

*Draft Final Report:*  
**2014 TOXICITY TESTING OF BALTIMORE  
HARBOR SEDIMENTS**

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## **Executive Summary**

In satisfaction of a request from the Chesapeake Bay Foundation (CBF) the Aquatic Toxicology Facility of the Environmental Science & Technology Department (ENST) at the University of Maryland, College Park, performed sediment toxicity tests on eleven sediment samples collected by the contractor EA Engineering from locations in Bear Creek proximate to Sparrows Point, Baltimore Harbor. Sediment toxicity was investigated using the estuarine amphipod *Leptocheirus plumulosus* 10 day sediment toxicity assay [ASTM, 1992]. Collection occurred October 13 – 14, 2014 and bioassays were performed October 28 – November 7, 2014. Sediment samples were split with one aliquot provided for toxicity testing and the remainder prepared by EA Engineering for chemical analysis.

Six of eleven sample sites demonstrated statistically significant toxicity with four producing 94% to 100% mortality in test organisms compared to a reference sediment which had 99% survival. Toxic stations were generally further from shore in areas of fine sediment deposition rather than near shore in areas of coarser sediments. A clear north-to-south toxicity gradient was indicated along the margin of this depositional region with the northernmost site not found to be toxic and the southernmost producing complete lethality. Unfortunately a significant spatial gap exists between sites with substantial toxicity and those with minimal toxicity. Additional attention is required to adequately assess toxicity in this region. Based on previous research, toxic sediments also extend west toward the far shore of Bear Creek and likely southwest into the mouth of the Patapsco River as well. Additional sampling and toxicity testing is necessary to fully define the extent and severity of toxicity in sediments within Bear Creek and vicinity.

Toxicity largely correlated with measured concentrations of metal and organic contaminants generally accepted as originating from Sparrows Point-related industrial activities. Metal and PAH concentrations frequently exceeded predicted effect concentration (PEC) levels (especially Zn, Cr, Ni, Cu, Cd) and generally increased along the north-to-south toxicity gradient. Correlations between observed toxicity and various categories of organic contaminants (e.g., PAHs, VOCs, SVOCs, PCBs, and oil & grease) were hampered by high and variable reporting limits causing significant censoring of data. For example, the election to treat nondetected PAH and PCB analytes as equivalent to zero when estimating totals necessarily produced underestimations of total PAHs and PCBs, respectively. Never-the-less, toxicity was found to correlate strongly with Total PAHs as well as with Cr, Ni, and Cu. Since these contaminants (and others like oil & grease and VOCs) all co-varied, no assessment of individual causality was undertaken. High total organic carbon (TOC) and acid volatile sulfide levels may have reduced bioavailability of organic and/or metal contaminants but did not eliminate toxicity. Toxicity in sediments where SEM/AVS was < 1.0 and TOC was very high was likely a result of multiple contaminant stressors and may require application of complex *Toxicity Identification Evaluation* (TIE) procedures to determine dominant stressors. Collection of sediments from areas with intermediate contaminant concentrations that produce moderate toxicity (i.e., sampled between the minimally and severely toxic sites mentioned previously)

## Methods

### *Sample Collection and Handling*

Sediment samples from twenty stations were taken from the eastern side (Sparrows Point) of Bear Creek on October 13 – 14, 2014 with eleven provided for sediment toxicity testing. Stations from which sediment was obtained for toxicity testing are designated: SD-A02, SD-A03, SD-B02, SD-C02, SD-C03, SD-D02, SSD-E02, SD-E03, SD-G02, SD-H01, and SD-H03 (*Figure 1*). Sediments were collected and stored for toxicity tests following American ASTM and USEPA protocols (ASTM, 1994; USEPA, 1995). Sediment samples were collected by boat using a full-size Ponar grab sampler and boat-mounted davit. The top 5 cm of multiple grab samples were homogenized to generate material for testing. Sufficient material was collected to perform toxicity tests and for chemical analysis of sediments. Equal aliquots from at least three replicate samples were thoroughly homogenized in a stainless steel bowl using a stainless steel spoon to form a composite sample for each station for toxicity testing and sediment chemical analysis. Sub-samples for sediment toxicity testing were apportioned from each composite sample directly into pre-cleaned 2.5 L HDPE containers and held on ice while in the field. Samples were subsequently refrigerated at 4°C until processing for sediment tests. Reference sediment was collected from Bigwood Cove, a small tributary to the Wye River, using a Ponar grab sampler on September 5, 2014.

