N00164.AR.000695
NSWC CRANE
5090.3a

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION 5
77 WEST JACKSON BOULEVARD
CHICAGO, IL 60604-3590

REPLY TO THE ATTENTION OF:

DW-8J

December 18, 2001

Ms. Christine Freeman
Naval Surface Warfare Center
EPD, Code 095 B-3260
300 Highway 361
Crane, IN 47522-5001

Re: Letter of Approval
Biofacility Reports

Dear Ms. Freeman:

The United States Environmental Protection Agency (U.S. EPA) has reviewed and approves the following reports pertaining to the Biofacility composting operations:

1) Windrow M-203 Full-Scale Batch Report for Rockeye Soils, SWMU 10/15 Revision 1 dated October 2001

2) Conditional Approval of Supplemental Toxicity Report, Revision 0, June 2001 (see enclosed comments).

3) Pilot-Scale Treatability Test Report for Rockeye Soils, SWMU 10/15 Revision 0 dated October 2001

4) Quarterly Interim Progress Report, 3rd Quarter 2001 (July 1 - September 30) Revision 0 dated November 2001.

If you have any questions regarding this matter, please contact me at (312) 886-7890.

Sincerely,

Peter Ramanauskas
Environmental Engineer
WMB, Corrective Action Section

Recycled/Recyclable . Printed with Vegetable Oil Based Inks on 100% Recycled Paper (50% Postconsumer)

cc: Bill Gates, SOUTHDIV (w/o encl)
Doug Griffin, IDEM (w/o encl)

F:\USER\PRAMANAU\Crane\Biofacility Docs\Various BF Repors Approval.wpd

Review of Draft Report
Full-Scaled Bioremediation
NSWC Crane,
Crane, IN
June 2001

The report presents toxicity and pathogen test results in explosives-contaminated soil previously treated by composting. Earthworm and Microtox® toxicity tests and fecal coliform and salmonella pathogen tests were used to determine effects of aging after compost treatment.

The report is a bit confusing with respect to sampling. It appears toxicity data from aged compost were compared to S-001 Day 26 data collected and analyzed 2.5 years earlier (Executive Summary, Para 3; Introduction, Sect 1.1, Para 2). Yet in some parts of the report it seems S-001 Day 26 samples were analyzed along with aged samples (Introduction, Sect 1.1.1, 1.1.2; Test Results, Para 1, Sent 4; Table 2-1). This should be stated more clearly. Much of this information is probably found in the Toxicity Report (MK, 1999) and Sampling Plan (MK, 2000). However, it would be helpful to the reader of this report to include identities of the samples analyzed.

The report should describe the differences between piles S-001, S-096, S-159, and S-178. Any differences between Mine Fill A and Mine Fill B should be described. What is the difference between sample PCS-001 and PCS-002 FC in Table 1-1?

It appears only duplicates were collected from aged compost piles. If correct, was there a specific reason to analyze duplicates while five replicates were available from S-001 Day 26?

Table 2-1 shows greatly reduced toxicity for windrows S-159, S-096, and S-178 relative to S-001 Day 26 (no statistics). However, aged (2.5 years) S-001 compost shows no decrease in toxicity to earthworms. This outcome is mentioned in the Summary (Sect 2.4) and Conclusion (Sect 5.0) where it states, "no explanation is provided for this dose response." I realize there is no good explanation for these data, but I recommend a little more discussion of them since the report implies aging of compost decreases toxicity.

I have made additional comments in the margins of the report itself.

