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Draft Technical Report

Puget Sound Naval Shipyard (PSNS): Sediment Monitoring Report May 2011

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For

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Data Quality Assurance:

- SSC Pacific Bioassay Laboratory is a certified Laboratory under the State of California Department of Health Services, Environmental Laboratory Accreditation Program (ELAP), Certificate No. 2601; State of Washington Department of Ecology, Laboratory ID. No. F893.
- All data have been reviewed and verified.
- Any test data discrepancies or protocol deviations have been noted in the summary report pages.

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EXECUTIVE SUMMARY

Sediment toxicity testing was conducted using standardized protocols with the marine amphipods, *Leptocheirus plumulosus* and *Ampelisca abdita*, the polychaete worm, *Neanthes arenaceodentata*, (USEPA 1994 and Farrar and Bridges 2011, respectively) and Mediterranean mussel (*Mytilus galloprovincialis*) embryos (Anderson et al. 1996) to evaluate the environmental risk of sediment samples collected from Puget Sound Naval Shipyard (PSNS). The results reported are from a single collection event (April 2011) and included a total of 6 test endpoints for two samples.

No toxicity was observed for either sediment samples, PS03 or PS09, for the whole sediment test with the marine amphipod, *Leptocheirus plumulosus*, or with the marine polychaete, *Neanthes arenaceodentata*. The whole sediment test with the marine amphipod, *Ampelisca abdita*, did not meet test acceptability criteria and analyses that were conducted are for informational purposes only. The sediment sample collected from PS09 showed a slight significant decrease from the control. The controls associated with the exposure at the sediment-water interface using embryos from the bivalve, *Mytilus galloprovincialis*, did not meet test acceptability criteria; however, all samples performed better than the control and a comparative analysis was conducted anyhow and showed no toxicity was present for either sediment samples.

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CETIS	Comprehensive Environmental Toxicity Information System
Cu	Copper
DO	Dissolved Oxygen
EC_{50}	Median Effective Concentration
ELAP	Environmental Laboratory Accreditation Program
FSW	Filtered Seawater
HDPE	High Density Polyethylene
LC_{50}	Median Lethal Concentration
NPDES	National Pollutant Discharge Elimination System
PSNS	Puget Sound Naval Shipyard
SPAWAR	Space and Naval Warfare
SSC Pac	SPAWAR Systems Center Pacific
TST	Test for Significant Toxicity
USEPA	United States Environmental Protection Agency

1. INTRODUCTION

Sediment toxicity testing using the marine amphipods *Ampelisca abdita, Leptocheirus plumulosus* and the polychaete *Neanthes arenaceodentata*, and embryos from the bivalve *Mytilus galloprovincialis* was performed to evaluate the environmental quality of sediments collected from Puget Sound Naval Shipyard (PSNS). The amphipods and polychaete worm were tested in homogenized sediment samples, whereas bivalve embryos were exposed in sediment-water interface (SWI) toxicity tests described by Anderson et al. (1996). Samples were collected April 27, 2011 and testing was conducted at the SPAWAR Systems Center Pacific (SSC Pac) Bioassay Laboratory in San Diego, CA from May 3 through 31, 2011. Sediment chemistry evaluating the metal content as well as grain size and organic content was performed on the samples and is presented herein. Diffusive gradients in thin films (DGTs) were also concurrently deployed to assess the bioavailability of metals associated with the sediment porewater as an additional line of evidence to assess the environmental quality of the sediments tested.

2. MATERIALS AND METHODS

To meet the defined objectives for the project, this study included a series of tasks to characterize toxicity, physico-chemical parameters on overlying water, porewater, sediment, and labile metal concentrations using DGTs. An overview of the approach is shown in Figure 2-1.

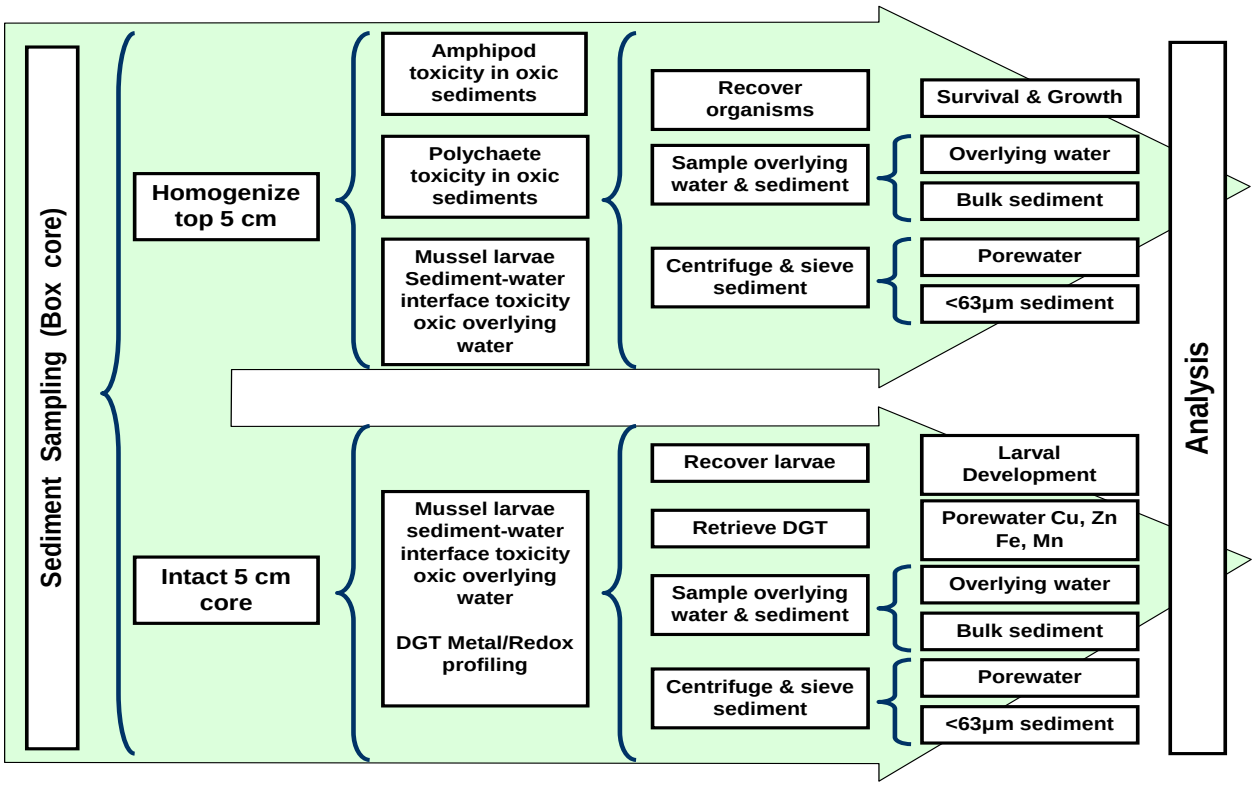

Figure 2-1. Schematic of generalized experimental design.

