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Feasibility of Bioremediation of Agent Orange/Dioxin in Vietnam

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ABSTRACT

Agent Orange residue, containing traces of dioxins, TCDD (tetrachlorodibenzodioxin) and OCDD (octachlorodibenzodioxin), remains in the soil and in lake sediment at the former air force bases in Da Nang, Bien Hoa, and Phu Cat, Vietnam, long after the war with the US. Natural attenuation has not detoxified the soil or sediment. In 2009, the US EPA and the Vietnam Academy of Science and Technology, conducted a 6-month pilot study in Da Nang. This study concluded that bioremediation treats Agent Orange effectively. Combined TCDD data for all aerobic Land Treatment Units (LTUs) showed a significant downward trend using the Mann-Kendall (M-K) trend test (p=0.0093; S=-398; n=63). TCDD degradation rate, determined by Ordinary Least Squares (OLS) regression slope, was -110 ng/kg per day (ppt./day). Buffered aerobic treatment (LTU No. 7) achieved a 70% TCDD reduction, with a significant downward trend (p=0.0503; S=-31; n=14). TCDD degradation rate was -274 ppt./day. LTU No. 7 also showed a reduction in the TCDD/OCDD ratio (p=0.0002; S=-65; n=14), indicating that TCDD was undergoing biodegradation, while the OCDD was degrading four times more slowly, making OCDD a potential biodegradation marker. Buffered anaerobic treatment (Cell No. 5) showed a significant downward trend (p=0.064; S=-75; n=27). TCDD degradation rate was -232 ppt./day. In both aerobic and anaerobic treatments, Agent Orange herbicides, 2,4-D and 2,4,5-T, were rapidly degraded, declining at rates of 9.2 and 11.8 mg/kg per day (ppm./day), respectively. It is estimated that there are 26 unaddressed dioxin sites in Vietnam. Bioremediation of these sites is not only cost-effective but is easily accomplished using in-country resources and manpower.

Key words: Vietnam, Agent Orange, dioxin, bioremediation

1. Introduction:

Da Nang air force base (AFB) was subject to significant contamination with the defoliant, Agent Orange, during the American occupation of the AFB. A cleanup operation, code named “Pacer Ivy”, was conducted at the AFB by the US after the peace accord of 1972. Stockpiles of herbicide were removed from Vietnam and destroyed; however, spilled Agent Orange residues containing traces of the dioxins: 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and octachlorodibenzodioxin (OCDD) remain in the soil on site and in lake sediment. Dioxins (50 ppb.) represent a tiny fraction (about 0.001%) of the residual 2,4-D and 2,4,5-T herbicides (about 500 mg/kg or ppm.) remaining in the soil in Da Nang; natural attenuation has not been effective in detoxifying the soil or sediment.

An Environmental Task Force (ETF) was formed as a subcommittee of the Vietnam – U.S. Joint Advisory Committee (JAC) and held its first set of meetings during November 17-25, 2008, in Hanoi. During the meetings, the ETF discussed plans to remediate contaminated areas in the northern part of the Da Nang Airport. The ETF proposed to remove soil and sediment from the contaminated areas and to place them in a secured holding, treatment, and on-site land disposal area away from human activities at the airport. The issue arose as to whether to construct a biologically-active landfill, which would provide some level of treatment, or a passive landfill, which would not.

A pilot study was authorized to determine what degree of removal could be expected in an active landfill and what operating conditions would enable an effective level of treatment. The pilot study was designed and put in place by June 1, 2009. It was funded in part by the Ford Foundation, with contributions from EPA's Office of Research and Development and the Office of Superfund Response and Technology Innovation.

Biodegradation of TCDD has been reported in the scientific literature, has been demonstrated in the laboratory and in pilot studies, and has been employed in full-scale cleanups. Early studies have shown long half-lives (at least 20 years) for both TCDD and OCDD.[1,2] The EPA Environmental Response Team (ERT) has been using aerobic bioremediation (land farming) to decontaminate soil containing pentachlorophenol (PCP) and chlorodioxins at wood treating waste sites for many years.[3,4] Over 13 PCP sites have been treated successfully using both biostimulation (altering the soil environment to stimulate indigenous microbial growth) and bioaugmentation (reintroducing an enriched culture of active microbes isolated from the same site). ERT also has demonstrated anaerobic bioremediation of soil at 32 sites contaminated with the persistent organochloride pesticide, toxaphene.[5,6] VAST's Institute of Biotechnology has published results of multiple studies showing biodegradation of dioxin.[7,8,9,10] This present study is a joint effort of VAST, EPA Region 9, and the ERT. EPA provided sampling devices for the aerobic and anaerobic reactors, field monitoring equipment, and fertilizers and chemicals required for the project in the field and in the VAST laboratory. EPA also provided VAST with a detailed design and sampling plan in the form of a Power Point presentation. This study did not address the mechanisms of biodegradation.

