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TECHNICAL REPORT 3167
SEPTEMBER 2018

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Water Body Number
WA-15-0040 Sinclair Inlet

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ADMINISTRATIVE INFORMATION

The work described in this report was performed for the Naval Sea Systems Command (NAVSEA), Naval Inactive Ships Program (SEA 21I) by the Environmental Sciences Branch (Code 71750) and the Energy and Environmental Sustainability Branch (Code 71760) of the Advanced Systems and Applied Sciences Division (Code 71700) of the Space and Naval Warfare Systems Center Pacific (SSC Pacific) San Diego, CA. The authors gratefully acknowledge the programmatic and management support provided by W. Boozer, G. Kitchen, and N. Tastad (SEA 21I); N. Gulch and D. Kopack (SEA 04R); J. Morrison (SEA 00L); and F. Cundiff (Cape Henry Associates). Sampling and logistical support was provided by K. Forris and J. Scheidt (NAVSEA Inactive Ships On-Site Maintenance Office, Bremerton, WA); A. Lewis and T. Beryle (Naval Facilities Engineering Command Northwest PRB314); and M. Aylward and J. Klinkert (Puget Sound Naval Shipyard & Intermediate Maintenance Facility c/106.32). Analytical chemistry analysis was conducted by ALS Global Inc, Kelso, WA under Contract Number/Purchase Order Agreement N6600117P6856.

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EXECUTIVE SUMMARY

The ex-INDEPENDENCE (CV 62), which had been moored at Mooring G at Naval Base Kitsap in Bremerton, WA (NBK-BREM) since decommissioning in September 1998 was towed on March 11, 2017 to Brownsville, TX, where the ship arrived on June 1, 2017 for dismantling. Based on a consultation with National Marine Fisheries Service (NMFS)(NOAA NMFS, 2016), the Navy was required to clean the ship's hull prior to towing in order to mitigate the possibility of transferring invasive species to other regions and thereby harm endangered species and habitats. This report presents the sediment sampling and analysis results to assess potential impacts to sediment quality from biofouling removal from CV 62 while it was moored at NBK-BREM and the Puget Sound Naval Shipyard & Intermediate Maintenance Facility (PSNS&IMF) in Sinclair Inlet, Puget Sound, WA. The data developed for this study provided a basis for

1- determining whether biofouling removal from CV 62 impacted sediment quality at Mooring G, and
2- assessing the nature and extent of any impact.

Sediment chemistry sampling was performed at six locations within the zone of influence near the ship prior to the start of cleaning operations (Pre-Removal) and another sampling event conducted approximately one month after the ship was moved from Sinclair Inlet and about two months after biofouling removal was completed (Post-Removal). Sediment chemistry samples were collected by ponar grab for the Pre-Removal samples and with divers for Post-Removal samples. The surface grab samples (0–10 cm) from each sampling event were composited and analyzed for total Copper (Cu), total Zinc (Zn), solid content (Solids %), grain size (Fines %, Gravel %, Sand %, Silt %, and Clay %), and total organic carbon (TOC %). During the Post-Removal sampling event, sediment cores (0-25 cm) were collected from each site to obtain samples for analysis of Acid Volatile Sulfide (AVS), Simultaneously Extracted Metals (SEM), total Cu, total Zn, solids, and TOC to evaluate metal bioavailability and potential toxicity at the site.

Data from the 0-10 cm grab samples taken before and after the project showed that there were no statistically significantly ($p \leq 0.05$) differences in concentrations of total Cu and Zn, grain size, and TOC at the site after biofouling removal was completed compared to before removal. However, sediment Zn concentrations measured after biofouling removal were about twice as high as concentrations prior to biofouling removal ($p \leq 0.10$). Data from the sediment cores were used to measure AVS, SEM, and the fraction of organic carbon (f_{OC} = TOC/100) to assess the bioavailability and potential toxic effects of metals by comparing the $(\Sigma SEM - AVS)/f_{OC}$ measured to benchmarks of adverse biological effects from metal toxicity to the benthic community developed by US EPA (2005). Based on average concentrations of metals and AVS measured in the 0-10 cm core sections and using the most conservative assumptions, the analysis showed that there was a low (8.9%) chance of possible impact, a medium chance (27.1%) of potential impact, and high chance (64%) of negligible impact to the benthic community from metal toxicity.

The variation in concentrations of total Cu and Zn and grain size parameters increased by at least a factor of two between the two sampling events indicating that the biofouling removal operations caused a disturbance in the sea floor conditions. However, it is unclear whether the disturbance also contributed contamination to the site or if the disturbance stirred up contamination that was already present. Under the anoxic conditions, the metal contaminants present would most likely be inert, bound up in insoluble metal sulfides. Whether the disturbance will contribute to the release of contamination depends on the natural rate of sediment reworking and oxidation. The findings from

this study showed that the potential impact of biofouling removal to the benthic community from the release of copper and zinc was low and that the benthic community in the area of the vessel was not adversely degraded.

ACRONYMS

ΣSEM	sum of Simultaneously Extracted Metals µmol/g
°C	degree Celsius
ADCP	acoustic Doppler current profiler
ANOVA	analysis of variance
AVS	Acid Volatile Sulfide (s) µg/mol
BACI	before-after-control-impact
BOD	biochemical oxygen demand
CASS-6	nearshore seawater certified reference material for trace metals
CdS	cadmium sulfide
CERCLA	Comprehensive Environmental Response Compensation and Liability Act
CH3D	hydrodynamic model: curvilinear hydrodynamics in 3 dimensions
Clay	clay, %
CoefV	coefficient of variation
CoefV*	coefficient of variation adjusted for small sample size ($n \leq 6$)
cm	centimeter (s)
cm^2	square centimeter (s)
CRM	certified reference material
CSa	coarse sand, %
Cu	copper
CuS	copper sulfide
CV 62	carrier vessel 62 (ex-INDEPENDENCE)
CVAA	cold vapor atomic absorption
DO	dissolved oxygen
DOC	dissolved organic carbon
DO-Sat	dissolved oxygen saturation
DQO	data quality objective (s)
DUP	duplicate (s)
DVR	digital video recorder
ENVVEST	Environmental Reinvestment
EPA	Environmental Protection Agency
FeS	iron sulfide
FIAS	flow injection for atomic spectroscopy

Fines	Fines = (Silt + Clay)/(Gravel +Sand + Silt + Clay), %
f_{OC}	fraction of organic carbon = TOC/100, unitless
ft	feet
ft^2	square feet
g	gram (s)
GNOME	general NOAA operational modeling environment
H_2S	hydrogen sulfide
HEPA	high-efficiency particulate air
HgS	mercury sulfide
ICP-MS	inductively coupled plasma – mass spectrometry
IMF	Intermediate Maintenance Facility
in	inch (es)
kg	kilogram (s)
K_{sp}	solubility product (ratio of dissolved : solid)
KW	Kruskal-Wallis
L	liter (s)
lb	pound (s)
LCS	laboratory control sample
Lipid	lipid content, %
LTM	long term monitoring
m	meter (s)
m^2	square meter (s)
MB	method blank
MCC	maximum criteria concentration
MDL	method detection limit
MDR	mixed-diamine reagent
Me	metal
MeS	metal sulfide
min	minute (s)
mL	milliliter (s)
MRL	method reporting limit
MS	matrix spike
mS/cm	milli-Siemens per centimeter, a measure of conductivity
MS	matrix spike

MSa	medium sand, %
MSD	matrix spike duplicate
NaOH	sodium hydroxide
NAVSEA	Naval Sea Systems Command
NBK	Naval Base Kitsap
NH_3	ammonia
NiS	nickel sulfide
NISMO	NAVSEA Inactive Ships Maintenance Office
NIST	National Institute of Standards & Technology
NMFS	National Marine Fisheries Service
NO_2	nitrite
NO_3	nitrate
NOAA	National Oceanic and Atmospheric Administration
NOD	Nature of Discharge
NSTM	Naval Ships' Technical Manual
NTU	nephelometric turbidity units
NUWC	Naval Undersea Warfare Center, Newport, RI
OUB	operable unit B
PEMES	Perkin-Elmer multi-element standard solution
PNNL	Pacific Northwest National Laboratory
ppb	parts per billion, same as µg/L
ppt	parts per thousand
PD	Percent Difference, %
PR	Percent Recovery, %
PSEP	Puget Sound Estuary Program
PSNS	Puget Sound Naval Shipyard
PSNS&IMF	Puget Sound Naval Shipyard & Intermediate Maintenance Facility
PSU	practical salinity units
PWP	Project Work Plan
QA	Quality Assurance
QAP	Quality Assurance Plan
QC	Quality Control
Q-HNO_3	quartz-still grade nitric acid
RPD	relative percent difference

SAP	sampling and analysis plan
SD	standard deviation
SEM	Simultaneously Extracted Metal(s), µmol/g
SEM_Cd	simultaneously extracted cadmium, µmol/g
SEM_Cu	simultaneously extracted copper, µmol/g
SEM_Hg	simultaneously extracted mercury, µmol/g
SEM_Ni	simultaneously extracted nickel, µmol/g
SEM_Pb	simultaneously extracted lead, µmol/g
SEM_Zn	simultaneously extracted zinc, µmol/g
Silt	silt, %
SIS	Surrogate Spike
SMS	Washington State Sediment Management Standards
Solids	solid content of sample, %
SPAWAR	Space and Naval Warfare
SRM	standard reference material
SSC Pacific	Space and Naval Warfare Systems Center Pacific, San Diego, CA
SW6010B	solid waste method 6010bB
TOC	total organic carbon, %
µg/g	microgram per gram, same as parts per million (ppm)
µmol/g	micro mole per gram, same as parts per million based on molecular weight
µg	microgram (s)
µg/L	micrograms per liter, same as part per billion (ppb)
µm	micron (s)
US	United States
VCSa	very coarse sand, %
VFSa	very fine sand, %
WAC	Washington Administrative Code
WQC	Water Quality Criteria (ion)
WQS	Water Quality Standard (s)
Zn	zinc
ZnS	zinc sulfide

CONTENTS

APPENDICES

Figures

Tables

1. INTRODUCTION

The ex-INDEPENDENCE (CV 62), which had been moored at Mooring G at Naval Base Kitsap in Bremerton, WA (NBK-BREM) since decommissioning in September 1998 (Figure 1, and Figure 2; Seaforces.org, 2017), was towed on March 11, 2017 to Brownsville, TX, where the ship arrived on June 1, 2017 for dismantling (Navytimes.com 2017). Based on a consultation with National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service (NMFS), the Navy was required to clean the ship's hull prior to towing in order to mitigate the possibility of transferring invasive species to other regions and thereby harm endangered species and habitats (NOAA NMFS, 2016; Naval Undersea Warfare Center [NUWC], 2016). While removing biofouling organisms prior to towing reduces the probability of spreading invasive species, there was concern that biofouling removal could have an impact on environmental quality in Sinclair Inlet. In order to address this concern, the Environmental Sciences and Advanced Systems & Applied Sciences Branches at the Space and Naval Warfare (SPAWAR) Systems Center Pacific, San Diego CA (SSC-Pacific) and NUWC Newport, RI undertook a series of studies to assess potential environmental quality impacts to Sinclair Inlet associated with the hull cleaning of CV 62 (Earley et al., 2018a; Earley et al., 2018b).

Prior to biofouling removal, comprehensive biological surveys of taxonomy and biomass present on the hull of CV 62 were conducted by SSC-Pacific and NUWC at randomly selected stations along transect belts on the hull, as well as other isolated areas of the hull where fouling was known to occur (Earley et al., 2018a). Additionally, an initial inspection and video of the biofouling present on the forward 300 ft of the vessel was conducted by Seaward Marine Services Inc. on November 5, 2016. Biofouling removal was conducted by Seaward Marine Services Inc., under contract to the Navy, from January 6 to 27, 2017. Biofouling removal was conducted using the self-propelled, diver driven SCAMP® cleaning machine in accordance with the Naval Ships' Technical Manual (NSTM) Chapter 081 (NAVSEA, 2006). Water quality monitoring (Earley et al., 2018b) was conducted to evaluate key water quality parameters at six sites located near the Ship (area of influence) and four Reference sites within western Sinclair Inlet during four sampling events conducted before removal (November 9 to 10, 2016), during removal (January 10, 2017), at the end of removal (January 31, 2017), and 40 days after removal was completed (March 7, 2017). Sediment monitoring was conducted before removal (Pre-Removal on December 13, 2017) and after the ship was towed from Sinclair Inlet (March 30, 2017).

