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PILOT METAL SEQUESTRATION DEMONSTRATION REPORT SITE 28 NSWC INDIAN  
HEAD MD  
12/1/2005  
NEPTUNE AND COMPANY, INC.

**PILOT METAL SEQUESTRATION DEMONSTRATION REPORT  
SITE 28 – Original Burning Ground  
NAVAL SUPPORT FACILITY, INDIAN HEAD (NSF-IH)  
INDIAN HEAD, MARYLAND**

**Prepared for**



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**Version 1.0  
December 2005**



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April 6, 2006

Installation Commanding Officer  
Naval Support Facility, Indian Head  
Attn: Shawn Jorgensen, Code HN2WSJ  
101 Strauss Avenue, Bldg. 289  
Indian Head, MD 20640-5035

RE: Final Pilot Metal Sequestration Demonstration Report, Site 28 – Original Burning  
Ground, Naval Support Facility Indian Head, December 2005

Dear Mr. Jorgensen:

The Federal Facilities Division of the Maryland Department of the Environment's Hazardous Waste Program has no comment on the above referenced document.

If you have any questions, please contact me at (410) 537-3791.

Sincerely,

Curtis DeTore  
Remedial Project Manager  
Federal Facilities Division

CD:mh

cc: Mr. Dennis Orenshaw  
Mr. Jeff Morris  
Mr. Horacio Tablada  
Mr. Harold L. Dye, Jr.





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November 29, 2005

Installation Commanding Officer  
Naval Support Facility, Indian Head  
Attn: Shawn Jorgensen, Code HN2WSJ  
101 Strauss Avenue, Bldg. 289  
Indian Head, MD 20640-5035

RE: Draft Pilot Metal Sequestration Demonstration Report, Naval District Washington  
Indian Head, July 2005

Dear Mr. Jorgensen:

The Federal Facilities Division of the Maryland Department of the Environment's Hazardous Waste Program has no comment on the above referenced document.

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Mr. Harold L. Dye, Jr.



## EXECUTIVE SUMMARY

Naval Support Facility, Indian Head (NSF-IH) is a Naval Support Activity, South Potomac facility within the Naval District Washington Region. Historical operations of a zinc recovery furnace at the NSF-IH in Indian Head, Maryland, have resulted in zinc contamination of sediments in Mattawoman Creek, a tidal freshwater creek adjacent to the Naval facility. A pilot demonstration project was conducted with Mattawoman Creek sediments to assess the efficacy of using a calcium phosphate apatite-biosolids mixture as an in-situ stabilization treatment for zinc contamination in sediments. Three 100-ft<sup>2</sup> contaminated test plots, designated Plot 3, Plot 4, and Plot 5, were established adjacent to the site of the former zinc recovery furnace. Two 100-ft<sup>2</sup> uncontaminated plots, designated Plot 1 and Plot 2, were established upstream of the contaminated test plots. The contaminated plots were amended with apatite and biosolids as follows: Plot 3, 0.5% apatite and 15% biosolids; Plot 4, unamended; and Plot 5, 1.0% apatite and 15% biosolids. Uncontaminated Plot 1 was left unamended, and uncontaminated Plot 2 was amended with 0.5% apatite and 15% biosolids. All plots were then tilled to a depth of 1 foot. Prior to amendment, sediment from all plots was collected for analysis of orthophosphate and zinc concentrations in pore water and zinc concentrations in sediment.

Approximately four months later, sediment from all plots was resampled for analysis of pore water orthophosphate and pore water and sediment zinc concentrations. Laboratory bioassays were conducted to evaluate acute and chronic toxicity of the post-amendment sediments to the amphipod *Hyalella azteca*. Bioaccumulation of zinc by the oligochaete *Lumbriculus variegatus* was also measured in a 28-day bioassay.

Concentrations of zinc in pore water in the uncontaminated plots did not change between pre-amendment and post-amendment sampling, while concentrations of pore water zinc in the contaminated plots decreased by a factor of 12 in Plot 3 (0.5% apatite) and a factor of 60 in Plot 5 (1.0% apatite).

Zinc in Pore Water (µg/L)		
Plot	Pre-amendment	Post-amendment
1	8.63	8.95
2	1.95	1.96
3	5600	486
4	7145	4000
5	7300	123

Survival of *Hyalella azteca* in both uncontaminated plots (Plots 1 and 2) was greater than 90%, while survival in the contaminated unamended plot (Plot 4) was 0%. *Hyalella* survival in Plot 3, which received an amendment of 0.5% apatite and 15% biosolids was 31%, while increasing the apatite amendment rate to 1.0% (Plot 5) resulted in *Hyalella* survival of 58%.

Growth of *Hyalella azteca*, measured as mean individual weight, followed a similar pattern of survival. Mean organism weights in the uncontaminated plots were 0.603 mg and 0.409 mg for Plots 1 and 2, respectively. Mean *Hyalella* weight in Plot 3, amended with 0.5% apatite and 15% biosolids, was 0.083 mg per individual, while mean weight in Plot 5, amended with 1.0% apatite, was 0.148 mg per individual. No weights could be obtained from the contaminated unamended Plot 4, because all individuals died before completion of the test.

Zinc concentrations in *Lumbriculus* tissue were highest (3070 mg/kg) in the contaminated, unamended Plot 4, decreasing to 1610 mg/kg at the 0.5% apatite amendment, and 654 mg/kg at the 1.0% apatite amendment. Zinc concentrations in *Lumbriculus* tissue in the two uncontaminated plots averaged 391 mg/kg.

Addition of apatite and biosolids (or amendments) to zinc-contaminated sediments resulted in a decrease in pore water zinc concentrations, increases in benthic invertebrate survival and growth, and decreases in benthic invertebrate bioaccumulation corresponding to the rate of apatite applied. None of the applied amendment rates resulted in changes to contaminated test plot performance equivalent to uncontaminated test plot performance, indicating that optimal rates of apatite and biosolids amendment have not been determined.

Apatite, as phosphate rock, was obtained from PCS Phosphate, a division of Potash Corporation. Their address is 1530 NC Hwy 306 South, Aurora, North Carolina, 27806. The biosolids (Orgro) were obtained from D.C. Materials of Glen Dale, Maryland.

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## ACRONYMS

ARO	Aquatic Research Organisms
CV	Coefficient of Variation
DO	Dissolved Oxygen
EPA	Environmental Protection Agency
IH	Indian Head
LDH	Layered Double Hydroxide
MSL	Marine Science Laboratory
NAVFAC	Naval Facilities
NSF-IH	Naval Support Facility, Indian Head
ORP	Oxidation Reduction Potential
QA	Quality Assurance
SPAWAR	Space and Naval Warfare
TOC	Total Organic Carbon
Trt	Treatment
YCT	Yeast-cerophyll-trout
XANES	X-Ray Absorption Near Edge Spectroscopy

## **1.0 INTRODUCTION**

Naval Support Facility, Indian Head (Figure 1-1) is a 3,500-acre facility located 25 miles south of Washington, D.C. in Charles County, Maryland. The Center is located in a tidal freshwater-estuary ecosystem. Salinity in the Potomac River ranges from 0.01 to 3.0 parts per thousand near Indian Head. The United States Navy has operated this facility for more than 100 years as a Naval proving ground, powder storage facility, and more recently, as a propellant production plant. In the early years, a zinc recovery furnace was built near the shore of Mattawoman Creek that borders the northeast side of the NSF, Indian Head. Mattawoman Creek is a tidal tributary to the lower Potomac River. Historical data indicate the furnace was built in 1928 in Building 415. This facility was used to recover zinc under the Navy's metal-recycling program during World War II. Reviews of station maps indicated the recovery building was removed in the early 1950s (CH2MHill, 2005). The location of the former zinc recovery furnace was designated as Site 28 by the Base Installation Restoration (IR) Program



**Figure 1-1. Naval Support Facility, Indian Head**

The soil at Site 28, and in particular, near the former Building 415 site, is void of much vegetation (Figure 1-2), possibly a result of the very high zinc contamination. As a result, significant erosion has occurred and zinc has eroded with the soil surrounding the former recovery furnace into the sediments of Mattawoman Creek, just downstream from Slavins Dock. A pilot demonstration metals sequestration project was conducted on sediments in the intertidal zone of Mattawoman Creek, just offshore from the former zinc furnace.



**Figure 1-2. Location of Site 28 former zinc recovery furnace adjacent to Mattawoman Creek**

This metals sequestration project was conducted for Naval Facilities (NAVFAC) Indian Head by Neptune and Company, Inc., and Battelle under Contract N47408-01-D-8207, with cooperation and assistance from NAVFAC Washington Indian Head; U.S. EPA National Risk Management Research Laboratory, Cincinnati; and the Navy's Space and Naval Warfare (SPAWAR) Systems Command, San Diego.

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The phosphate rock used in this project was obtained from PCS Phosphate, a division of Potash Corporation. Their address is 1530 NC Hwy 306 South, Aurora, North Carolina, 27806. Mr. Barry Winn was our point of contact at PCS Phosphate.

The biosolid material was purchased from D.C. Materials of Glen Dale, Maryland. David Hill (Baltimore City Composting), the point of contact for the Biosolid material, recommended D.C. Materials as a supplier.

## **2.0 SAMPLING AND ANALYSIS**

This section provides a summary of the survey design and pre-amendment and post-amendment sampling activities. Additional information on the survey design is presented in the Indian Head Metals Sequestration work plan (Neptune and Company and Battelle, 2004).

### **2.1 Plot Selection and Dimensions**

The metal sequestration demonstration project along Mattawoman Creek at NSF-IH followed the work plan with very few exceptions noted in this report. Five demonstration plots were selected on June 28. The location of these plots was based on preliminary fieldwork conducted in April 2004 and the analytical results that were obtained from samples collected at that time. These results are available in Appendix C of the work plan (Neptune and Company and Battelle, 2004). The two uncontaminated plots were selected upstream of Slavins Dock. The control plots are shown in Figures 2-1 and 2-2 with Slavins Dock in the background. Slavins Dock is approximately 50 meters upstream of the edge of Plot 2. Plot 2 for this demonstration project covers the area that was sampled in April. Plot 1 for this demonstration project was selected approximately 14

feet upstream of Plot 2. Plots 1 and 2 for this demonstration project were arranged in rectangles, five feet wide (perpendicular to the shore line) and 20 feet in length (parallel to the shore line). This arrangement was necessary due to the slope of the sediments and the aquatic vegetation beds that cover parts of the uncontaminated area. This area is shown in Figures 2-1 and 2-2.