2.1. *Test Material*

Sediments from PSNS were collected using standard sediment collection, sampling, and storage procedures (ASTM 2008). Sediment samples were collected using a Van Veen sampler to preserve the integrity of *in situ* conditions as best as possible. The sampling equipment was pre-cleaned, and scrubbed and rinsed with site water between grabs, with careful attention not to sample from the sides of the device to avoid cross-contamination. Sampling occurred on the top 5 cm of sediment, focusing on the oxic and suboxic layers. Sediment was composited in pre-cleaned 2 L HDPE wide-mouth bottles for later homogenization and coarse press-sieving (2 mm) at the laboratory to remove native organisms and potential predators. Additionally, intact cores were collected for the SWI toxicity tests using pre-cleaned polycarbonate core tubes following specifications in Anderson et al. (1996). SCUBA divers manually collected the intact core samples from the field by completely filling the tubes and capping the ends of the tube. Caps were taped and shipped to the SSC Pac Bioassay Laboratory in insulated ice chests containing blue ice. Upon receipt in the laboratory, sediments were stored in the dark at 4^{o}C until use, and were used for experimentation as soon as possible. Sediment within the cores was dropped down to a 5cm mark on the side of each core on the day prior to initiation. Test initiation was targeted for 48 h within collection, with a maximum holding time of two weeks (USEPA, 1994). Sample collection and receipt times are summarized in Table 2-1. Copies of chain of custody forms are provided in Appendix D.

Table 2-1. Sediment Sample Collection and Receipt Times.

Sample/ Station ID	Latitude	Longitude	Type	Sample Collection Date	Sample Receipt Date/Time	Sample Receipt Temperature (°C)
PS03	47.555783	-122.651925	Grab	4/27/2011 11:25	4/29/2011 09:00	6.1
			Intact Core	4/27/2011 10:50	4/29/2011 09:00	6.1
PS09	47.560127	-122.636493	Grab	4/27/2011 12:35	4/29/2011 09:00	6.1
			Intact Core	4/27/2011 12:20	4/29/2011 09:00	6.1

2.2. *Test Organisms and Acclimation*

Toxicity testing included the following experimental organisms: the two amphipods (*Ampelisca abdita* and *Leptocheirus plumulosus*), one the polychaete (*Neanthes arenaceodentata*) and embryos from the bivalve embryos (*Mytilus galloprovincialis*).

Selection of test organisms was based on the desire to assess the responses in benthic invertebrates that differ in sensitivity to Cu and Zn, contaminant exposure route, and geographical location. *A. abdita* (Figure 2-2) is a suspension feeding, sediment ingesting amphipod that builds tubes out of sand grains (Redmond et al., 1994), while *L. plumulosus* is a free burrowing species (USEPA 1994). *N. arenaceodentata* (Figure

2-2) is a surface deposit feeding/predatory omnivorous polychaete, and builds mucoid tubes in surficial sediments (Dillon et al., 1993). All three species occur extensively in North America, are exposed to a combination of overlying water and porewater, in addition to sediment particles, detritus, and prey that might be an exposure source for Cu and Zn, and are frequently employed in testing for regulatory programs.

M. galloprovincialis embryo-larval development tests were incorporated in sediment-water interface (SWI) toxicity exposures (Anderson et al. 1996; Anderson et al. 2001). The relevancy of SWI tests in the assessment of sediment bioavailability and toxicity is high; 1) embryos are negatively buoyant and therefore directly exposed to sediment-associated contaminants during critical phases of cell differentiation; 2) the endpoint plays a major role in the development of saltwater WQC for Cu (USEPA 1995a); 3) the endpoint has served as the primary test for the development of site specific WQC for Cu in water effect ratio (WER) studies (e.g. Rosen et al. 2005, 2009; Earley et al. 2007), and for the development of predictive models of Cu toxicity in surface waters (e.g. Arnold et al. 2006; Chadwick et al. 2008); 4) the SWI toxicity test with *M. galloprovincialis* is a recommended test for the assessment of sediment quality as part of recently derived sediment quality objectives (SQOs) for the state of California (Bay et al. 2007); and 5) the lack of feeding during embryogenesis simplifies the interpretation of data towards the dissolved water concentration only.

Sub-adult *L. plumulosus*, approximately 2-4 mm in length, were obtained from Chesapeake Cultures, Inc. (Hayes, VA). *A. abdita*, approximately 0.71 – 1.18 mm in length, were obtained from Aquatic Research Organisms, Inc. (Hampton, NH). Juvenile *N. arenaceodentata* were obtained from a culture maintained by Aquatic Toxicology Support (Bremerton, WA). Gravid *M. galloprovincialis* were obtained from Carlsbad Aquafarm (Carlsbad, CA).

Amphipods and polychaetes were received at least one day prior to test initiation to allow for acclimation to appropriate test conditions (salinity, temperature, and lighting). Gravid mussels and urchins were received on the morning of the test initiation day. Mussel embryos were obtained from thermal-shock induced spawning from gravid mussels and sea urchins injected with potassium chloride to induce spawning. All organisms were visually inspected to confirm that they were of the proper size, and in good health, prior to use in toxicity testing.

Figure 2-2. Toxicity endpoints for this study included a) polychaete (*Neanthes arenaceodentata*) survival and growth, b) amphipod (*Leptocheirus plumulosus*) survival, c) amphipod (*Ampelisca abdita*) survival, and d) bivalve (*Mytilus galloprovincialis*) embryo-larval development and survival. Photos are not to scale.

2.3. *Toxicity Testing Procedures*

Testing was conducted in accordance with standard methods (USEPA 1994, USEPA 1995, ASTM 1996). The 10-day amphipod survival tests with whole sediment, the 28-day polychaete survival and growth test, and the 2-day sediment-water interface (SWI) bivalve embryo development test were conducted on the samples listed in Table 2-1. Negative controls consisting of sediment from the amphipod collection site was included in the 10-day whole sediment test. For the 2-day SWI test, a chamber control (screen tube) and a seawater negative control were also tested concurrently. Summaries of the test conditions are provided in Table 2-2, Table 2-3, and Table 2-4.

For both the whole sediment and sediment-water interface (SWI) toxicity tests, samples from the overlying water were collected at the beginning and end of the exposures, while porewater, DGT samplers and sediment samples were collected and analyzed at the test termination only. All test chambers were set up with sediment, water and aeration on the day prior to test initiation. Screen tubes for the SWI test were gently introduced to each core tube on the day of test initiation. Water quality parameters including pH, dissolved oxygen (DO), salinity, temperature and ammonia were measured in the overlying water prior to organism addition to ensure that conditions were within those tolerated. Daily observations of water quality, aeration and sediment condition (e.g. anoxia, microbial growth, etc.) were made. All instruments used for water quality measurements were calibrated daily according to manufacturer specifications.