### *Sediment Toxicity Bioassays*

Toxicity of collected sediments was investigated using methods described in the *Standard Guide for Conducting 10-day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods* [ASTM, 1992]. The test species was the estuarine amphipod *Leptocheirus plumulosus*. Organisms were purchased from Chesapeake Cultures, Hayes, VA and shipped priority overnight for use in the assay on arrival. The 10-day exposure, which started on October 28, 2014, used *Leptocheirus* that were 2 – 4 mm in length (i.e., passed through a 750 µm screen but retained on a 500 µm screen). Prior to introducing organisms Bear Creek and reference sediments were sieved through a 500 µm mesh to remove debris, resident amphipods, competitors, and predators. For each sediment sample five replicate 1 L glass beakers were loaded with 175 mL aliquots of sieved sediment using a stainless steel spoon. Filtered aerated temperature (24°C) and salinity (15‰) adjusted Wye River water was added to test chambers bringing the final volume to 1 L. Overlying water was introduced by d over a baffle to minimize sediment suspension. Immediately prior to test initiation overlying water was siphoned from chambers and renewed. Test chambers were maintained static with gentle aeration using 1 mL glass pipettes at a rate of approximately 5 bubbles/second. Tests were conducted in a temperature controlled room maintained at 24 ± 1°C under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles at the surface of the test chamber). General water chemistry (DO, pH, NH<sub>3</sub>, and salinity) was performed on all replicate chambers prior to introducing organisms (Day-0) and prior to test conclusion (Day-9). On all other test days water chemistry was performed on only one replicate per sediment. Porewater ammonia was also measured prior to beginning the tests. Porewater was extracted from sediment samples by placing bulk sediments into duplicate 50 mL conical-bottom HDPE tubes and centrifuging at 3,500 rpm for 15 minutes. Separated porewater was decanted from compacted sediment into glass beakers and NH<sub>3</sub> analyzed immediately. Toxicity tests were initiated by loading 20 *L. plumulosus* into each test chamber on Day-0. *Leptocheirus* were unfed for the duration of the exposure.

Observations of test chambers were made daily. At the conclusion of the 10-day exposure overlying water and sediments from test beakers was rinsed through a 500  $\mu\text{m}$  sieve to collect and count surviving organisms. Test acceptability criteria for *Leptocheirus* required  $\geq 90\%$  survival in the control treatment.

### *Statistical Analysis*

The only endpoint for the *Leptocheirus* test was survival. Statistical analysis of arc-sine square root transformed survival data was performed by one-way analysis of variance followed by a Holm-Sidak multiple-comparison of treatment means with the control mean. Statistical tests were performed using SigmaStat v.3.5 (Systat Software, Inc.) with significance set at a minimum probability level of 0.05.

## **Results and Discussion**

### *Sediment Toxicity Tests*

All water quality measures within test chambers were within acceptable parameters during the 10-day exposure interval except ammonia in SD-C02 which progressively increased from 2.2 mg/L to 35 mg/L over the test interval (water quality data is available on request). *Leptocheirus* survival of 99% in the control treatment exceeded the minimum test acceptability criteria of  $\geq 90\%$ . Survival after 10 days in six of eleven Bear Creek samples was significantly reduced compared to the control (*Table 1*). Survival in the four most southerly stations (SD-E03, SD-G02, SD-H01, and SD-H02) at 10 days was 0 to 6%. Survival in SD-B02 and SD-C03 sediments (78% and 76%, respectively) were also found to be modestly (but statistically significantly) reduced compared to the Bigwood Cove control (APPENDIX A: ANOVA Results). Sediments from remaining stations (SD-A02, SD-A03, SD-C02, SD-D02, and SD-E02) had survival rates of 88% to 98% and were not found to differ significantly from the control. Sediments from all but one of the non-toxic stations (SD-A03) were comprised predominantly of sand, suggesting they were collected from non-depositional regions. These, and similar sandy areas, are of questionable value when investigating toxicity as they lack sufficient surface area and TOC to sorb metal and organic contaminants and tend to lose/exchange porewater during collection by Ponar.