**Figure 2-1. Uncontaminated zone with emergent aquatic vegetation, upstream of Slavins Dock (visible in background). Contaminated plots are approximately 800 meters downstream as shown by the arrow.**



**Figure 2-2. Setting up plots in uncontaminated zone**

Plots 3 through 5 (designated 0X-06292004, where X = 3, 4, or 5) were prepared in the contaminated zone just offshore from the former zinc recovery furnace. This area is shown in Figure 2-3. These plots were constructed in 10 feet by 10 feet dimensions with 12 to 14 feet between each plot. All plots were constrained on the offshore sides with silt fencing to reduce the opportunity for sediment suspension and release. They are located 6 to 9 feet offshore from the high water mark. The most upstream edge of Plot 03-06292004 is just downstream from the metal stake position (in Figure 2-3), perpendicular to the shore. This area is very close to sample location 05-04222004 (April sampling). Plot 04-06292004 is in approximately the same location as sample location 02-04222004 (April sampling) and Plot 05-06292004 is located approximately 15 feet upstream of the upper most edge of sample location 03-04222004 (April sampling). Plots 3 through 5 (June sampling) are all within the area that had zinc concentrations of 8082 to 12120 ppm, as measured in the samples collected in April. However, the plots constructed for amendment are larger and extend farther offshore than those areas used during the April sampling.



**Figure 2-3. Plots in contaminated zone at low tide after tilling, prior to sampling**

## **2.2 Pre-amendment Plot Sampling**

All five plots were sampled prior to adding amendments as described in the work plan. Sampling was conducted, after the plot was tilled, by obtaining approximately 13 scoops of sediment down to a depth of 5 to 7 inches across the 100-ft<sup>2</sup> area. This set of grab samples was then homogenized via mixing in a stainless-steel bowl. Another 13 scoops were then collected, added to the mixture, and the sediment was again thoroughly mixed. From this homogenized composite, samples were collected and immediately placed in a sampling container. Between each plot, the stainless-steel bowl was cleaned with non-phosphate detergent. Between Plots 4 and 5, a rinsate blank was collected by rinsing deionized water over the cleaned stainless-steel mixing bowl and collecting the water in a sample bottle. All sample containers were then cleaned on the outside, sealed, labeled, and stored in the cooler with ice. Prior to shipping, the samples were bubble-wrapped, chain-of-custody forms completed, ice added to the coolers, and submitted for overnight shipping. The samples sent to Battelle Marine Sciences Laboratory in Sequim, Washington, were shipped on June 28<sup>th</sup> and arrived the following day. Samples for U.S. EPA and Applied Marine Sciences (AMS) were shipped on June 29<sup>th</sup> and arrived the following day. The EPA and AMS samples were stored overnight on ice, until ready for shipment the following day. Unfortunately, the meter that was to be used for pH and ORP measurement was not functioning so no pH or redox measurements were obtained.

bioassays were conducted with *Lumbriculus variegatus*, a burrowing freshwater oligochaete worm. Total organic carbon and grain size measurements were conducted on samples from each plot in a manner identical to that of the pre-amendment samples.



**Figure 2-4. Adding apatite on top of biosolids, Plot 2**



Figure 2-5. Plot 3 with apatite on top of biosolids and half of plot tilled

Uncontaminated Sediment (upstream of Slavins Dock):



Contaminated sediment (just offshore from the former zinc recovery furnace):

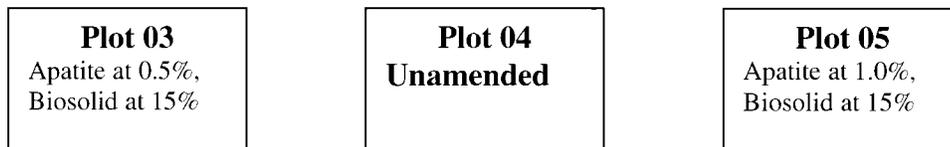


Figure 2-6. Summary of uncontaminated and contaminated plot amendment rates

### 3.0 ANALYTICAL RESULTS AND DISCUSSION

#### 3.1 Data Set Preparation

The analytical results for the Indian Head Metals Sequestration Study were compiled and loaded into a database prior to data evaluation. The data include the sediment, tissue and

bioassay results collected for this investigation. The *Hyalella* bioassay results were taken from Battelle data tables (Appendix A, Draft Indian Head Solid Phase Report, 2005). The sediment, *Lumbriculus* tissue, and pore water chemistry results were compiled from analytical laboratory electronic deliverables in Excel format. All site characterization for pre- and post-amendment analyses were based on measurements using identical sampling and analytical methods performed by the same analytical laboratory.

Prior to the evaluation, sediment and tissue chemistry data results were qualified in two ways using the following guidelines:

- No datum was removed during validation. There were no rejected results qualified with “R” in the data sets.
- All results were classified as detected.

When a location had both an original sample and a field duplicate sample, the original sample was used to characterize the location. Field duplicates were used for quality assurance (QA) review, but not included in the data evaluation. Use of both the original and field duplicate results introduces bias in area-wide estimation by doubling the weight of locations with field duplicates.

### **3.2 Sediment Chemistry Results**

Prior to application of amendments, a composite sediment sample was collected from each of the five test plots. The pre-amendment composite sample from each plot was homogenized and split into three subsamples and analyzed for sediment zinc concentration to characterize the initial condition in each plot. After four months, one post-amendment sample from each plot was analyzed for zinc. Post-amendment samples were held in cold storage at 4°C for 8 weeks before bioassay testing was initiated. The pre-amendment and post-amendment sediment zinc concentrations are presented in Table 3-1. The initial sediment concentrations of zinc can be quantitatively categorized into two groups. The group comprised of uncontaminated plots upstream of Slavins Dock having sediment zinc concentrations at background levels (Plot 1 and Plot 2) was significantly different from the group of contaminated plots located just offshore from the former zinc recovery furnace (Plot 3, Plot 4, and Plot 5). Within each group, there were no statistically significant differences.

**Table 3-1. Summary of Pre-amendment and Post-amendment Pore Water and Sediment Zinc Concentrations**

	Pore Water Zinc ( $\mu\text{g/L}$ )		Sediment Zinc ( $\text{mg/kg dry wt}$ )	
	Pre-amendment	Post-amendment <sup>1</sup>	Pre-amendment <sup>1</sup>	Post-amendment
Plot 1	8.63	8.90	62.4	39.0
Plot 2	1.95	1.96	75.6	140
Plot 3	5600	486	37239	21780
Plot 4	7145	4000	35834	18206
Plot 5	7300	123	72567	16368

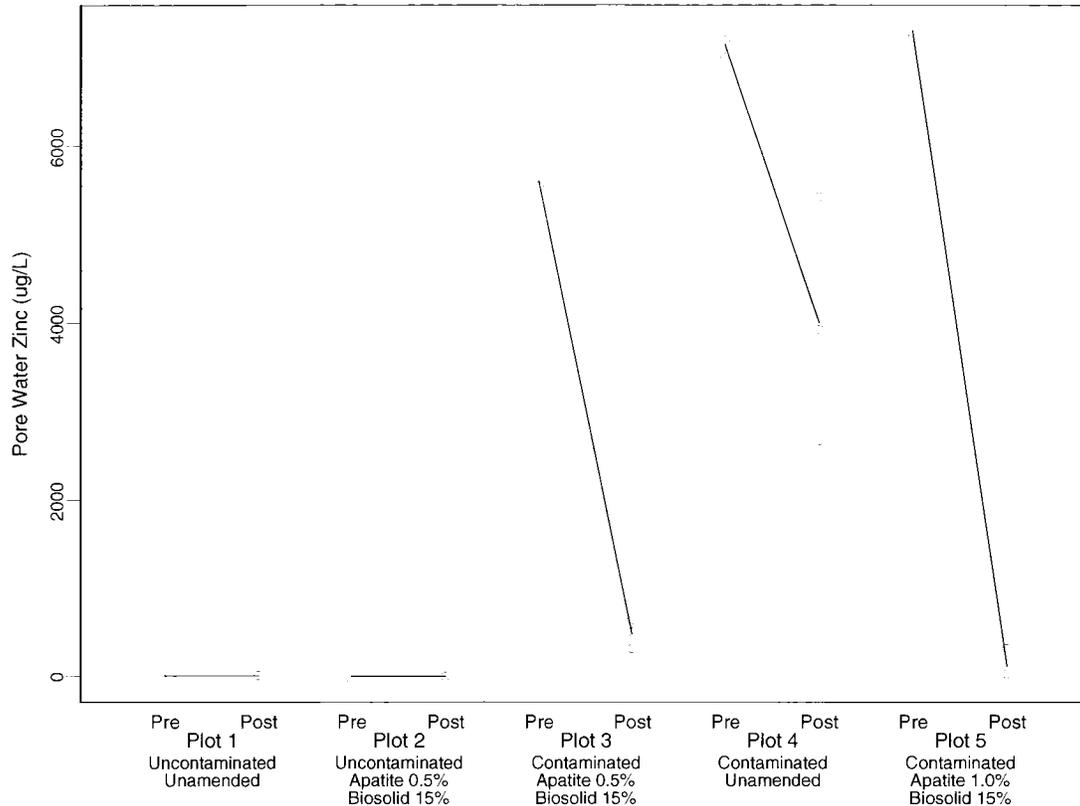
<sup>1</sup>Mean of three subsamples collected from each plot.

Post-amendment sediment zinc concentrations were not expected to decline significantly due to apatite binding the zinc, because the acid extraction method (nitric, hydrochloric, and hydrofluoric acids) used for sediment analysis should dissolve zinc from apatite as well as sediment particles. Zinc concentrations were expected to be on the order of 17% lower in the post-amendment samples because the apatite and biosolid amendments totaled approximately 17% of the sediment by weight. Actual post amendment sediment zinc concentrations decreased in all contaminated plots (3, 4 and 5). This drop may be the result of natural variability of the sediments within each plot; this cannot be adequately determined due to the small number of samples collected.

### 3.3 Pore Water Chemistry Results

Zinc concentrations were measured in the pore water after the collected sediment was centrifuged and filtered. Pore water concentrations were analyzed for zinc in a single pre-amendment sample and in three post-amendment samples at each of the amended plots. Results are summarized in Table 3-1. The change between the pre- and post-amendment concentrations was reviewed to look for an effect due to amendment applications. Figure 3-1 presents the zinc pore water concentrations at each location. The pre-amendment results are depicted by “x” and the post-amendment results are depicted by “o”. A straight line connects the average concentrations at pre- and post-amendment conditions for each test plot to identify the change in concentration. The uncontaminated plots changed so little that a difference was not observed visually. But each of the contaminated plots showed a large drop in zinc pore water concentration, with the largest drop noted at the highest apatite amendment (Plot 5). The size of the drop in pre- to post-amendment concentrations in the contaminated plots can be ordered from largest to smallest as follows: 7177  $\mu\text{g/L}$  at Plot 5 (Apatite at 1%), 5114  $\mu\text{g/L}$  at Plot 3 (apatite at 0.5%), and 3145  $\mu\text{g/L}$  at Plot 4 (no amendment). Plot 4 was not amended but the area was tilled to homogenize the surface sediment with deeper layers to be consistent with the actions in Plot 3 and 5. The fraction of zinc in pore-water remaining is ordered from greatest reduction to least reduction: Plot 5 (1.6%), Plot 3 (8.7%), and Plot 4 (56%). Again, the high-dose amendment application produced the maximum change. The drop in both sediment and pore-water zinc for all contaminated plots is not completely understood. The decrease is potentially due to tilling but would mean the tide is also necessary since the pre-amendment samples were collected after tilling. Additional

sediment deposition between sampling periods is also a potential contributor to this observed decrease in sediment zinc between sampling events. Zinc speciation via spectroscopy should provide some information to help understand this behavior.