This report presents the sediment sampling and analysis results used to assess potential impacts to sediment quality from biofouling removal from the ex-INDEPENDENCE (CV 62) while it was moored at NBK-BREM and the Puget Sound Naval Shipyard & Intermediate Maintenance Facility (PSNS&IMF) in Sinclair Inlet, Puget Sound, WA. The results of sediment sampling prior to biofouling removal on December 12, 2016 and sediment sampling conducted after the ship was towed from Sinclair Inlet on March 30, 2017 were used for this assessment. The objectives of the sediment quality study were to determine whether biofouling removal caused significant impacts to the sediment quality from:

(a) the release of Cu and Zn
(b) degradation of the benthic community

Figure 1. Location of the ex-INDEPENDENCE (CV62) while she was moored in Sinclair Inlet prior to her departure to Brownsville, TX for dismantling.

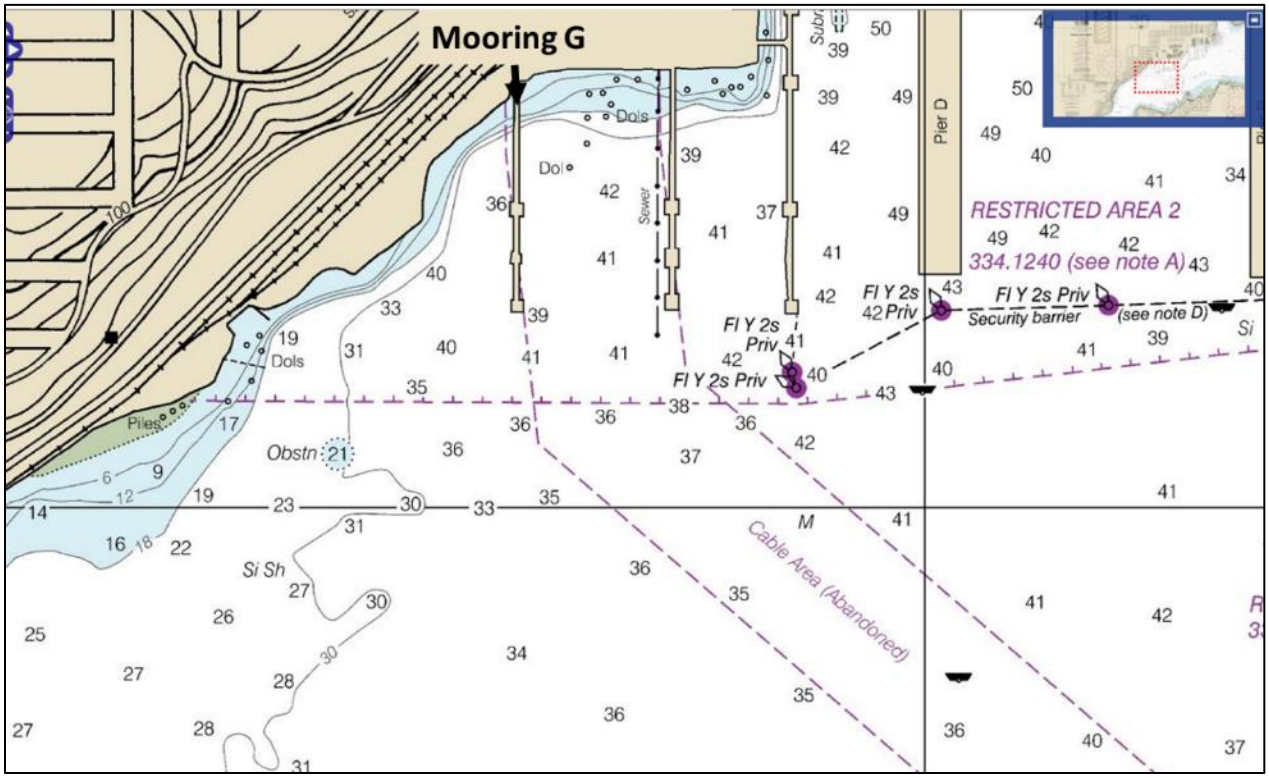

Figure 2. Bathymetry in the vicinity of Mooring G from NOAA Chart 18452.

This report presents the field observations and data analyses used to assess any adverse environmental impacts associated with the hull cleaning event based on benchmarks of sediment quality defined by the Washington State Department of Ecology (Ecology) and the US Environmental Protection Agency (US EPA). The data quality objectives (DQOs, Table 1) and Quality Assurance and Quality Control (QA/QC) procedures were identified in the Project Work Plan (PWP) and Sampling and Analysis Plan (SAP) prepared for the study (SSC-Pacific and NUWC, 2016).

Benchmarks of sediment quality for protection of the marine benthic community in Washington State are defined in WAC 173-204-320 (Table 2). Additional data to assess the bioavailability and potential toxicity of sediment metals were also evaluated to assess the protection of benthic organisms from adverse effects of metal exposure (US EPA, 2005).

The spatial boundaries of the study were defined as the zone of influence near the ship. The zone of influence was defined as the area most likely to be impacted by material released during biofouling removal (see PWP, SSC-Pacific and NUWC, 2016). Sediment sampling was confined to the area immediately below the location where the ship was moored as that is where the vast majority of material was expected to settle to the bottom. This is based on the modeling results (SSC-Pacific and NUWC, 2016) and the fact that the hulls of CV 62 and ex-KITTY HAWK (CV 63) that were moored at Mooring G during biofouling removal (Figure 3) likely prevented transport of suspended particles away from Mooring G. Mooring G is located within Operable Unit B (OUB) Marine as defined in the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) response actions for the Bremerton Naval Complex (BNC) and is adjacent to areas remediated by cleanup actions (Figure 4; Navy, 2012).

Data were evaluated to assure accuracy, precision, completeness, comparability, and representativeness of the sampling results. Sampling and analytical methods for sediment sampling followed the sampling procedures recommended for the Puget Sound Estuary Program (PSEP, 1997; Ecology, 2015), Ecology's sediment monitoring program (Dutch et al., 2009, 2015), and the procedures recommended for assessing metal bioavailability and toxicity in marine sediments (US EPA, 2005).

Data from the sampling were evaluated in the context of historical Cu and Zn data available for the site which includes investigations for Long Term Monitoring (LTM) of Operable Unit B Marine grids (OUB Marine; US Navy, 2012; URS Group, 2015 and 2016a), Charleston Beach Monitoring (URS Group, 2016b), ENVVEST (Environmental Reinvestment, US Navy, US EPA, and Ecology, 2000) studies conducted by the Navy and participating stakeholders (ENVVEST, 2006), an activated carbon sediment amendment demonstration project conducted at Pier 7 of PSNS&IMF (Kirtay et al., 2017), and Sinclair Inlet monitoring conducted by Ecology's sediment monitoring program for the Urban Waters Initiative (UWI, Weakland et al., 2013).

Figure 3. Location of sediment sampling stations that are co-located with the water quality monitoring stations directly adjacent to CV 62 at Mooring G (blue circles). CV63 (USS Kittyhawk) is located adjacent to CV-62. The CH3D model grid and ADCP mooring location are also shown.

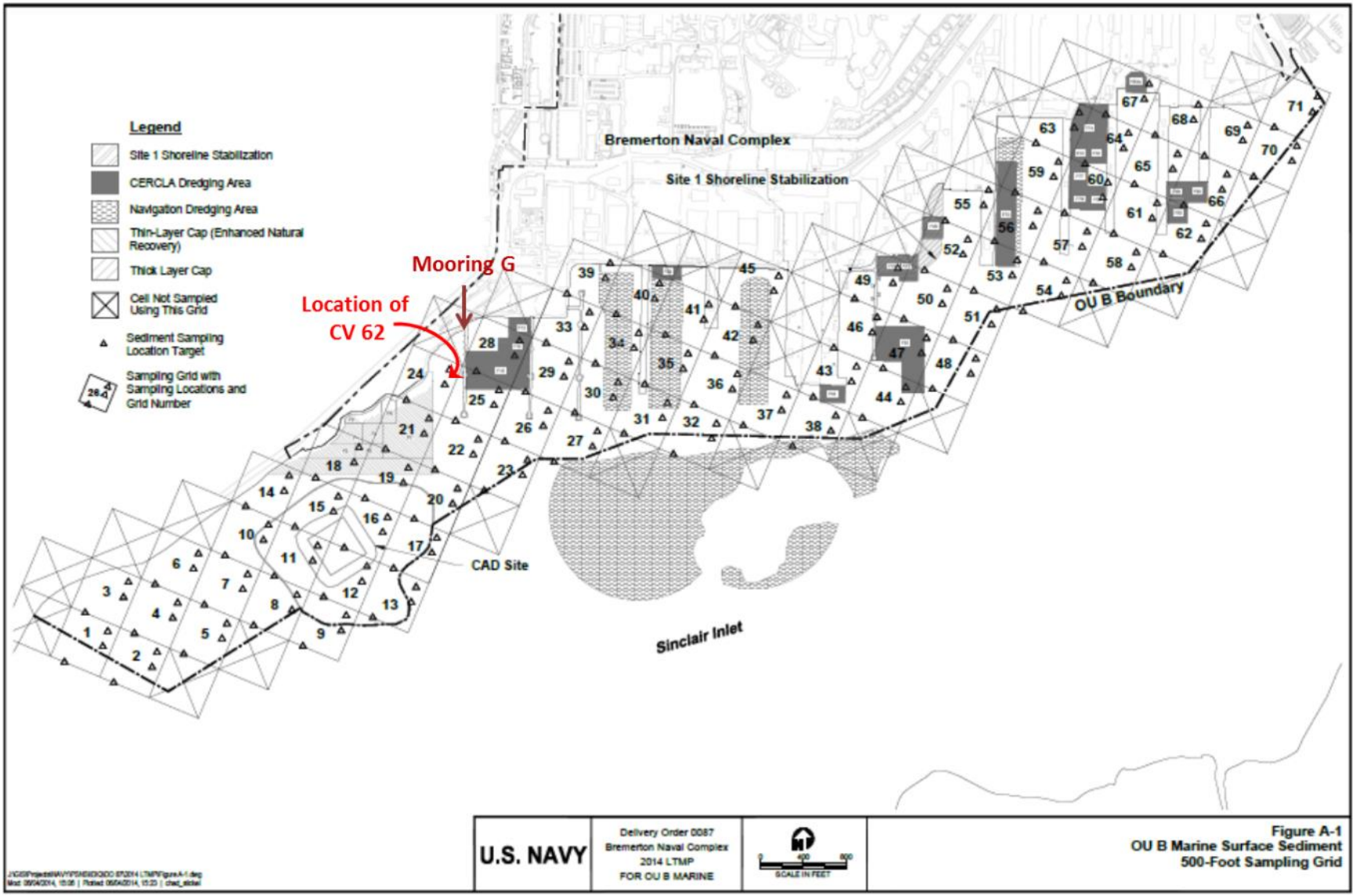

Figure 4. Location of Mooring G and CV 62 in relation to Remedial Actions and Long Term Monitoring within OUB Marine (numbered grids) conducted for the Bremerton Naval Complex (Figure from US Navy 2012).

Table 1. Data quality objectives (DQOs) for assessing sediment quality impacts from biofouling removal from the ex-INDEPENDENCE (CV62) moored in Sinclair Inlet, Puget Sound, WA.

Data Quality Objectives
STEP 1: State the Problem The ex-INDEPENDENCE (CV62), moored in Sinclair Inlet since decommissioning in Sept. 1998, was towed to Brownsville, TX for dismantling in March 2017. Based on a consultation with the National Marine Fisheries Service, the Navy was required to clean the ship's hull prior to towing in order to mitigate the transfer of invasive species to other regions. While removing biofouling organisms prior to towing may reduce the probability of spreading invasive species, there was concern that biofouling removal may have a detrimental impact on sediment quality in Sinclair Inlet. Beneficial uses for the protection of aquatic life that may be impacted include: 1) degrading sediment quality from the potential release of Cu and Zn that may be present in the hull coating on the ship; and 2) potential adverse impacts to the benthic community on the sea floor adjacent to the ship. A sediment quality assessment study was needed to assess the potential impacts to sediment quality from biofouling removal and determine if the action degrades the environmental quality of Sinclair Inlet.
STEP 2: Identify the Decision Will biofouling removal cause adverse impacts to sediment quality after removal from: a) the release of Cu and Zn? b) degradation of the benthic community?
STEP 3: Identify Inputs to the Decision 1. Identify zone of influence or area most likely to be impacted by biofouling removal in Sinclair Inlet. 2. Conduct sediment sampling within the zone of influence before and after biofouling removal. Data to be collected include: a) Sediment concentrations of Cu and Zn b) Sediment concentrations of total organic carbon (TOC) and grain size c) Acid volatile sulfide (AVS) and simultaneously extracted metal (SEM) concentrations to evaluate metal bioavailability and toxicity d) benthic community composition 3. Compare data from study to other local and regional monitoring being conducted within Sinclair/Dyes Inlet and Puget Sound ecosystem.