Considering only those sites comprised of fine sediments, all were toxic to *Leptocheirus* except the northern-most (i.e., SD-A03). A clear north-to-south toxicity gradient was indicated along the margin of the depositional region: toxicity was not detected at SD-A03; minimal to moderate toxicity occurred at SD-B02 and SD-C03 (24 – 26% mortality); significant toxicity occurred at SD-E03 (94% mortality); and complete lethality occurred at SD-G02, SD-H01, and SD-H03 (*Figure 1*). Moreover, daily observations from sediment assays indicate total amphipod mortality occurred within one to three days in samples from SD-H01 and SD-H03. Unfortunately there is a significant spatial gap between the northern-most site with substantial toxicity (SD-E02) and the southern-most site with minimal toxicity (SD-C03). Additional attention is required to adequately assess toxicity in the region between these sites (*Figure 3*). Toxic sediments likely also extend west toward the far shore of Bear Creek and southwest into the mouth of the Patapsco River. Additional sampling and toxicity testing will be necessary to fully define the extent and severity of toxic sediments within Bear Creek and vicinity.

## *Chemical Analyses*

Concentrations of numerous metal and organic contaminant as well as grain size analysis, percent moisture, total organic carbon (TOC), and acid volatile sulfides (AVS) were determined for Bear Creek split-sediment samples and reported by EA Engineering [EA Engineering Technical Memorandum, January 14, 2015]. Categories of contaminants included metals, polycyclic aromatic hydrocarbons (PAH), N - hexane extractable materials (HEM; oil and grease), semivolatile organic compounds (SVOC), volatile organic compounds (VOC), and polychlorinated biphenyls (PCB). This suite of contaminants has been previously associated with steel manufacturing, ship building and related industrial activities on the Sparrows Point Peninsula [Ashley and Baker, 1999; EA Engineering, 2011]. Mechanisms of transport of persistent contaminants to adjacent sediments via groundwater migration, surface runoff, and atmospheric deposition have also been described [Ashley and Baker, 1999; EA Engineering, 2011]. Toxic metals (particularly Zn, Cr, Ni, Cu, and Cd) are common environmental contaminants of steel manufacture either released as fine particles during smelting or leached from slags discarded on site [Harber and Forth, 2001]. Formation and release of PAHs result from the pyrolysis of coal during steel production as well as leaching from coal piles near the shoreline [Ashley and Baker, 1999]. Steel manufacturing on Sparrows Point has long been recognized as a discharge source of oil and grease to surrounding waters [Wheeler, 1991]. Categories of contaminant are discussed in relation to observed toxicity for the eleven split-sediment samples tested for toxicity.

*Metals* – Reported concentrations of several metals exceeded predicted effect concentration (PEC) values suggesting possible contributions to toxicity (*Table 2; Figure 2*). The most notable exceedances were for zinc (Zn), chromium (Cr), nickel (Ni), copper (Cu), and cadmium (Cd). As expected, sandy sites had comparatively low metals levels while sites with fine silt had proportionally higher metals levels. With few exceptions concentrations of metals were highly correlated between sites [APENDIX B: Pearson Product Moment Correlation], such that when one metal was particularly high at a given site, other metals were also elevated. Metals concentrations within depositional areas were generally equal to or greater than those measured in surface sediments around Coke Point as part of a 2011 Risk Assessment [EA Engineering, 2011].