**Figure 3-1. Comparison of pre- and post-amendment concentrations of zinc in pore water**

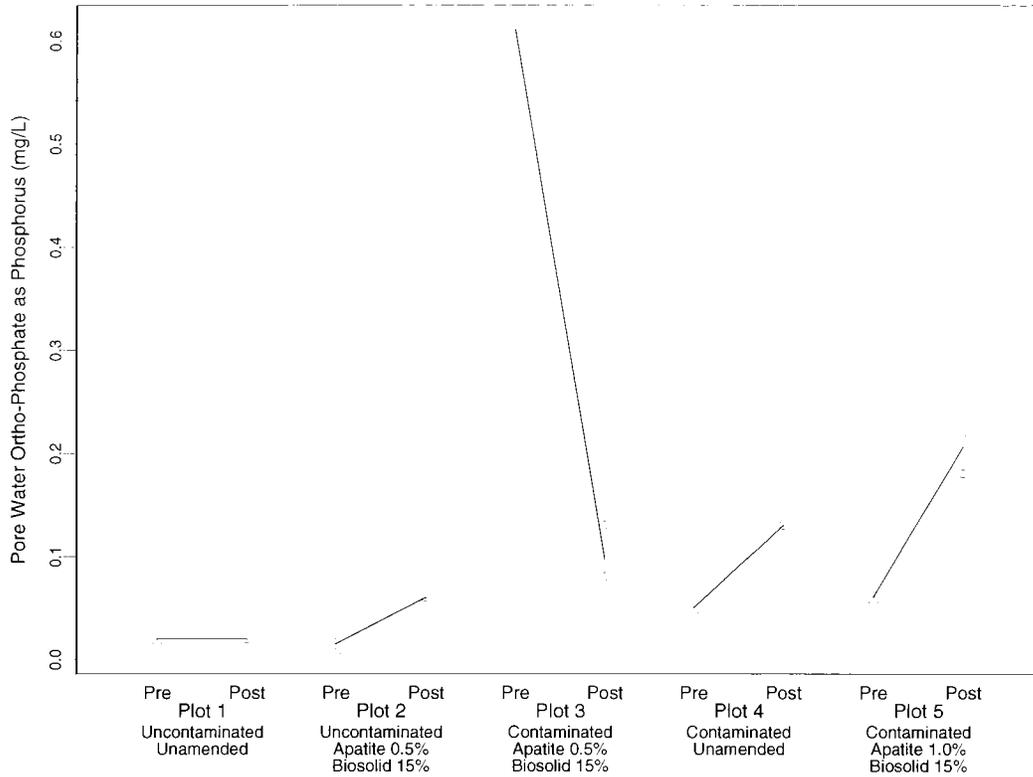
A summary of total organic carbon (TOC) and sediment grain size (% fines) in the pre-amendment samples vs. the post-amendment samples is presented in Table 3-2. TOC concentrations in post-amendment samples were significantly higher in Plots 2, 3, and 5 when compared to pre-amendment concentrations. This was expected due to the addition of the organic-rich biosolid material to these plots. TOC decreased slightly in Plot 1, which received no biosolid amendments. TOC in the Plot 4 post-amendment sample was two times higher than in the pre-amendment sample at Plot 4, but still significantly lower than the post-amendment samples from Plots 2, 3, and 5. The increase in Plot 4 post-amendment TOC may be due to the location of Plot 4 between Plots 3 and 5, and potential tidal transport of fine-grained biosolids from those plots to Plot 4. Another potential explanation is that a fine algal film was noted on the surface of the sediment in Plots 3, 4, and 5 during the post-amendment sampling, and this film may be the explanation for the increased TOC concentrations in Plot 4. The increase in fines in all plots is not completely understood but may also be due to sediment deposition.

**Table 3-2. Summary of Pre-amendment and Post-amendment Total Organic Carbon and % Fines**

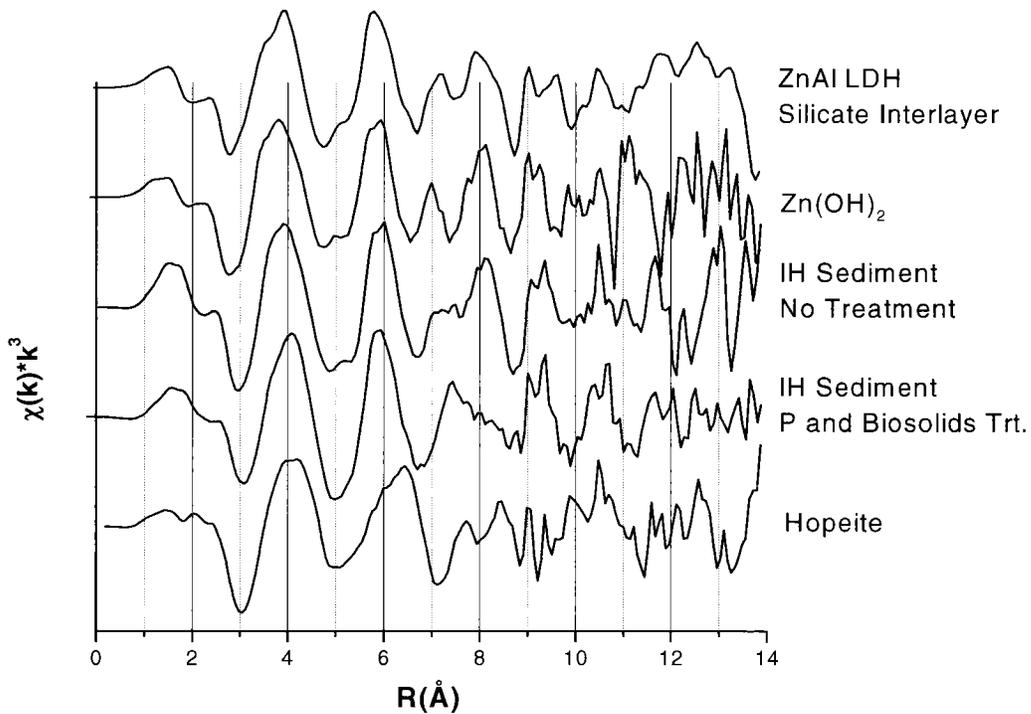
	<b>TOC – Pre-amendment (mg/kg)</b>	<b>TOC – Post-amendment (mg/kg)</b>	<b>TOC % Change</b>	<b>% Fines – Pre-amendment</b>	<b>% Fines – Post-amendment</b>	<b>Fines % Change</b>
Plot 1	20000	14000	-30%	2.14%	5.11%	+140%
Plot 2	15900	65900	+314%	19.65%	31.72%	+61%
Plot 3	8500	34700	+308%	10.93%	15.65%	+43%
Plot 4	8800	17500	+99%	15.35%	31.50%	+105%
Plot 5	5400	60100	+1010%	7.66%	15.10%	+97%

Ortho-phosphate concentrations were measured in both pre-amendment and post-amendment pore water samples to determine if the apatite or biosolids contained enough soluble phosphorous to promote algal growth and eutrophication. Figure 3-2 shows that orthophosphate concentrations in Plot 1 did not change, while Plots 2, 4, and 5 saw an increase in ortho-phosphate between pre-amendment and post-amendment sampling. Plot 3 saw a sharp decrease in orthophosphate concentrations over the same time period. The effect of this slight (up to 200 µg/L) increase in orthophosphate concentration observed in two of three plots cannot be assessed without an understanding of the current water quality and orthophosphate load in Mattawoman Creek.

X-Ray Absorption Near Edge Spectroscopy (XANES) analysis of pre and post-amendment sediments is underway at the EPA. Initial results indicate the amendments are changing the overall speciation of the zinc. This is indicated by the change in the spectra as shown in Figure 3-3. Hopeite is a form of zinc phosphate (hydrated). Slight changes in the 7-9 inverse angstroms region of the spectra are indicative of a transformation in zinc. The exact nature of this change is being investigated at this time. The theory of XANES analysis is beyond the scope of this report (see [http://www.rigakumsc.com/exafs/about\\_tech.html](http://www.rigakumsc.com/exafs/about_tech.html) for additional information).



**Figure 3-2. Pre- and post-amendment concentrations of ortho-phosphate in pore water**



**Figure 3-3. XANES spectra of Indian Head sediments with and without amendments**

### 3.4 Bioassay Results

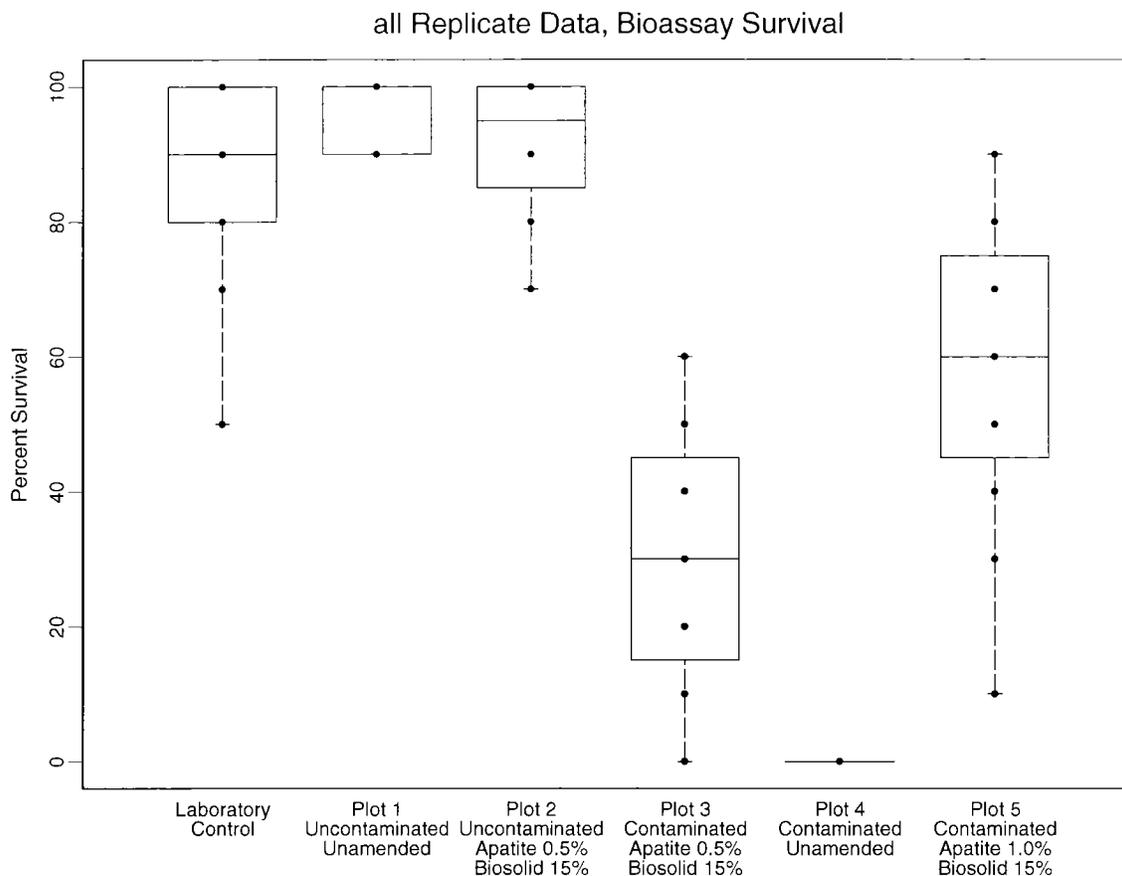
#### 3.4.1 *Hyalella azteca* Survival Bioassay

A solid-phase 28-day chronic toxicity test with the amphipod *Hyalella azteca* was conducted from December 28, 2004, to January 25, 2005. The results of the test are summarized in Table 3-3. Complete test results and laboratory documentation are presented in Appendix A. All water quality parameters were within acceptable limits during the test with the exception of temperature, which dipped below the recommended minimum of 20°C for a period of about two hours because of a power outage on January 8, 2005, and salinity, which rose as high as 8 ppt for approximately one day as a result of the inadvertent opening of a seawater feed at the conclusion of the January 8 power outage. Details of these deviations are presented in Section A-2.3 of Appendix A. No significant adverse affect on data quality was expected from this deviation.