Table 1. Data quality objectives (DQOs) ... (Continued).

Data Quality Objectives
STEP 4: Define the Study Boundaries The spatial boundaries are defined as the zone of influence near ship and reference locations outside the zone of influence but within the northwestern portion of Sinclair Inlet. The zone of influence is defined as the area most likely to be impacted by material released during biofouling removal.
STEP 5: Develop a Decision Rule The data collected will be used to assess the impact to environmental quality from biofouling removal and determine if the action will significantly degrade the environmental quality of Sinclair Inlet. Sediment quality benchmarks as defined in WAC 173-204, US EPA (2005) provide guidelines to assess the protection of benthic organisms from adverse effects of metal exposure, and the antidegradation policy is described in WAC 173-201A-300. Data from the assessment will help inform the decision making process for maintaining environmental quality in Sinclair Inlet.
STEP 6: Evaluate Decision Errors Data will be evaluated to assure accuracy, precision, completeness, comparability, and representativeness. Sampling and analytical methods for Cu and Zn in seawater will follow ultra-clean sampling procedures and performance-based QA/QC for trace metal analysis in sediment. Data will also be evaluated to determine effects from other sources not related to biofouling removal such as effects on water column DO levels from naturally occurring plankton and algae blooms in the Inlet, surface runoff of Cu and Zn during storm events that may occur during the project, redistribution of existing contamination in sediment and nearshore locations at the site, and other unforeseen processes or events that might occur during the project.

Table 1. Data quality objectives (DQOs) ... (Continued).

Data Quality Objectives
STEP 7: Optimize the Design for Obtaining Data Sediment chemistry sampling will be performed at six locations within the zone of influence near the ship prior to the start of operations and approximately one month after the ship is moved from Sinclair Inlet. Baseline Sediment samples of top 10 cm will be composited and analyzed for total Cu, total Zn, TOC, and grain size. During the post-project sampling additional sediment cores will be obtained and sectioned to evaluate metal bioavailability at the site. Benthic community samples will also be collected in a similar fashion prior to the start of operations at the six sediment chemistry sites. The benthic community samples will be sieved and preserved for benthic community analysis. Results from the benthic community samples will also be available for comparison to future benthic community analysis conducted in the area. Sediment chemistry data before and after removal operations will be used to determine whether there are significantly higher concentrations of Cu and Zn that can be attributed to the hull's cleaning of the ex-INDEPENDENCE. In addition, data to assess bioavailability and potential metal toxicity impact on sediment quality will be evaluated to assess the ecological risk impact to the benthic community. Overall, the samples and their subsequent analysis will be used to identify potential impacts to sediment quality from biofouling removal, assess potential ecological effects of metals to the benthic community, and compare the results to known thresholds within the anti-degradation policy. Current meter and other modeling data collected during the study will be available to inform the decision making process for maintaining environmental quality in Sinclair Inlet.

Table 2. Sediment benchmarks for evaluating sediment quality for Cu and Zn (Ecology, 2013).

	A. Chemical Criteria	
	Marine Sediment	
Element	Maximum Chemical Criteria (MCC) dry weight (ppm)	Sediment Quality Standard (SQS) dry weight (ppm)
Cu	390 μg/g	390 μg/g
Zn	960 μg/g	410 μg/g

2. METHODS

2.1 FIELD SAMPLING PROCEDURES

Sampling and analytical methods for sediment sampling followed the sampling procedures recommended for the Puget Sound Estuary Program (PSEP, 1997; Ecology, 2015) and Ecology's sediment monitoring program (Dutch et al., 2009, 2015). Baseline sediment surface samples (0–10 cm) were collected prior to biofouling removal on December 12, 2016 at six stations co-located with the water quality monitoring stations at Mooring G (Table 3, Figure 3). Post-Removal sediment surface samples (0–10 cm depth) and core samples (0–25 cm depth) were collected at the same locations by PSNS&IMF Divers on March 30, 2017 approximately 3 weeks after CV 62 was towed from Sinclair Inlet on March 11, 2017 and eight weeks after biofouling removal was completed on January 27, 2017. In addition, adult mussel (*Mytilus* spp.) specimens were collected from the hull of CV 62 during the biofouling surveys conducted in December 2016 (SSC-Pacifc and NUWC, 2017) following the national mussel watch protocols (Kimbrough et al., 2008; Johnston et al., 2014; Johnston, 2017). Details of the sampling are provided below.

Table 3. Target sediment quality sampling stations co-located with water quality stations adjacent to the ship and before and after sampling dates.

Station	Description	Latitude	Longitude	Pre-Removal	Post-Removal
CV62-1	Bow of ship at Mooring G	47.55400	122.65698	12/13/17	3/30/17
CV62-2	Midship forward starboard side at Mooring G	47.55334	122.65666	12/13/17	3/30/17
CV62-3	Midship aft starboard side at Mooring G	47.55204	122.65662	12/13/17	3/30/17
CV62-4	Stern of ship at Mooring G	47.55108	122.65695	12/13/17	3/30/17
CV62-5	Midship aft port side at Mooring G	47.55206	122.65753	12/13/17	3/30/17
CV62-6	Midship forward port side at Mooring G	47.55330	122.65731	12/13/17	3/30/17

The Pre-Removal samples were collected to establish a baseline of sediment conditions prior to any hull cleaning conducted on CV 62. Pre-Removal baseline samples were collected using a ponar grab sampler (Ben Meadows Company, Janesville, WI; 6 in x 6 in x 4 in depth, surface area 0.2 m^2) from a small boat moored at each station (Table 4). Grab samples were inspected after retrieval to insure the sediment surface was undisturbed. The sediment was then composited, placed in glass jars and transported to SSC-Pacific where the samples were stored in a walk-in cooler at 4°±2°C. They were shipped to the contract laboratory (ALS Global, Kelso, WA) for trace metal chemistry, grain size, moisture content, and total organic carbon (TOC) analysis. An additional sample from each station was collected and immediately sieved through a 1 mm screen; the material collected was preserved with 10% formalin solution and the samples were sent to a taxonomic laboratory (EcoAnalyst, Moscow, ID) for benthic community analysis (Dutch et al., 2009).

The Post-Removal samples were collected to assess any changes in of sediment conditions after hull cleaning of CV 62 was completed. Approximately two months after the biofouling removal was completed and three weeks after CV 62 was towed from Mooring G, another round of sediment samples were collected at the same stations previously sampled (Table 3, Figure 3). Divers collected

surface grab samples (0–10 cm) using 4.5-inch (12 cm) core liners that were pressed into the sediment, capped on both ends and removed to the water surface. Two sediment cores of about 0–25 cm in length were also collected from each station by pressing 2.5 ft core (76 cm) liners into the sediment and capping both ends and removing the intact core with overlying water to the water surface without disturbing the sediment profile (Table 4A).

Table 4. Analytical parameters, container type, number of samples and field duplicates, storage conditions, and allowable holding times prior to analysis for parameters measured in samples collected during the study.

A. Pre-Removal Sediment						
Parameter	Sample Type[a]	Con-tainer [b]	Number of Samples	Field Dups	Storage	Holding Time
Total Cu	Grab[a]	Glass[b]	6	1	4º±2°C	1 year
Total Zn	Grab	Glass	6	1	4º±2°C	1 year
Moisture	Grab	Poly	6	1	4º±2°C	1 year
TOC	Grab	Poly	6	1	4º±2°C	1 year
Grain Size	Grab	Poly	6	1	4º±2°C	1 year
Benthic Infauna	Grab	Glass	6	1	Fixed with Formalin	1 year
B. Post-Removal Sediment						
Parameter	Sample Type[a]	Con-tainer [b]	Number of Samples	Field Dups	Storage	Holding Time
Total Cu	Grab, CS[a]	Poly	6	1	Frozen (-18ºC)	1 year
Total Zn	Grab, CS	Poly	6	1	Frozen (-18ºC)	1 year
Moisture	Grab, CS	Poly	6	1	Frozen (-18ºC)	1 year
Grain Size	Grab	Poly	6	1	Frozen (-18ºC)	1 year
TOC	Grab, CS	Poly	6	1	Frozen (-18ºC)	1 year
Total Cu & Zn	CS	Poly	23	6[d]	Frozen (-18ºC)	1 year
AVS	CS	Poly	23	6[d]	Frozen (-18ºC)	14 days[c]
SEM	CS	Poly	23	6[d]	Frozen (-18ºC)	14 days[c]

a. Grab = 0–10 cm composite; CS = 0–25 cm core, sectioned at 2-3 cm intervals in the top 10 cm and 5 cm intervals > 10 cm.

b. Glass = pre-cleaned glass with Teflon-lined lid; Poly = polypropylene; Bag = clean Ziploc® plastic bag

c. The holding time may be extended if the samples are frozen and the oxidized layer is removed prior to analyses.

d. Duplicate cores were collected from each station. The duplicate cores were archived frozen intact, complete with overlying water. Holding time is considered indefinite.

Table 4. Analytical parameters ... (Continued).

C. Mussels						
Parameter	Sample Type[a]	Con-tainer [b]	Number of Samples	Field Dups	Storage	Holding Time
Total Cu	Specimens	Bag	4	1	Frozen (-18ºC)	1 year
Total Zn	Specimens	Bag	4	1	Frozen (-18ºC)	1 year
Moisture	Specimens	Bag	4	1	Frozen (-18ºC)	1 year
Lipid Content	Specimens	Bag	4	1	Frozen (-18ºC)	1 year

a. Grab = 0–10 cm composite; CS = 0–25 cm core, sectioned at 2-3 cm intervals in the top 10 cm and 5 cm intervals > 10 cm.

b. Glass = pre-cleaned glass with Teflon-lined lid; Poly = polypropylene; Bag = clean Ziploc® plastic bag

The divers were equipped with SuperLite® 17 helmets (Kirby Morgan Dive Systems, Inc., Santa Maria, CA) and 7 mm neoprene wet suits with surface supplied air and warm water through an umbilical tether system from the dive boat. The dive team consisted of two divers, two tether handlers, a dive supervisor and backup, standby divers. The divers were in constant communication with the dive supervisor and scientific team with audio communications and an underwater video camera (UWS-3200, Outland Technology, Slidell, LA) with a light emitting diode that was either attached to the diver's helmet or hand held. The video was displayed on a monitor onboard the dive boat and the video and audio from the divers were recorded with a digital video recording (DVR) device. The direct communication with the divers was very valuable to the scientific team, as the divers were able to communicate information about sea floor conditions and provide feedback on equipment performance and sampling conditions. The divers recorded video of the bottom conditions at the station locations and conducted a video transect that traversed from Mooring G, across the berthing area that was formerly below the hull of the vessel, extending to the bottom area west of where the vessel was berthed. The video provided a record of bottom conditions approximately 3 weeks after CV 62 was towed from Sinclair Inlet and 8 weeks after biofouling removal was completed.

The 0–10 cm surface sample from the short cores from each station were frozen until they could be sent to the contract laboratory (ALS Global, Kelso, WA) for compositing and analysis of total Cu and Zn, grain size, moisture, and TOC. One of the long sediment cores (0–25 cm) from each station was brought to the PSNS&IMF chemistry laboratory, Building 59, where they were held on ice and allowed to settle overnight and then sectioned to obtain samples for analysis of Acid Volatile Sulfide (AVS) and Simultaneously Extracted Metals (SEM) to assess metal bioavailability at the site (US EPA, 2005). After carefully siphoning off the overlying water, the cores were sectioned by slowly extruding the remaining sediment and slicing it into 2–3 cm sections for the top 10 cm and about 5 cm sections for the bottom 10–25 cm of core. Samples of each core section were placed into polypropylene jars and held frozen until they were transferred to the contract lab (ALS Global, Kelso, WA) for AVS, SEM, total Cu, total Zn, moisture, and TOC analysis The remaining long cores from each station were frozen intact with overlying water and archived to preserve anoxic conditions in the sediment cores (Table 4B).