Metals concentrations also correlated with sediment toxicity; higher concentrations generally occurring in the more southerly sites where toxicity was greatest. Zinc was somewhat of an exception with high concentrations occurring in all depositional sediments (*Figure 2A*). Ratios of simultaneously extracted metals to acid volatile sulfides (SEM/AVS) from several of the toxic sites were  $\geq 1.0$  suggesting bioavailable metals played some part in observed toxicity. However, toxicity was also observed at several sites where SEM/AVS ratios were  $< 1.0$  suggesting other contaminants likely also contributed to toxicity.

*PAHs* – A suite of 16 PAH compounds were quantified. Naphthalene, fluoranthene, and pyrene were detected in all eleven samples on which toxicity tests were conducted (ranges 4.1  $\mu\text{g}/\text{kg}$  – 4,000  $\mu\text{g}/\text{kg}$ , 14  $\mu\text{g}/\text{kg}$  – 4,900  $\mu\text{g}/\text{kg}$ , and 6.3  $\mu\text{g}/\text{kg}$  – 5,000  $\mu\text{g}/\text{kg}$ , respectively) (*Table 3*). Other analytes were only reported sporadically in the eleven tested sediments. However, inconsistent dilution of samples (presumably due to significant matrix interference) produced reporting limits (RL) that were highly variable between samples for individual analytes. For example, the RL for most analytes ranged from 18  $\mu\text{g}/\text{kg}$  in SD-D02 to 1,900  $\mu\text{g}/\text{kg}$  in SD-H03. These high and variable reporting limits make meaningful comparison between stations difficult. More important, setting values for nondetected analytes at zero likely produces a significant under-estimation when determining *Total PAH* values (*Table 3*). A more defensible estimate of *Total PAH* should use

one-half the detection limit (DL) for all analytes [Clarke 1995; see Appendix C: Dealing with nondetects]. Given the difficulty in analysis associated with these complex and heavily contaminated sediments, a conservative estimate would actually include the entire DL for all analytes in determining *Total PAH*. Despite this limitation, *Total PAH* values as reported correlated with toxicity (as well as with metals); concentrations increased along the north-to-south toxicity gradient. It is probable that metal and PAH contaminants both contributed in some measure to observed toxicity.

*HEM* – Reporting limits for N - hexane extractible materials (HEM; oil and grease) were also highly variable. Sites with the greatest toxicity and highest TOC had oil and grease reporting limits of  $\geq 100,000$  mg/kg (i.e., 10%). These very high reporting limits preclude meaningful interpretation of the data. If actual HEM values were even half the reporting limit in the southernmost sites this would represent oil and grease contaminant levels greater than 5%. Quantification of this category of contaminant requires significantly improved sensitivity.

*SVOCs & VOCs* – Analyses of suites of semivolatile and volatile organic compounds were only performed on six of eleven samples for which toxicity tests were run and quantitation above reporting limits were very infrequent. Notable exceptions were the SVOC bis(2-ethylhexyl)-phthalate (range 18 $\mu$ g/kg – 33,000  $\mu$ g/kg) and the VOC toluene (range 1.7  $\mu$ g/kg – 16  $\mu$ g/kg). Again, highly variable reporting limits hamper meaningful interpretation of these results.

*PCBs* – Polychlorinated biphenyls are of little toxicological concern to benthic organisms but may bioaccumulate/biomagnify within edible aquatic resources (e.g., fish and shellfish) to levels that pose a significant risk for human consumption. This is of particular concern given the proximity of residential communities and level of regional recreational (and subsistence) fishing activity.

## Recommendations

1. Additional chemical analysis and toxicity testing within Bear Creek is warranted to determine more fully the spatial extent and severity of surface sediment toxicity in the region (*Figure 3*).
  - a. The segment within the depositional zone between SD-C03 and SD-E03 requires particular attention.
  - b. Contaminant gradients should be investigated west across Bear Creek and southwest to the mouth of the Patapsco River.
2. Legacy contaminants should be investigated by collecting sediment cores from depositional areas within Bear Creek. This is particularly important if development plans for the region include disturbance of sediments for marine construction or dredging.
3. Application of a modified *Toxicity Identification Evaluation* (TIA) procedure would help determine which categories of contaminants are predominantly responsible for observed toxicity. Briefly, organic contaminants are extracted from toxic Bear Creek sediments (via accelerated solvent extraction) and “spiked” (either in entirety or after fractionation) into non-toxic reference sediments.