An overlapping large-scale (3,384 m^3) study was conducted in Bien Hoa by the Vietnamese from April 2009 to July 2011.[11] The results from the averages of 12 composite samples at the start and 64 composite samples distributed over the 13 month study showed a 99.5% reduction in TCDD toxic equivalents from 10,865 ppt. to 52 ppt. This translates into a linear degradation rate of about -30 ppt./day (R^2= 93.9%) using least squares analysis (MS Excel @linest function). The Mann-Kendall test yielded a significant downward trend (p=0.0002; S=-94; n=114). These results show reduction rates for TCDD similar to those seen at Da Nang, where the VAST aerobic treatments evidenced a linear trend of about -81 ppt./day. Reproducing the results obtained in Da Nang at Bien Hoa proved that the dioxin remediation goal of 1,000 ppt. could be readily achieved by bioremediation. In addition to the third major Agent Orange site at Phu Cat, it is estimated that there are 26 other sites in Vietnam which have not been addressed.

2. Results:

Eleven 2-m^3 soil treatment units were constructed on the Da Nang AFB using two modes of biological treatment: aerobic and anaerobic. The treatment details are further specified in the Materials and Methods section. VAST personnel managed the treatment units and collected soil samples for time series dioxin analysis. Five cells, unit numbers 1-5, including a reference cell (Cell No. 1) were used for the anaerobic treatment, and six (6) LTUs, unit numbers 6-11, including a reference unit (LTU No. 6) were used for the aerobic treatment.

2.1. TCDD Results:

The analytical results for TCDD are summarized in Table 1 (Aerobic LTUs) and Table 2 (Anaerobic Cells). The data shown were all obtained by GC/MS analysis from a NELAC-certified EPA contract laboratory in the US. EPA results were obtained using Method SW-8290A. Because of budgetary limitations, not all the samples were analyzed every month, but priority was given to certain treatment schemes. The tables reflect this judgment.

2.2. OCDD Results:

OCDD is the second most abundant dioxin congener found in Agent Orange. It is not toxic nor very biodegradable. OCDD is less biodegradable than TCDD because of its lower solubility and higher chlorine content, so it might be a potential biomarker for the TCDD biodegradation. The analytical results for OCDD are summarized in Table 3 (Aerobic LTUs) and Table 4 (Anaerobic Cells).

2.3. Agent Orange Herbicide Results:

In a later transmission, VAST provided results on the herbicides in soil samples previously analyzed for dioxin. The analysis was done by the Russian Tropical Research Laboratory. EPA received no QA/QC information on these results, but they are consistent with the literature on herbicide biodegradation. Agent Orange was originally formulated as 50% 2,4-D and 50% 2,4,5-T, and the initial concentrations in the environmental samples approximate this ratio (2,4-D = 43%). During treatment, both herbicides degraded at approximately the same rate regardless of treatment mode, so for the purpose of this paper all the non-reference results were combined to make the following summary table:

3. Discussion:

In order to determine whether or not significant downward trends could be observed in dioxin data collected during the pilot study, graphical displays of the data were prepared and trend evaluations were performed under an EPA Superfund support contract.[12, 13]

The following null and alternative hypotheses were tested to determine potential trends in TCDD concentrations obtained using the aerobic, anaerobic, and reference treatments:
Null Hypothesis, H_0: Dioxin data do not exhibit any significant downward (upward) trend in the TCDD or OCDD concentrations collected during the 6 month period; versus the alternative hypothesis;
Alternative Hypothesis, H_1: Dioxin data exhibit a significant downward (upward) trend in TCDD or OCDD concentrations collected during the 6 month period.

The Mann-Kendall (M-K) nonparametric test was used to determine significant trends (e.g., at 0.05 or 0.1 level of significance) during the 6-month experimental period. When significant trends were detected, Ordinary Least Squares (OLS) regression was used to estimate the strength of the trends and ultimately to determine rates based on the regression slope. When multiple observations were collected during the various sampling events, trend evaluations were performed based both upon the raw data without averaging as well as on the averages of coincident multiple observations. It is important to remember that the results remain probabilistic estimates, and conclusions based upon statistical tests must also include assessment of the graphics.

In this discussion, for simplicity, OLS regression analyses use graphics generated using Windows Excel: only data generated by EPA's NELAC-certified laboratory are used. The contractor's statistical reports uses all results and ProUCL 5.0 software; the differences are minor and do not affect the conclusions.