Biological samples of fouling organisms growing on the hull were collected for chemical residue analysis to characterize to the potential loading of Cu and Zn from biomass removal. During the biological surveys conducted in December 2016 (SSC-Pacific and NUWC, 2017), divers collected adult mussel (*Mytilus* spp.) specimens about 3–6 in (6–15 cm) in length from the random sampling locations sampled along each transect belt which included samples from the top, middle, and bottom

of the hull on both the port and starboard sides (Figure 5). Specimens collected from each transect belt were combined into a single bag (approximately 7–15 individuals). Due to logistics constraints, it was only possible to sample one location on transect belt 4, therefore, specimens from transect belts 4 and 5 were combined into a single sample. The samples were stored frozen whole in the shell until they were transferred to the contract laboratory (ALS Global, Kelso, WA), where they were thawed, shucked, and composited for analytical analysis of total Cu and Zn, moisture, and lipid content (Table 4C).

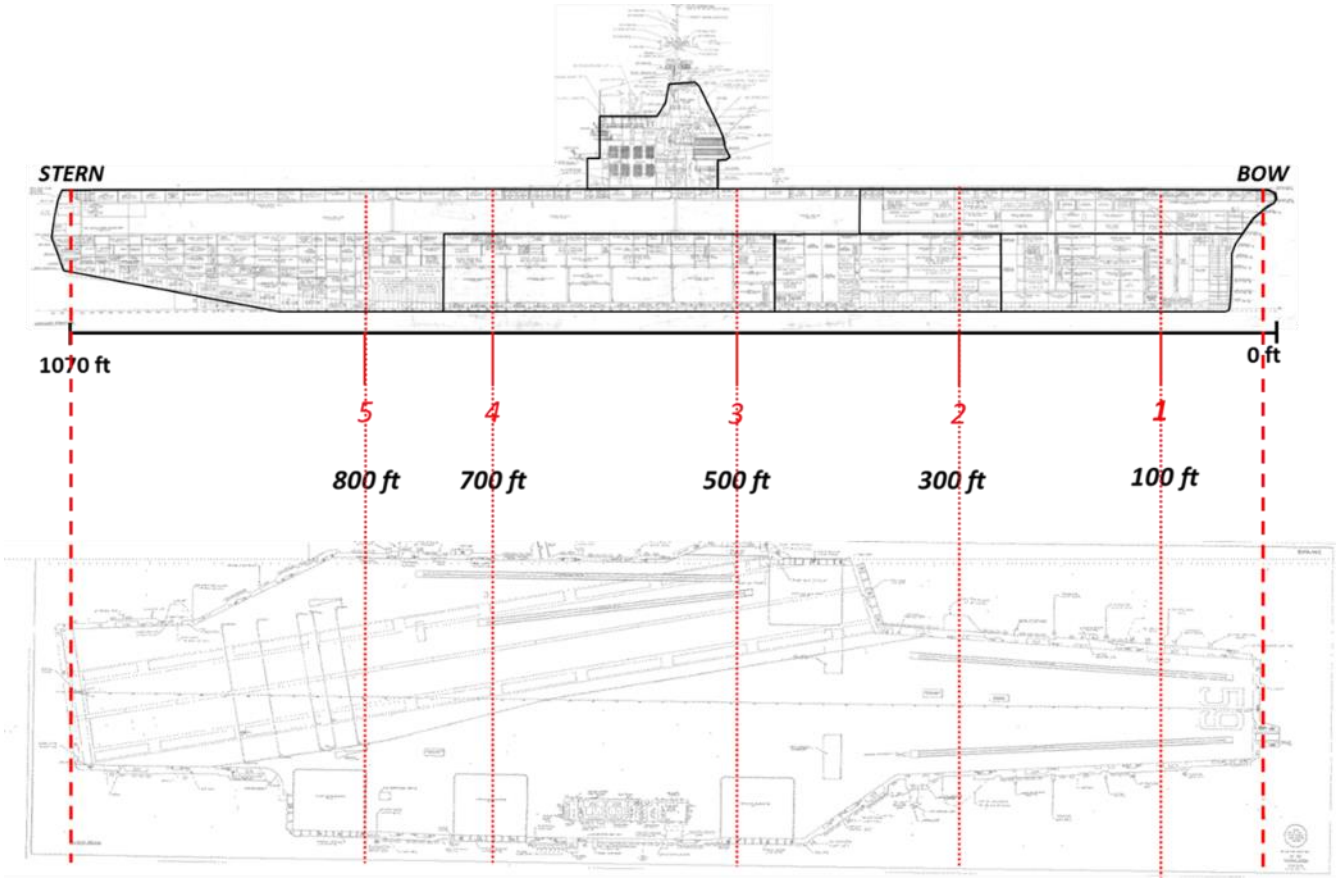

Figure 5. Transect belts around the hull of CV 62 that were sampled during the biological surveys of CV 62 which corresponded to locations were mussel (*Mytilus* spp.) were collected for residue analysis of Cu, Zn, moisture, and lipid content.

2.2 ANALYTICAL METHODS

Sediments from the Pre- and Post-Removal 0–10 cm surface samples were composited by station and analyzed for total Cu, total Zn, TOC, and grain size (Table 4). The core section samples were submitted for analysis of AVS, SEM, total Cu, total Zn, moisture content, and TOC (Table 4). The mussel tissue samples were submitted for analysis of total Cu and Zn, moisture, and lipid content. All analyses were conducted by ALS Global (Kelso, WA) under contract to SSC-Pacific (Contract Number/Purchase Order Agreement N6600117P6856, awarded June 2, 2017). The quality assurance/quality control (QA/QC) samples used for the project are defined in Table 5, the recommended acceptance criteria and corrective actions are listed in Table 6, the calculations used to determine QA/QC statistics are defined in Table 7, and data qualifiers are provided in Table 8. The details of the analytical analysis and QA/QC procedures followed are detailed below.

Samples selected for total Cu and Zn analyses were stored in a cooler (4º±2°C) or frozen (-18°C) until submitted for analysis. Archived sample material, if available, was held frozen. Upon submittal for analysis, samples were thawed, wet homogenized, subsampled for percent moisture, grain size, and TOC analysis, then freeze-dried and homogenized using a ball-mill prior to digestion. The subsamples for moisture and grain size were analyzed using Puget Sound Estuary Protocols (PSEP), TOC was analyzed by ATSM Method D4129M, and the metal samples underwent total digestion and analysis by Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) using procedures recommended by EPA Method 6010B (SW6010B).

The sediment core samples were extracted and analyzed for AVS using US EPA Method EPA-821-R-100 (Allen et al., 1991; Allen and Fu, 1993). In this method, sulfide in the sample is converted to hydrogen sulfide by the addition of hydrochloric acid at room temperature. The hydrogen sulfide (H_2S) is purged from the sample by an inert gas and trapped in a sodium hydroxide (NaOH) solution. With the addition of a mixed-diamine reagent (MDR), the sulfide is converted to methylene blue and quantified on a spectrometer. The AVS results were reported in units of µmol/g on a dry-weight basis. The QA/QC samples for the AVS included a MB and LCS. Because AVS is a reactive substance, MS and SRM are not applicable. The LCS was generated by homogenizing a large composite field sample and conducting repeated analysis of the sample (n=6) to provide an estimate of the analytical variance and precision of the method (Johnston, 1993).

The SEM extracts were analyzed for Cd, Cu, Ni, Pb, and Zn by ICP-MS using SW6010B. The SEM extracts were also analyzed for SEM Hg by Cold Vapor Atomic Absorption (CVAA) using EPA Method 7470A (US EPA, 1994). The QA/QC samples for SEM analysis included MB, LCS, MS, MSD, Lab DUPs and SRMs for the analytes of concern. The SEM metal solution concentrations were determined in units of µg/L and then converted to µg SEM/g of sediment extracted for AVS-SEM determination. These data were further converted to µmol/g for each SEM metal.

When the molar concentration of AVS are greater than the molar concentration of the sum of the SEM metals, bioavailability and toxicity of the metals are not expected because the metals are likely bound as non-soluble sulfides (US EPA, 2005). If sum of SEM (ΣSEM) metals are greater than AVS, then the metals would be released in order of their sulfide solubility product (K_{sp}) that expresses the ratio of dissolved : solid species, where the lower the K_{sp} the more tightly bound is the metal-sulfide compound (Morse, Millero, Cornwell, and Rickard, 1987). The SEM metals analyzed were metals of interest because they have lower sulfide ratios than FeS (Table 9), therefore if the sum of the SEM metal were greater than AVS, then the metals with the largest sulfide solubility would be present as potentially toxic free metal (US EPA, 2005). SEM Hg was also analyzed because Hg is a contaminant of concern for OUB Marine (US Navy, 2012); however, HgS has a much lower sulfide solubility (2×10^{-53}; URI, 2006) than the other metals so it is very unlikely free Hg would exist in the presence of AVS.

Table 5 Definitions of QA/QC samples and frequency of analysis.

Sampling Quality Control		
QA/QC Sample	Definition	Frequency
Field Duplicate (DUP)	The field duplicate is a collocated sample defined as a sample collected as near in space and time to the original field sample as the sampling equipment and procedure allows.	1 every ten samples
Equipment Blank (EB) or Field Blank (FB)	The equipment or field blank is collected to assure that sampling apparatus and equipment are free of contamination and that the sampling procedures did not contaminant the sample	1 every sampling event or when specific equipment is used
Laboratory Quality Control		
QA/QC Sample	Definition	Frequency
Method or Procedural Blank (MB)	A combination of solvents, surrogates, and all reagents used during sample processing, processed concurrently with the field samples. Monitors purity of reagents and laboratory contamination.	1/sample batch[1]. A processing batch MB must be analyzed with each sequence.
Standard Reference Material (SRM)	An external reference sample which contains a certified level of target analytes; serves as a monitor of accuracy. Extracted and analyzed with samples of a like matrix (may not be available for all analytes)	1/ batch[1]
Laboratory Control Sample (LCS)	An internal reference sample of known concentration prepared by the performing lab which contains target analytes in similar matrix; serves as a monitor of accuracy when SRM is not available. Extracted and analyzed with samples of a like matrix.	1/ batch[1]
Matrix Spike (MS)	A field sample spiked with the analytes of interest is processed concurrently with the field samples; monitors effectiveness of method on sample matrix; performed in duplicate.	1/sample batch[1]
Lab Duplicate Sample	Second aliquot of a field sample processed and analyzed to monitor precision; each sample set should contain a duplicate.	1/sample batch[1]
Recovery Internal Standards (RIS)	All field and QC samples are spiked with recovery internal standards just prior to analysis; used to quantify surrogates to monitor extraction efficiency on a per sample basis. (Only applicable for GM/MS analysis)	Each sample analyzed for organic compounds
Surrogate Internal Standards (SIS)	All field and QC samples are spiked with a known amount of surrogates just prior to extraction; recoveries are calculated to quantify extraction efficiency. (Only applicable for GC/MS analysis of organic compounds)	Each sample analyzed for organic compounds

[1] A batch is defined as 10–20 field samples processed simultaneously and sharing the same QC samples.

Table 6. Measurement QA/QC parameters, acceptance criteria, and suggested corrective actions.

QA/QC Parameter	Acceptance Criteria	Corrective Action
Method Blank (MB)	MB≤MRL If MB>MRL; sample values ≤10X MB, then perform corrective action	Perform corrective action re-process (extract, digest) sample batch. If batch cannot be re-processed, notify client and flag data.
Standard Reference Material (SRM)	Metals: ≤20% RPD Determined vs. certified range. Analyte concentration must be > 10xMDL to be used for QC criteria.	Review data to assess impact of matrix. Reanalyze sample and/or document corrective action. If other QC data are acceptable then flag associated data if sample is not reanalyzed.
Matrix Spike (MS)/MS Duplicate (MSD)	Organic compounds: 40–120% recovery Metals: 70–130% recovery Method criteria for all other parameters	Review data to assess impact of matrix. If other QC data are acceptable and no spiking error occurred, then flag associated data. If QC data are not affected by matrix failure or spiking errors occurred, then re-process MS. If not possible, then notify client and flag associated data.
Surrogate Spike (SIS)	Organic compounds: 40–120% recovery	Review data. Discuss with Project Manager. Reanalyze, re-extract, and/or document corrective action and deviations.
Laboratory Control Sample (LCS)	AVS: 40–120% recovery Metals: 70–130% recovery Method criteria for all other parameters	Perform corrective action. Re-analyze and/or re-process sample batch. If batch cannot be re-processed: notify client, flag data, discuss impact in report narrative.
Precision: Laboratory Duplicates	Organic compounds (MSD): <30% RPD Metals: <30% RPD Method criteria for all other parameters	Review data to assess impact of matrix. If other QC data are acceptable, then flag associated data. If QC data are not affected by matrix failure, then re-process duplicate. If not possible, then notify client and flag associated data.

Table 7. Calculation of QA/QC Statistics.