Figure 1. Stations sampled within Bear Creek, October 13 – 14, 2014 and tested for toxicity (percent survival of amphipods at the end of the 10-day *Leptocheirus plumulosus* sediment tests included). The red line indicates the approximate transition from coarse sandy sediment to the depositional region comprised of fine grained silt. Within the depositional region (west of the red line) a clear north-to-south toxicity gradient was revealed.

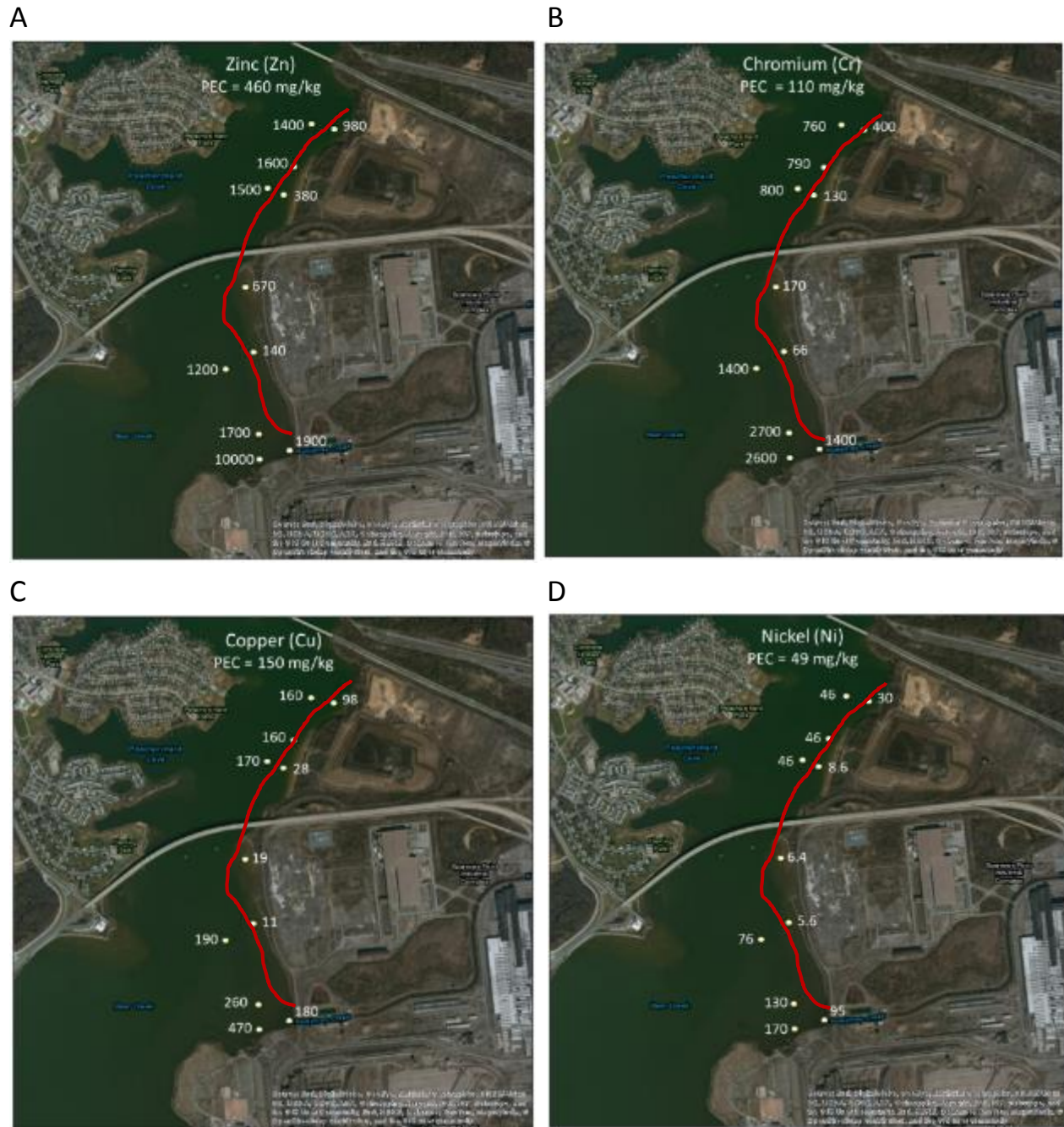


Figure 2. Measured concentrations of zinc (A), chromium (B), copper (C), and nickel (D) at the eleven stations tested for sediment toxicity [values from EA Engineering Technical Memorandum January 14, 2015]. Concentrations in fine sediments from depositional areas (west of red line) often exceeded predicted effect concentration (PEC) levels and revealed a clear north-to-south concentration gradient. Levels in course sandy sediment (east of red line) were generally lower and showed no clear gradient.





0 0.175 0.35 0.7 Miles



**Legend**

● Sampling Locations

Figure 3. Regions within Bear Creek requiring additional investigation (i.e., chemical analysis and toxicity testing) to fully characterize the extent of contamination and potential of toxicity to regional biota. Sediments should be collected in broad east-to-west transects traversing the entirety of shaded areas.

Table 1. Survival of *Leptocheirus plumulosus* in 10-day sediment tests and statistical significance of results. Treatments with an asterisk (\*) differed significantly from the control.