DRAFT

SUPPLEMENTAL TOXICITY REPORT

FULL-SCALE BIOREMEDIATION
NSWC CRANE
CRANE, INDIANA

Revision 0

June 2001

CONTRACT N68950-96-D-0052
DELIVERY ORDER #FC08

Prepared for

SOUTHERN DIVISION
NAVAL FACILITIES ENGINEERING COMMAND
2155 Eagle Drive
P.O. Box 190010
North Charleston, South Carolina 29419-9010

Prepared by:

TOLTEST Inc
1915 North 12th Street
Toledo, Ohio 43603
419/241-7175

PREPARED/APPROVED BY:

Peter J. Chevalier — Date
Project Manager/Environmental Specialist

APPROVED BY:

Lance Parsons — Date
General Manager

EXECUTIVE SUMMARY

Explosives contaminated soil at Naval Surface Warfare Center (NSWC) Crane, Indiana, is being treated through a composting bioremediation process. While treatment of the soil in this process results in contaminant reduction to industrial use clean-up levels as established by the United States Environmental Protection Agency Region V (U.S. EPA Region V), the toxic effect of the treated soil, or compost, on the environment is a concern.

What about MFB?

effects

Acute toxicity testing was performed on composted soil from Mine Fill A (MFA) to determine the toxic affects of the composted soil on the environment. The findings were reported in the Toxicity Report Full-Scale Bioremediation NSWC Crane, Crane, Indiana [MK, 1999].

The previous toxicity testing performed at MFA [MK, 1999] verified that treated contaminated soil compost did not show a greater toxicity than treated non-contaminated soil compost. However, the testing program was not designed to evaluate the effects on toxicity of aged compost. Consequently supplemental toxicity testing was developed to determine if there is a reduction in the toxicity of treated compost over time when compared to the initial full–scale windrow toxicity testing. To determine these effects, four windrows of varying age, which had been placed in the field as backfill, were sampled and analyzed for toxicity parameters. The results of this analysis were compared with results from the first full-scale windrow, S-001, which was sampled on the last day it was processed (Day 26).

this is unclear. Was S-001 generated just prior to testing or were the original piles

Environmental effects were evaluated using two toxicity methods: Earthworm and Microtox® Toxicity; and two pathogen tests: fecal coliform and salmonella.

The Earthworm Toxicity analysis subjected earthworms, *Eisenia foetida,* to various concentrations of compost of different ages to determine the concentration of compost that would result in 50% mortality of the sample population (LC-50). Results show a decrease in toxicity with increasing age of the compost except for the oldest windrow.

Microtox® analysis was used to evaluate the toxic effects of the composted soil on the illuminance of the marine bacteria, *Photobacterium phosphoreum.* This organism was subjected to various concentrations of compost to determine the concentration of compost which resulted in a 50% reduction in the illuminance of the bacteria (EC-50). The results indicated that all aged compost was less toxic to this organism than freshly processed compost.

Pathogen analysis was performed based on specifications found in 40 CFR 503.32, which state that sewage sludge may be used in land application if it meets established requirements for fecal coliform and salmonella analysis. The compost resulting from full-scale biofacility operations is intended to be used for land application, therefore, these same standards were applied to the compost. The results from all samples met the requirements for use in land application.

Given these results, the composted soil should be considered acceptable for use as a topsoil or backfill for general land application.

1.0 INTRODUCTION

1.1 PURPOSE AND OBJECTIVES

Full-scale operations of the Soils Bioremediation Facility began April 13, 1998 at the Naval Surface Warfare Center (NSWC) Crane Division in Crane, Indiana. Acute toxicity testing was performed by Morrison Knudsen, Corp. (MK) on composted soil from Mine Fill A (MFA), Windrow S-001, to determine the toxic affects of the composted soil on the environment. This toxicity testing [MK, 1999] verified that treated contaminated soil compost did not show a greater toxicity than treated non-contaminated soil compost. However, the testing program was not designed to evaluate the effects on toxicity of aged compost. Consequently, a Sampling and Analysis Plan for Supplemental Toxicity Sampling of Composted Material (STS-SAP) from Mine Fill A and Mine Fill B [MK, 2000] was developed and approved by the EPA to determine if there is a reduction in the toxicity of the treated compost over time. Need to define

This report summarizes the activities for the supplemental toxicity sampling and analysis performed on four select windrows. These windrows were of varying age and had been previously placed as backfill material at MFA and Mine Fill B (MFB). The toxic~~ity~~ effects were studied using two toxicity methods: Earthworm and Microtox® Toxicity; and two pathogen tests: fecal coliform and salmonella. The results of this analysis were then compared with the initial samples obtained from Windrow S-001 on the last day it was processed (Day 26). ~~Are samples from S-001 Day 26 valid~~

Validity comparison should be discussed briefly.