Percent Recovery

The percent recovery (PR) is a measurement of accuracy, where one value is compared with a known/certified value. The formula for calculating this value is:

$$PR = \frac{amountdetected}{amountexpected} x\,100$$

Table 7. Calculation of QA/QC Statistics. (Continued)

Percent Difference

The percent difference (PD) is a measurement of precision as an indication of how a measured value is difference from a "real" value. It is used when one value is known or certified, and the other is measured. The formula for calculating PD is:

$$PD = \frac{X_2 - X_1}{X_1} x\,100$$

where: X_1 = known value (*e.g.*, SRM certified value)

X_2 = determined value (*e.g.*, SRM concentration determined by analyst)

Relative Percent Difference

The relative percent difference (RPD) is a measurement of ***precision***; it is a comparison of two similar samples (matrix spike/matrix spike duplicate pair, field sample duplicates). The formula for calculating RPD is:

$$RPD = \left| \frac{(X_1 - X_2)}{(X_1 + X_2)/2} \right| x\ 100$$

where: X_1 is concentration or percent recovery in sample 1

X_2 is concentration or percent recovery in sample 2

Note: Report the absolute value of the result — the RPD is always positive.

Relative Standard Deviation

The relative standard deviation (RSD) is a measurement of ***precision***; it is a comparison of three or more similar samples (*e.g.*, field sample triplicates, initial calibration, MDLs). The formula for calculating RSD is:

$$RSD = \frac{Standard\ Deviation\ of\ All\ Samples}{Average\ of\ All\ Samples} \times 100$$

Table 8. Data Qualifiers used by the contract lab (ALSGlobal).

Inorganic Data Qualifiers	
*	The result is an outlier. See case narrative.
#	The control limit criteria is not applicable. See case narrative.
B	The analyte was found in the associated method blank at a level that is significant relative to the sample result as defined by the DOD or NELAC standards.
E	The result is an estimate amount because the value exceeded the instrument calibration range.

Table 8. Data Qualifiers used by the contract lab (ALSGlobal). (Continued)

Inorganic Data Qualifiers	
J	The result is an estimated value.
U	The analyte was analyzed for, but was not detected (“Non-detect”) at or above the MRL/MDL.
	DOD-QSM 4.2 definition: Analyte was not detected and is reported as less than the LOD or as defined by the project. The detection limit is adjusted for dilution.
i	The MRL/MDL or LOQ/LOD is elevated due to a matrix interference.
Inorganic Data Qualifiers	
X	See case narrative.
Q	See case narrative. One or more quality control criteria was outside the limits.
H	The holding time for this test is immediately following sample collection. The samples were analyzed as soon as possible after receipt by the laboratory.
Metals Data Qualifiers	
#	The control limit criteria is not applicable. See case narrative.
J	The result is an estimated value.
E	The percent difference for the serial dilution was greater than 10%, indicating a possible matrix interference in the sample.
M	The duplicate injection precision was not met.
N	The Matrix Spike sample recovery is not within control limits. See case narrative.
S	The reported value was determined by the Method of Standard Additions (MSA).
U	The analyte was analyzed for, but was not detected (“Non-detect”) at or above the MRL/MDL.
	DOD-QSM 4.2 definition: Analyte was not detected and is reported as less than the LOD or as defined by the project. The detection limit is adjusted for dilution.
W	The post-digestion spike for furnace AA analysis is out of control limits, while sample absorbance is less than 50% of spike absorbance.
i	The MRL/MDL or LOQ/LOD is elevated due to a matrix interference.
X	See case narrative.
+	The correlation coefficient for the MSA is less than 0.995.
Q	See case narrative. One or more quality control criteria was outside the limits.

Table 9. Metal-sulfide* solubility products and ratios (from US EPA, 2005).

Metal Sulfide	$Log_{10}K_{sp,2}$ [a]	$Log_{10}K_{sp}$ [b]	$Log_{10}(K_{MS}/K_{FeS})$
FeS	-3.64	-22.39	—
NiS	-9.23	-27.98	-5.59
ZnS	-9.64	-28.39	-6.00
CdS	-14.10	-32.85	-10.46
PbS	-14.67	-33.42	-11.03
CuS	-22.19	-40.94	-18.55
Ag_2S	-36.14	-54.71	-32.32

[a]Solubility products, $K_{sp,2}$ for the reaction $M^{2+} + HS^- = MS(s) + H^+$ for FeS (mackinawite), NiS (millerite), and CdS (greenockite) from Emerson et al. (1983). Solubility products for ZnS (wurtzite), PbS (galena), CuS (covellite), and Ag_2S (acanthite) and $pK_2 = 18.57$ for the reaction $HS^- = H^+ + S^{2-}$ from Schoonen and Barnes (1988).
[b]K_{sp} for the reaction $M^{2+} + S^{2-} = MS(s)$ is computed from log $K_{sp,2}$ and pK_2.

*Metal-Sulfide (MeS) equilibrium of the type:

$$MeS(s) + H^+ \rightleftharpoons Me^{2+}(aq) + HS^-(aq)$$

Sediment quality benchmarks for the protection of benthic organisms from metal exposure have been developed based on the knowledge of AVS, the sum of the simultaneously extracted metals (ΣSEM), and fraction of organic carbon (f_{OC}) in the sediment (US EPA, 2005):

Low risk of adverse biological effects

Equation 1: $(\Sigma SEM - AVS)/f_{OC} \leq 130$ μmol/g OC

May have adverse biological effect

Equation 2: 130 μmol/g OC < $(\Sigma SEM - AVS)/f_{oc} \leq 3000$ μmol/g OC

Adverse biological effects expected

Equation 3: $(\Sigma SEM - AVS)/f_{oc} > 3000$ μmol/g OC

2.3 DATA ANALYSIS

Statistical analyses were performed to determine if there were statistically-significant differences between Pre- and Post-Biofouling Removal for total Cu, total Zn, grain size fractions, solids content, and TOC. Data from the sampling were evaluated in the context of historical Cu and Zn data available for the site which includes investigations for Operable Unit B Marine (OUB Marine, US Navy; 2012), the ENVVEST (Environmental Reinvestment) program (US Navy, US EPA, and Ecology, 2000), studies conducted by the Navy and participating stakeholders (ENVVEST 2006), an activated carbon sediment amendment demonstration project conducted at Pier 7 of PSNS&IMF (Kirtay et al., 2017), and Sinclair Inlet monitoring conducted by Ecology's sediment monitoring program (Weakland et al., 2013).

2.3.1.1 Statistical Analysis

The raw data were validated based on the pre-defined performance based QA/QC procedures identified in the PWP (SSC-Pacific and NUWC, 2016) and all useable data were combined into a flat file (MS Excel, Microsoft Inc., Redman, WA) for statistical analysis. The flat file was imported into

R-Studio (v98.1091, r-studio.com, Boston, MA) running R (v3.01.1, R Foundation for Statistical Computing, www.r-project.org) for statistical analysis. Descriptive statistics were compiled for the data for the Pre- and Post- sampling events and corresponding tables and plots showing the data were prepared. A hypothesis was developed based on the traditional Before – After design:

$\boldsymbol{H_o}$: There are no differences between variables measured prior to biofouling removal (Pre-) and the same variables measured after the ship was cleaned and towed (Post-) from Sinclair Inlet.

Prior to conducting statistical tests, histograms of the data were plotted to determine whether the data distribution conformed to a normal or lognormal distribution and were suitable for parametric analysis of variance (ANOVA) or would be better evaluated using non-parametric statistical tests that do not require assumptions of normality. For practicality, both parametric and non-parametric tests were calculated to test the hypothesis.

For $\boldsymbol{H_o}$ the following tests were used

Equation 4: Parametric $ANOVA_{Normal}$: F = aov(Y ~ Event, data = Sediment)

Equation 5: Parametric $ANOVA_{Logormal}$: F = aov(log(Y) ~ Event, data = Sediment)

Equation 6: Non-Parametric: KW = kruskal.test(Y ~ Event, data = Sediment)

Where

Y = variable of interest

log(Y) = log base 10 transformed data for variable Y

Event = Pre- or Post-Biofouling Removal

Sediment = sediment raw data set

And

F and KW = statistical result for ANOVA or Kruskal-Wallis non-parametric tests, respectively

p(F) and p(KW) = probability of random result, and

$p \leq 0.05$ denotes statistical significance

Box and whisker plots (Figure 6) by event were constructed to visualize statistical comparisons and evaluate the magnitude of the differences detected. Core profiles were constructed with the data from the core sections collected Post-Removal to illustrate the distribution of moisture content, TOC, total Cu, total Zn, AVS, and SEM present in the sediments of the site. The probability of exceeding sediment quality benchmarks for the protection of benthic organisms was calculated by pooling the sediment core data by depth, calculating the mean (μ) and standard deviation (σ) for Y = (ΣSEM - AVS)/f_{oc}, and estimating the probability of exceeding the benchmarks using the Excel NORM.DIST function:

Equation 7: $P(B_x) = 1 - NORM.DIST(B_x,\mu,\sigma,TRUE)$

Where

B_x = Sediment benchmark of interest {130 μmol/g OC, 3000 μmol/g OC}

μ = mean of the data distribution, and

σ = standard deviation of the data distribution, and

NORM.DIST(B_x,μ,σ,TRUE) returns the cumulative distribution with a mean of μ, standard deviation of σ for B_x

The probability of exceeding the benchmarks was calculated assuming a normal distribution {μ, σ} and lognormal distribution {$\mu^{\wedge}$, $\sigma^{\wedge}$}

Equation 8: $P(B_x^{\wedge}) = 1 - NORM.DIST(B_x^{\wedge},\mu^{\wedge},\sigma^{\wedge},TRUE)$

where the data were log transformed using the following transformation:

Equation 9: $Y^{\wedge} = \log[(\Sigma SEM - AVS)/f_{OC} + 2000]$

The probability obtained from the lognormal distribution is more conservative than the probability calculated from the normal distribution because the lognormal distribution includes the values that form a long tail to the right of the distribution.

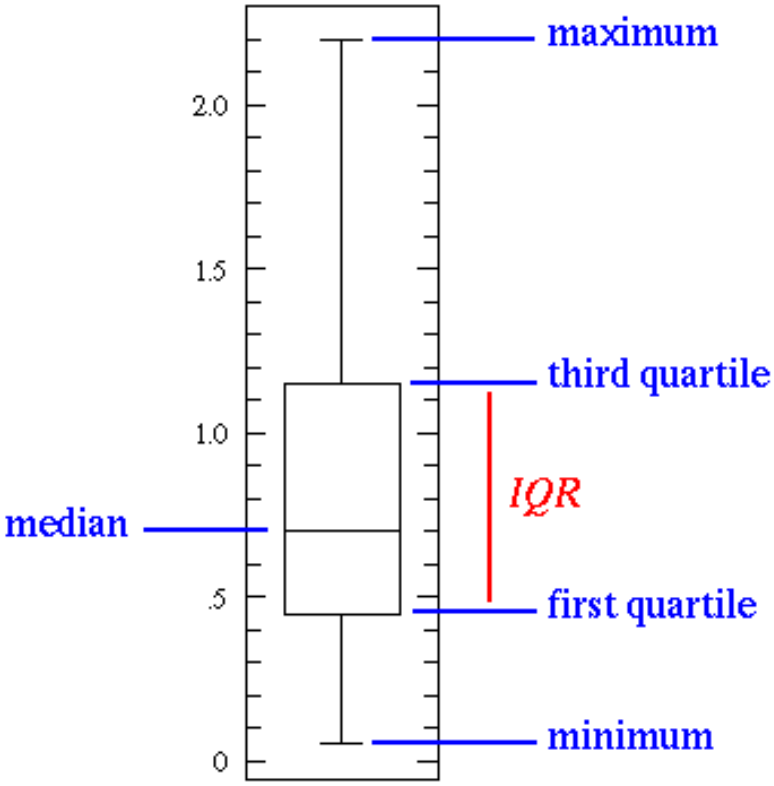

Figure 6. Statistical parameters depicted in a typical box and whisker plot showing the median (line), the first and third quartile (box), the inner quartile range, and range of the data (whiskers). Figure from http://www.physics.csbsju.edu/stats/box2.html.