Station	Replicate					Total	Statistical results
	A	B	C	D	E		
CONTROL	20	20	19	20	20	99	---
SD-A02 <sup>a</sup>	17	19	18	19	20	93	NO
SD-A03 <sup>a</sup>	18	16	18	17	19	88	NO
SD-B02 <sup>a</sup>	14	14	15	19	16	78*	0.003
SD-C02 <sup>a</sup>	19	17	18	14	20	88	NO
SD-C03 <sup>a</sup>	17	18	14	11	16	76*	<0.001
SD-D02 <sup>a</sup>	20	20	19	20	15	94	NO
SD-E02 <sup>a</sup>	20	19	19	20	20	98	NO
SD-E03 <sup>a</sup>	0	2	0	2	2	6*	<0.001
SD-G02 <sup>b</sup>	0	0	0	0	0	0*	NOT TESTED
SD-H01 <sup>b</sup>	0	0	0	0	0	0*	NOT TESTED
SD-H03 <sup>b</sup>	0	0	0	0	0	0*	NOT TESTED

**Notes:**

Dark shaded rows are from samples that were predominantly sandy (sand/gravel > 75% as reported in EA Engineering Technical Memorandum January 14, 2015)

Light shaded rows are from samples that were moderately sandy (grain size was not reported for SD-A02 and SD-D02 but samples were noted as having a moderate to high sand content during sieving for toxicity testing)

<sup>a</sup> Treatments with partial survival were included in one-way ANOVA and subsequent Dunnett's multiple-comparison to the control ( $p < 0.05$ )

<sup>b</sup> Treatments with zero survival were excluded from statistical analysis

Table 2. Concentrations of select metals and SEM/AVS ratios in Bear Creek sediments (values provided in EA Engineering Technical Memorandum, January 14, 2015) as well as probable effect concentration (PEC) values for individual metals [MacDonald et al. 2000].