Details of the earthworm and Microtox® test procedures and results can be found in the Microtox® and Earthworm Toxicity Report from Aqua Survey, Inc. (ASI) provided in Appendix A. Pathogen test results from Pure Earth Environmental Labs, Inc. (PE) are provided in Appendix B.

1.1.1 Earthworm Toxicity

The objective of earthworm toxicity testing is to assess the acute toxicity of the composted soil on earthworms. For this test, the lethal concentration (LC) of compost was estimated to determine the concentration of compost which causes 50% mortality of earthworms. This lethal concentration, referred to as the LC-50, was averaged for the five samples collected from Windrow S-001 Day 26 and compared to the LC-50 of the four previously backfilled windrows. The differences were then compared to determine if the LC-50 values for the aged compost were greater or less than the LC-50 values for Windrow S-001 Day 26.

2.5 yr ago, this is confusing the way it is written

1.1.2 Microtox® Analysis

The objective of Microtox® toxicity testing is to assess the acute toxicity of the composted soil on the marine bacteria *Photobacterium phosphoreum*. For this test, the effective concentration (EC) of compost was estimated to determine the concentration of compost which causes a 50% decrease in the illuminance of the test organisms. This effective concentration, referred to as the EC-50, was averaged for the five samples collected from Windrow S-001 and compared to the EC-50 of the four aged windrows. The differences were then compared to determine if the EC-50 values for the aged compost were greater or less than the EC-50 values for Windrow S-001 Day 26.

1.1.3 Pathogen Testing and Analysis

The objectives for pathogen testing were based on specifications found in 40 CFR 503.32, which pertain to the use or disposal of sewage sludge. These specifications state that sewage sludge may be used for land application if the sludge contains less than the regulated amounts of fecal coliform and salmonella. The composted soil has been returned to the land, therefore these same standards will be applied to the compost. Objectives for the density of fecal coliform in the compost are less than 1000 Most Probable Number (MPN) per gram of dry solids on a dry weight basis, and salmonella is less than three MPN per four grams of dry solids on a dry weight basis.

1.2 SAMPLE COLLECTION

For this study, samples were collected from four windrows that had been previously used as backfill material at either MFA or MFB (i.e. aged compost). Details about the four windrows and their identifying sample numbers are listed in Table 1-1. Figures 1 through 4 depict the placement locations for these windrows.

Table 1-1
Windrow Identifiers

Sample ID	Windrow #	Day Zero	Day Last	# of Days Processed	Cleanup Goal Achieved	Date Placed in Field	Approximate Compost Age	Burial Depth	Placement Area
PCS-001	S-001	4/15/98	5/11/98	26	Residential	5/98	2.5 years	8 feet	MFA, B-159
PCS-002 FD	S-001	4/15/98	5/11/98	26	Residential	5/98	2.5 years	8 feet	MFA, B-159
PCS-003	S-096	8/5/99	8/11/99	6	Residential	8/17/99	15 months	Surface	MFA, South PPA*
PCS-004	S-159	5/1/00	5/10/00	9	Residential	5/16/00	6 months	Surface	MFB, PPA
PCS-005	S-178	7/25/00	8/4/00	10	Residential	8/10/00	3 months	Surface	MFB, B-166

what's the difference?

*PPA – Permanent Placement Area

The sampling procedures outlined in Appendix D of the STS-SAP were followed while collecting the compost samples. Windrow S-001 was exposed with a backhoe prior to sampling since it was buried at the bottom of the berm. The other three windrows were at the surface and the top foot was exposed with a shovel prior to sampling. Samples for Microtox® and Pathogens analysis were contained in 8 ounce glass jars. The samples for earthworm toxicity were contained in a 3.5 gallon HDPE pail lined with two plastic bags.