2.3.2 Decision Framework

A decision matrix (Table 10) was used to evaluate whether biofouling removal caused any impacts to sediment quality in Sinclair Inlet. The decision takes into account whether there were statistical differences between the Pre- and Post- Removal sampling events, the magnitude of the difference,

and the potential of exceeding sediment quality benchmarks for the protection of benthic organisms from metal exposure. Obviously, if there is no difference between the Pre- and Post- sampling events for a parameter, or the data from Post- sampling indicates better sediment quality at the sites than Pre-, then the conclusion would be no impact from biofouling removal. If there are statistically significant differences showing that sediment quality conditions at the site worsened Post-Removal, the conclusion about impact would depend upon the magnitude of the difference and the probability of exceeding benchmarks for the protection of benthic organisms from metal exposure (Table 10). The decision matrix allows the degree of impact to be evaluated in a quantitative manner and is similar to approaches commonly used in environmental risk and assessment studies (Johnston et al., 2002; Thom et al., 2005; Labisoa et al., 2014; Diefenderfer et al., 2016).

Table 10. The decision matrix (A) and probability of outcome (B) used to assess the impact of biofouling removal on sediment quality at the site. The conclusion is based on the magnitude of statistical differences between the Pre- and Post-Removal conditions and the potential of metal bioavailability in sediment to cause adverse biological effects (US EPA, 2005). The range of colors within the table inform the predicted impacts include a range of negligible (green), potential (light yellow), possible (light pink), probable (bright pink) and adverse (red) impacts.

A. Decision Matrix.

			Metal Bioavailability In Sediment		
			$(\Sigma$SEM-AVS)/f_{OC}		
			≤ 130 umol/g OC	130 umol/g OC > and ≤3000 umol/g OC	> 3000 umol/g OC
	Magnitude of Difference		Low Risk of Adverse Biological Effects	May have Adverse Biological Effects	Adverse Biological Effects May be Expected
Statistical Difference from Reference	No Difference or Better than Pre-Removal	**None**	**No Impact**	**No Impact**	**No Impact**
	≤2x Pre-Removal	**Slightly Different**	**Negligible Impact**	**Potential Impact**	**Possible Impact**
	>2x and <5x Pre-Removal	**Different**	**Negligible Impact**	**Possible Impact**	**Probable Impact**
	≥5x and <10x Pre-Removal	**Highly Different**	**Negligible Impact**	**Probable Impact**	**Adverse Impact Likely**
	>10x Pre-Removal	**Very Highly Different**	**Negligible Impact**	**Adverse Impact Likely**	**Adverse Impact Likely**

B. Probability of Outcome.

Probability of Outcome	P
Very Low	< 2%
Low	≥ 2% < 10%
Medium	≥ 10% < 50%
High	≥ 50% < 80%
Very High	≥ 80%

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3. RESULTS

The raw data and QA/QC narrative from chemistry analysis reported by ALS Global, Inc. are provided in Appendix A for Pre-Removal sediment grabs (Appendix A-1), Post-Removal sediment grabs (Appendix A-2), AVS cores (Appendix A-3), and mussel tissues (Appendix A-4). The raw taxonomic data obtained from the Pre-Removal sampling are provided in Appendix B. Video clips from the underwater surveys of the hull of CV 62 prior to biofouling removal and the sea floor after CV 62 was towed from Sinclair Inlet are provided in Appendix C. Appendix D provides all the data from the study in the electronic data deliverable (EED) format required for submission to Ecology's Environmental Information Management (EIM) system (Ecology, 2017). Printed versions of the report include a CD that contains additional content for Appendices A–D.

3.1 QA/QC REVIEW

The analytical chemistry data were evaluated to assure accuracy, precision, completeness, comparability, and representativeness of the analytical results. The data were validated according to the QA/QC procedures defined in the QAP and the raw data were flagged with appropriate qualifier codes, if applicable (see Appendix A). The analytical parameters, holding times prior to analysis, the methods used, the method reporting limits (MRL), and method detection limits (MDL) are summarized in Table 11. No anomalies were reported for the analytical results for the Pre- and Post-Removal sediment surface samples, mussel tissue samples, and TOC, and moisture analysis of the core sections.

For the AVS analysis of the cores sections, the contract lab reported that acceptable recovery levels for sulfide SRM was not achieved, however, the AVS digestion is less aggressive than normally used for total sulfide analysis and low recovery of the sulfide SRM was expected. To address sulfide accuracy and precision, the contract lab was requested to perform AVS analysis on 6 replicate aliquots from one field sample to demonstrate proficiency and accuracy (Laboratory Control Sample – LCS). The results from the repeated analysis showed that AVS was highly variable with an adjusted coefficient of variation ($CoefV^* = \sigma/\mu \times (1 + 1/4n) \times 100$; Sokal and Rohlf, 1995) of 41% (Table 12). The variability was likely due to sample inhomogeneity and laboratory variation. Sample inhomogeneity was also the likely cause of the high RPD outside of control limits, obtained for laboratory duplicates in the metals analysis of the AVS cores (Appendix A-3).

The AVS is very susceptible to oxidation (US EPA, 2005), so the samples were stored frozen in tightly sealed polycarbonate jars. However, there was head space in the jars and the amount of material collected in the core sections was highly variable due to shells, rocks, and other debris present in the samples. This resulted in varying amounts of exposed surface of the sample that could have been oxidized during storage. The contract lab was requested to remove the oxidized surface level prior to homogenization, but because of limited sample size and standardized sample processing procedures performed by the lab it is not clear how well this request was carried out. The ideal situation would be to process the fresh cores immediately after collection or after thawing and sectioning intact cores (Johnston, 1993; US EPA, 2005), however, this was not possible due to contracting procedures and logistical limitations.

The sediment surface samples (0–10 cm) were collected prior to biofouling removal on December 12, 2016 (Pre-Removal) and on March 30, 2017 after the ship was towed from Sinclair Inlet (Post-Removal). In addition, the Post-Removal sampling included collecting 0–25 cm core samples from the stations. The analytical chemistry results for the sediment grabs are provided in Table 13 and Table 14 presents the results obtained for the sediment core sections.

Table 11. The analytical parameters, dates collected and analyzed, holding times prior to analysis, methods used, the method reporting limit (MRL), method detection limit (MDL), and reporting units achieved for Pre-Removal sediment (A), Post-Removal sediment (B), and mussel tissue samples (C) collected during the study.

					Holding Time					
					Extraction or Analysis	Analysis				
Parameter	Date Colleted	Storage	Date Extracted	Date Analyzed	Months	Days After Extraction	Method	MRL	MDL	units
A. Pre-Removal Sediment Samples										
Cu	12/13/2016	4°±2°C	6/9/2017	6/27/2017	5.93	18	SW6010C	2.4	0.9	ug/g dry
Zn	12/13/2016	4°±2°C	6/9/2017	6/27/2017	5.93	18	SW6010C	2.5	0.5	ug/g dry
Solids	12/13/2016	4°±2°C		6/9/2017	5.93		PSEP TS			% wet
TOC	12/13/2016	4°±2°C		6/29/2017	6.60		ASTMD4129M	0.05	0.02	% dry
Grain Size	12/13/2016	4°±2°C		6/15/2017	6.13		PSEP Grain Size			% dry
B. Post-Removal Sediment Samples										
Cu Grab	3/30/2017	Frozen(-18°C)	6/9/2017	6/27/2017	2.37	18	SW6010C	1.25	0.48	ug/g dry
Zn Grab	3/30/2017	Frozen(-18°C)	6/9/2017	6/27/2017	2.37	18	SW6010C	1.56	0.31	ug/g dry
Solids	3/30/2017	Frozen(-18°C)		6/9/2017	2.37		PSEP TS			% wet
TOC	3/30/2017	Frozen(-18°C)		6/29/2017	3.03		ASTMD4129M	0.05	0.02	% dry
Grain Size	3/30/2017	Frozen(-18°C)		6/15/2017	2.57		PSEP Grain Size			% dry
Cu Core Sections	3/30/2017	Frozen(-18°C)	6/28/2017	6/29/2017	3.00	1	SW6010C	1.6	0.6	ug/g dry
Zn Core Sections	3/30/2017	Frozen(-18°C)	6/28/2017	6/29/2017	3.00	1	SW6010C	2.0	0.4	ug/g dry
AVS	3/30/2017	Frozen(-18°C)	6/28/2017	6/29/2017	3.00	1	821/R-91-100	3.55	0.97	umol/g dry
SEM Cd	3/30/2017	Frozen(-18°C)	6/28/2017	6/29/2017	3.00	1	SW6010C	0.0017	0.0006	umol/g dry
SEM Cu	3/30/2017	Frozen(-18°C)	6/28/2017	6/29/2017	3.00	1	SW6010C	0.058	0.014	umol/g dry
SEM Pb	3/30/2017	Frozen(-18°C)	6/28/2017	6/29/2017	3.00	1	SW6010C	0.009	0.004	umol/g dry
SEM Hg	3/30/2017	Frozen(-18°C)	6/28/2017	6/29/2017	3.00	1	7470A	0.00034	0.00007	umol/g dry
SEM Ni	3/30/2017	Frozen(-18°C)	6/28/2017	6/29/2017	3.00	1	SW6010C	0.009	0.003	umol/g dry
SEM Zn	3/30/2017	Frozen(-18°C)	6/28/2017	6/29/2017	3.00	1	SW6010C	0.050	0.020	umol/g dry
C. Mussel Tissue Samples										
Cu	12/10/2016	Frozen(-18°C)	7/3/2017	7/10/2017	6.83	7	SW6010C	0.07	0.04	ug/g wet
Zn	12/10/2016	Frozen(-18°C)	7/3/2017	7/10/2017	6.83	7	SW6010C	0.07	0.04	ug/g wet
Solids	12/10/2016	Frozen(-18°C)	6/30/2017	6/30/2017	6.73		FreezeDry			% wet
Lipids	12/10/2016	Frozen(-18°C)	6/29/2017	7/6/2017	6.70	7	NLIPIDS	0.16		% wet

Table 12. The results of AVS analysis of six replicate aliquots conducted to determine laboratory proficiency and accuracy.

Replicate	AVS μmol/g wet
CV62AVS-19 Field SRM1	12.7
CV62AVS-19 Field SRM2	10.7
CV62AVS-19 Field SRM3	9.2
CV62AVS-19 Field SRM4	11.3
CV62AVS-19 Field SRM5	9.7
CV62AVS-19 Field SRM6	22.6
Mean (μ)	12.7
Standard Deviation (σ)	5.0
CoefV*	41%

Table 13. Analytical chemistry results for Pre-Removal (A) and Post-Removal (B) 0-10 cm surface samples. Data are shown for the concentration of metals (Cu and Zn), and percent of gravel, sand, clay, very coarse sand (VCSa), coarse sand (CSa), medium sand (MSa), very fine sand (VFSa), fines (Fines = Silt + Clay), total organic carbon (TOC), and solids in each sample. The results of the field duplicate are also provided to assess field variability. The results are on a dry weight basis, except for solids, which are on a wet-weight basis.

A. Surface sampling (0–10 cm) results Pre-Removal.

			Parameter													
		Collection	Cu	Zn	Gravel	Sand	Silt	Clay	VCSa	CSa	MSa	FSa	VFSa	Fines	TOC	Solids
Location_ID	Sample_ID	Date	ug/g		percent											
CV62-1	CV62SED-01	12/13/2016	200	229	0.00	1.50	68.37	33.04	0.1	0.2	0.2	0.2	0.8	98.5	3.78	22.40
CV62-2	CV62SED-02	12/13/2016	170	420	32.34	14.98	34.03	13.68	3.9	2.0	1.2	1.8	6.0	50.2	2.73	41.20
CV62-3	CV62SED-03	12/13/2016	180	203	0.98	4.28	69.41	28.53	1.0	0.4	0.6	0.4	1.8	94.9	3.30	27.00
CV62-4	CV62SED-04	12/13/2016	146	163	0.36	2.07	72.58	26.27	0.4	0.2	0.2	0.2	1.2	97.6	3.23	27.70
CV62-5[1]	CV62SED-05,06	12/13/2016	207	181	0.28	2.07	69.23	30.94	0.3	0.2	0.2	0.4	1.0	97.7	3.89	23.45
CV62-6	CV62SED-07	12/13/2016	206	215	1.83	2.72	69.85	31.43	0.5	0.4	0.2	0.5	1.2	95.7	3.54	28.90
		n	6	6	6	6	6	6	6	6	6	6	6	6	6	6
		min	146.0	163.0	0.0	1.5	34.0	13.7	0.1	0.2	0.2	0.2	0.8	50.2	2.7	22.4
		mean	184.8	235.2	6.0	4.6	63.9	27.3	1.0	0.6	0.4	0.6	2.0	89.1	3.4	28.4
		stdev	24.1	93.6	12.9	5.2	14.7	7.1	1.5	0.7	0.4	0.6	2.0	19.1	0.4	6.7
		max	206.5	420.0	32.3	15.0	72.6	33.0	3.9	2.0	1.2	1.8	6.0	98.5	3.9	41.2
		CV*	13.6%	41.5%	226.0%	117.1%	24.0%	27.0%	147.2%	131.1%	101.9%	110.1%	104.3%	22.3%	12.9%	24.7%
Field Duplicate																
CV62-5	CV62SED-05	12/13/2016	242	175	0.15	1.67	70.53	32.24	0.23	0.19	0.19	0.21	0.85	102.8	3.89	22.8
CV62-5DUP	CV62SED-06	12/13/2016	171	187	0.40	2.47	67.92	29.64	0.27	0.24	0.17	0.56	1.23	97.6	3.88	24.1
		RPD	34.38%	6.63%	90.91%	38.65%	3.77%	8.40%	16.00%	23.26%	11.11%	90.91%	36.54%	5.20%	0.26%	5.54%