3.1 TCDD Aerobic Biodegradation

The combined GC/MS TCDD results from all aerobic treatments were analyzed by M-K Trend analysis (Figure 19 in Reference 12), which demonstrated a high probability (p=0.0093; S=-398; n=63) that there is a significant downward trend in the aerobic results. OLS regression analysis estimated the rate of the linear downward trend -110 ppt. per day with an R^2 of 80%. Where averaged data are used for each interval the graphic is clearer but the conclusion is the same: there is a significant downward trend (at all levels of significance ≥ p-value =0.005) (Figure 1, OLS Regression Trend for All Aerobic Land Treatment Units Combined). The statistics and the graphical display clearly demonstrate a significant linear downward trend in the combined aerobic LTUs.

As shown in Figure 2, OLS Regression Trend for Buffered Aerobic Land Treatment Unit (No. 7), the buffered aerobic treatment achieved a 70% TCDD reduction. LTU No. 7 employed the approach developed by ERT for remediating soil at pentachlorophenol sites. GC/MS TCDD results from buffered, aerobic treatment were analyzed by M-K Trend analysis demonstrated a high probability (p=0.0214; S=-46; n=14) that there is a significant downward trend in the aerobic results (α=0.05). Where averaged data are used for each interval the graphic is clearer but the conclusion is the same: M-K Trend analysis demonstrated a high probability (at all levels of significance ≥ p-value =0.005, n=7) that there is a significant downward trend in EPA TCDD concentrations suggesting that EPA aerobic treatment is effective. OLS regression analysis estimates the rate of the linear downward trend of TCDD was -274 ppt./day (R^2=67%). At this rate achievement of a 1,000 ppt. remediation goal would take approximately 250 days. At a starting concentration of 42,673 ppt. (t-0 avg.), the remediation goal would be reached in 152 days.

The statistics and graphs support the hypothesis that there is a significant linear downward trend in TCDD concentrations for aerobic bioremediation in general and a highly significant downward trend for buffered, aerobic treatment (LTU No. 7), specifically.

3.2 TCDD Anaerobic Biodegradation

Buffered anaerobic treatment (Cell No. 5) showed a significant downward trend (at all levels of significance ≥ p-value ~ 0.02) in TCDD concentration suggesting that the EPA buffered anaerobic bioremediation method is effective (raw data). Figure 3, OLS Regression Trend for Buffered Anaerobic Treatment Cell (No. 5). OLS regression analysis estimates the rate of the linear downward trend of TCDD was -232 ppt./day (R^2=61%). Cell No. 5 was fully-hydrated and achieved anaerobic conditions quickly, unlike the other anaerobic cells, which were not fully hydrated for 3 months. The t-0 sample for Cell No. 5 (23,900 ppt.) was not representative and was not included in the OLS regression analysis.

In summary, the EPA buffered aerobic and anaerobic data exhibit significant downward trends in TCDD concentration. The biological treatment processes developed by ERT, respectively, for PCP and toxaphene sites appear to be effective for TCDD and Agent Orange contaminated soil and sediment sites as well.

3.3. Reference Units and Abiotic Loss

The reference units were shared by the US EPA and VAST. Cell No. 1 and LTU No. 6 were constructed to be the aerobic and anaerobic reference units, respectively. Both units were intended to remain dry and untouched during the course of the study. However, Cell No. 1 leaked and was hydrated and LTU No. 6 was watered because the soil became too hard for the crew to sample. Because the soil was wet, some biological activity may be included with abiotic losses. Since both reference units essentially were managed the same way, the results are similar and Cell No. 1 and LTU No. 6 can be combined to produce the Figures 4 and 5 for TCDD and OCDD Reference Trends, respectively.

No significant trend was observed in TCDD concentration time series data collected from reference cells during the 6 month period (p=0.3285; S=-20; n=25). Average data yielded a slope of -118 ppt./day (R^2= 14%). No significant trend was observed in OCDD concentration time series data collected from reference cells during the 6 month period (p=0.4535; S=-6; n=25). Average data yielded a slope of -54 ppt./day (R^2= 14%). Data from the reference units shows no significant trend in TCDD or OCDD concentrations, so we may conclude that abiotic losses are insignificant.

3.4 OCDD as a Biomarker

Figure 6, OCDD Aerobic Biodegradation, shows the linear reduction in average OCDD concentration (36%) in the buffered aerobic LTU over 6 months. This may be compared with a TCDD reduction of 70% over the same period. Because OCDD appears to degrade four times more slowly than TCDD, it is an imperfect biomarker, but, combined with the conclusion that abiotic losses are insignificant, it should be sufficient to prove that biodegradation, not chemical weathering, is the principal cause of the removal of TCDD.

