

Bench Test of AO and TCDD Treatment

Joint Venture Test of Bioremediation and Enhanced Treatment with BCx 900
and BCx 900+2, September 2022

HLAllen³ Environmental

Purpose and Scope

- A Standard Recipe has been shown to be effective in bioremediating soil contaminated with Agent Orange at 500 mg/kg and TCDD at 40,000 ng/kg.
- New products (BCx 900 and BCx 900+2) have been proposed to accomplish the same job.
- This Study seeks to demonstrate the performance of these three methods using contaminated soil from the Vietnam War.
- This is a joint venture between HLAllen³ Environmental and EkoGea-na.

Soil to be Treated

- 14 years ago, EPA and the Vietnam Academy of Science and Technology conducted a successful pilot study in Danang, VN.
 - The overall removal efficiency of the treatment was 25.6%, but the treatment efficiency of the Standard Recipe was 60.3% after 6 months. 119 sample jars containing 80-100 grams of soil were recovered from the study and were composited for this project. The average TCDD concentration was 35,000 ng/kg (PPT) after treatment.
 - These samples were mixed together thoroughly, making a wet mixture of about 15 kg. Using an average dry weight estimated from the samples, the total dry weight should be about 11 kg.
 - Using a sampling trowel the stockpile was evenly distributed into 12 portions of about 2 kg (4.4 lbs) each, which is the basis for the amendment loading. The entire stockpile was used.

Loading and Nutrients

- The Standard Recipe is based upon EPA studies of bioremediation of Pentachlorophenol-contaminated soil, which has proven very effective. Loading is based on a dry soil weight.
- The Standard Recipe consists of 50 g/kg sawdust, 10 g/kg CaCO_3 , 2.4 g/kg NH_4NO_3 (substituted K_2NO_3) and a pinch of Bloodmeal (12% organic Nitrogen), 0.3 g/kg of Na_2HPO_4 *
- *Note: The phosphorus is needed to metabolize the sawdust and other incidental organics. These soils were treated 13 years ago and should still contain phosphorous, which is a conservative element.

The Treatments were amended as follows

- The sawdust was amended dry with KNO_3 as a nitrogen source and CaCO_3 to moderate pH as the sawdust degrades, because this soil is poorly buffered. The Control plots contained unamended sawdust only.
- 50 grams of sawdust was placed in 3 piles in each of 4 - 10"x18" (0.118 sq.m.) aluminum pans. (The 9"x12" pans are 0.071 sq.m.)
- Using a sampling trowel the stockpile was evenly distributed into 12 portions of about 2 kg (4.4 lbs) each, which is the basis for the amendment loading.
- The loading factor for the BCx was 250 g/sq.m. Since each portion occupied 0.039 sq.m. I added 10 ml of BCx 900 or BCx 900+2 to the appropriate pile.

The Stockpile of Soil from 119 Samples and 50 Grams of Hardwood Sawdust in Each Pile



Loading the Wet Soil into the Plots



After the soil dried out a bit I remixed the treatments and spread them in 9"x12" pans and relocated them to the randomly chosen plots.

Tray with Plots with BCx 900+2 Showing 10 ml Infusion



Empty Stockpile Container with Sampling Trowel



Layout of the Nested Design, Three Treatment Plots and One Control, With 3 Replicates Each



- Red Flags are the Standard Recipe, Yellow Flags contain BCx, White Flags are the Controls.

Framework to Support Cover. Note Flags



Ground has been prepared for possible direct application of the plots.

Rain Cover was Applied None Too Soon!