2.3.1. Sediment-Water Interface Toxicity Tests

The *M. galloprovincialis* embryo-larval development toxicity tests were conducted according to USEPA (1995b) and Anderson et al. (1996). Test conditions and acceptability criteria are summarized in Table 2-2. For the SWI test, early stage (< 4 hour old) embryos were placed at the interface using a screen tube (25 µm mesh) that rests ~1 cm above a 5 cm sediment core (Figure 2-3). Developing larvae were exposed to contaminant flux from the sediment in both intact core and homogenized core tubes (2.5 inches in diameter), which were filled with 300 mL of overlying uncontaminated FSW. The number of surviving normal D-shaped larvae (% normal alive) was determined on an inverted microscope at the end of the test.

Each sample consisted of six replicates, four for organism exposure, one for destructive sampling of the sediments at the beginning of the test, and one for placement of a diffusive gradient in thin-film (DGT) to measure the profile of metal (Cu, Zn, Fe and Mn) concentrations in the porewater and overlying water. The mussel embryos never came into direct contact with the sediment and do not feed, so are exposed primarily to dissolved substances that partition out of the sediment. This test is required in newly established California SQOs (Bay et al. 2007; SSCWRP 2014), and the embryo-larval development endpoint of this species independently dictates ambient saltwater WQC for Cu (USEPA, 1995a) and was used in

marine Cu BLM development (Chadwick et al. 2008), and therefore, provides a nice linkage between water and sediment metal bioavailability assessment.

At the end of the exposure period for the SWI toxicity test, screen tubes were carefully removed from the sediment and the embryos were washed into glass scintillation vials, and preserved in 10% buffered formalin for later microscopic examination.

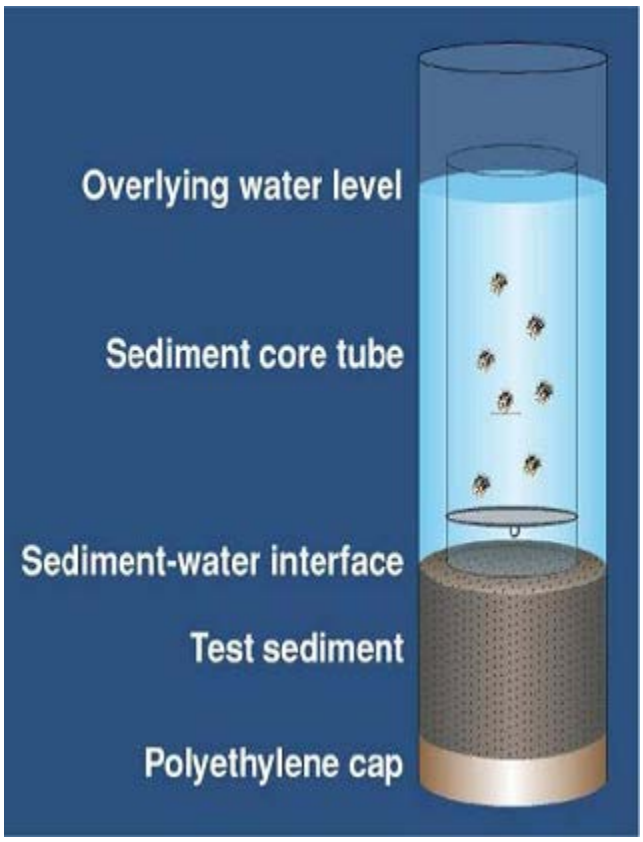

Figure 2-3. Diagram of the sediment-water interface toxicity test.

Table 2-2. Specifications for 2-day Chronic Exposure Using the Mediterranean Mussel Embryo-Larvae at the Sediment-Water Interface.

Test Periods	5/3-5/5/2011
Test organism	Mediterranean mussel – *Mytilus galloprovincialis*
Test organism source	Carlsbad Aquafarm, Carlsbad, CA
Test duration; endpoints	48 hr; embryo-larval survival and development success (proportion normal-alive)
Test solution renewal	None
Feeding	None
Test Chamber size/type	1L glass mason jar w/ polycarbonate screen tubes with 25 µm mesh
Test sediment depth	5 cm
Test sediment manipulation	Undiluted sediment exposed as intact cores
Overlying water volume	300 ml
Test temperature	15 ± 1 °C
Test salinity	30 ± 2 ppt
Light quality	10-20 µE/m^2/s (Ambient laboratory levels)
Photoperiod	16 hr light/ 8 hr dark
Aeration	Laboratory filtered air, continuous (1-2 bubbles per second delivered through a Pasteur pipette) , maintain >90% saturation
No. of organisms per chamber	~250 eggs, appropriate sperm density to provide > 90% fertilization success (determined in a pre-test trial)
No. of replicates	5
Overlying water source	Filtered (0.45 µm) natural seawater collected from near the mouth of San Diego Bay at SSC PAC Laboratory
Test acceptability criteria	≥ 80% mean normal-alive in control
Reference toxicant	Copper sulfate, standard EPA laboratory method only; 48 hr water only exposure; five concentrations (5 replicates each)
Test protocol	EPA 600/R-95/136 (USEPA 1995)

2.3.2. Whole Sediment Toxicity Tests

The *A. abdita* and *L. plumulosus* exposures were conducted using minor modifications of standard methods (USEPA, 1994). Recently, a new protocol was published for *N. arenaceodentata* (Farrar and Bridges, 2011) that employs an earlier life stage (≤ 7 day old emergent juveniles) than other standard methods with this species (e.g. ASTM 2000). This method was demonstrated to be considerably more sensitive than methods employing 2-3 week old organisms in comparative round robin testing. In addition, the growth endpoint using the new procedure described by Farrar and Bridges (2011) was among the most sensitive in a multi-species comparison of acute and chronic toxicity in marine sediments (Greenstein et al., 2008).

Summaries of the test conditions and test acceptability criteria for the whole sediment toxicity tests are shown in Table 2-3 and Table 2-4. Briefly, the amphipod tests included approximately 150g of homogenized wet sediment in 1 L glass jars, with 700 mL of overlying uncontaminated 0.45 µm FSW. The polychaete tests contained 75 g of wet sediment and 175 mL of FSW (Farrar and Bridges, 2011). Overlying water in all tests was continuously aerated with filtered laboratory air at a rate of approximately 100 bubbles per minute. A 24-h equilibration period with the overlying water was allowed prior to addition of test organisms (Day 0). Exposures were static for *A. abdita* and *L. plumulosus* for 10 days (acute exposure), while weekly renewals of the overlying water were made in the 28 day exposures with *N. arenaceodentata* (chronic exposure). The organisms were recovered on 0.5 mm sieves at the end of the test and enumerated for survival. For *N. arenaceodentata,* recovered organisms were purged overnight in FSW prior to drying for growth assessment, and then transferred into microcentrifuge vials for acid digestion (nitric acid under heat) and measurement of Cu and Zn in the tissues (Rosen et al., 2008).