2.0 EARTHWORM TOXICITY

2.1 TEST METHOD DESCRIPTION

Earthworm Toxicity analysis was performed at ASI using the test organism, *Eisenia foetida,* supplied by Carolina Biological Supply Company. The laboratory standard operating procedure (SOP) followed in performing the analysis is included in the Full-Scale Quality Assurance Project Plan [MK, 1998] Appendix G, page A-4. Adult earthworms which were greater than 60 days of age were received at the laboratory in a healthy condition by overnight delivery from the supplier. The earthworms were maintained by the laboratory in artificial soil consisting of a mixture of sphagnum moss, clay and silica sand during the one day acclimation period. The earthworm environment was kept at a temperature of 20 ± 2 degrees Centigrade (°C) during which time the earthworms were fed with Magic Worm Food® prior to use in this analysis. Feedings were documented on the culture laboratory livestock record.

The laboratory SOP specifies that the earthworms should be allowed to acclimate to the experimental environment for five days prior to initiating any tests, but the earthworms were subjected to only a one-day acclimation period before testing began. The discrepancy in SOP protocol was due to supply and shipping problems. The Laboratory Manager for ASI concluded that the shortened acclimation period did not effect the results of the testing (see the letter from ASI's Laboratory Manager in Appendix C).

After the stabilization period, the earthworms were exposed to five concentrations of the compost mix: 6.25%, 12.5%, 25%, 50%, and 100%. The varying concentrations of compost were prepared using a grinder to homogenize the appropriate quantity of compost with artificial soil from the laboratory. Artificial soil was prepared from a homogenous mixture of 10% sphagnum peat, 20% colloidal kaolinite clay, and 70% grade 70 silica sand. Prior to initializing the test, the water holding capacity of the compost was determined and the test media was hydrated with deionized water to 75% of its water holding capacity.

For each exposure concentration, three testing vessels, each containing 200 grams of compost and artificial soil were prepared (total of 15 test vessels). Ten earthworms were placed in each testing vessel by sequential randomization where no more than two earthworms were added into each vessel at a time. The vessels consisted of a one liter cleaned canning jar with screw top lids with 1/16 inch diameter holes in the center top for air. The test was conducted under continuous illumination at a temperature of 20+/-2°C. The earthworms were exposed to the compost for 14 days in order to permit a more accurate and complete assessment of the environmental impact of the compost.

The earthworms were evaluated at 7-day and 14-day intervals from project initiation. The earthworms were not fed during the duration of the analysis.

2.2 TEST RESULTS

Each test vessel was analyzed separately at 7-day and 14-day intervals. The two replicates from a single concentration were added together to determine a live count for the thirty worms initially subjected to the concentration. Then using the five concentrations for a particular sample, the LC-50 was calculated for each sample. The results for the earthworm toxicity analysis on the four aged windrows and S-001 Day 26 are summarized in Table 2-1. The values reported represent the concentration of compost which resulted in the mortality of 50% of the earthworms. Results for five samples collected from Windrow S-001 Day 26 have been averaged to provide a representative value for the windrow. The minimum and maximum LC-50 values for S-001 Day 26 samples were 11.5 and 68.3 respectively. Std. error?

In addition to the compost sample vessels, a positive control and a negative control was analyzed to demonstrate that the earthworms for this test reacted similar to other earthworms. The positive control utilized potassium chloride (KCl) as a reference toxicant. The LC-50 for KCl was 6,372.8 parts per million (ppm) at Day 7 and 5,747.6 ppm at Day 14 which is comparable to the published guideline of 6,340 mg/L. Mean survival of the negative control was 97% which equates to a mortality rate that is less than the 10% mortality rate permitted by the method.

Std Error?