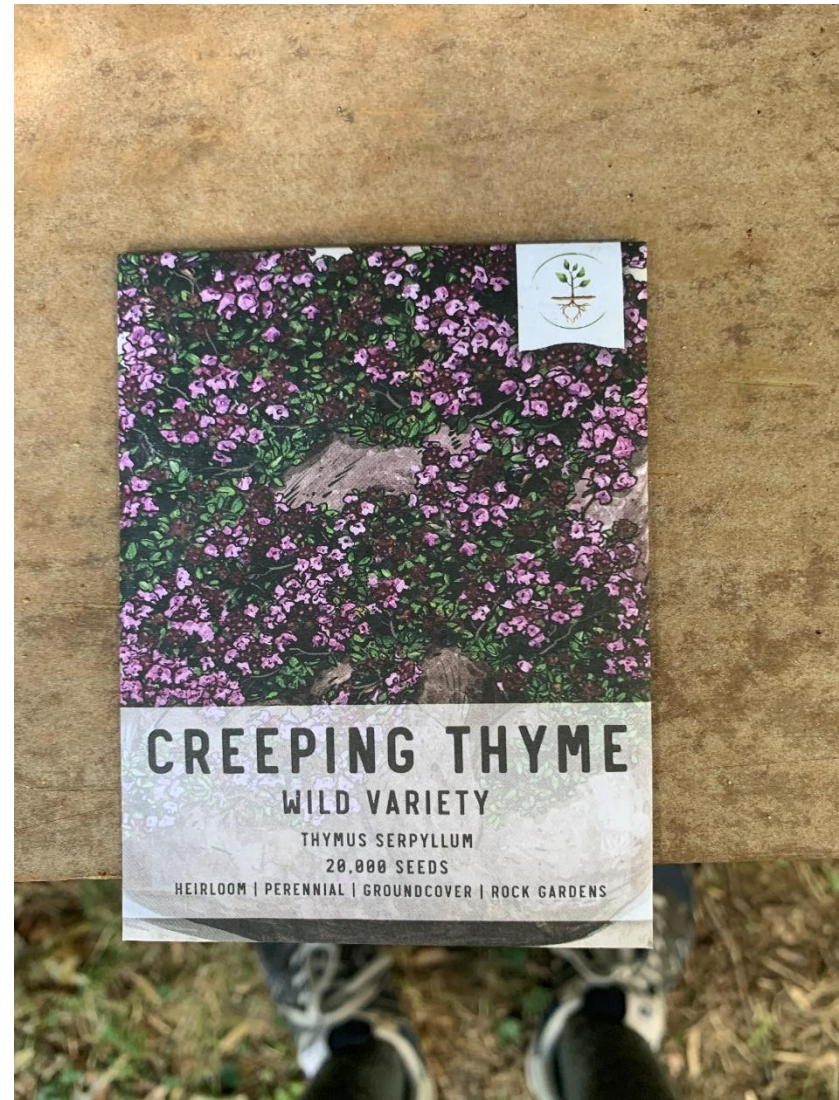


Some rain got in anyway, and I had to let the soil dry for a day before I could mix and repot them.

Creeping Thyme is Sensitive to Agent Orange in Soil



Creeping Thyme – 2 pkgs. Tiny Seeds, Need Sunlight to Germinate



Additives BCx 900 and BCx 900+2

Application Rate 250 g/sq.m. (20 ml for 0.071 sq.m.)



3 Portions of Soil Partially Dried and Amended with BCx 900+2



Partially Dry Soil Divided into 9x12 Inch Pans and Refreshed
with 10 ml BCx 900+2 (total added 20 ml)



Final Layout of Trays Using Predetermined Random Distribution



Plots labeled left to right front to back A-L
EPA A,E,I; Control B,D,L; BCx900 C,G,K; BCx900+2 F,H,J

Sampling and Analysis

- When the soil became less soupy, I mixed each plot thoroughly and relocate the plots. I will prepare composites from each of the 3 treatments, with one replicate, and the control and send them to the selected lab.
- A second analysis can be performed 2 months later.
- We need to analyze for the herbicides 2,4-D, 2,4,5-T, and 2,4,5-TP. Dioxin congener analysis by GC/MS can be limited to TCDD and OCDD, because other congeners are almost absent.
- Because biodegradation is linear rather than first-order, there must be cosubstrates supporting identified microbial growth. In previous studies, the Vietnamese did a comprehensive analysis of probable cosubstrates.