Station	Metal conc. (mg/kg)							SEM/AVS	Survival (%)
	Zn	Cr	Cu	Ni	Cd	Pb	Hg		
SD-A02	<b>980</b>	<b>400</b>	98	30	<b>5.4</b>	<b>160</b>	0.26	0.5	93
SD-A03	<b>1400</b>	<b>760</b>	<b>160</b>	46	<b>7.1</b>	<b>240</b>	0.36	0.5	88
SD-B02	<b>1600</b>	<b>790</b>	<b>160</b>	46	<b>9.2</b>	<b>260</b>	0.46	0.6	78*
SD-C02	380	<b>130</b>	28	8.6	3	51	0.09	0.6	88
SD-C03	<b>1500</b>	<b>800</b>	<b>170</b>	46	<b>8.5</b>	<b>250</b>	0.42	1.0	76*
SD-D02	<b>670</b>	<b>170</b>	19	6.4	<b>4.8</b>	25	na	nc	94
SD-E02	140	66	11	5.6	0.7	16	na	nc	98
SD-E03	<b>1200</b>	<b>1400</b>	<b>190</b>	<b>76</b>	<b>5.3</b>	<b>190</b>	na	0.6	6*
SD-G02	<b>1700</b>	<b>2700</b>	<b>260</b>	<b>130</b>	<b>5.7</b>	<b>130</b>	0.53	1.0	0*
SD-H01	<b>1900</b>	<b>1400</b>	<b>180</b>	<b>95</b>	4.9	110	0.38	2.0	0*
SD-HO3	<b>10000</b>	<b>2600</b>	<b>470</b>	<b>170</b>	<b>45</b>	<b>260</b>	<b>0.83</b>	21	0*
PEC	460	110	150	49	5.0	130	0.64		

**Notes:**

Bold metal concentrations exceed PECs.

SEM/AVS = ratio of simultaneously extracted metals to acid volatile sulfides

Dark shaded rows indicate samples that were predominantly sandy (sand/gravel > 75% as reported in EA Engineering Technical Memorandum January 14, 2015)

Light shaded rows indicated samples that were moderately sandy (grain size was not reported for SD-A02 and SD-D02 but samples were noted as having a moderate to high sand content during sieving for toxicity testing)

\* = samples acutely toxic to *Leptocheirus plumulosus*

na = not analyzed

nc = not calculated

Table 3. Concentrations of three PAH analytes and of Total PAHs in Bear Creek sediments (all values from EA Engineering Technical Memorandum, January 14, 2015). Total PAHs are reported as provided by EA Engineering with nondetected analytes excluded, tabulated at ½ the reporting limit, and tabulated at the entire reporting limit. Probable effect concentration (PEC) values for individual analytes and Total PAHs are provided for reference [MacDonald et al. 2000].

Station	Naphthalene	Fluoranthene	Pyrene	Total PAH (µg/kg)		
				ND = 0	ND = ½ RL	ND = RL
SD-A02	200	410	270	2,749	2,773	2,798
SD-A03	310	390	380	1,449	2,949	4,449
SD-B02	380	490	490	2,309	3,709	5,109
SD-C02	74	110	90	356	1,126	1,896
SD-C03	560	680	690	5,630	6,170	6,710
SD-D02	4.2	14	6.3	24.5	141	258.5
SD-E02	17	17	18	60.5	217	372.5
SD-E03	530	1900	<b>2000</b>	10,360	12,550	14,740
SD-G02	430	<b>4900</b>	<b>2800</b>	14,330	17,300	20,270
SD-H01	220	1400	1300	2,920	10,720	18,520
SD-HO3	<b>4000</b>	<b>2600</b>	<b>5000</b>	11,600	23,950	<b>36,300</b>
PEC	561	2230	1520	22800	22800	22800

**Notes:**

Bold values exceed PECs.

Dark shaded rows are from samples that were predominantly sandy (sand/gravel > 75% as reported in EA Engineering Technical Memorandum January 14, 2015)

Light shaded rows are from samples that were moderately sandy (grain size was not reported for SD-A02 and SD-D02 but samples were noted as having a moderate to high sand content during sieving for toxicity testing)

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## APPENDIX A: Statistical Analysis via ANOVA of Survival Data

### One Way Analysis of Variance

Monday, December 22, 2014, 11:43:39 AM

Normality Test (Shapiro-Wilk) Passed (P = 0.026)

Equal Variance Test: Passed (P = 0.741)