Table 2-1
Earthworm Toxicity Results (%)

	Windrow # and Age				
	S-001 Day 26	S-178 3 months	S-159 6 months	S-096 15 months	S-001 2.5 years
7 Day LC50	36.8	58.8	> 100	> 100	28.1
14 Day LC50	33.0	57.3	> 100	> 100	27.8
Average	34.9	58.1	> 100	> 100	28.0

S-001 appears to have become more toxic at 2.5 yr

2.3 DATA QUALITY CONTROL

All data from the earthworm toxicity analysis were verified, and at least 10% of the samples were validated by TolTest Inc. (TT) personnel per the procedures outlined in Section 9.0 of the *FS-QAPP* [MK, 1998]. Data were compared with field and laboratory quality control (QC) sample data to assess its usability for supporting operational decisions. The results of data verification and validation are presented in this section.

Data associated with the earthworm toxicity were verified by reviewing chain-of-custody forms, sample preservation records, analytical holding times, requested turnaround times, and sample data in comparison to analytical quality assurance objectives. In addition, at least 10% of the samples were validated by performing a thorough review of the analytical data utilizing laboratory analytical records to assess laboratory performance in relation to the quality control criteria and procedural requirements. All samples were received by the laboratory in good condition (i.e., there were no broken containers, and custody seals were intact) and at a recorded temperature of 8°C.

To monitor the conditions in which this analysis took place, the pH of the testing vessels was monitored. Initially, and at the completion of the analysis, the pH of the testing material was recorded. The pH of the compost samples ranged from 6.6 to 8.6 standard units (su). Recommended test conditions for the earthworm are between 4 and 10 su. These values are considered acceptable for the test organisms and are not likely to have caused any adverse reactions.

The temperature of the testing vessels was monitored daily during analysis. Temperatures did not show fluctuations on any day of analysis. Temperature data logs are not available for 11/15/00 and 11/16/00, the first and second day of testing, due to an oversight by ASI. The laboratory manager for ASI concluded that this oversight did not affect the outcome of the test (refer to the ASI letter in Appendix C).

The laboratory recommended test temperature is 20°C ± 2°C with fluctuation not to exceed more than 3°C on any day of analysis. The highest temperature level recorded was 24.0°C and the lowest was 17.0°C. Although these temperatures exceeded the target temperatures for this test, it is not believed to have affected the outcome of the test.

The laboratory performed method blank, laboratory control sample (LCS) and replicate analyses to evaluate accuracy and precision. A laboratory method blank was prepared from the artificial soil prepared in the laboratory which was utilized as a dilutant to the compost mixture. This blank was prepared and analyzed with the samples in order to evaluate potential interference from laboratory equipment as well as to determine if the earthworms met established laboratory criteria. Two blanks were analyzed during this test, one showed 0% mortality of the earthworms in the artificial soil and the other showed 3% mortality. Both blanks were less than the 10% blank mortality rate defined for this project. Results of the method blanks are acceptable for all analysis.

The LCS is a sample prepared in the laboratory, consisting of artificial soil spiked with known amounts of target analytes which is processed through the same preparation as field samples. In this analysis, KCl was used as a reference toxicant. The KCl was utilized as a positive control to evaluate the sensitivity of each batch of organisms. Varying concentrations of KCl contaminated soil were prepared to determine the LC-50 of the KCl contaminated soil. The LC-50 for the KCl was calculated to be 6,372.8

milligrams per liter (mg/L) of KCl for the 7-day test period and 5,747.6 mg/L for the 14-day testing period. This value represents the concentration of KCl which results in 50% mortality of the earthworms. This value is comparable with published guideline of 6,340 mg/L of KCl, as well as previous laboratory data and verifies that the earthworms reacted at similar sensitivities as other batches of *Eisenia foetida*. Temperature and pH monitoring of the KCl are provided in Appendix A. The accuracy of this LCS meets established acceptance limits for this procedure.