**Table 3-3. Mean and standard deviation (sd) survival and growth in the 28-day *Hyalella azteca* Chronic Toxicity Test, Indian Head sediments**

Sample ID	Condition	Percent Survival			Weight per Individual (mg)		
		Mean	sd	CV	Mean	sd	CV
Initial Weight	–	–	–	–	0.019	0.003	18%
Laboratory Control Sediment	–	88	15	18%	0.331	0.081	25%
Plot 1	Uncontaminated; not amended	96	5	5%	0.603	0.057	9%
Plot 2	Uncontaminated; 0.5% apatite, 15% biosolids	92	10	11%	0.409	0.043	11%
Plot 3	Contaminated; 0.5% apatite, 15% biosolids	31	21	67%	0.083	0.048	57%
Plot 4	Contaminated; not amended	0	0	–	–	–	–
Plot 5	Contaminated; 1.0% apatite, 15% biosolids	58	24	41%	0.148	0.078	53%

Mean survival of *H. azteca* in the laboratory control sediment was 87.5%, which is greater than the acceptability criterion of 80% and validates the test. *H. azteca* mean survival in each of the uncontaminated Indian Head sediments (reference sediment) was >90% and was significantly reduced at all contaminated plots (Table 3-3). *H. azteca* mean survival in the unamended zinc-contaminated sediment (Plot 4) was 0%. Survival in the zinc-contaminated plot amended with 0.5% apatite (Plot 3) was 31%, while the 1% apatite amendment increased survival in zinc-contaminated Plot 5 to 58% (Table 3-3). Bioassay survival results are presented in Figure 3-4. The bioassay data analysis that was performed is consistent with the procedures described in the literature (EPA, 1994). The reference describes techniques for comparing the treated and reference sediments to the sediment control.



**Figure 3-4. Survival of *Hyaella azteca* by replicate**

In Figure 3-4, each box plot shows the survival results of the 12 replicates for each test treatment plotted as filled points along the vertical center of the box. There are fewer than 12 points seen when multiple replicates produced the same percent survival. The median line (horizontal midline across the box plot) is a good indication of the overall survival rate at a test plot. The scatter or spread of the dots shows the variability of the 12 replicate survival results for each test plot.

The distribution assumptions of the survival data were tested using the Shapiro Wilks W test. The bioassay survival results from the control sediment and Plots 1 and 2 did not conform to a normal distribution and could not be transformed to a normal distribution using the arcsin transformation. The replicates from Plot 3 and Plot 5 were tested for outliers using the Dixon test, but no statistically significant outliers were identified (both p-values > 0.10). A Kruskal-Wallis test, the nonparametric equivalent of the Analysis of Variance test, was performed to test for differences in survival results between test plot samples and the laboratory control. The Kruskal-Wallis test was significant (p-value < 0.00001), indicating that at least one test plot location was significantly different from the laboratory control. Follow-up comparisons using the Wilcoxon Rank Sum test were performed to identify which test plots have reduced survival compared to the laboratory control. The survival rates at Plot 1 and Plot 2 were not significantly lower

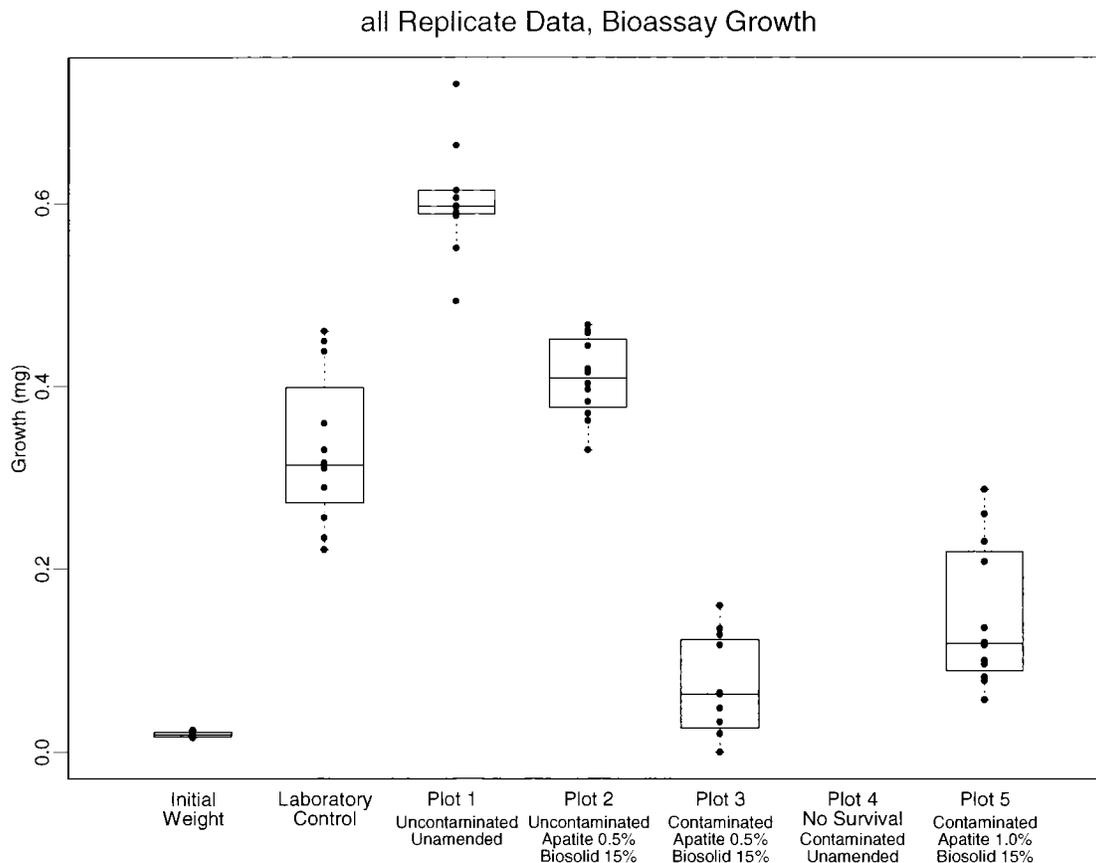
than the laboratory control (Plot 1,  $p=0.92$ ; Plot 2,  $p=0.72$ ). There were no survivors at Plot 4 ( $p=0.00005$ ; also biologically significant by default due to no survival). The survival rates at Plot 3 and Plot 5 were significantly lower than the laboratory control (Plot 3,  $p=0.00003$ ; Plot 5,  $p=0.0013$ ). Plot 5 had a significantly greater survival rate than Plot 3 (Wilcoxon Rank Sum test  $p=0.0048$ ; also t-test  $p=0.0030$  for comparison since both groups conformed to a normal distribution).

In comparing test plot survival to laboratory control, a one-sided hypothesis test was performed, namely  $H_0$ : test plot survival  $\geq$  control survival vs.  $H_1$ : test plot survival  $<$  control survival. When the test is significant ( $p$ -value  $< 0.05$ ), as in the case of Plot 5, we reject the null hypothesis ( $H_0$ ) and conclude the alternative hypothesis ( $H_1$ ) and summarize our conclusion as Plot 5  $<$  Laboratory Control or, equivalently, Laboratory Control  $>$  Plot 5. When the test is not significant, as in the case of Plot 1, we accept the null hypothesis and summarize our conclusion as Plot 1  $\geq$  Laboratory Control. Using this format, significant differences in survival rates can be summarized as follows: Plot 1 and Plot 2  $\geq$  Laboratory Control  $>$  Plot 5  $>$  Plot 3  $>$  Plot 4. The statistical test  $p$ -values provide the significance levels for the differences observed in Figure 3-4.

### **3.4.2 Hyalella azteca Growth Bioassay**

A sublethal endpoint, growth, was also evaluated by the 28-day chronic exposure. Average dry weight per individual *Hyalella* at test initiation was 0.019 mg (standard deviation = 0.003 mg). Amphipods in all amendments and the control sediment, showed some degree of growth over the 28 days. At the end of the 28-day period, the average weight of amphipods in the laboratory control sediment was 0.331 mg (Table 3-3). The average weight of amphipods in the Indian Head sediments ranged from 0.083 mg to 0.603 mg per individual (Table 3-3). Mean *Hyalella* growth at test plots was significantly greater at uncontaminated plots (0.60 mg at Plot 1 and 0.41 mg at Plot 2), and significantly reduced at all contaminated plots (0.08 mg at Plot 3 and 0.15 mg at Plot 5). Bioassay growth results for all replicates are presented in Figure 3-5. In Plot 4, all the organisms died and therefore no growth measures were collected.

In Figure 3-5, each box plot shows the growth results for the 12 replicates for each test treatment plotted as filled points along the vertical center of the box. There appear to be fewer than 12 points when multiple replicates produce nearly identical growth. The median line (horizontal midline across the box plot) is a good indication of the overall survival rate at a test plot. The scatter or spread of the points shows the variability of the growth results for each test plot.



**Figure 3-5. Bioassay results for *Hyaella azteca* – growth endpoint**

The growth results from the laboratory control sediment and all test plots conformed to a normal distribution (Shapiro Wilks test for normality p-values ranged from p=0.09 for Plot 5 to p=0.76 for Plot 2). The variability of growth results from the laboratory control sediment and all test plots were not significantly different (p-value of the F-test for the maximum/minimum ratio of variances is 0.065). An Analysis of Variance, which tests for equality in growth between the control sediment and all test plots, indicated there were significant differences (p-value < 0.00001). Dunnett's multiple comparison procedure, which compares all test plots to the laboratory control sediment at a simultaneous significance value of 0.05, indicated the following significant differences: Plot 1 and Plot 2 have greater growth than the laboratory control sediment (though the difference may not be meaningful); and Plot 3 and Plot 5 have significantly less growth than the laboratory control sediment. The estimated differences in growth and simultaneous 95% confidence intervals about the differences are reported in Table 3-4. Confidence intervals not containing zero indicate a significant difference in growth for the two groups being compared. When Tukey multiple comparisons for all possible pairs are performed, Plot 5 growth is significantly greater than Plot 3 growth (Table 3-4).