Table 2-3. Specifications for 10-day Whole Sediment Acute Exposure Using the Marine Amphipods *Ampelisca abdita* and *Leptocheirus plumulosus*.

Test Periods	5/3-13/2011
Test organism	Marine amphipods – *Ampelisca abdita* and *Leptocheirus plumulosus*
Test organism size at initiation	Adult 3-5 mm
Test organism source	Aquatic Research Organisms, Inc. and Chesapeake Cultures, Inc.
Test duration; endpoint	10-day; survival
Test solution renewal	None
Feeding	None
Test Chamber size/type	1L glass mason jar
Test sediment depth	5 cm (approximately 150 g)
Test sediment manipulation	Homogenized and sieved to <2.0 mm
Overlying water volume	700 ml
Control sediment source	Sediment from amphipod collection site, Yaquina Bay, OR
Test temperature	15 ± 1 °C
Test salinity	30 ± 2 ppt
Light quality	Ambient laboratory illumination
Photoperiod	Continuous light (24 hr), ambient laboratory lighting
Aeration	Laboratory filtered air, continuous (1-2 bubbles per second delivered through a Pasteur pipette), maintain >90% saturation
No. of organisms per chamber	20
No. of replicates	*Leptocheirus* – 3; *Ampelisca* - 4
Overlying water source	Filtered (0.45 µm) natural seawater collected from near the mouth of San Diego Bay at SSC PAC Laboratory
Test acceptability criteria	≥ 90% mean survival in control sediment
Reference toxicant	Copper sulfate, standard EPA laboratory method only; 96-h water only exposure; five concentrations (4 replicates each)
Test protocol	EPA 600/R-94/025 (USEPA 1994)

Table 2-4. Specifications for 28-day Whole Sediment Chronic Exposure Using the Marine Polychaete *Neanthes arenaceodentata* (adapted from Farrar and Bridges 2011).

Test Periods	5/3-31/2011
Test organism	Marine polychaete – *Neanthes arenaceodentata*
Test organism size at initiation	Juvenile ≤ 7 d post-emergent
Test organism source	Aquatic Toxicology Support
Test duration; endpoint	28-day; survival and growth
Test solution renewal	50% once weekly
Feeding	Twice weekly, 2 mg of ground Tetramin © per organism
Test Chamber size/type	400 mL glass beaker
Test sediment depth	2 cm (approximately 75 g)
Test sediment manipulation	Homogenized and sieved to <2.0 mm
Overlying water volume	175 ml
Control sediment source	Sediment from amphipod collection site, Yaquina Bay, OR
Test temperature	15 ± 1 °C
Test salinity	30 ± 2 ppt
Light quality	Ambient laboratory illumination
Photoperiod	Continuous light (24 hr), ambient laboratory lighting
Aeration	Laboratory filtered air, continuous (1-2 bubbles per second delivered through a Pasteur pipette), maintain >90% saturation
No. of organisms per chamber	20
No. of replicates	10
Overlying water source	Filtered (0.45 µm) natural seawater collected from near the mouth of San Diego Bay at SSC PAC Laboratory
Test acceptability criteria	≥ 80% mean survival in control sediment and positive growth in control organisms
Reference toxicant	Copper sulfate, standard EPA laboratory method only; 96-h water only exposure; five concentrations (4 replicates each)
Test protocol	Farrar and Bridges (2011)

2.4. *General Chemistry*

All glassware, plasticware and associated equipment were cleaned thoroughly prior to use by soaking in 10% nitric acid (HNO_3) for 24 h, followed by rinsing in de-ionized water. Glassware used as test chambers also underwent a 24 h soak in 30 ppt 0.45 µm FSW.

2.5. *Total and Dissolved Metal Measurements*

Assessment of metal concentrations was made following methodology recommended by USEPA, including use of trace metal clean sampling techniques in the collection, handling and analysis (USEPA, 1996). Water and porewater samples were collected in 30-mL acid-cleaned low-density polyethylene bottles. Samples were acidified to pH ≤2 with quartz still-grade nitric acid (Q-HNO_3) in a High Efficiency Particle Air (HEPA) class-100 all polypropylene working area.

2.5.1. Metal Concentrations in Water

Overlying Water Sample Collection

Overlying water samples were taken from test chambers at the beginning and end of exposure periods (i.e. time zero and time final). The water samples were decanted from the test chamber using a peristaltic pump, without disturbing the sediment, into acid-cleaned 30 mL high density polyethylene (HDPE) bottles. Samples were collected in duplicate from each test chamber; one replicate was acidified to measure total metals, while the other replicate was filtered at a clean bench with a 0.45 µm filter attached to the pump tubing, and then acidified for quantification of dissolved metals. Each water sample was acidified to a pH of ≤ 2 with 50 µL of $QHNO_3$.

Pore Water Sample Collection

Pore water samples were collected from the test sediments at test termination only. After the overlying water was sampled and/or discarded, replicates of each sediment treatment were combined into a centrifuge tube in an anaerobic chamber. The combined replicate samples were ultra-centrifuged at 9000 rpm for 15 minutes leaving the pore water as a supernatant. The pore water was sampled from the centrifuge tube using a peristaltic pump with a 0.45 µm filter into acid cleaned 15 mL HDPE bottles. Each water sample was acidified to a pH ≤ 2 with 50 µL of $QHNO_3$.

Overlying Water and Porewater Metal Analysis

Metal concentrations in overlying and pore water samples were measured using in-line pre-concentration Flow Injection Analysis and a Perkin-Elmer SCIEX ELAN DRC II inductively coupled plasma with detection by mass spectrometry (ICP-MS; USEPA, 1994b). Each sample first ran through a Flow Injection Analysis System (FIAS) to pre-concentrate the metals, and to reduce the salt-content of the sample. The sample was then directly transferred into the Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for quantification. Blanks were analyzed every 5 samples to make sure the system was clean and to give a

reference point for the background level. A Standard Reference Material (SRM) was analyzed after each blank to ensure that the instrument was measuring accurately and precisely. The blank was NASS 2 (open ocean sea water) and the SRM was CASS 4 (coastal seawater) both from the National Research Council of Canada.

When deemed necessary for samples with high metal concentrations, samples were diluted with 0.1 N Q-HNO_3 made up in high-purity (18 MΩ cm^{-1}) water in order to minimize matrix related interferences. The diluted samples were injected directly into the ICP-MS via a Perkin-Elmer Autosampler 100. Analytical standards were made in CASS4 Nearshore Seawater Reference Material for Trace Metals, National Research Council Canada, with Perkin-Elmer multi-element standard solution (PEMES-3) diluted in 1N Q-HNO_3, and were analyzed at the beginning and end of the run. The analysis also included measurement of the Standard Reference Material (SRM) 1643e from the National Institute of Standards & Technology (NIST), and analytical blanks made up of 1N Q-HNO_3 after every five samples. A coefficient of variation (CV) of ≤5% for replicate measurements was observed, as well as a recovery within 15% for direct injection of SRM 1643e. The method limit of detection is defined as three times the standard deviation of the analytical blanks made of 1N Q-HNO_3.