Soil composition change before and after treatment

	1.5DN5C (Before treatment)	1.5DN5T (After two year treatment)	Fold
I	2,4,5-T Methyl ester; 2,4-D methyl ester; Acetic acid, (2,4-dichlorophenoxy)	2,4,5-T Methyl ester	51.0
IIA	Phenol, 2,4-dichloro-; Phenol, 2,4,5-trichloro-	Phenol, 2,4-dichloro-; Phenol, 2,4,5-trichloro-	1.6
IIB	Phenol, 4,5-dichloro-2-methoxy-; Phenol, 2,4,6-trichloro-; Phenol, trichloro-; Phenol, 2,6-bis(1-methylpropyl); Phenol, 2,3,4,6-tetrachloro-; Phenol, 2,3,5,6-tetrachloro-; Phenol, 2,3,5-trichloro-; Phenol, 2,3,6-trichloro-	Phenol, 2,4,6-trichloro-; Phenol, trichloro-; Phenol, 2,6-bis (1,1-dimethylethyl); Phenol, dichloro-; Phenol, 2,6-dichloro-	5.4
III	Benzene, 1,2,4-trichloro-3-methoxy; 1,2-Dimethoxy-4,5-dichloro-benzene; Benzene, dichlorodimethoxy- ; Naphthalene, 1,3,7-trichloro-; Benzene, 1,2,4-trichloro-5-ethoxy-	Benzene, dichlorodimethoxy-; Benzene, 1,2,3-trichloro-4-methoxy	20.3
IVA	1-Nonadecene; 9-Tricosene, (Z)-; Nonadecane, 2-methyl-; Heptadecane; Tetradecane; Nonadecane; Eicosane; Pentadecane; Octadecane; Hexadecane	1-Octadecene; 1-Nonadecene; Heptadecane; Tetradecane; Nonadecane; Eicosane; Octadecane; Docosane	1.1
IVB	9-Octadecenamide, (Z)-; 9-Octadecenoic acid, (E)-; Dodecanoic acid; Dodecanoic acid, 1-methylethyl ester; Octadecanoic acid; n-Hexadecanoic acid; Tetradecanoic acid	9-Octadecenoic acid, (E)-; Dodecanoic acid; Dodecanoic acid, 1-methylethyl ester; Octadecanoic acid; n-Hexadecanoic acid; Tetradecanoic acid; Nonanoic acid; Hexadecanoic acid, methyl ester; Z-7-Hexadecenoic acid	2.9

Sampling Plan

- There are 3 different treatments and 1 control (no treatment). Each is replicated 3 times - meaning we set up 3 plots for each condition. We will use plant germination with wild creeping thyme as a visual cue but the analytical is also important.
- There is a need for soil sampling for 2 parameters: dioxins and herbicides in 2 sampling rounds. We recommend analyzing samples at the beginning of the study ("round 1" or as soon as reasonably possible) and when we are ready to conclude the study ("round 2" estimated 3-4 months from now).
- Sample Type and general considerations:
- These will be composite solids/soils samples. Samples will be prepared for 2 analytical methods (both methods are GC/MS). Note: For all samples we want the lab to run each sample 3 times (do 3 extracts from the material in the jar) and provide results so we can gauge lab error.

Analytical Methods

Methods:

Dioxin method: EPA Method 8290/8290A. Ask the lab for 2,3,7,8-TCDD and OCDD only. We don't believe the method should be hi-res. If there is an option, check with us.

Herbicides method: Pretty sure we want EPA 8151A. Looking for 2,4D and 2,4,5-T and possibly Silvex.

We will combine or composite samples from each treatment type (each treatment type combined into 1 sample) plus a duplicate.