In the growth comparisons of test plots to laboratory control, a two-sided hypothesis test is being performed, namely  $H_0$ : test plot growth – control growth = 0 (growth is not

different) vs.  $H_1$ : test plot growth – control growth  $\neq 0$  (either  $< 0$  or  $> 0$ , growth response is different). When the comparison is significant (p-value  $< 0.05$ ), the associated 95% confidence interval does not contain zero, and the direction of the conclusion is captured by the estimate of the difference.

**Table 3-4. Multiple Comparisons of *Hyaella* Growth Results**

	Estimate of Difference	95% Confidence Interval on Difference	Growth Conclusion
<b>Comparison (Dunnett's)</b>			
<b>Plot 1 – Control</b>	0.27 mg	(0.21 mg, 0.34 mg)	Plot 1 > Control
<b>Plot 2 – Control</b>	0.08 mg	(0.012 mg, 0.14 mg)	Plot 2 > Control
<b>Plot 3 – Control</b>	-0.26 mg	(-0.33 mg, -0.20 mg)	Plot 3 < Control
<b>Plot 5 – Control</b>	-0.18 mg	(-0.25 mg, -0.12 mg)	Plot 5 < Control
<b>Comparison (Tukey's)</b>			
<b>Plot 5 – Plot 3</b>	0.078 mg	(0.15 mg, 0.004 mg)	Plot 5 > Plot 3

### 3.4.3 *Lumbriculus* Bioaccumulation Bioassays

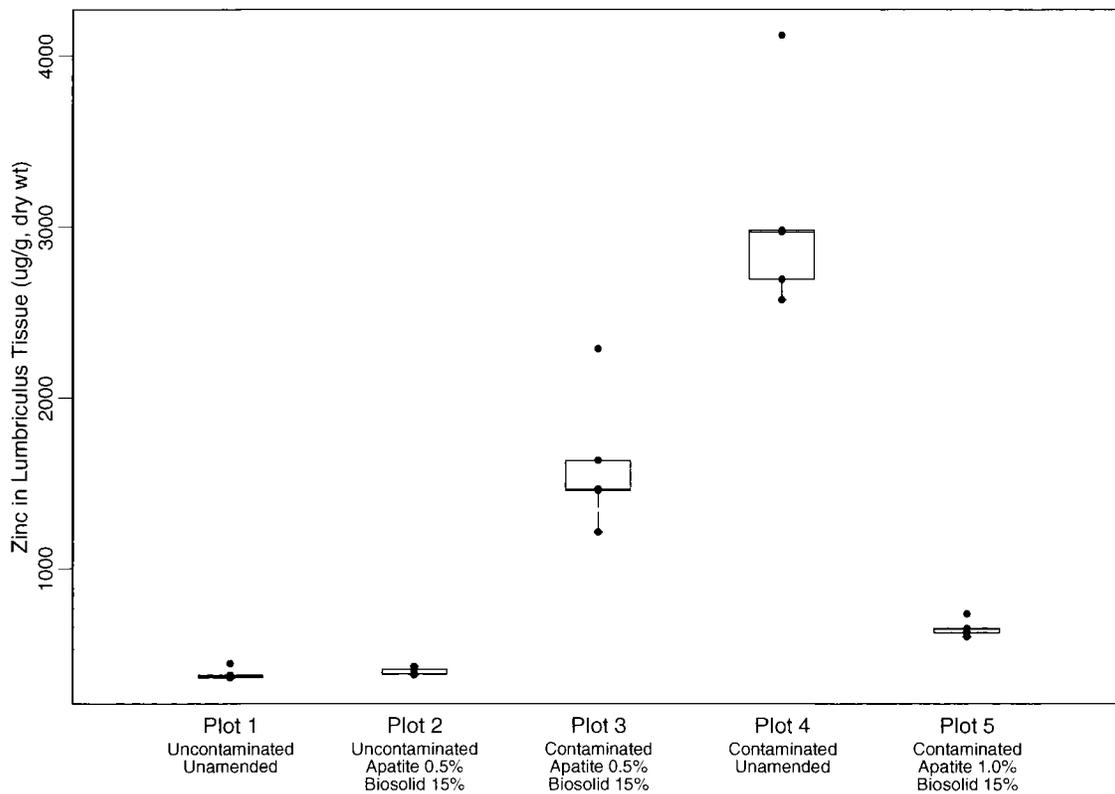
A 4-day toxicity screening test was conducted with the oligochaete *Lumbriculus variegatus* December 16–20, 2004. *Lumbriculus* survival in the Indian Head sediments and the control sediment ranged from 93% to 100% (Appendix A). The worms were buried in all sediments. Therefore, it was appropriate to conduct the 28-day bioaccumulation test with all sediments. The 28-day bioaccumulation test was conducted from December 21, 2004, to January 18, 2005. All water quality parameters, with the exception of one DO measurement for one replicate from Plot 3, were within acceptable limits during the test. Aeration to that replicate was increased. Worms in all sediments scattered throughout the aquarium and showed typical burying behavior.

Comparisons between test plots indicated that tissue concentrations following exposure to sediments from Plot 1 and Plot 2 are not different. The remainder of the plots comparisons produced significant differences in tissue uptake. A summary of *Lumbriculus* zinc concentrations is presented in Table 3-5. All replicate results for tissue concentrations at test plots are presented in Figure 3-6.

**Table 3-5. *Lumbriculus* Tissue Zinc Concentration Summary**

	Plot 2	Plot 1	Plot 5	Plot 3	Plot 4
<b>mean estimates (µg/g)</b>	398	384	654	1610	3070
<b>mean comparisons</b>	<b>t-test p-values<sup>1</sup></b>				
vs Plot 1	0.09				
vs Plot 5	<0.0001	0.006			
vs Plot 3	0.001	0.006	0.003		
vs Plot 4	0.0003	0.006	0.0004	0.002	
<b>variance estimates (µg/g)</b>	478	1260	2090	132000	377000
<b>variance comparisons</b>	<b>F-test p-values</b>				
vs Plot 1	0.37				
vs Plot 5	0.13	0.51			
vs Plot 3	<0.0001	0.0003	0.001		
vs Plot 4	<0.0001	<0.0001	0.0002	0.44	

<sup>1</sup> t-test used if variances not significantly different; Welch's modified t-test used if variances are significantly different.



**Figure 3-6. Bioassay results for *Lumbriculus variegates* – tissue bioaccumulation endpoint for Indian Head Metals Sequestration Project**

Plot 1 results did not conform to a normal distribution (Shapiro Wilks test for normality  $p=0.02$ ). All other test plots were not significantly different from a normal distribution ( $p$ -values ranged from 0.08 for Plot 4 to 0.23 for Plot 2). Due to non-normality of Plot 1, the nonparametric Wilcoxon Rank Sum test was used to compare Plot 1 to all other test plots. For those plots conforming to a normal distribution, F-tests were used to check the assumption of equality of variance between test plots. F-tests indicated that the variability at Plot 2 and Plot 5 did not differ ( $p=0.13$ ), the variability at Plot 3 and Plot 4 did not differ ( $p=0.44$ ), but variability in Plot 2 and Plot 5 were significantly less than the variability at Plot 3 and Plot 4 (all  $p$ -values  $\leq 0.002$ ). Differences in tissue uptake of zinc at test plots were assessed using a t-test to compare test plots with equal variability and using a Welch modified t-test to compare test plots with unequal variability.

Table 3-5 lists the estimates of means and variability in zinc tissue concentrations for the test plots (rounded to 3 significant digits). Also reported in Table 3-5 are the  $p$ -values from statistical comparisons between test plots. For example, the estimate of zinc concentration variability for Plot 5 is  $2090 \mu\text{g}/\text{g}^2$  and for Plot 3 is  $132000 \mu\text{g}/\text{g}^2$ . The F-test comparison of Plot 5 and Plot 3 variability is reported in the variance comparisons section of Table 3-5 (in the column labeled "Plot 5" and row labeled "vs Plot 3") and lists a  $p$ -value of 0.001, indicating significantly different variability ( $p$ -value  $<0.05$ ). Similarly, the estimate of mean (average) zinc tissue concentration for Plot 5 is  $654 \mu\text{g}/\text{g}$  and for Plot 3 is  $1610 \mu\text{g}/\text{g}$ . The comparison of Plot 5 and Plot 3 mean zinc tissue concentrations is reported in the mean comparisons section of Table 3-5 (in the column labeled "Plot 5" and row labeled "vs Plot 3") and lists a  $p$ -value of 0.003, indicating a significant difference in mean tissue concentrations for these test plots. Since the estimate of mean concentration for Plot 5 is smaller than for Plot 3 and the mean comparison indicates that the difference is significant, the conclusion is recorded as an inequality as Plot 5  $<$  Plot 3.

### **3.5 Conclusions**

Prior to amendment, the test plot sediment concentrations of zinc were characterized as Plot 1 = Plot 2  $<$  Plot 3 = Plot 4 = Plot 5. Pore water concentrations of zinc in pre-amendment samples were low in uncontaminated plots and high in contaminated plots. A semi-quantitative comparison (no formal statistical comparison) of pore water zinc concentrations in post-amendment to pre-amendment samples at the contaminated test plots suggests the following ordering of reduction: Plot 5  $>$  Plot 3  $>$  Plot 4. There was no compelling qualitative evidence to infer an increase in ortho-phosphate concentration with application of amendments at the rates applied in this metals sequestration project. Significant differences in acute toxicity observed in *Hyalella* survival rates were characterized as Plot 1, Plot 2  $\geq$  Laboratory Control  $>$  Plot 5  $>$  Plot 3  $>$  Plot 4. Significant differences in chronic toxicity observed in *Hyalella* growth rates were characterized as Plot 1, Plot 2  $>$  Laboratory Control  $>$  Plot 5  $>$  Plot 3  $>$  Plot 4. Significant differences in bioaccumulation of zinc in *Lumbriculus* tissue were characterized as Plot 1 = Plot 2  $<$  Plot 5  $<$  Plot 3  $<$  Plot 4. The findings from all lines of evidence support the conclusion that contaminated sediments amended with 1% apatite (Plot 5) performed better than contaminated sediments amended with 0.5% apatite

(Plot 3), which performed better than the unamended contaminated sediments (Plot 4). All improvements are in the anticipated direction and indicate that the treatment is effective, but that the optimum dose rate has not been determined.

#### **4.0 REFERENCES**

EPA. 1994. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants in Freshwater Invertebrates. Chapter 14, Data Recording, Data Analysis and Calculations, and Reporting. EPA 600/R-94/024. June 1994.