2.5.2. Metal Concentration in Sediment

Sediment Sample Collection

Sediment samples were collected at the test termination from exposure beakers for both sediment samples. After overlying and pore waters were removed from multi-replicate composites, approximately 120 mL sediment was transferred to a HDPE bottle for bulk metal analysis. The remaining sediment was wet-sieved through a 63 µm sieve, and transferred to a separate 120 mL HDPE bottle for metal and TOC analysis of the <63 µm size fraction (Spadaro et al. 2008).

Sediment Metal analysis – ICP-MS and SEM-AVS

Bulk sediment samples were analyzed using both ICP-MS and SEM-AVS. The <63 µm fraction was only analyzed by ICP-MS. ICP-MS analyses were conducted at SSC Pacific. SEM-AVS analyses were conducted by the Engineer Research and Development Center Laboratory (ERDC).

For ICP-MS analyses, empty 30 mL HDPE bottles were labeled and dried at 60°C in a drying oven for at least 24 hours. The dried bottles were then weighed and the tare mass (g) recorded. Enough wet sediment to get a dry mass of approximately 0.25 g was transferred to each 30 mL bottle. The bottles were placed in the oven with no caps at 60°C for at least 24 hours, followed by verification of complete dryness. The bottles with dry sediment were weighed again and the mass (g) was recorded. One mL of concentrated trace metal grade (TMG) Hydrochloric Acid (HCL) and 0.5 mL of concentrated TMG HNO_3 were added to each sediment sample. The samples were allowed to digest for 24 hours at room temperature capped loosely and put on a warm heating plate (≈60°C) for at least 1 hr. Subsequently, about 30 mL of 1 N TMG HNO_3 was added to each sample and the final mass (g) recorded. After particles were allowed to settle,

sample dilutions of the overlying digestate were made. A 5-fold dilution of each sample was made before metal concentration analysis by transferring 2 mL of sample digestate solution (no particles) to a 15 mL centrifuge tube and adding 8 mL of 1N TMG HNO_3 for a total volume of 10 mL.

Metal concentrations were measured using an ICP-Optical Emissions Spectrometer (ICP-OES). Three duplicate samples were chosen at random for each run. For every 5 samples, a blank was run to make sure the system was clean and to give a reference point for the background level of metals. A SRM was run after each blank to ensure that the instrument was measuring accurately and precisely. The blank was either 1N TMG HNO_3 or 18 MΩ cm^{-1} water and the SRM was 1643e (trace metals in water) from the National Bureau of Standards. In addition, six blanks were prepared from empty 30 mL HDPE bottles which were treated in the same manner as the sediment digestions. All acid additions and dilutions were carried out identically.

2.5.3. Metal Concentration in Tissues

Tissue Collection and Analysis

Tissue from the *Neanthes* whole sediment test were evaluated for metal concentration following the exposure period of 28 d. At the termination of the bioassay, organisms were allowed to depurate for a minimum of 24 hr. Organisms were examined following the depuration period for debris in their gut and were gently palpated to further remove debris. Organisms were gently rinsed with Milli-Q DI water to remove salts, blotted dry, and then placed into pre-cleaned, dried and pre-weighed polypropylene microcentrifuge tubes (1.5 mL). Wet tissue was then dried at 60°C. Once the tissues were dry, the vials were weighed again. Concentrated Q-HNO3 (50 µL) was added to each vial making sure to cover the tissue as much as possible. The vials were allowed to digest for at least three days at room temperature at a clean bench. Finally, 1500 µL 1N Q-HNO3 was added to each vial and the vial weighed again. One mL of acid was taken from each digestion and analyzed by ICP-MS.

2.5.4. Metal Concentration in DGTs

DGTs were positioned in surrogate test vessels for each of the sediment types, allowing for both DGT determination of overlying water and pore water Cu, Zn, Fe, and Mn measurements in the oxic and suboxic zones. Suboxic zones were defined as those layers of sediment where either or both Fe or Mn was present in the (0.45 µm) porewater.

DGT Collection and Analysis

At test termination, DGTs were recovered and rinsed with DI water. The DGT gel was extracted from the plastic housing and the DGT gel was set at the bottom of pre-cleaned, dried and weighed centrifuge tubes. The gel was then allowed to dry in a class-100 clean bench for several days at room temperature. Once dry, the vials were weighed again. Concentrated Q-HNO3 (50 µL) was added to each vial making sure to cover the DGT gel film as much as possible. The vials were allowed to digest for at least three days at room

temperature in the clean bench. Finally, 1500 µL 1N Q-HNO3 was added to each vial and the vial weighed again. One mL of acid was taken from each digestion and analyzed by ICP-MS.

3. RESULTS

Summaries of statistical, toxicity and raw test results for tests are provided in Table 3-2 through Table 3-9. Complete statistical summaries and bench water quality sheets are provided in Appendix A.

3.1. *Sediment-Water Interface Toxicity Results*

The chamber control associated with the SWI exposures with *M. galloprovincialis* was slightly outside of test acceptability criteria at 75.6% (acceptability criteria: ≥ 80% mean normal-alive). However, the tests were deemed acceptable based on the responses of the site sediments all performing better than the control. All water quality parameters were within the recommended range for the duration of the test. Table 3-2 summarizes the results of the SWI tests with *M. galloprovincialis*. Mean normal ranged from 80 to 95 percent and mean normal-alive ranged from 79 to 95 percent (Table 3-2). For statistical analyses, each sample was compared to the chamber control using the statistical software Comprehensive Environmental Toxicity Information System (CETIS) v1.8.7.16 (Tidepool 2012). No significant differences were observed for intact cores or homogenized field samples relative to the chamber control tested (all p-values >0.05).

Table 3-1. Summary of Statistical Results for the Sediment-Water Interface Test.