Figure 7, OLS Regression Trend for OCDD Biomarker Determination, shows the ratio TCDD/OCDD in LTU No. 7 over 6 months. It shows a downward trend with a decrease of 49% over 6 months. The M-K p-value for aerobic TCDD/OCDD ratios equals 0.11/0.12. This suggests there is a significant downward trend (at all levels of significance ≥ p-value ~ 0.11/0.12) in EPA aerobic ratios. The trend is not significant at 0.05 level of significance. OLS regression shows an R^2 of 55% (n=14).

3.5. Herbicide Biodegradation:

Agent Orange defoliant was factory-formulated as 50% 2,4-Dichlorophenoxyacetic acid (2,4-D) and 50% 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T). Chlorodioxins are formed coincidentally during the manufacture of 2,4,5-T, where they occur in trace amounts. Both herbicides are considered biodegradable to some extent.[14] Biodegradation rates for each herbicide were similar for both aerobic and anaerobic treatment modes in this study, so the results were combined to prepare Figure 8, Herbicide Degradation Linear Trends for Combined Aerobic and Anaerobic Units. Note that the initial 2,4-D concentration is about 43% of the residual formulation, indicating that some attenuation of 2,4-D may have occurred over the years. Figure 8 shows the trend for herbicide degradation as linear. Least-squares analysis gives degradation rates of 9.2 and 11.8 ppm/day for 2,4-D and 2,4,5-T, respectively. The scientific literature suggests that TCDD degrades linearly as a co-metabolite, while the microbial population utilizes other organic contaminants in the soil for first-order growth.[15] These herbicides apparently degrade linearly also; a logarithmic degradation model yields half-lives of 27 and 37 days, respectively, for 2,4-D and 2,4,5-T, but with R^2 values about 10% lower. Non-herbicide co-substrates in Da Nang soil include dichlorophenols, trichlorophenols, polyaromatic hydrocarbons (PAH), and others, which could be supporting first-order microbial growth.

4. Materials and Methods:

Eleven pilot treatment/sampling units were constructed at Da Nang International Airport (the former Da Nang AFB). Each reactor contained approximately 2-m^3 (about 4 metric tons) of Da Nang site soil. The soil was chosen on the basis of several extent of contamination studies in the defoliant storage area, and it was pre-mixed with a backhoe to achieve a target dioxin concentration of about 100,000 ppt. (The average of the actual starting concentrations was about 42,673 ppt. The units included:

- Two EPA Units: Cell No. 5 (anaerobic, buffered, biostimulated, bioaugmented) and LTU No. 7 (aerobic, buffered, biostimulated).
- Seven (7) VAST Units: Cell No. 2 (anaerobic, biostimulated), Cell No. 3 (anaerobic, bioaugmented with microbial group 1), Cell No. 4 (anaerobic, bioaugmented with microbial group 2), LTU No. 8 (aerobic, biostimulated, vented), LTU No. 9 (aerobic, bioaugmented, mixed), LTU No. 10 (aerobic, biostimulated, mixed), LTU No. 11 (aerobic, bioaugmented, mixed).
- Two (2) shared Reference Units: Cell No. 1 (anaerobic treatment) and LTU No. 6 (aerobic treatment), were designed to remain dry to account for abiotic losses.

EPA Cell No. 5 was prepared using a buffered anaerobic formula developed for soil contaminated with the chlorinated pesticide, toxaphene. The treatment includes the addition of a few percent of dried bloodmeal (an iron and nitrogen rich fertilizer), starch (to help maintain anaerobic conditions) and buffers to maintain a near neutral pH. Flooding the cell provides an organic-rich water blanket, which consumes oxygen and protects against reaeration. The 4 metric tons of soil in EPA Cell No. 5, was to be amended with 1% (40 kg) organic fertilizer, 0.12% (5 kg) sodium phosphate, 0.9% disodium phosphate (36 kg), 0.4% starch (16 kg). The cost of these agricultural grade chemicals was about $221. Only about 5 kg of blood meal and about 90 kg of moderately contaminated lake sediment were available in Da Nang.

EPA LTU No. 7 was prepared using a buffered aerobic system developed for bioremediating soil contaminated with PCP at wood treating waste sites. This technique involves engineering a mixed LTU augmented with a cellulosic bulking agent (hardwood sawdust or rice hulls), a phosphate buffer and lime (to control acid production in non-calcareous soil). Where biodegrading microbes are scarce, a bioaugmenting culture may be prepared from microbes isolated from the site, but this is not always necessary. The 4 metric tons of soil in LTU No. 7 was amended with 5% bulking agent (rice hulls), 0.24% ammonium nitrate (10 kg), 0.03% disodium phosphate (1 kg), and 1% lime (40 kg). The cost of these agricultural grade chemicals was about $81.