Number of Samples (Continued)

At Time = t-1 (pilot study start); 4 samples are needed (x2 analyses above)

EG1 - 1 sample + 1 duplicate (BCx900)

EG2 - 1 sample (BCx900+2)

EPA - 1 sample (EPA)

CONTROL- 0

At Time = t-2 (end of study e.g., 385 days); 5 samples are needed (x2 analyses)

EG1 - 1 sample (BCx900)

EG2 - 1 sample + 1 duplicate (BCx900+2)

EPA - 1 sample (EPA)

CONTROL - 1

We have 21 - 8 oz. Amber sample jars.

t-1 Results from EKO-GEA Agent Orange Study

Sampling Date 09/20/2022					
Sample Name	Dioxins (ng/kg) (ppt)		Herbicides (ug/kg)(ppb)		
	2,3,7,8-TCDD	OCDD	2,4-D	2-4-5-T	2-4-5-TP
EPA t-1 (AEI)	20,000	21,000	170,000	320,000	ND
EPA MS t-1 (AEI)	18,000	18,000	170,000	310,000	
BCx 900 t-1 (CGK)	18,000	18,000	160,000	270,000	ND
BCx 900+2 t-1 (FHJ)	24,000	24,000	160,000	280,000	ND
Average =	20,000	20,250	165,000	295,000	
Std Deviation =	2,828	2,872	5,774	23,805	
n=	4	4	4	4	

t-2 Results from EKO-GEA Agent Orange Study

Sampling Date 10/10/2023					
Sample Name	2,3,7,8-TCDD	OCDD	2,4-D	2,4,5-T	2,4,5-TP
Control t-2 (BD)	18,400		130,000	130,000	
EPA t-2 (AEI)	17,300		4,000	16,000	
BCx900 t-2 (CGL)	17,000		57,000	190,000	
BCx900 t-2 (K)	16,100		110,000	120,000	
BCx9002 t-2 (FHJ)	16,100		54,000	95,000	
BCx9002 t-2 Duplicate (FHJ)	16,400		4,300	130,000	
Averages =	16,580		45,860	110,200	
Standard Deviation =	545		44,113	63,168	
n =	5		5	5	
Df =	4		4	4	
Confidence (0.1) =	401		32,449	46,466	
Average Decrease =	17%		72%	63%	

Treatment Performance Results from EKO-GEA Agent Orange Study

Removal Percentages by Treatment Type	TCDD t-1	TCDD t-2	Removed	2,4-D t-1	2,4-D t-2	Removed	2,4,5-T t-1	2,4,5-T t-2	Removed
EPA	20,000	17,300	13.5%	170,000	4,000	97.6%	320,000	16,000	95.0%
BCD-900	18,000	17,000	5.6%	160,000	57,000	64.4%	270,000	190,000	29.6%
BCD-900+2	24,000	16,100	32.9%	160,000	54,000	66.3%	280,000	95,000	66.1%
BCD-900+2 Duplicate		16,400	31.7%		4,300	97.3%		130,000	53.6%
Control		18,400			130,000			130,000	

Due to a sampling error, BCD-900 t-2 results include a portion of the Control Plot L.
Plot K was analyzed separately
As a result the performance efficiency of BCD-900 is underestimated by perhaps 5% .

New Set-up After Last Sampling Event



These are the 5 remaining treatment plots.

Recommendation

- The results clearly demonstrate the EPA method and both the BCx900 and BCx900+2 effectively treat TCDD in DaNang soil and even more effectively treat Agent Orange Compounds 2,4-D and 2,4,5-TP.
- Depending upon the wishes of the group, we could be at the finish line.
- If we decide to go on, I will remix the 5 treatment plots and add additional nutrients, including sawdust, potassium nitrate, and calcium carbonate for the EPA method. For the BCx treatments, I will add whatever amount of amendment Dr. Dordic suggests.
- We would go one more round in 6 months.

Contact Information

- Harry Allen 3, 908-565-2329, Harryallen3@gmail.com
- Harry Allen 4, 626-319-8493, Harryallen4@gmail.com
- Jerry Wade, Jerry.wade@usecology.com
- Michael Richardson, Mrichx@hotmail.com
- Dr. Goran Dordic, gorandordic@yahoo.com