Station ID	Mean % Normal (SD)	% Difference from Control	p-value	Mean % Normal-Alive (SD)	% Difference from Control	p-value
Negative Control – Screen Tube	80.4 (7.3)	-	-	75.6 (13.8)	-	-
PS-03 Intact Core	89.4 (8.1)	11.2	0.9909	85.8 (11.1)	13.5	0.9642
PS-03 Homogenized	95.8 (2.0)	19.2	0.9996	97.4 (3.4)	28.8	0.9994
PS-09 Intact Core	92.0 (4.6)	14.4	0.9986	79.1 (11.9)	4.6	0.8005
PS-09 Homogenized	92.5 (3.3)	15.1	0.9988	83.4 (11.4)	10.3	0.9225

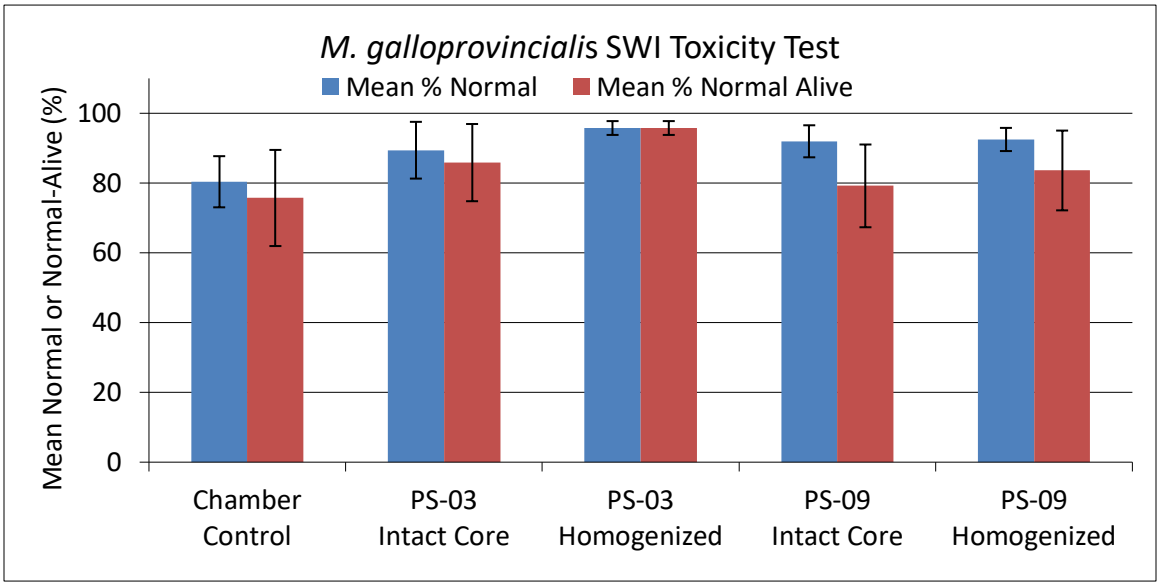

Figure 3-1. Mean percent normal and mean percent normal-alive *M. galloprovincialis* larvae for the sediment-water interface test.

3.2. *Whole Sediment Toxicity Results*

3.2.1. Leptocheirus plumulosus

The control associated with the 10-day whole sediment test with *L. plumulosus* met test acceptability criteria of 90 % survival. All water quality parameters measured were within the recommended range for the duration of the test. Survival was 86 and 93 % for samples PS-03 and PS-09, respectively. Each sample was compared statistically against the laboratory control sediment that was tested concurrently using a CETIS. No significant differences were observed in any of the sediment samples tested compared to the control (all p-values >0.05, Table 3-3, Figure 3-2. Mean percent normal and mean percent normal-alive *M. galloprovincialis* larvae for the sediment-water interface test.).

3.2.2. Ampelisca abdita

For the 10-day whole sediment test with *A. abdita*, dramatic mortality was observed two days into the exposure period. It was decided that ten additional organisms would be added to two of the four replicates (replicates A & B) and based on the average number of mortalities observed across all replicates a normalization of the initial number of organisms in each replicate would be made; 26 for the A & B replicates and 16 organisms for the C & D replicates. The *A. abdita* toxicity test as a whole did not meet test acceptability criteria for the mean survival (90% survival) in the controls. However, samples were compared against the control for interest's sake. Sample PS-09 was significantly decreased from the control sediment (p = 0.0493, Table 3-3, Figure 3-2) and while PS-03 did not show significance relative to the control, the trend for toxicity is similar to PS-09.

Table 3-2. Summary of Statistical Results for the Whole Sediment Tests with *L. plumulosus* and *A. abdita*.

Station ID	*Leptocheirus plumulosus*			*Ampelisca abdita*		
	Mean % Survival (SD)	**% Difference from Control**	**p-value from Student's t-test**	**Mean % Survival (SD)**	**% Difference from Control**	**p-value from Student's t-test**
Laboratory Control[A]	90.0 (8.7)	-	-	78.5 (17.9)	-	-
PS-03 Homogenized	86.7 (2.9)	-3.7	0.2317	59.3 (12.1)	-24.5	0.0686
PS-09 Homogenized	93.3 (5.8)	3.7	0.6788	58.9 (4.4)	-25.0	**0.0493**

[A] – Control sediment was sediment from Sequim Bay.
Values in **bold** indicate a statistically significant decrease compared to the control.

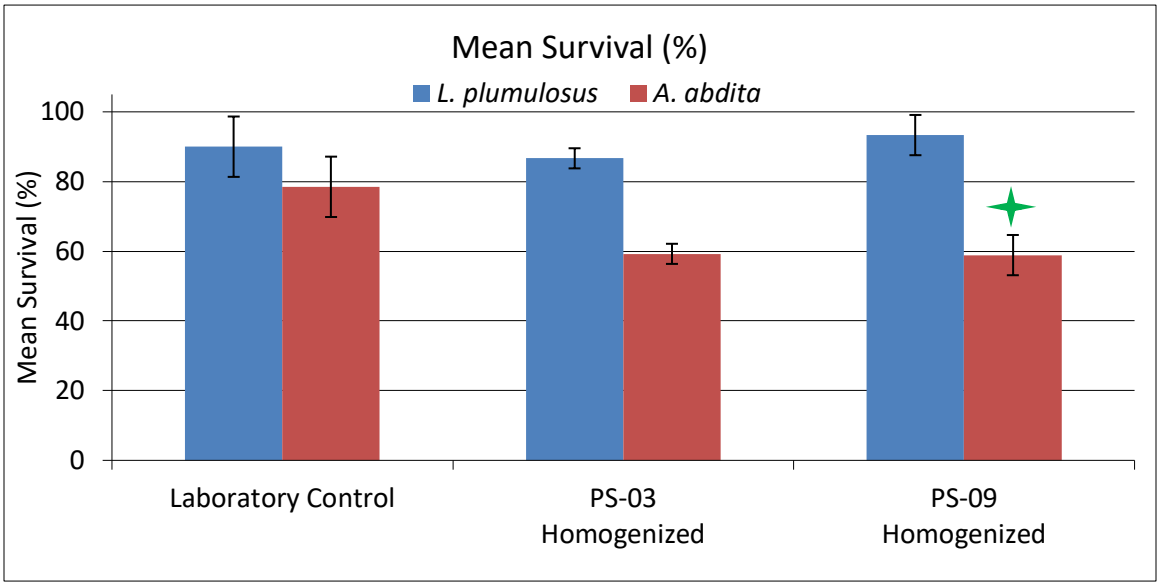

Figure 3-2. Mean percent survival of *L. plumulosus* and *A. abdita.* The star indicates statistical decrease from the respective laboratory control.