[1] Average of Field Duplicates

B. Surface sampling (0–10 cm) results Post-Removal.

			Parameter													
		Collection	Cu	Zn	Gravel	Sand	Silt	Clay	VCSa	CSa	MSa	FSa	VFSa	Fines	TOC	Solids
Location_ID	Sample_ID	Date	ug/g		percent											
CV62-1	CV62SED-51	3/30/2017	240	208	0	2.36	76.34	31.39	0.0	0.1	0.3	0.6	1.4	97.9	3.72	11.80
CV62-2	CV62SED-52	3/30/2017	82	245	60.09	28.46	6.93	3.27	9.3	8.8	7.1	1.5	1.7	10.3	0.59	62.00
CV62-3	CV62SED-53	3/30/2017	778	314	19.12	9.47	55.74	22.65	2.9	1.4	1.3	1.7	2.2	73.3	3.60	27.60
CV62-4	CV62SED-54	3/30/2017	169	1200	1.44	2.03	75.41	24.31	0.4	0.2	0.2	0.3	0.9	96.6	3.34	26.10
CV62-5	CV62SED-55	3/30/2017	432	242	1.21	5.42	67.09	27.60	0.4	0.4	0.4	1.3	3.0	93.5	3.50	27.90
CV62-6[1]	CV62SED-56,57	3/30/2017	472	252	8.93	6.41	60.63	27.79	2.6	1.2	0.7	0.7	1.3	85.2	4.28	24.25
		n	6	6	6	6	6	6	6	6	6	6	6	6	6	6
		min	82.0	208.0	0.0	2.0	6.9	3.3	0.0	0.1	0.2	0.3	0.9	10.3	0.6	11.8
		mean	362.1	410.2	15.1	9.0	57.0	22.8	2.6	2.0	1.7	1.0	1.7	76.1	3.2	29.9
		stdev	253.2	388.5	23.2	9.9	25.8	10.1	3.5	3.4	2.7	0.6	0.7	33.5	1.3	16.8
		max	778.0	1200.0	60.1	28.5	76.3	31.4	9.3	8.8	7.1	1.7	3.0	97.9	4.3	62.0
		CV*	72.8%	98.7%	159.6%	114.4%	47.2%	45.9%	139.6%	172.3%	170.4%	59.7%	44.1%	45.8%	42.8%	58.5%
Field Duplicate																
CV62-6	CV62SED-56	3/30/2017	678	284	9.67	4.16	58.74	27.09	1.35	0.72	0.47	0.45	1.17	85.8	4.11	25.3
CV62-6DUP	CV62SED-57	3/30/2017	265	220	8.19	8.65	62.51	28.49	3.85	1.66	0.89	0.89	1.36	91.0	4.44	23.2
		RPD	87.59%	25.40%	16.57%	70.10%	6.22%	5.04%	96.15%	78.99%	61.76%	65.67%	15.02%	5.85%	7.72%	8.66%

[1] Average of Field Duplicates

Table 14. Results for individual core sections (A), core sections averaged for 0–10 cm depth interval (B), core sections averaged for depth > 10 cm (C) of AVS, SEM metals, TOC, solids, and total Cu and Zn. Shaded cells indicate parameter not detected at the MDL shown.

A. Core section results Post-Removal.

	Core Depth	Avg Depth	AVS	SEM_Cd	SEM_Cu	SEM_Pb	SEM_Hg	SEM_Ni	SEM_Zn	ΣSEM	ΣSEM-AVS	(ΣSEM-AVS)/foc	TOC	Solids	Cu	Zn
Location	cm	cm	µmol/g dry weight									µmol/g OC	% dry	% wet	µg/g dry weight	
CV62-1	0	1.5	0.11	0.021	1.80	0.262	0.00012	0.176	2.42	4.68	4.57	120.24	3.80	19.0	184	186
	3	4.5	7.30	0.016	1.25	0.253	0.00006	0.101	2.40	4.02	-3.28	-85.63	3.83	23.8	268	279
	6	8	0.41	0.021	1.86	0.326	0.00005	0.121	3.22	5.55	5.14	135.56	3.79	27.9	250	296
	10	12.5	15.20	0.013	0.72	0.216	0.00006	0.089	1.83	2.87	-12.33	-322.02	3.83	25.4	232	261
	15	17.5	12.40	0.015	1.31	0.197	0.00007	0.111	1.84	3.47	-8.93	-293.64	3.04	33.1	171	156
	20	23	16.50	0.010	0.59	0.203	0.00006	0.080	1.71	2.59	-13.91	-374.92	3.71	26.8	240	257
	26															
CV62-2	0	2	46.00	0.011	0.33	0.189	0.00006	0.078	1.82	2.42	-43.58	-1174.56	3.71	25.0	236	287
	4	7	2.33	0.007	0.68	2.020	0.00004	0.098	8.25	11.05	8.72	1207.79	0.72	75.7	106	248
	10															
CV62-3	0	2.5	4.80	0.005	0.37	0.899	0.00004	0.135	17.00	18.41	13.61	3057.47	0.45	83.9	127	585
	5	7.5	13.00	0.059	7.27	0.213	0.00009	0.124	21.40	29.07	16.07	586.37	2.74	24.4	23400	7400
	10	12	45.00	0.017	0.36	0.142	0.00006	0.110	4.94	5.57	-39.43	-1355.01	2.91	37.1	201	257
	14															
CV62-4	0	1.5	18.20	0.021	2.31	0.166	0.00006	0.104	6.70	9.30	-8.90	-436.22	2.04	40.2	183	227
	3	4.5	0.05	0.012	1.05	0.165	0.00009	0.084	1.35	2.66	2.61	82.89	3.15	18.5	124	137
	6	8	0.06	0.012	1.40	0.156	0.00012	0.098	1.55	3.22	3.16	100.20	3.15	22.5	163	165
	10	12.5	0.26	0.012	1.24	0.165	0.00006	0.213	1.53	3.16	2.90	92.06	3.15	24.3	376	151
	15	19	25.10	0.015	0.81	0.187	0.00006	0.114	1.54	2.67	-22.43	-686.01	3.27	26.0	147	147
	23															
CV62-5	0	2.5	45.20	0.011	0.25	0.139	0.00006	0.092	1.63	2.12	-43.08	-1398.57	3.08	28.2	138	232
	5	7.5	8.70	0.019	2.53	0.213	0.00010	0.130	3.36	6.25	-2.45	-55.12	4.44	24.7	1510	287
	10	14.5	41.60	0.018	1.09	0.222	0.00008	0.126	2.08	3.54	-38.06	-1093.78	3.48	30.0	240	203
	19															
CV62-6	0	2.5	64.00	0.011	0.50	0.424	0.00005	0.147	16.30	17.38	-46.62	-1857.35	2.51	42.7	1810	282
	5	7.5	10.30	0.009	0.87	0.151	0.00007	0.078	1.34	2.45	-7.85	-487.66	1.61	38.7	163	121
	10	12.5	24.30	0.012	1.22	0.156	0.00006	0.106	1.77	3.26	-21.04	-703.54	2.99	36.8	354	173
	15	17.5	32.50	0.019	1.64	0.261	0.00009	0.151	2.35	4.42	-28.08	-630.98	4.45	25.7	452	264
	20															

	Summary	AVS	SEM_Cd	SEM_Cu	SEM_Pb	SEM_Hg	SEM_Ni	SEM_Zn	ΣSEM	ΣSEM-AVS	(ΣSEM-AVS)/foc	TOC	Solids	Cu	Zn
	n	23	23	23	23	23	23	23	23	23	23	23	23	23	23
	min	0.05	0.005	0.25	0.14	0.00004	0.08	1.34	2.12	-46.62	-1857.35	0.45	18.50	106	121
	average	18.84	0.016	1.37	0.32		0.12	4.71	6.53	-12.31	-242.28	3.04	33.06	1351	548
	median	13.00	0.013	1.09	0.20		0.11	2.08	3.54	-8.90	-322.02	3.15	26.80	232	248
	max	64.00	0.059	7.27	2.02	0.00012	0.21	21.40	29.07	16.07	3057.47	4.45	83.90	23400	7400

Table 14. Results for individual core sections ... (Continued).

B. Core section results Post-Removal average for 0–10 cm depth.

B. Average for 0-10 cm depth

	AVS	SEM_Cd	SEM_Cu	SEM_Pb	SEM_Hg	SEM_Ni	SEM_Zn	ΣSEM	ΣSEM-AVS	(ΣSEM-AVS)/foc	TOC	Solids	Cu	Zn
Location	**umol/g dry weight**									**umol/g OC**	**% dry**	**% wet**	**ug/g dry weight**	
CV62-1	2.61	0.019	1.64	0.28		0.13	2.68	4.75	2.14	56.73	3.81	23.57	234	254
CV62-2	24.17	0.009	0.50	1.10		0.09	5.04	6.74	-17.43	16.61	2.22	50.35	171	268
CV62-3	8.90	0.032	3.82	0.56		0.13	19.20	23.74	14.84	1821.92	1.59	54.15	11764	3993
CV62-4	6.10	0.015	1.59	0.16		0.10	3.20	5.06	-1.04	-84.38	2.78	27.07	157	176
CV62-5	26.95	0.015	1.39	0.18		0.11	2.50	4.19	-22.76	-726.85	3.76	26.45	824	260
CV62-6	37.15	0.010	0.68	0.29		0.11	8.82	9.91	-27.24	-1172.51	2.06	40.70	987	202
Overall average 0-10 cm	17.65	0.017	1.60	0.43		0.11	6.91	9.06	-8.58	-14.75	2.70	37.05	2356	859
Overall stdev 0-10 cm	13.75	0.008	1.18	0.36		0.02	6.47	7.48	16.42	1022.92	0.92	13.24	4623	1536

C. Core section results Post-Removal average for > 10 cm depth.

		AVS	SEM_Cd	SEM_Cu	SEM_Pb	SEM_Hg	SEM_Ni	SEM_Zn	ΣSEM	ΣSEM-AVS	(ΣSEM-AVS)/foc	TOC	Solids	Cu	Zn
Location	**Max Depth cm**	**umol/g dry weight**									**umol/g OC**	**% dry**	**% wet**	**ug/g dry weight**	
CV62-1	26	14.70	0.013	0.87	0.21		0.09	1.79	2.98	-11.72	-330.19	3.53	28.43	214	225
CV62-2															
CV62-3	14	45.00	0.017	0.36	0.14		0.11	4.94	5.57	-39.43	-1355.01	2.91	37.10	201	257
CV62-4	23	12.68	0.013	1.03	0.18		0.16	1.54	2.91	-9.77	-296.97	3.21	25.15	262	149
CV62-5	19	41.60	0.018	1.09	0.22		0.13	2.08	3.54	-38.06	-1093.78	3.48	30.00	240	203
CV62-6	20	28.40	0.016	1.43	0.21		0.13	2.06	3.84	-24.56	-667.26	3.72	31.25	403	219
Avg Core depth	20.4														
Overall average >10 cm		28.48	0.015	0.96	0.19		0.12	2.48	3.77	-24.71	-748.64	3.37	30.39	264	210
Overall stdev >10 cm		14.87	0.002	0.39	0.03		0.03	1.39	1.08	14.03	467.05	0.31	4.39	81	40

3.2 PRE-AND POST-REMOVAL SEDIMENT SURFACE SAMPLES

The sediment surface samples were collected to document conditions in the surface sediment before and after biofouling removal. The Pre-Removal surface samples collected by ponar grabs were observed to be very soft, unconsolidated material, with a black jelly-like consistency, smelling strongly of sulfide, and no evidence of any oxidation or bioturbation. Shell material and other debris were also observed in the samples. Samples with similar consistency were reported by the divers when they collected the Post-Removal surface samples and cores. The divers encountered shell hash and other debris on the sea floor which was recorded on the video feed. While different sampling methods were used for the Pre- (ponar grab) and Post-Removal (diver-collected 0–10 cm core) sampling events, both methods provided equivalent results as they uniformly sampled the 0–10 cm surface layer present. The advantage of using divers to collect the samples Post-Removal was that the divers were able to avoid any obstruction from biological material that was present on the sea floor and thus, avoided problems of shell and debris that could prevent the ponar-sampler from obtaining an intact sample by not closing properly. Both sampling methods met the Data Quality Objective of collecting an undisturbed uniform sample of the 0–10 cm surface sediment layer, which was verified by the field sampling team.