Group Name	N	Missing	Mean	Std Dev	SEM
CONTROL	5	0	19.800	0.447	0.200
SD-A02	5	0	18.600	1.140	0.510
SD-A03	5	0	17.600	1.140	0.510
SD-B02	5	0	15.600	2.074	0.927
SD-C02	5	0	17.600	2.302	1.030
SD-C03	5	0	15.200	2.775	1.241
SD-D02	5	0	18.800	2.168	0.970
SD-E02	5	0	19.600	0.548	0.245
SD-E03	5	0	1.200	1.095	0.490

Source of Variation	DF	SS	MS	F	P
Between Groups	8	1334.800	166.850	57.097	<0.001
Residual	36	105.200	2.922		
Total	44	1440.000			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Holm-Sidak method):  
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
CONTROL vs. SD-E03	18.600	17.204	<0.001	Yes
CONTROL vs. SD-C03	4.600	4.255	<0.001	Yes
CONTROL vs. SD-B02	4.200	3.885	0.003	Yes
CONTROL vs. SD-A03	2.200	2.035	0.223	No
CONTROL vs. SD-C02	2.200	2.035	0.183	No
CONTROL vs. SD-A02	1.200	1.110	0.618	No
CONTROL vs. SD-D02	1.000	0.925	0.592	No
CONTROL vs. SD-E02	0.200	0.185	0.854	No

**APENDIX B: Pearson Product Moment Correlation**

Correlation Coefficient

P Value

Number of Samples

	<b>Zn</b>	<b>Cr</b>	<b>Ni</b>	<b>Cu</b>	<b>Pb</b>	<b>PAH</b>	<b>PCB</b>
Survival	-0.534 0.0907 11	-0.893 <b>0.00022</b> 11	-0.892 <b>0.00023</b> 11	-0.755 <b>0.00717</b> 11	-0.257 0.446 11	-0.817 <b>0.00215</b> 11	-0.628 0.182 6
Zn		0.678 <b>0.0219</b> 11	0.791 <b>0.00376</b> 11	0.887 <b>0.00028</b> 11	0.496 0.121 11	0.535 0.0896 11	0.971 <b>0.0013</b> 6
Cr			0.976 <b>0.000003</b> 11	0.902 <b>0.00015</b> 11	0.440 0.175 11	0.917 <b>0.00007</b> 11	0.623 0.186 6
Ni				0.953 <b>0.000006</b> 11	0.487 0.129 11	0.852 <b>0.00086</b> 11	0.796 0.0584 6
Cu					0.677 <b>0.0221</b> 11	0.796 <b>0.00340</b> 11	0.883 <b>0.0199</b> 6
Pb						0.420 0.199 11	0.384 0.453 6
PAH							0.460 0.358 6

Pair(s) of variables with positive correlation coefficients and P values below 0.050 tend to increase together. For pairs with negative correlation coefficients and P values below 0.050, one variable tends to decrease while the other increases. For pairs with P values greater than 0.050, there is no significant relationship between the two variables.

Statistically significant results (e.g.,  $P \leq 0.05$ ) are indicated in **bold**. Note strongly positive correlation coefficients between Zn, Cr, Ni, and Cu. Note also significant positive correlation coefficients between Total PAH concentrations and Cr, Ni, and Cu. Sources of contaminants and sites of deposition appear highly dependent. There are significant negative correlations between survival and Cr, Ni, Cu and Total PAH concentrations (i.e., survival decreases as concentrations of these contaminants increase). Lead (Pb) concentrations did not correlate with survival or generally with other metals concentrations suggesting sources for Pb differ from other contaminants. Concentrations of PCBs also did not correlate with toxicity or with most other contaminants.

## **APPENDIX C: Dealing with nondetects**

Nondetected results are treated either by 1) substitution of the detection limit as the quantified concentration, or 2) substitution of one-half the detection limit as the quantified concentration. Clarke [1995] recommends a steps in selecting the substitution method that simplify to “substitution method 1) should generally be used where the number of censored data results are less than 40% of the data set, and method 2) where the censored data is greater than 40%.”