4.1 Sampling and Analysis Methods

Owing to the fact that this study was being conducted half-way around the world, management of the site was left primarily to VAST. EPA left an explicit power point presentation covering sampling and management of the treatment units and several videos with the Vietnamese as guidance. EPA also provided VAST with sampling equipment, sample jars and laboratory equipment. The Ministry of Defense (MOD) provided oversight as quality control for the Vietnamese.

Samples from the 11 treatment units were collected monthly for 6 months by VAST and sent to the United States for dioxin congener analysis. Sampling, sample handling, sample preparation (mixing, drying, screening, packaging), and shipping were overseen by the MOD for quality assurance. The code for numbering the repackaged samples (jar number vs. sample identification number) was retained by MOD until they received the results from EPA.

Composite soil samples were taken initially (t-0, or time zero days) and at 30-day intervals for six months (t-180). These soils were thoroughly mixed, dried, and subsampled (split) for analysis. VAST analyzed the soil composites for pH and soil moisture content (%). The samples which came to the US were received by the ERT and transshipped to a NELAC certified contract laboratory (SGS Systems, Wilmington, NC). The samples were extracted and analyzed using high resolution gas chromatography/ mass spectrometry (GC/MS) for dioxin congeners (EPA Method SW-8290A) with a 30-day delivery date. After the results and the quality assurance packages were received by the ERT and transmitted to Vietnam, MOD revealed the sample codes to ERT and VAST, and the results were reduced to descriptive form.

4.2. Monitoring of soil parameters:

Herbicide analysis and operating parameters (pH and moisture content) were performed by VAST and the MOD independent of EPA. Tables 6 and 7 summarize the soil moisture and pH results reported during the course of treatment. Ideal soil moisture for aerobic treatment should be about 20% by volume depending upon soil type. The specific amount depends on soil characteristics, such as, porosity, organic content, and grain size. Anaerobic treatment requires saturated soil, plus standing water as previously discussed. The soil in Da Nang is fine-grained, but the actual porosity was not reported.

5. Conclusions:

The pilot study posed a null hypothesis that neither aerobic nor anaerobic microbial bioremediation would be effective in achieving a TCDD standard of 1,000 ppt. (ng/kg) in the soil and sediment in Da Nang. Rejecting that hypothesis would confirm the feasibility of bioremediation. The hypothesis was tested using Mann-Kendall analysis, and the slope of the time series was determined by ordinary least squares regression. The null hypothesis was rejected at the 0.05 and 0.1 levels of significance.

Biodegradation was observed in both aerobic and anaerobic treatments. The combined EPA and VAST aerobic TCDD concentrations data exhibit a significant downward trend. The results reveal a TCDD linear trend of more than -110 ppt./day aerobically and a -47 ppt./day anaerobically. Individual treatment recipes and management schemes yielded different rates. The most effective rates were obtained with buffered, biostimulated treatments. The linear biodegradation trends for these were -274 ppt./day aerobically and -232 ppt./day anaerobically.

The results for the reference units for the two treatments (individually and combined) do not evidence significant TCDD trends.

M-K and OLS analyses evidence a significant linear downward trend for the TCDD/OCDD ratio in the buffered aerobic LTU, thus demonstrating that biodegradation of TCDD is the principal mechanism for the reduction over the experimental period.

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Notes on supplemental information:

The VAST Bien Hoa study was reported on in a Power Point presentation at the JAC meeting in Hanoi in September 2012, and an author-approved summary is included as supplemental information.

Graphics describing the statistics are included in the report, “Draft Trend Evaluations of EPA Agent Orange/Dioxin Bioremediation Pilot Study Data Da Nang Air Force Base, Vietnam,” Reference 12, which is included as supplemental information.

About the author:

Harry L. Allen III has a Doctorate in Environmental Science from Rutgers University. He worked as a scientist for the USEPA for over 40 years, the last 30 as a specialist with the EPA’s Environmental Response Team. He has been focusing on bioremediation of hazardous waste sites contaminated with wood treating chemicals, pesticides, solvents and petroleum for much of his 1career. Now retired, he teaches.

TABLES:

Table 1 Summary of Aerobic Land Treatment Unit GC/MS Results for TCDD (in ng/kg or ppt.)							
LTU Number	Time-0	t-30 Days	t-60 D	t-90 D	t-120 D	t-150 D	t-180 D
M6 (Reference)	12,500	61,100	24,833	34,133	21,567	34,400	26,150
M7 (EPA, Buffered)	67,600	83,800	37,960	34,967	34,133	23,800	25,350
M8 (VAST, Vented)	45,400	50,100	No Sample	No Sample	No Sample	29,000	32,700
M9 (VAST, Vented)	61,700	47,900	No Sample	No Sample	No Sample	40,800	25,850
M10 (VAST, Mixed)	37,000	19,100	No Sample	43,433	32,800	35,600	38,100
M11 (VAST, Mixed)	25,400	33,200	50,033	41,367	20,067	31,400	30,350