3.2.3. Neanthes arenaceodentata

The control associated with the 28-day whole sediment test with *N. arenaceodentata* met test acceptability criteria of 90 % survival and positive growth. All water quality parameters measured were within the recommended range for the duration of the test. Survival was 100 % for both samples (Table 3-3). Growth was 5.1 and 4.7 mg for PS-03 and PS-09, respectively. Each sample was compared statistically against the laboratory control sediment that was tested concurrently using CETIS. No significant differences were observed in any of the sediment samples tested compared to the control for either survival or growth (all p-values >0.05, Table 3-3, Figure 3-3, Figure 3-4).

Table 3-3. Summary of Statistical Results for 28-day Whole Sediment Test with *N. arenaceodentata.*

Station ID	Survival			Growth		
	Mean % Survival (SD)	% Difference from Control	p-value from Student's t-test	Mean Growth (mg) (SD)	% Difference from Control	p-value from Student's t-test
Laboratory Control[A]	90.0 (31.6)	-	-	4.928	-	-
PS-03 Homogenized	100.0 (0.0)	11.1	1.000	5.106	3.6	0.5905
PS-09 Homogenized	100.0 (0.0)	11.1	1.000	4.682	-4.99	0.3840

[A] – Control sediment was sediment from Sequim Bay.

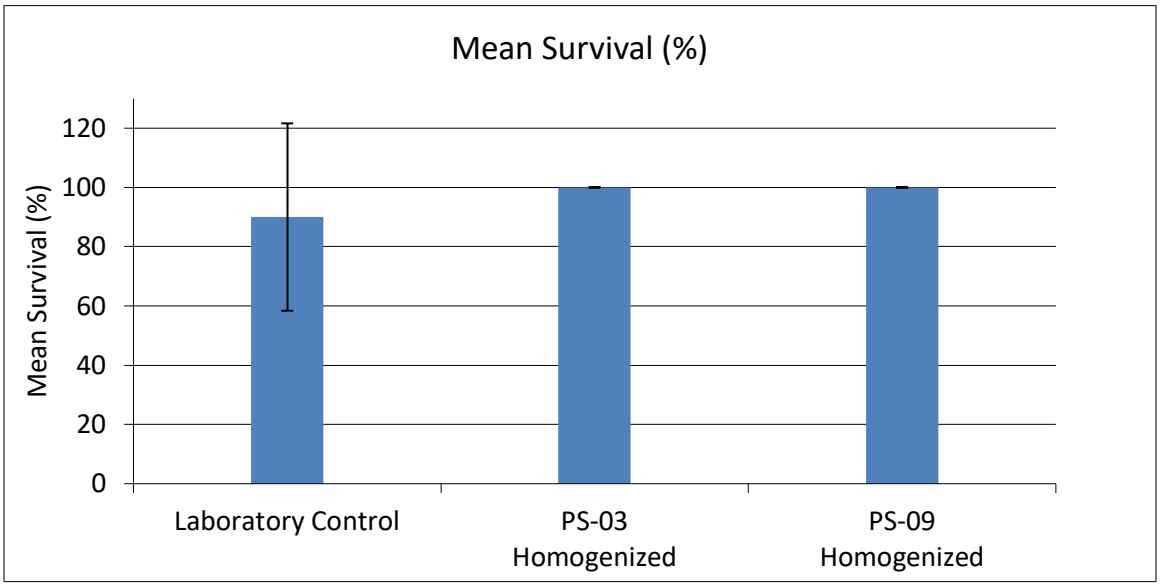

Figure 3-3. Mean percent survival of *N. arenaceodentata*.

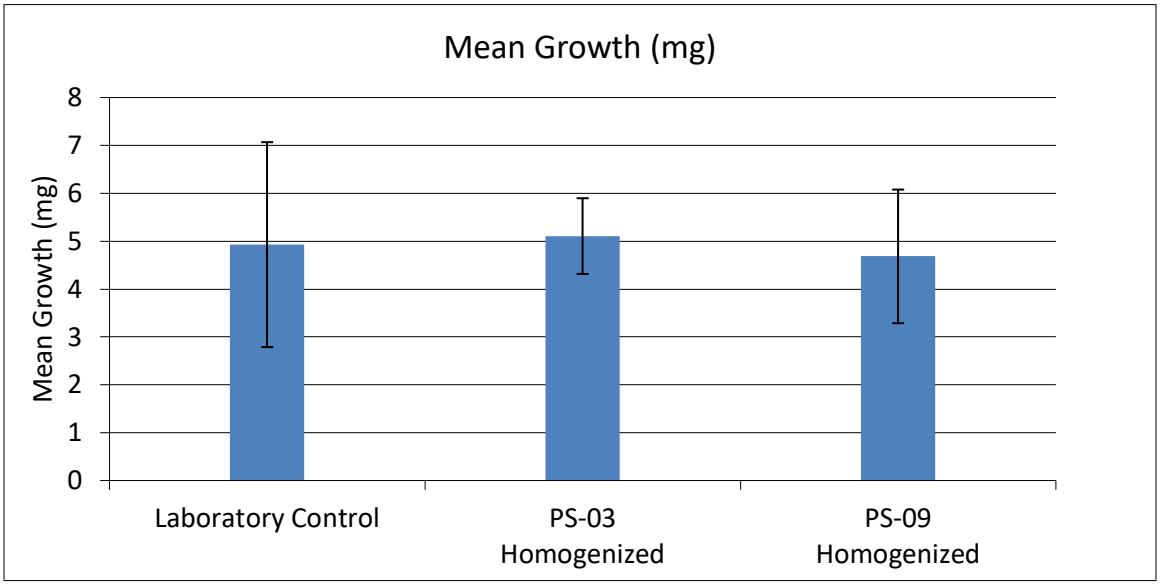

Figure 3-4. Mean growth of *N. arenaceodentata*.

3.3. *Water, Pore Water and Sediment Chemistry*

Concurrent measurements of physicochemical parameters in the dissolved and particulate phases were completed and are presented below in Table 3-4 to Table 3-12.

3.3.1. Bulk Sediment Chemistry

As stated previously, bulk sediment samples were collected following decanting of the overlying waters. Bulk samples were analyzed for Cu and Zn and total organic carbon (TOC). Remaining sediment was then processed through a 63 µm sieve for additional metal and TOC analysis. Bulk sediment samples were also evaluated using SEM-AVS by the Army's research lab ERDC and results were normalized to the amount of organic carbon.

Table 3-4. Sediment Chemistry Results – Bulk Sample.