CH2MHill. 2005. Final Remedial Investigation Report Site 28, Naval District Washington, Indian Head. Prepared for Department of the Navy, Naval Facilities Engineering Command Atlantic. April 2005.

Neptune and Company and Battelle. 2004. Work Plan for the Pilot Metals Sequestration Demonstration Project at Indian Head Naval Surface Warfare Center. Prepared for NAVFAC Washington, Indian Head. May 2004.

## **APPENDIX A BIOASSAY TESTING REPORT**

### **A-1.0 BIOASSAY TESTING**

Sediment composites from the Mattawoman Creek in Indian Head, Maryland, underwent bioassay and bioaccumulation testing following general guidance provided in *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, 2<sup>nd</sup> Edition* (USEPA 2000). Chronic toxicity was assessed through direct sediment exposures to a species of freshwater amphipod for 28 days. Bioaccumulation of pollutants of concern was assessed through a 28-day exposure of a freshwater worm.

#### **A-1.1 Methods**

##### **A-1.1.1 Amphipod (Solid Phase) Toxicity Tests**

The species of test organisms used to evaluate solid phase toxicity of the Indian Head samples was *Hyalella azteca*, a freshwater amphipod. Test organisms were obtained from the commercial supplier Aquatic Research Organisms (ARO) of Hampton, New Hampshire.

All test organisms were delivered to Battelle's Marine Science Laboratory (MSL) in freshwater from the original cultures. Upon arrival at MSL, the test organisms were placed into separate 10-L aquaria and acclimated slowly (Table A-1) to test conditions. The aquaria were provided with slow flow-through water exchange and aeration. During holding, *Hyalella azteca* were fed a ground Tetramin® fish food slurry daily. Organisms exhibiting abnormal behavior or appearance were not used in toxicity tests. Receipt, acclimation, and animal care records are part of the raw data files for this project.

##### **A-1.1.1.1 Sediment Sample Preparation**

Six sediment samples were prepared for biological analysis under the Indian Head tests: four river test composites, one reference sediment, and one artificial control sediment. A formulated sediment was used as the laboratory control sediment for the amphipod exposure. The formulation was based on one alternative described in EPA (2000) and Kemble et al. (1999). The formulated sediment was comprised of washed sand (1.24 kg) that had been passed through a 500-micron-mesh sieve and trapped on a 250-micron-mesh sieve was mixed with alpha cellulose (0.07 kg; Sigma Chemical), a silt-clay mixture (0.22 kg; ASP 400, Engelhard Corporation), and granular dolomite (7.5 g; Lily Miller Brands). The control sediment was not amended or conditioned prior to testing. All sediment samples, except the artificial control sediment, were stored in MSL's walk-in cold room at  $4 \pm 2$  °C until used in toxicity or bioaccumulation tests.

**Table A-1. Target Conditions for the Amphipod Solid-Phase Acute Toxicity Tests, Indian Head Sediments, December 2004–January 2005**

<b>Test Organism Species:</b>	<i>Hyalella azteca</i>
Holding Conditions:	Measure temperature and dissolved oxygen on arrival Acclimate to test temperature at 2° C/24 h Observe daily for mortality and condition during holding Hold at test conditions listed below Feed 5 mL ground Tetramin® slurry
<b>TEST TYPE/ SETUP:</b>	<b>28-day Chronic Toxicity</b>
General Method:	EPA/600/R-99/064 (modified from 42-d test)
Duration:	28 days
Test Material:	Sediment (Freshwater)
Endpoint(s):	Survival, growth
Number of Treatments:	6 (5 test, 1 laboratory control)
Number of Replicates:	12
Test Population:	10 per replicate; total <i>n</i> = 120
Age of Organisms:	7–14-days old
Test Chamber:	400-mL polypropylene flow-through jar
Test Volume:	100 mL sediment; fill to outflow port with freshwater (~ 300 mL)
Flow Conditions:	<u>Static Renewal</u> : 2 volume exchanges per day.
Other Setup Notes:	Renewals via timed flow through
<b>TEST CONDITIONS:</b>	
Lighting:	16 h light, 8 h dark
Light Quality/Intensity:	Wide-spectrum fluorescent lights/~100–1000 lux
pH:	No standard
Temperature:	23 °C ± 3 °C (instantaneous) 23 °C ± 1 °C (daily test average)
Dissolved Oxygen:	≥ 2.5 mg/L
Aeration:	Gentle at ~1 bubble/second
Hardness:	Should not vary by > 50% during test
Alkalinity:	Should not vary by > 50% during test
Conductivity:	No standard
NH <sub>3</sub> (overlying):	Should not vary by > 50% during test
<b>INITIATION NOTES:</b>	Isolate and obtain dry weights of 80 individuals (4 reps); dry 24 h at 60 °C
<b>MONITORING FREQUENCY:</b>	

<b>Test Organism Species:</b>	<i>Hyalella azteca</i>
pH:	Day 0, Day 27; measure to 0.1 pH unit
Temperature:	<u>Test jars:</u> Daily; 1 container per treatment <u>Water Table:</u> min/max thermometer; record daily measure to 1 °C
Dissolved oxygen:	Day 0, Day 27; 3 times per week during test; 1 container per treatment measure to 0.1 mg/L
Hardness:	Day 0, Day 27; 1 container per treatment
Alkalinity:	Day 0, Day 27; 1 container per treatment
Conductivity:	Day 0; Day 9 (10-d replicates); Day 27; weekly during test; 1 container per treatment
NH <sub>3</sub> (overlying):	Day 0, Day 27; 1 container per treatment
Observations:	1 hr post initiation, then daily
Feeding:	Daily: 1.0 mL/chamber YCT; 3 times per week: ~0.5 mL/chamber ground Tetramin® fish food slurry; do not overfeed
Other Monitoring Notes:	Individuals that fail to bury within 1 hr of initiation may be removed and replaced
<b>TERMINATION NOTES:</b>	May pipette survivors before sieving Use 250- $\mu$ m sieve Count and isolate survivors for weighing Dry at 60 °C for 24 h
Test Validity Criteria:	Control survival $\geq$ 80%; measurable growth in control sediments

During test initiation, 80 individuals (4 replicates of 20) of *Hyalella azteca* were removed from the holding containers and used to determine the average dry weight of an individual at the start of the test. At the 28-day test termination, all surviving individuals from the treatments were retrieved for dry weight analyses. Dry weights for *Hyalella* were determined by placing individuals from each control replicate into individual weighing boats and placing the boats in a drying oven (~60 °C) for 24 hours.

#### **A-1.1.1.3 Water Quality Monitoring**

General water quality parameters measured as part of the *Hyalella* assays included temperature, dissolved oxygen, pH, hardness, conductivity, and alkalinity. All water quality parameters were measured in each replicate on Day 0 and Day 27 of the assay. Temperature and dissolved oxygen in each replicate were measured daily. Temperature was measured by using a Fluke 52 K/J Thermometer. Sample pH was measured by using an Orion Research model SA250 pH meter. Dissolved oxygen was measured by using YSI Model 57 oxygen meter. Conductivity was measured by using an Oakton TDSTestr™ hand-held meter. Alkalinity was monitored by using either a Hatch model AL-AP test kit or Titrets hand-held titration cells. Hardness was checked by using Titrets hand-held titration cells. Pore water ammonia concentration was measured on Day 0 by using an Orion Model 920A ammonia meter and on Day 28 by using a Hach model DR100 Colorimeter.

#### **A-1.1.2 Oligochaete Bioaccumulation Assay**

The species of organism used to evaluate bioaccumulation of the Indian Head sediments was *Lumbriculus variegatus*, a burrowing freshwater oligochaete worm. Test organisms were obtained from ARO. Test organisms were shipped to MSL in their original culture water to ensure viability. Upon arrival at MSL, the test organisms were placed into a 10-L aquarium and acclimated slowly (Table A-2) to test conditions. The aquarium was provided with slow, flow-through water renewal and aeration. During the holding period, the worms were fed coarsely ground trout chow (1–2% of worm body mass) once daily. Organisms exhibiting abnormal behavior or appearance were not used in the toxicity tests. Receipt, acclimation, and animal care records are part of the data files for this project.

##### **A-1.1.2.1 Bioaccumulation Testing**

Prior to conducting the 28-day bioaccumulation test, a 4-d toxicity screening test was run as specified in USEPA (2000). For this test, *Lumbriculus variegatus* was exposed to small volumes (~100 ml) of test and control sediments (the control sediment was the same formulated sediment that was used for the *Hyalella* test, except that it contained more alpha cellulose–0.77 kg). The purpose of this preliminary screening was to determine the potential toxicity of the test sediments to the worms, and whether or not the worms would burrow into the test sediments. Four replicates, to which 10 worms were added, of each sediment were used. To be used in the 28-day bioaccumulation study, a

test should show no significant mortality versus the control sediment and test organisms must burrow into it. General target conditions for the 4-d test were similar to those specified for the 28-day test (Table A-2).

After completion of the 4-d toxicity screening test, the bioaccumulation test was initiated. *Lumbriculus variegatus* were exposed to the five Indian Head site sediments and the formulated laboratory control sediment for 28 days under static renewal conditions. The test chambers were 9.5-L (2.5-gal.) glass aquaria. Five replicates per treatment were run. The test was initiated by placing about 5 g (wet-weight) *Lumbriculus variegatus* into each aquarium. The weight was obtained by placing a mass of worms in a tared weighing dish and by using a small-bore pipette to remove as much extraneous water as possible. To allow for the weight of the water retained within the mass of worms, the target wet weight for initiation was 6.65 g, as specified in EPA (2000). The stocking weight for each replicate was recorded. Three samples (~6.6-g wet weight) of *Lumbriculus variegatus* were collected and frozen at test initiation to provide tissues for background chemistry analyses.

Renewal of the overlying water was accomplished via timed (1 hour), twice-daily automatic flow-through. At the end of the 28-day testing period, *Lumbriculus variegatus* was allowed to depurate in clean freshwater for about 24 hours. Following depuration, organism tissues were transferred into the appropriate, labeled chemistry jars for tissue chemical analyses and stored frozen (~20°C) until analysis. Target test conditions for the 28-day bioaccumulation test are provided in Table A-2.

#### **A-1.1.2.2 Water Quality Monitoring**

General water quality parameters measured as part of the *Lumbriculus* assay included temperature, dissolved oxygen, pH, hardness, conductivity, and alkalinity. All water quality parameters were measured in each replicate on Day 0, Day 7, Day 14, Day 21, and Day 27 of the assay. Temperature and dissolved oxygen in each replicate were measured daily.

Temperature was measured by using a Fluke 52 K/J Thermometer. Sample pH was measured by using an Orion Research model SA250 pH meter. Dissolved oxygen was measured by using YSI Model 57 oxygen meter. Conductivity was measured by using an Oakton TDSTestr™ hand-held meter. Alkalinity was monitored by using either a Hach model AL-AP test kit or Titrets hand-held titration cells. Hardness was checked by using Titrets hand-held titration cells. Pore water ammonia concentration was measured on Day 0 by using an Orion Model 920A ammonia meter and on Day 28 by using a Hach model DR100 Colorimeter.