Table 2 Summary of Anaerobic Treatment Cell GC/MS Results for TCDD (in ng/kg or ppt.)							
Cell Number	Time-0	t-30 Days	t-60 D	t-90 D	t-120 D	t-150 D	t-180 D
M1 (Reference)	101,000	47,600	No Sample	55,767	71,000	24,300	40,000
M5 (EPA, Buffered)	21,600	63,300	44,167	25,667	18,083	28,800	33,400

Table 3 Summary of Aerobic Land Treatment Unit GC/M Results for OCDD (in ng/kg or ppt.)							
LTU Number	Time-0	t-30 Days	t-60 D	t-90 D	t-120 D	t-150 D	t-180 D
M6 (Aerobic Reference)	5,640	71,500	29,500*	32,633	25,333	22,100	22,200
M7 (EPA, Buffered)	34,400	33,800	23,600	30,333	26,967	20,300	21,300
M8 (VAST, Vented)	16,600	35,200	No Sample	No Sample	No Sample	18,200	22,450
M9 (VAST, Vented)	19,400	27,400	No Sample	No Sample	No Sample	19,000	18,000
M10 (VAST, Mixed)	19,100	8,590	No Sample	28,400	22,900	21,400	20.150
M11 (VAST, Mixed)	10,100	17,100	32,533	24,933	20,067	16,800	18,100

*Sample analyzed with t-90 samples.

Table 4 Summary of Anaerobic Treatment Cell GC/MS Results for OCDD (in ng/kg or ppt.)							
Cell Number	Time-0	t-30 Days	t-60 D	t-90 D	t-120 D	t-150 D	t-180 D
M1 (Reference)	46,400	27,600	No Sample	26,100	45,900	19,800	33,500
M5 (EPA, Buffered)	23,900	42,700	26,300	19,817	17,717	18,300	24,300

Table 5 Average Herbicide Totals for All Non-Reference Units (in mg/kg or ppm)			
Days of Treatment	2,4,5-T	2,4-D	% 2,4-D
0	2,409	1,823	43.1%
30	2,666	1,736	39.4%
60	1,042	720	40.9%
90	1,293	845	39.5%
120	283	175	38.2%
150	536	279	34.2%
180	780	408	34.3%

Table 6 Monitoring of Soil Moisture Content During Bioremediation Treatment							
Treatment Unit	Moisture (%)						
	Initial	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Cell No. 1 (Reference)	9.4	15.6	19.8	19.2	17.47	21.96	18.77
Cell No. 5A (Buffered)	10.5	17.5	18	21.4	17.53	20.13	20.62
Cell No. 5B (Buffered)	10.2	18	18.5	20.1	18.47	21.73	16.8
LTU No. 6 (Reference)	10.1	3.1	4.23	6.9	11.93	13.35	12.31
LTU No. 7 (Buffered)	11	11.5	11.4	14	17.33	17.5	15.17

Table 7 Monitoring of pH During Bioremediation Treatment							
Treatment Unit	pH (Units)						
	Initial	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Cell No. 1 (Reference)	4.09	4.16	3.76	3.72	4.00	3.72	3.88
Cell No. 5A (Buffered)	7.45	7.28	7.27	6.65	6.65	6.37	6.68
Cell No. 5B (Buffered)		7.43	7.45	7.07	6.57	6.85	
LTU No. 6 (Reference)	3.83	3.81	3.61	3.62	4.9	3.6	3.6
LTU No. 7 (Buffered)	7.6	7.9	7.3	7.2	6.98	7.36	6.9

Observations regarding pH which should be noted, as follows:

- Heavily contaminated soil in Da Nang is acidic as shown in the Cell No. 1 and LTU No. 6 soil samples.
- The pH in buffered Cell No. 5 and LTU No. 7 remained neutral for the duration of the study.