Sample ID	Silt & Clay <63µm (%)	pH	TOC (%)	Cu (mg/kg)	Zn (mg/kg)	Bulk Sed/TOC Cu (mg/g OC)	Bulk Sed/TOC Zn (mg/g OC)
PS03	71.3	7.26	2.9	199.6	232.0	6.9	8.0
PS09	81.0	7.42	2.6	213.9	258.7	8.2	10.0

Table 3-5. Sediment Chemistry Results – < 63µm Fraction and SEM-AVS.

Sample ID	TOC (%)	Cu (mg/kg)	Zn (mg/kg)	<63µm/TOC Cu (mg/g OC)	<63µm/TOC Zn (mg/g OC)	∑SEM-AVS (µmol/g)	$(\sum SEM\text{-}AVS)/f_{oc}$
PS03	3.5	213.1	229.5	6.1	6.6	-8.96	-309
PS09	4.4	223.7	260.5	5.1	5.9	-12.1	-466

3.3.2. Overlying and Porewater Chemistry

Table 3-6. Overlying and Porewater Chemistry Results – *L. plumulosus*.

	Overlying Water – Time Final				Pore Water	
Sample ID	Total Cu (µg/L)	Dissolved Cu (µg/L)	Total Zn (µg/L)	Dissolved Zn (µg/L)	Dissolved Cu (µg/L)	Dissolved Zn (µg/L)
PS03	1.2	0.8	3.0	7.2	0.8	10.4
PS09	1.7	0.8	9.4	6.1	0.4	1.0

Table 3-7. Overlying and Porewater Chemistry Results – *A. abdita*.

	Overlying Water – Time Final				Pore Water	
Sample ID	Total Cu (µg/L)	Dissolved Cu (µg/L)	Total Zn (µg/L)	Dissolved Zn (µg/L)	Dissolved Cu (µg/L)	Dissolved Zn (µg/L)
PS03	1.1	0.8	17.2	20.8	0.8	10.4
PS09	1.6	1.0	17.6	13.4	0.4	1.0

Table 3-8. Overlying and Porewater Chemistry Results – *N. arenaceodentata*.

	Overlying Water – Time Final				Pore Water		
Sample ID	Total Cu (µg/L)	Dissolved Cu (µg/L)	Total Zn (µg/L)	Dissolved Zn (µg/L)	Dissolved Cu (µg/L)	Dissolved Zn (µg/L)	DOC (mg/L)
PS03	1.2	0.8	1.8	0.5	0.4	ND	<5.0
PS09	0.8	0.8	3.1	3.1	0.4	16.5	<5.0

Table 3-9. Overlying Water Chemistry Results – *M. galloprovincialis*.

	Overlying Water – Time 0					Overlying Water – Time Final				
Sample ID	Total Cu (µg/L)	Dissolved Cu (µg/L)	Total Zn (µg/L)	Dissolved Zn (µg/L)	Total Cu (µg/L)	Dissolved Cu (µg/L)	Total Zn (µg/L)	Dissolved Zn (µg/L)	DOC (mg/L)	TOC (mg/L)
PS03 - C	1.4	0.7	4.5	4.3	1.7	0.6	2.4	0.4	<0.5	<0.5
PS03 - H	1.3	0.6	1.7	1.9	2.6	0.6	3.1	ND	<0.5	<0.5
PS09 - C	0.9	0.6	2.4	2.2	1.3	0.6	2.2	ND	<0.5	<0.5
PS09 - H	1.1	0.6	1.6	2.5	4.7	0.6	5.3	ND	<0.5	<0.5

Table 3-10. Porewater Chemistry Results – *M. galloprovincialis*.

Sample ID	Dissolved Cu (µg/L)	Dissolved Zn (µg/L)	DOC (mg/L)
PS03 - C	0.3	153.6	168.0
PS03 - H	0.3	68.6	<0.5
PS09 - C	0.2	49.4	2600
PS09 - H	0.3	32.7	0.6

Table 3-11. Overlying Water Chemistry Results – Ammonia (mg/L).

Sample ID	Initiation			Termination		
	SWI test	10-d whole sediment test	28-d whole sediment test	SWI test	10-d whole sediment test*	28-d whole sediment test
PS03 - C	2.2	-	-	1.0	-	-
PS03 - H	0.2	0.7	1.1	ND	1.6/ND	ND
PS09 - C	0.5	-	-	0.4	-	-
PS09 - H	1.3	ND	0.4	1.1	1.6/ND	0.12

* - first and second values are for *L. plumulosus test* and *A. abdita* tests, respectively
ND – Non-detect

Table 3-12. Field Contaminated Sediments – *Neanthes* Tissue Chemistry Results

Sample ID	Cu (mg/kg)	Zn (mg/kg)
PS03	20.8	99.9
PS09	8.7	50.9

4. QA/QC

A thorough review of the data and test procedures did not identify any likely impacts on test results as a result of these deviations; therefore, all presented data were deemed acceptable. Additionally, all test acceptability criteria were met.

All tests were conducted within the recommended 1-month holding time (initiated within three days of receipt). While the temperatures of the samples upon receipt were slightly outside the EPA recommended range of 0-6 °C, the samples were in a state of cooling and this exceedence was not deemed an issue.

Control test acceptability criteria were met for the *Leptocheirus* amphipod and the *Neanthes* polychaete toxicity tests. Control test acceptability criteria for the SWI exposure with embryos of *M. galloprovincialis* was just under the 80% mean normal-alive. However, the tests were deemed acceptable based on the responses of the site sediments all performing better than the control. For the *Ampelisca* amphipod toxicity tests, acceptability criteria were not met (mean survival of controls ≥ 90% survival). However, samples were compared against the control for interest's sake.

The Total ammonia concentrations were below those that would be anticipated to be toxic to the test endpoints. A glossary of the qualifier codes used on the test datasheets is provided in Appendix E.

4.1. *Reference Toxicant Testing*

A 2-day copper sulfate ($CuSO_4$) reference toxicant test was conducted concurrently for the bivalve embryo-larval development test. The lab controls associated with this test did not met test acceptability criteria (TAC) and therefore is not deemed official. However, since the dose response observed was typical and the 2.9µg/L concentration was above the TAC, the reference toxicant test is shown below and reported for comparative and informational purposes.

The median effective concentration (EC_{50}) was 10.2 and 9.9 µg/L for the proportion normal and proportion normal-alive endpoints, respectively. Each of these endpoints fell within two standard deviations of the laboratory's historical means (Table 4-1); indicating sensitivity to copper was consistent with that historically observed for this species.

Table 4-1. Results Summary for the Copper Reference Toxicant Tests Concurrently Conducted with the NBPL RWM Samples Collected on May 11, 2016.

Species & Endpoint	NOEC (µg/L copper)	LC_{50} or EC_{50} (µg/L copper)	Historical mean ± 2 SD (µg/L copper)
Mediterranean Mussel Embryo-Larval Development:			
Proportion Normal	8.4	9.9	7.1 ± 3.7
Proportion Normal-Survival	8.4	10.2	7.0 ± 4.3

5. REFERENCES

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