The laboratory SOP specifies that the number of replicates analyzed for this series should be three, but due to an oversight by the laboratory, only two replicates were tested. The laboratory manager for ASI concluded that this oversight did not affect the outcome of the test (refer to the ASI letter in App. C).

The consistency between the three vessels created for each compost concentration was evaluated to determine the precision of this analysis. A linear correlation should be seen in the dose responses and the observed mortality for the test samples. This would be demonstrated by an increased mortality as a higher concentration of compost is utilized in the testing vessels. This correlation is not observed in the sample results. There is actually a slight increase in survivability in S-001 (and its duplicate) as the concentration increases from 12.5% to 25%. The survivability then drops to zero at 50% and 100% concentrations. The survivability of S-096 (15 months) and S-159 (6 months) varies little throughout the test, and indeed the LC50 is calculated to be >100%. S-178 (3 months) exhibits similar characteristics to S-001 and complete extinction is not observed until the concentration reaches 100%.

The QC data demonstrate that analyses exhibit acceptable accuracy and precision when compared to the project's data quality objectives specified in Appendix G of the *FS-QAPP* [MK, 1998]. Acceptable values for both positive and negative controls as well as consistent values between replicates demonstrate that project data quality objectives have been met for the earthworm analysis. The data meets the data quality objectives established for this project and are, therefore, considered usable to support project decisions.

2.4 SUMMARY

The data from Table 2-1 suggests that the toxicity of compost decreases with age, as indicated by the lower LC-50 in the S-001 Day 26 samples than in progressively older samples. However this dose response is not observed in the S-001 2.5 year sample. No explanation is provided for this dose response.

3.0 MICROTOX® ANALYSIS

3.1 TEST METHOD DESCRIPTION

In this analysis, the marine bacterium *Photobacterium phosphoreum* was exposed to various concentrations of elutriate from compost for 5 and 15 minutes according to the SOP in FS-QAPP Appendix G page A-11. However, the SOP for preparation of the elutriate found in the FS-QAPP is appropriate for use on sediments but not on compost. This discrepancy is addressed in a letter from ASI's laboratory manager, presented in Appendix C. The correct SOP, which the laboratory used in this and previous toxicity testing, is provided in Appendix D. Sections 1.7 through 1.9 of this SOP are applicable to preparation of the elutriate from compost. Field Change Request (FCR) FS-032, presented in Appendix E, is a request to replace the old, incorrect SOP for use on sediment, with the correct SOP for use on compost. This FCR has been submitted and approved by the U.S. EPA.

The elutriate was prepared by adding 50 grams dry weight of compost to 500 milliliter (ml) of deionized water which was agitated in the dark for 48 hours at 20± 2°C. After agitation, the suspension was centrifuged and the elutriate fraction was decanted and used to prepare the test solutions.

Test organisms were obtained from Azur Environmental in a freeze-dried culture. The organisms were reconstituted and maintained in a reagent well at a temperature of 5°C for a period of less than two hours prior to analysis. During that time, volumes of organisms were transferred to test cuvettes and acclimated to Mircotox® diluent at the test temperature of 15°C for 15 minutes prior to test initiation. The test began as the organisms were introduced to four elutriate test concentrations of 5.63%, 11.25%, 22.5%, and 45.0% and a blank. Observations of light output for each sample were made five and fifteen minutes after test initiation using a Model 500 Microtox® Unit.

3.2 TEST RESULTS

The results of the Microtox® Toxicity Analysis performed by ASI are summarized in Table 3-1. The raw data from ASI is provided in Appendix A. The values reported represent the effective concentration of compost which resulted in a 50% reduction in the light output (EC50) of the marine bacterium *Photobacterium phosphoreum*. Results for five samples collected from Windrow S-001 Day 26 have been averaged to provide a representative value for the windrow.

how many samples from sites were averaged? Dupes?
REW

3.3 DATA QUALITY CONTROL

All data from the Microtox® Toxicity Analysis were verified, and at least 10% of the samples were validated by TT personnel per the procedures outlined in Section 9.0 of the *FS-QAPP* [MK, 1998]. All data were compared with field and laboratory QC sample data to assess its usability for supporting operational decisions. The results of data verification and validation are presented in this section.