FIGURES

Figure 1. OLS Regression Trend for All Aerobic Land Treatment Units Combined

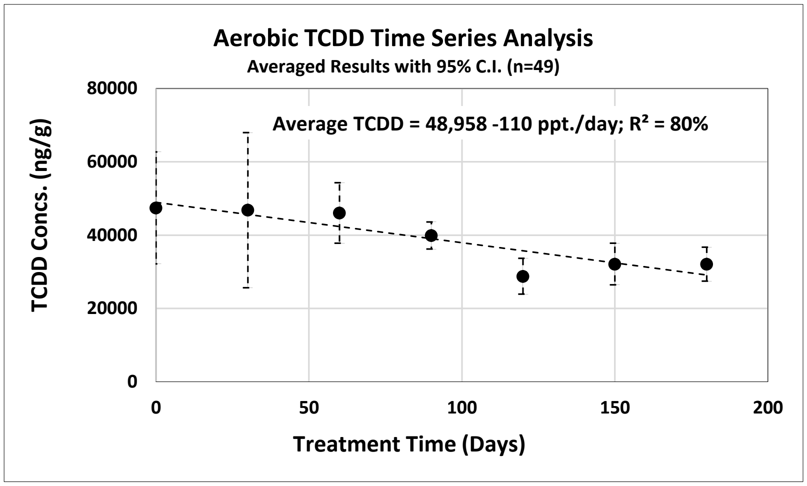

Figure 2. OLS Regression Trend for Buffered Aerobic Land Treatment Unit (No. 7)

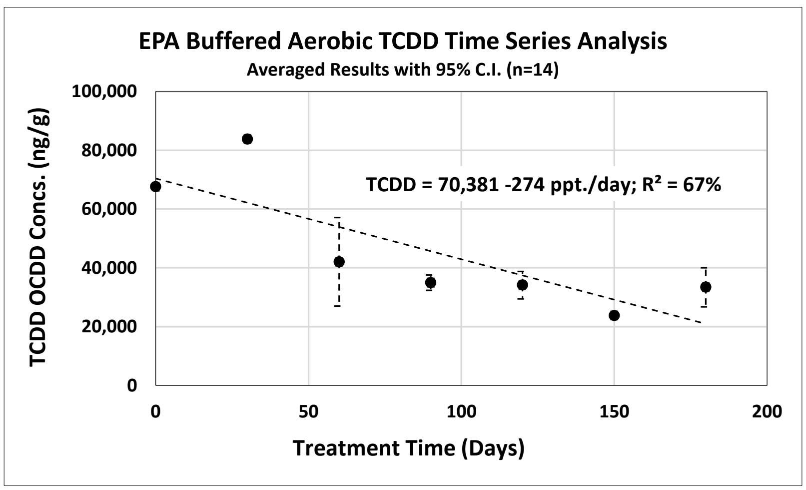

Figure 3. OLS Regression Trend for Buffered Anaerobic Treatment Cell (No. 5)