**Table A-2. Target Conditions for the Bioaccumulation Test, Indian Head Sediments, October-November 2004**

<b>Test Organism Species:</b>	<i>Lumbriculus variegatus</i>
Age/size:	Adult
Holding Conditions:	Measure temperature and dissolved oxygen on arrival Acclimate to test temperature at 2° C/24 h Observe for mortality and condition during holding Hold at test conditions listed below Feed coarsely ground trout chow; feed at 1–2% of body weight every couple of days, check for left over food before feeding
<b>TEST TYPE/ SETUP:</b>	<b>4-day Toxicity Screening</b>
General Method:	EPA/600/R-99/064
Duration:	4 days
Flow Conditions:	Static Renewal: 2 volume exchanges per day.
Endpoint(s):	Survival; burial in test sediments during test
Test Material:	Sediment
Number Treatments:	6 (5 test, 1 laboratory control)
Number of Replicates:	4
Test Population	10 per replicate; total $n = 40$
Test Chamber:	300 mL container
Test Volume:	100 mL sediment; 175 mL overlying water
Other Setup Notes:	Renewals were accomplished via an automatic timed (1-hour) flow through.
<b>TEST CONDITIONS:</b>	
Lighting:	16 h light, 8 h dark
Light Quality/Intensity:	Wide-spectrum fluorescent lights/~100–1000 lux
pH:	No standard
Temperature:	23 °C ± 3 °C (instantaneous) 23 °C ± 1 °C (daily test average)
Dissolved Oxygen:	≥ 2.5 mg/L
Aeration:	None, unless DO below target, then 1 bubble/second
Hardness:	Should not vary by > 50% during test
Alkalinity:	Should not vary by > 50% during test
Conductivity:	No standard
NH <sub>3</sub> (overlying):	Should not vary by > 50% during test
Feeding:	None during test
<b>MONITORING:</b>	
pH:	Day 0; Day 3; 1 container per treatment measure to 0.1 pH unit

<b>Test Organism Species:</b>	<i>Lumbriculus variegatus</i>
Temperature:	Test jars: Daily; 1 container per treatment Water Table: min/max thermometer; record daily measure to 1 °C
Dissolved oxygen:	Daily (Days 0–4); 1 container per treatment measure to 0.1 mg/L
Hardness:	Day 0; Day 3; 1 container per treatment
Alkalinity:	Day 0; Day 3; 1 container per treatment
Conductivity:	Day 0; Day 3; 1 container per treatment
NH <sub>3</sub> (overlying):	Day 0; Day 3; 1 container per treatment
Observation Frequency:	1 hr, then daily
Feeding:	None during test
Other Monitoring Notes:	NA
<b>Test Type/ Setup:</b>	<b>28-day Bioaccumulation</b>
General Method:	EPA/600/R-99/064
Ammonia Purging:	None
Duration:	28 days
Flow Conditions:	Static Renewal: 2 volume exchanges per day
Endpoint(s):	Survival, tissue contaminant level
Test Material:	Sediment freshwater
Number Treatments:	6 (5 test, 1 laboratory control)
Number of Replicates:	5
Test Population:	Add 6.65 g wet weight to each replicate to meet target of 5 g wet weight per replicate
Test Chamber:	2.5-gal L aquarium
Test Volume:	1 L sediment; 1 L overlying water or fill to outflow port with freshwater
Other Setup Notes:	Layer sediments and start overlying water renewal one day prior to testing.
<b>TEST CONDITIONS:</b>	
Lighting:	16 h light, 8 h dark
Light Quality/Intensity:	Wide-spectrum fluorescent lights/~100–1000 lux
pH:	No standard
Temperature:	23°C ± 3°C (instantaneous) 23°C ± 1°C (daily test average)
Dissolved Oxygen: Aeration	≥ 2.5 mg/L Gentle at ~1 bubble/second
Hardness:	Should not vary by > 50% during test
Alkalinity:	Should not vary by > 50% during test
Conductivity:	No standard
NH <sub>3</sub> (overlying):	Should not vary by > 50% during test

<b>Test Organism Species:</b>	<i>Lumbriculus variegatus</i>
<b>INITIATION NOTES:</b>	Remove background sample Stock <b>6.65</b> g wet weight per aquarium (this is 1.33 × target weight of 5 g and is allowable to account for water content within the mass of worms)
<b>MONITORING:</b>	
pH:	Days 0; 7, 14, 21, 27; 1 container per treatment
Temperature:	Test jars: Daily; 1 container per treatment Water Table: min/max thermometer; record daily
Dissolved oxygen:	Daily; 1 container per treatment
Hardness:	Days 0; 7, 14, 21, 27; 1 container per treatment
Alkalinity:	Days 0; 7, 14, 21, 27; 1 container per treatment
Conductivity:	Days 0; 7, 14, 21, 27; 1 container per treatment
NH <sub>3</sub> (overlying):	Days 0; 7, 14, 21, 27; 1 container per treatment
Observation Frequency:	1 hr, then daily
Feeding:	None during test
Other Monitoring Notes:	Measure WQ parameters to level indicated for 4-d test
<b>TERMINATION NOTES:</b>	Prep for depuration prior to termination Use # 40 (425 μm) sieve; transfer worms to shallow dish quickly to keep them from crawling through sieve Depurate 24 hours
Test Validity Criteria:	Water quality parameters <u>typically</u> within guidelines Worms should burrow into sediments
<b>REFERENCE TOXICANT:</b>	None

## A-2.0 RESULTS

### A-2.1 Amphipod (Solid Phase) Toxicity Test

#### A-2.1.1 *Hyalella azteca* Solid Phase 28-day Chronic Toxicity Test

The solid phase 28-day chronic toxicity test with *H. azteca* was conducted from December 28, 2004, to January 25, 2005. The results of the test are summarized in Tables A-3 and A-4. Complete test results by replicate are presented in Appendix B. Mean survival in the laboratory control sediment was 88%. Average dry weight per individual *Hyalella* at test initiation was 0.019 mg (standard deviation = 0.003 mg).

All water quality parameters were within acceptable limits during the test (Table A-5) with the exception of those parameters that are described in Section A-2.3.

**Table A-3. Summary of water quality measurements for the 28-day *Hyalella azteca* Chronic Toxicity test, Indian Head sediments, December 2004–January 2005. (Values for January 8–10, 2005 are not included, see Section A-2.3.)**

Treatment	Temperature (°C)		pH		Dissolved Oxygen (mg/L) Min	Hardness (mg/L CaCO <sub>3</sub> )		Alkalinity (mg/L CaCO <sub>3</sub> )		Conductivity (mS)	
	Min	Max	Min	Max		Day 0	Day 27	Day 0	Day 27	Day 0	Day 27
Target Range:	22 20	24 <sup>1</sup> 26 <sup>2</sup>	No Guideline <sup>3</sup>		>2.5	No Guideline <sup>3</sup>		No Guideline <sup>3</sup>		No Guideline <sup>3</sup>	
Plot 1	21	24	8.2	8.6	6.6	153	170	130	150	0.34	0.41
Plot 2	21	23	8.2	8.6	7.1	153	170	130	160	0.35	0.50
Plot 3	21	23	8.1	8.4	7.0	153	170	125	140	0.31	0.47
Plot 4	21	24	7.9	8.5	6.7	136	170	125	140	0.34	0.44
Plot 5	21	23	7.9	8.4	6.4	136	153	140	160	0.34	0.51
Control Sediment	21	24	7.8	8.5	3.5	153	170	130	140	0.36	0.50

<sup>1</sup> Daily average for duration of test.

<sup>2</sup> Allowable day-to-day variation.

<sup>3</sup> Must not vary more than 50% during test.

**Table A-4. Mean and standard deviation (sd) survival and growth in the 28-day *Hyalella azteca* Chronic Toxicity Test, Indian Head sediments, December 2004–January 2005**

Sample ID	Condition	Percent Survival			Weight per Individual (mg)		
		Mean	sd	CV	Mean	sd	CV
Initial Weight	–	–	–	–	0.019	0.003	18%
Laboratory Control Sediment	–	88	15	18%	0.331	0.081	25%
Plot 1	Uncontaminated; not amended	96	5	5%	0.603	0.057	9%
Plot 2	Uncontaminated; 0.5% apatite, 15% biosolids	92	10	11%	0.409	0.043	11%
Plot 3	Contaminated; 0.5% apatite, 15% biosolids	31	21	67%	0.083	0.048	57%
Plot 4	Contaminated; not amended	0	0	–	–	–	–
Plot 5	Contaminated; 1.0% apatite, 15% biosolids	58	24	41%	0.148	0.078	53%

**Table A-5. Summary of water quality measurements for the 28-day *Lumbriculus variegatus* Bioaccumulation Assay, Indian Head sediments, December 2004–January 2005. (Values for January 8–10, 2005 are not included, see Section A-2.3.)**

Treatment	Temperature (°C)		pH		Dissolved Oxygen (mg/L) Min	Hardness (mg/L CaCO <sub>3</sub> )		Alkalinity (mg/L CaCO <sub>3</sub> )		Conductivity (mS)	
	Min	Max	Min	Max		Day 0	Day 27	Day 0	Day 27	Day 0	Day 27
Target Range:	22 20	24 <sup>1</sup> 26 <sup>2</sup>	No Guideline <sup>3</sup>		>2.5	No Guideline <sup>3</sup>		No Guideline <sup>3</sup>		No Guideline <sup>3</sup>	
Plot 1	22	23	7.9	8.4	3.3	170	204	150	160	0.34	0.47
Plot 2	22	23	7.7	8.1	2.7	170	238	165	300	0.36	0.76
Plot 3	22	24	7.8	8.2	<b>2.1</b>	170	121	160	300	0.34	0.74
Plot 4	22	24	7.6	8.1	2.5	170	121	155	350	0.36	0.74
Plot 5	22	23	8.0	8.3	4.0	170	187	175	200	0.34	0.40
Control Sediment	22	23	8.4	8.6	5.1	170	170	175	160	0.34	0.38

<sup>1</sup> Daily average for duration of test.

<sup>2</sup> Allowable day-to-day variation.

<sup>3</sup> Must not vary more than 50% during test.

*H. azteca* mean survival in each of the uncontaminated Indian Head sediments was >90% (Table A-4). *H. azteca* mean survival in the zinc-contaminated sediment ranged from 0% (unamended) to 58% (amended with 1% apatite) (Table A-4).

A sublethal endpoint, growth, was also evaluated by the 28-day chronic exposure. Amphipods in all treatments, including the control sediment, showed some degree of positive growth over the 28 days. At the end of the 28-day period, the average weight of amphipods in the laboratory control sediment was 0.331 mg (Table A-4). The average weight of amphipods in the Indian Head sediments ranged from 0.083 mg to 0.603 mg per individual (Table A-4).