Table 3-1
Microtox® Results(%)

	Windrow # and Age				
	S-001 Day 26	S-178 3 months	S-159 6 months	S-096 15 months	S-001 2.5 years
5 Min. EC50	36.0	>45	>45	>45	>45
15 Min. EC50	37.8	>45	>45	>45	>45
Average	36.9	>45	>45	>45	>45

Data associated with the Microtox® toxicity were verified by reviewing chain-of-custody forms, sample preservation records, analytical holding times, requested turnaround times, and sample data in comparison to analytical quality assurance objectives. In addition, at least 10% of the samples were validated by performing a thorough review of the analytical data utilizing laboratory analytical records to assess laboratory performance in relation to the quality control criteria and procedural requirements. All samples were received by the laboratory in good condition (i.e., there were no broken containers, and custody seals were intact) and at a recorded temperature of 8°C. This temperature would not have adversely affected either the Microtox analysis since it was performed at 20°C.

Laboratory method blanks consisting of dilutant water were analyzed with each sample. Blank ratios were calculated by dividing the initial blank reading by the blank reading at the appropriate time interval. Blank ratios for the 5-minute interval ranged from 1.00 1.44. Blank ratios for the 15-minute interval ranged from 1.02 to 1.57. These values demonstrate consistent readings between analyses and also meet method criteria for blank ratios which should be greater than 0.75.

The results of all Microtox® EC-50 values fell outside the range of the test concentrations. Concentrations up to 45% elutriate were analyzed during this analysis.

An approximated value of greater than 45% was reported by the laboratory for all samples.

The quality control performed for the Microtox® analysis has met method requirements for completion and acceptable precision for this analysis. The data meets the data quality objectives established in Appendix G of the *FS-QAPP* [MK, 1998] for this project and are therefore, considered usable to support project decisions.

3.4 SUMMARY

Federal guidelines have not been established for evaluating EC-50 values. The only comparison which can be made is between windrow S-001 Day 26 sample results and the remaining sample results. The results of this testing indicate that aging, even for a short period of time, has a marked influence on the toxicity to this marine bacterium.

4.0 PATHOGEN TESTING AND ANALYSIS

4.1 TEST METHOD DESCRIPTION

4.1.1 Salmonella Analysis

Salmonella analysis was performed according to the Pure Earth Environmental Laboratory, Incorporated SOP located in Appendix G of the FS-QAPP. For the salmonella analysis, each sample was prepared with three dilution series. Each dilution consisted of a series of five replicate tubes for analysis for a total of 15 tubes for each sample. Tubes were incubated for 24 hours in a water bath at 35.0°C after which time they were examined for turbidity and/or gas production which would indicate a positive result for salmonella.

4.1.2 Fecal Coliform Analysis

Fecal Coliform analysis was performed according to the Pure Earth Environmental Laboratory, Incorporated SOP located in Appendix G of the FS-QAPP. In the fecal coliform procedure, four dilutions were performed and a series of five replicate tubes were prepared for each dilution for a total of 20 tubes for each sample. Tubes were incubated for 24 hours in a water bath at 44.5°C after which time they were examined for turbidity and/or gas production which would indicate a positive result for fecal coliform.

4.2 TEST RESULTS

The analytical results from Pure Earth Environmental Lab are included in Appendix B. The results for Windrow S-001 Day 26 [MK,1999] are an average of the samples obtained from each of the five cross sections. The results for salmonella analyses were less than three Most Probable Number (MPN) per four grams of solid for all samples. The value of the fecal coliform analyses for Windrow S-001 Day 26 were initially reported as less than 1,000 MPN per gram of dry solid. Positive values for fecal coliform were detected in the four aged windrow samples but all were below 1,000 MPN per gram of dry solids. A summary of these results is shown in Table 4-1.