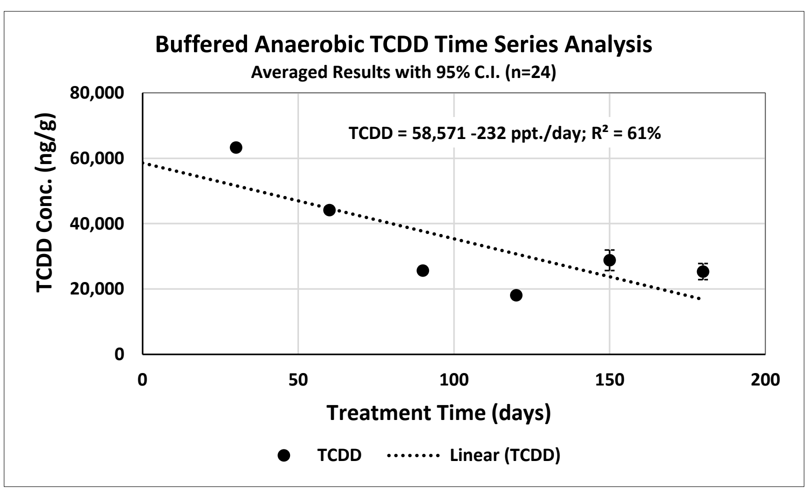

Figure 4. TCDD Reference Trend

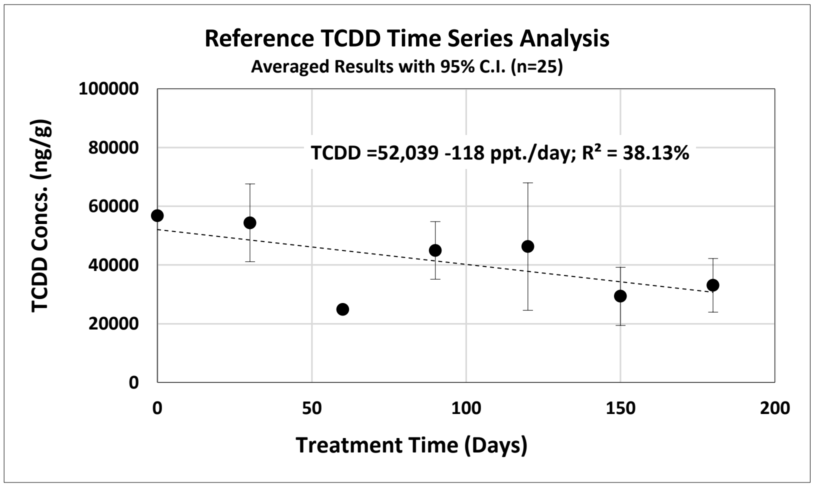

Figure 5: OCDD Reference Trend

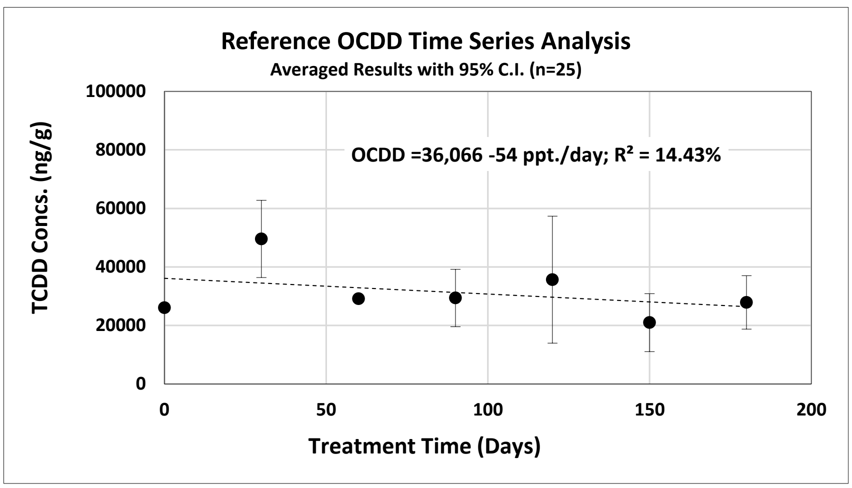

Figure 6. OCDD Aerobic Biodegradation

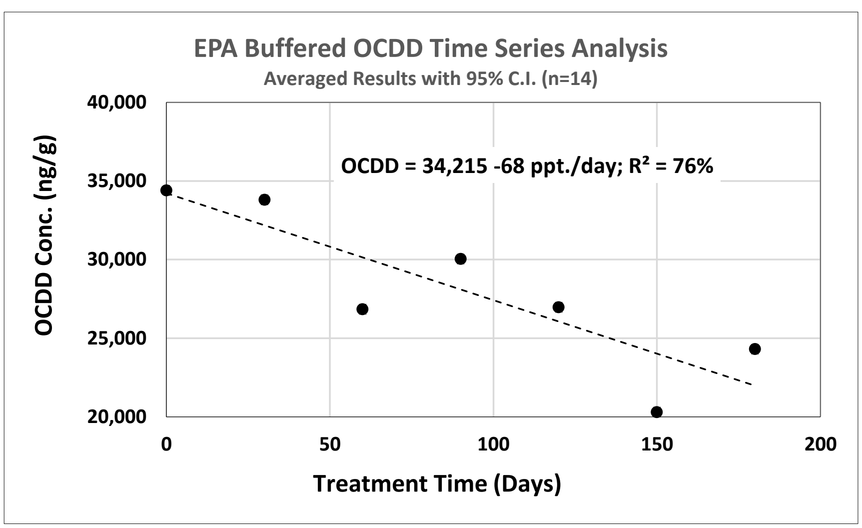

Figure 7. OLS Regression Trend for OCDD Biomarker Determination

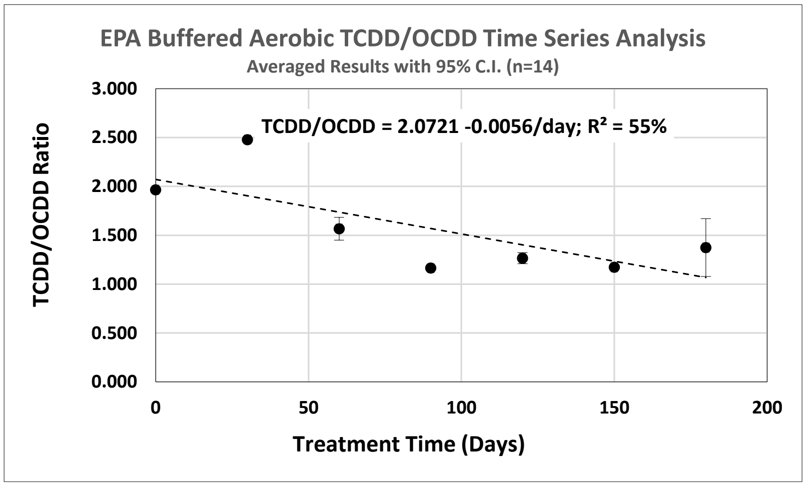

Figure 8. Herbicide Degradation Linear Trends for Combined Aerobic and Anaerobic Units