The reference toxicant tests were not successful in estimating a zinc LC<sub>50</sub> for this test population.

### **A-2.2 *Lumbriculus variegatus* 28-day Bioaccumulation Test**

The 4-day toxicity screening test was conducted December 16–20, 2004. *Lumbriculus* survival in the Indian Head sediments and the control sediment ranged from 93% to 100% (Appendix B). The worms buried in all sediments. Therefore, it was appropriate to conduct the 28-day bioaccumulation test with all treatments.

The 28-day bioaccumulation test was conducted from December 21, 2004, to January 18, 2005. All water quality parameters, with the exception of one DO measurement for one replicate from Plot 3, were within acceptable limits during the test (Table A-5). Aeration

to that replicate was increased. Worms in all treatments scattered throughout the aquarium and showed typical burying behavior.

The 28-day bioaccumulation test resulted in sufficient tissue mass for analysis of zinc. Results of the zinc tissue concentrations will be submitted separately to Neptune and Company for incorporation into a data report for the Indian Head Metals Sequestration Demonstration Project.

### **A-2.3 Deviation**

A widespread power outage in the early morning on January 8, 2005, caused by a snow storm, caused the laboratory to be without power for about two hours. The flow system to the water tables was shut down to prevent damage to the temperature controllers that could have resulted from a power surge as power was restored. Temperature on the water tables was measured at 17.4°C (*Lumbriculus*) and 16.7°C (*Hyalella*) about 1.5 h into the outage. About 25 minutes after power was restored, temperatures for the two tables were 16.2°C and 16.8°C, respectively. When power was restored, a seawater feed into the head tank supplying the freshwater tests was inadvertently opened. This error was not discovered until the morning of January 10, 2005, when routine conductivity and hardness measurements indicated unusually high readings. At this point salinity was measured at about 8 ‰ in both tests. The source of the problem was found and corrected shortly before the regularly scheduled morning renewal. About two hours after the renewal, the salinity in the *Hyalella* test was measured at 0 ‰, but at 2 ‰ in the *Lumbriculus* test. Unusual *Lumbriculus* behavior was noticed in that many worms were on the surface of the sediments, crawling up the sides of the aquaria, or congregating in groups in the corners of the tanks. An additional one-hour flow-through renewal for the *Lumbriculus* test was performed that afternoon. On January 11, 2005, there was no evidence of mortality in the *Hyalella* test and the worms appeared to be returning to normal behavior (spreading through the tank and burying). There was no evidence of increased mortality in the *Lumbriculus* test. The results presented below indicate that the inadvertent increase in salinity did not adversely affect either test.

### **REFERENCES**

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- Kemble, N.E., F.J. Dwyer, C.G. Ingersoll, T.D. Dawson, and T.J. Norberg-King. 1999. Tolerance of freshwater test organisms to formulated sediments for use as control materials in whole-sediment toxicity tests. *Environmental Toxicology and Chemistry* 18:222-230.
- USEPA (U.S. Environmental Protection Agency). 2000. *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, 2<sup>nd</sup> Edition*.

APPENDIX B  
REPLICATE DATA

**Table B-1. Number of Surviving Amphipods and Proportion Survival per Replicate for the 28-day *Hyalella azteca* Chronic Toxicity test, Indian Head sediments, December 2004–January 2005**

Sediment	Rep.	Number Alive	Proportion Survival	Mean	SD	CV
Plot 1	1	9	0.90			
Plot 1	2	9	0.90			
Plot 1	3	10	1.00			
Plot 1	4	10	1.00			
Plot 1	5	10	1.00			
Plot 1	6	10	1.00			
Plot 1	7	10	1.00			
Plot 1	8	9	0.90			
Plot 1	9	10	1.00			
Plot 1	10	10	1.00			
Plot 1	11	9	0.90			
Plot 1	12	9	0.90	96%	5%	5%
Plot 2	1	10	1.00			
Plot 2	2	10	1.00			
Plot 2	3	10	1.00			
Plot 2	4	10	1.00			
Plot 2	5	7	0.70			
Plot 2	6	10	1.00			
Plot 2	7	8	0.80			
Plot 2	8	9	0.90			
Plot 2	9	9	0.90			
Plot 2	10	8	0.80			
Plot 2	11	9	0.90			
Plot 2	12	10	1.00	0.92	0.103	11%
Plot 3	1	0	0.00			
Plot 3	2	5	0.50			
Plot 3	3	6	0.60			
Plot 3	4	6	0.60			
Plot 3	5	1	0.10			
Plot 3	6	3	0.30			
Plot 3	7	0	0.00			
Plot 3	8	4	0.40			
Plot 3	9	3	0.30			
Plot 3	10	3	0.30			
Plot 3	11	2	0.20			
Plot 3	12	4	0.40	0.31	0.207	67%

*Pilot Metal Sequestration Report, NSF Indian Head*

<b>Sediment</b>	<b>Rep.</b>	<b>Number Alive</b>	<b>Proportion Survival</b>	<b>Mean</b>	<b>SD</b>	<b>CV</b>
Plot 4	1	0	0.00			
Plot 4	2	0	0.00			
Plot 4	3	0	0.00			
Plot 4	4	0	0.00			
Plot 4	5	0	0.00			
Plot 4	6	0	0.00			
Plot 4	7	0	0.00			
Plot 4	8	0	0.00			
Plot 4	9	0	0.00			
Plot 4	10	0	0.00			
Plot 4	11	0	0.00			
Plot 4	12	0	0.00	0.00	0.000	–
Plot 5	1	7	0.70			
Plot 5	2	6	0.60			
Plot 5	3	7	0.70			
Plot 5	4	5	0.50			
Plot 5	5	9	0.90			
Plot 5	6	1	0.10			
Plot 5	7	3	0.30			
Plot 5	8	6	0.60			
Plot 5	9	9	0.90			
Plot 5	10	5	0.50			
Plot 5	11	4	0.40			
Plot 5	12	8	0.80	0.58	0.241	41%
Control	1	7	0.70			
Control	2	5	0.50			
Control	3	9	0.90			
Control	4	8	0.80			
Control	5	9	0.90			
Control	6	10	1.00			
Control	7	9	0.90			
Control	8	8	0.80			
Control	9	12	1.00			
Control	10	10	1.00			
Control	11	10	1.00			
Control	12	10	1.00	0.88	0.154	18%

**Table B-2. Average amphipod dry weight per replicate for the 28-day *Hyaella azteca* Chronic Toxicity test, Indian Head sediments, December 2004–January 2005**

Sediment	Replicate	Weight per Individual (mg)	Mean	Standard Deviation	Coefficient of Variation
Initial Weight	1	0.016			
Initial Weight	2	0.020			
Initial Weight	3	0.024			
Initial Weight	4	0.018	0.019	0.003	18%
Plot 1	1	0.551			
Plot 1	2	0.606			
Plot 1	3	0.730			
Plot 1	4	0.590			
Plot 1	5	0.586			
Plot 1	6	0.614			
Plot 1	7	0.597			
Plot 1	8	0.493			
Plot 1	9	0.590			
Plot 1	10	0.596			
Plot 1	11	0.614			
Plot 1	12	0.663	0.603	0.057	9%
Plot 2	1	0.330			
Plot 2	2	0.362			
Plot 2	3	0.403			
Plot 2	4	0.415			
Plot 2	5	0.444			
Plot 2	6	0.458			
Plot 2	7	0.396			
Plot 2	8	0.467			
Plot 2	9	0.370			
Plot 2	10	0.461			
Plot 2	11	0.419			
Plot 2	12	0.383	0.409	0.043	11%
Plot 3	1	– <sup>†</sup>			
Plot 3	2	0.064			
Plot 3	3	0.048			
Plot 3	4	0.128			
Plot 3	5	0.020			
Plot 3	6	0.063			
Plot 3	7	– <sup>†</sup>			
Plot 3	8	0.135			
Plot 3	9	0.117			
Plot 3	10	0.033			
Plot 3	11	0.160			

*Pilot Metal Sequestration Report, NSF Indian Head*

<b>Sediment</b>	<b>Replicate</b>	<b>Weight per Individual (mg)</b>	<b>Mean</b>	<b>Standard Deviation</b>	<b>Coefficient of Variation</b>
Plot 3	12	0.065	0.083	0.048	57%
Plot 4	1	- <sup>1</sup>			
Plot 4	2	- <sup>1</sup>			
Plot 4	3	- <sup>1</sup>			
Plot 4	4	- <sup>1</sup>			
Plot 4	5	- <sup>1</sup>			
Plot 4	6	- <sup>1</sup>			
Plot 4	7	- <sup>1</sup>			
Plot 4	8	- <sup>1</sup>			
Plot 4	9	- <sup>1</sup>			
Plot 4	10	- <sup>1</sup>			
Plot 4	11	- <sup>1</sup>			
Plot 4	12	- <sup>1</sup>			
Plot 5	1	0.208			
Plot 5	2	0.287			
Plot 5	3	0.096			
Plot 5	4	0.136			
Plot 5	5	0.120			
Plot 5	6	0.260			
Plot 5	7	0.057			
Plot 5	8	0.117			
Plot 5	9	0.230			
Plot 5	10	0.078			
Plot 5	11	0.100			
Plot 5	12	0.082	0.148	0.078	53%
Control	1	0.449			
Control	2	0.256			
Control	3	0.289			
Control	4	0.330			
Control	5	0.316			
Control	6	0.221			
Control	7	0.359			
Control	8	0.234			
Control	9	0.460			
Control	10	0.311			
Control	11	0.438			
Control	12	0.310	0.331	0.081	25%

<sup>1</sup> No surviving amphipods.

**Table B-3. Number of Surviving Worms and Proportion Survival per Replicate for the 4-d *Lumbriculus variegatus* Toxicity Screening Test, Indian Head Sediments, December 2004**

	Rep.	Number Alive	Number Dead	Number Missing	Survival	Mean	SD
Plot 1	1	8	0	2	0.80		
Plot 1	2	10	0	0	1.00		
Plot 1	3	10	0	0	1.00		
Plot 1	4	10	0	0	1.00	0.95	0.10
Plot 2	1	10	0	0	1.00		
Plot 2	2	10	0	0	1.00		
Plot 2	3	10	0	0	1.00		
Plot 2	4	10	0	0	1.00	1.00	0.00
Plot 3	1	10	0	0	1.00		
Plot 3	2	11	0	0	1.00		
Plot 3	3	9	0	1	0.90		
Plot 3	4	10	0	0	1.00	0.98	0.05
Plot 4	1	10	0	0	1.00		
Plot 4	2	10	0	0	1.00		
Plot 4	3	10	0	0	1.00		
Plot 4	4	10	0	0	1.00	1.00	0.00
Plot 5	1	9	0	1	0.90		
Plot 5	2	8	0	2	0.80		
Plot 5	3	10	0	0	1.00		
Plot 5	4	10	0	0	1.00	0.93	0.10
Control	1	10	0	0	1.00		
Control	2	10	0	0	1.00		
Control	3	10	0	0	1.00		
Control	4	10	0	0	1.00	1.00	0.00