4.3 DATA QUALITY CONTROL

All data from the pathogen analysis were verified by TolTest personnel per the procedures outlined in Section 9.0 of the *FS-QAPP* [MK, 1998]. Data was compared

with field and laboratory QC sample data to assess its usability for supporting operational decisions. The results of data verification are presented in this section.

Table 4-1
PATHOGEN ANALYSIS

	Windrow #				
	S-001 Day 26	S-178 3 months	S-159 6 months	S-096 15 months	S-001 2.5 years
Salmonella	<3	<0.117	<0.125	<0.129	<0.109
Fecal Coliform	<1,000	19	371	806	<2.73

Data associated with the pathogen analysis were verified by reviewing chain-of-custody forms, sample preservation records, analytical holding times, requested turnaround times, and sample data in comparison to analytical quality assurance objectives. All samples were received by the laboratory in good condition (i.e., there were no broken containers, and custody seals were intact) and at a recorded temperature of 4°C ± 2°C.

Other quality control testing performed for both pathogen tests include the analysis of replicates through the use of dilution series. All replicates showed no presence of salmonella or fecal coliform which demonstrates acceptable precision for this analysis.

4.4 SUMMARY

Pathogen tests were performed to evaluate the compost against "503 Class A" sludge. The 40 CFR 503.32 has established standards for pathogenic bacteria in sewage sludge for both salmonella and fecal coliform of less than 3 MPN per 4 grams dry solid and less than 1,000 MPN per 1 gram dry solids, respectively. The compost samples obtained for this study have met the established criteria for salmonella and fecal coliform.

5.0 CONCLUSION

Acute toxicity testing was performed on aged compost generated from the NSWC Crane BioFacility to determine its relative toxicity. This was determined using two toxicity methods: Earthworm and Microtox® Toxicity; and two pathogen tests: fecal coliform and salmonella. Comparisons were made between Windrow S-001 Day 26 samples and four samples obtained from aged compost. The results for Windrow S-001 Day 26 were obtained from the Toxicity Report prepared by Morrison Knudsen, Corp. (MK, 1999).

The present earthworm toxicity analysis suggests that the toxicity of compost decreases with age, as indicated by the lower LC-50 in the S-001 Day 26 samples than in progressively older samples. However this dose response in not observed in the S-001 2.5 year sample. No explanation is provided for this dose response.

The Microtox® results were less remarkable in that all aged compost was shown to be less toxic to the marine bacterium used in this test than the freshly processed compost.

The results of the two pathogen tests meet the specifications pertaining to the disposal and use of sewage sludge for both fecal coliform and salmonella. Fecal coliform results were less than 1,000 MPN per gram of solid and salmonella results were less than three MPN per four grams of solid for all samples.

The composting process has demonstrated that the bioremediation technology is a safe and effective treatment alternative for detoxifying soil contaminated with explosive compounds. The composted soil has been shown, over a period of time, to support the re-establishment of plants (e.g. grass) in land applications. The compost is suitable for recycling and reuse in accordance with 40 CFR 503.32, and it meets the same requirements as sewage sludge, therefore it should be considered acceptable for use as backfill or a top soil in approved land application locations.

6.0 REFERENCES

MK, 1998 *Quality Assurance Project Plan for Full-Scale Operations,* Soils Bioremediation Facility, NSWC Crane, Crane, Indiana. Delivery Order Number 0009, Contract Number N62467-93-D-1106. Prepared by Morrison Knudsen Corporation, March 12, 1998.

MK, 1999 Toxicity Report, Full-Scale Bioremediation, NSWC Crane, Crane, Indiana. Morrison Knudsen Corp., February 1999.

MK, 2000 Sampling and Analysis Plan for Supplemental Toxicity Sampling of Composted Material from Mine Fill A and Mine Fill B, NSWC Crane, Crane, Indiana. Morrison Knudsen Corp., June, 2000.