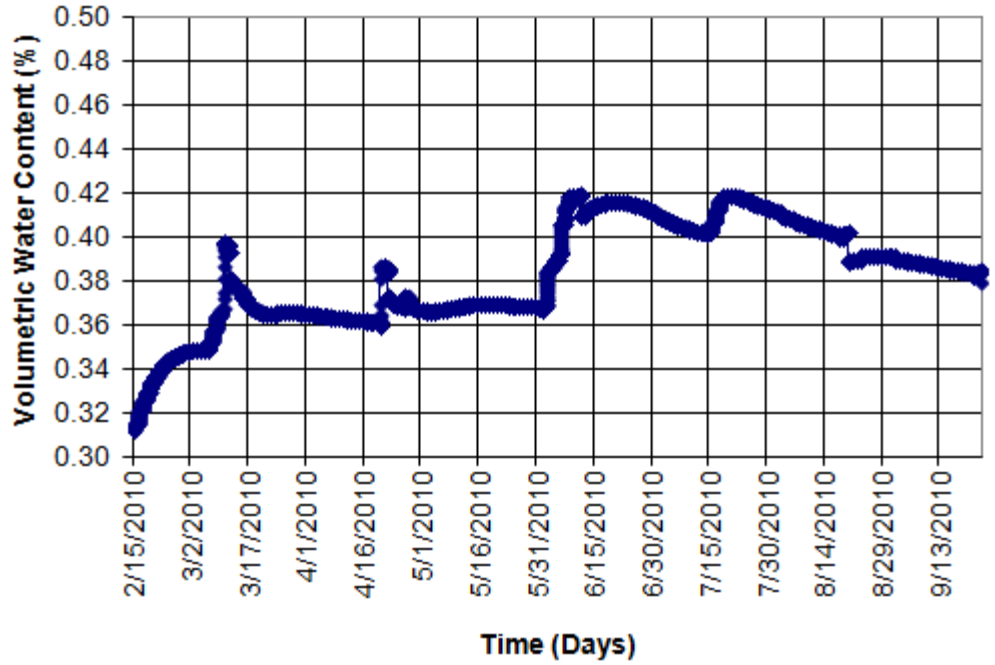

Figure 7: Change in moisture content recorded by the moisture sensor (T-2) in the treatment plot at a depth of 31 ft.

CHAPTER 4. CONCLUSION

The presence of HMX and RDX in subsurface soil and pore water samples down to a depth of 20 ft or more at MMR confirms the potential of these chemicals to pollute ground water and migrate off site. This study showed that treatment with a molasses water mixture (1:40 dilution) was very effective (p<0.001) in enhancing biodegradation of RDX in explosive contaminated subsurface Hawaiian soils to at least a depth of 13 ft at MMR. Unlike in the previous study, the treatment also appeared to help in removing HMX in shallow depths (5 ft). The degradation product MNX was present in both control and treatment plot pore water samples, however, DNX, and TNX were only found in treatment plot samples. The presence of only the first degradation product (MNX) in the control plot samples could be indicative of incomplete stimulation of indigenous microorganisms due to wetting only. Conversely, the presence of DNX and TNX in the treatment plot samples indicates enhanced biodegradation due to molasses treatment. Absence of all three degradation products in soil samples (collected before flooding events started) and their presence in pore water samples is a strong indicator of biodegradation rather than washout. The mean TOC concentrations between test plots differed very highly significantly (p <0.001). TOC concentrations in the control plot samples were relatively low (mostly <50 mg/l) and stable throughout the depths whereas higher average concentrations (~ 350 mg/l) were found in samples from the molasses treated plot. The difference in mean concentration of TOC with depth was also highly significant ($p < 0.01$). The mean TN concentrations also differed highly significantly with treatment ($p = 0.0004$). The highest TOC and TN concentrations were found at 5 to 13.5 foot depths which correlates with the zone of higher RDX degradation. The infiltration rate of molasses mixture in 1: 40 dilution is similar to the infiltration rate of water. The flood irrigation technique can be used to rapidly supply molasses in sufficient concentration to subsurface soils to enhance biodegradation of HMX, RDX and their degradation products. In this study, a 1:40 molasses dilution was able to deliver a carbon source effectively to at least a depth of 13.5 feet; however, in order to reach deeper depths, higher molasses concentrations or greater frequency of flooding treatments may be required.

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