The analytical chemistry results for the 0–10 cm Pre- and Post-Removal surface samples are presented in Table 13. Data are shown for the concentration of total metals (Cu and Zn), and percent of gravel, sand, clay, very coarse sand (VCSa), coarse sand (CSa), medium sand (MSa), very fine sand (VFSa), Fines (Fines = (Silt + Clay)/(Gravel + Sand + Silt + Clay)), total organic carbon (TOC), and Solids in each sample. The results of the field duplicates were used to assess field variability; the RPD reported indicates the range of difference needed to differentiate one sample from another. There were three stations (CV62-3, CV62-5, and CV62-6) where the Post-Removal total Cu concentration was greater than Pre-Removal concentration at levels above the RPD for field duplicates and these stations exceeded the Maximum Criteria Concentration (MCC) benchmark for Cu (Figure 7A). For total Zn, four stations (CV62-3, CV62-4, CV62-5, and CV62-6) had Post-Removal concentrations that were greater than Pre-Removal concentrations at levels above the RPD for field duplicates, but only CV62-4 exceeded the MCC benchmark for Zn (Figure 7B).

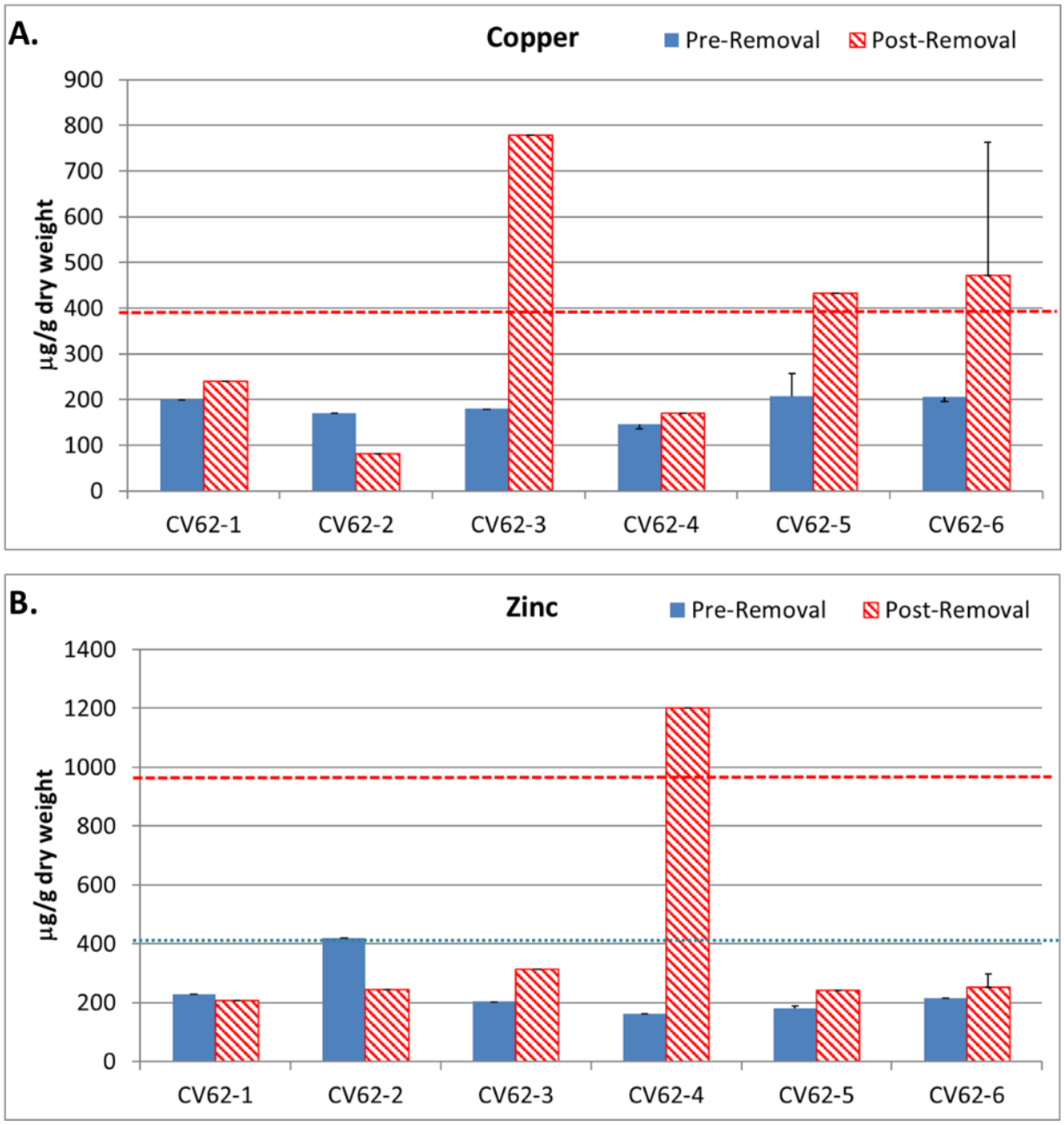

Figure 7. The concentration of total Cu (A) and Zn (B) measured in Pre- and Post-Removal 0–10 cm surface samples collected at each station location. The sediment quality benchmarks are shown for the Sediment Quality Standard (SQS, green dotted line) and Maximum Chemical Criteria (MCC, dashed red lines). Error bars are standard deviation of field duplicates.

On average, Pre-Removal grab samples contained lower total Cu (average 191 µg/g Cu dry weight) and lower total Zn (average 234 µg/g Zn dry weight) than the Post-Removal concentrations (average 397 µg/g Cu dry weight and 416 µg/g Zn dry weight). However, these differences were not statistically significant at $p \leq 0.05$ confidence level (Table 15). The lack of statistical significance between the two sampling periods was primarily due to the high variability of the results for both sampling periods (Figure 8). Significance tests calculated for the $ANOVA_{Normal}$ (Equation 4), $ANOVA_{Lognormal}$ (Equation 5), and nonparametric KW (Equation 6) distributions were all in agreement (Table 15). The difference in Zn concentrations Pre- and Post- were statistically significant at the $p \leq 0.10$ level, the difference was driven by the Post-Removal sample from CV62-4 that was much higher than all the other samples (Figure 7B, Figure 8B).

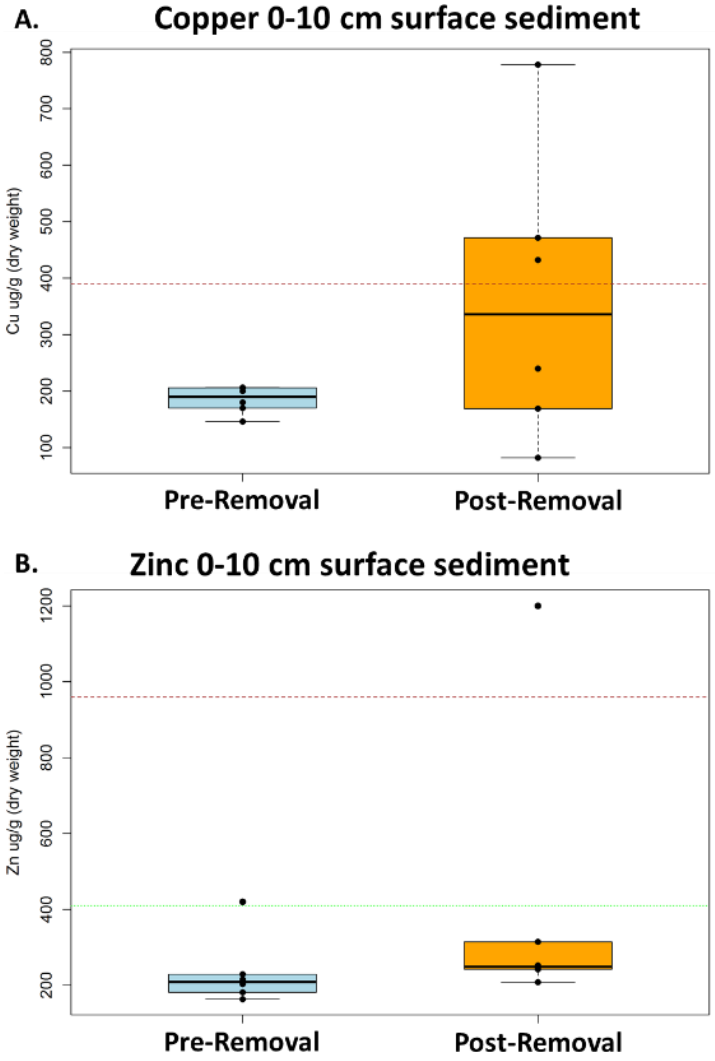

Figure 8. Box and whisker plot for concentrations of Cu (A) and Zn (B) measured in in Pre- and Post-Removal 0-10 cm surface sediment samples collected at each station location. The data (black points), Sediment Quality Standard (SQS, green dotted line), and Maximum Chemical Criteria (MCC, red dashed lines) are also shown. See Figure 6 for description of box and whisker statistical parameters.

Table 15. Results from statistical tests to determine if there were significant differences between variables measured in 0–10 cm grabs assuming normal, lognormal, and non-parametric distributions for the data. The relative difference between average Pre- and Post-Removal variables is also tabulated.

	Distribution			
	$ANOVA_{Normal}$	$ANOVA_{Lognormal}$	Non-Parametric	
Variable	**P(F)**	**P(F)**	**P(KW)**	**Difference**
Cu ug/g	0.118	0.221	0.262	2.1
Zn ug/g	0.309	0.240	**0.078**	1.8
Gravel %	0.417	0.221	0.298	2.6
Sand %	0.356	0.265	0.262	1.9
Silt %	0.583	0.461	0.631	0.9
Clay %	0.393	0.391	0.200	0.8
TOC %	0.677	0.489	0.749	0.9
Solids %	0.843	0.850	0.873	1.1

There were no statistically significant differences ($p \leq 0.05$) for TOC and Solids between sampling events (Figure 9, Figure 10, and Table 15), however, station CV62-2 had much less TOC and greater percent solids in the sample collected Post-Removal (Figure 9). There were also no statistically significant differences ($p \leq 0.05$) for the content of gravel, sand, silt, and clay between the Pre- and Post- samples (Figure 11, Figure 12, Table 15), although changes to more coarse material and less fines were evident in the samples from CV62-2, CV62-3, and CV62-6 (Figure 11).

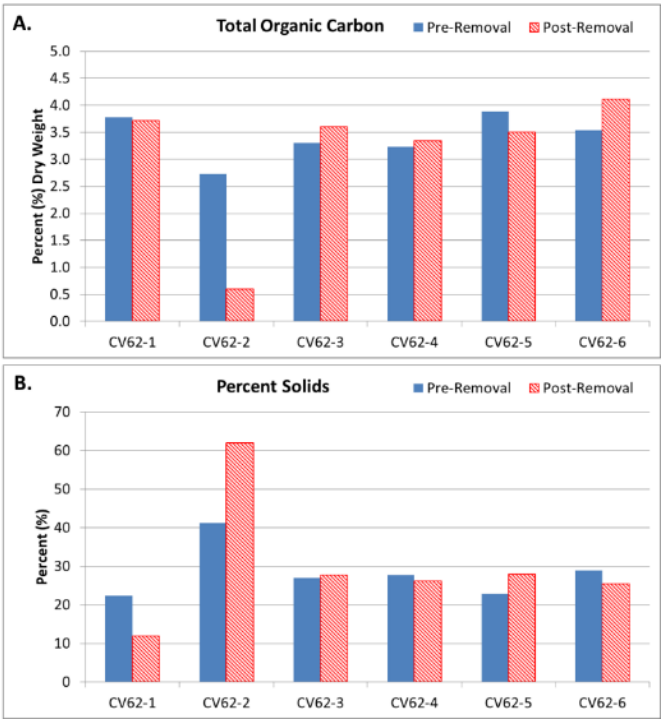

Figure 9. The percent TOC (A) and solids (B) measured in Pre- and Post-Removal 0–10 cm surface sediment samples collected at each station location.

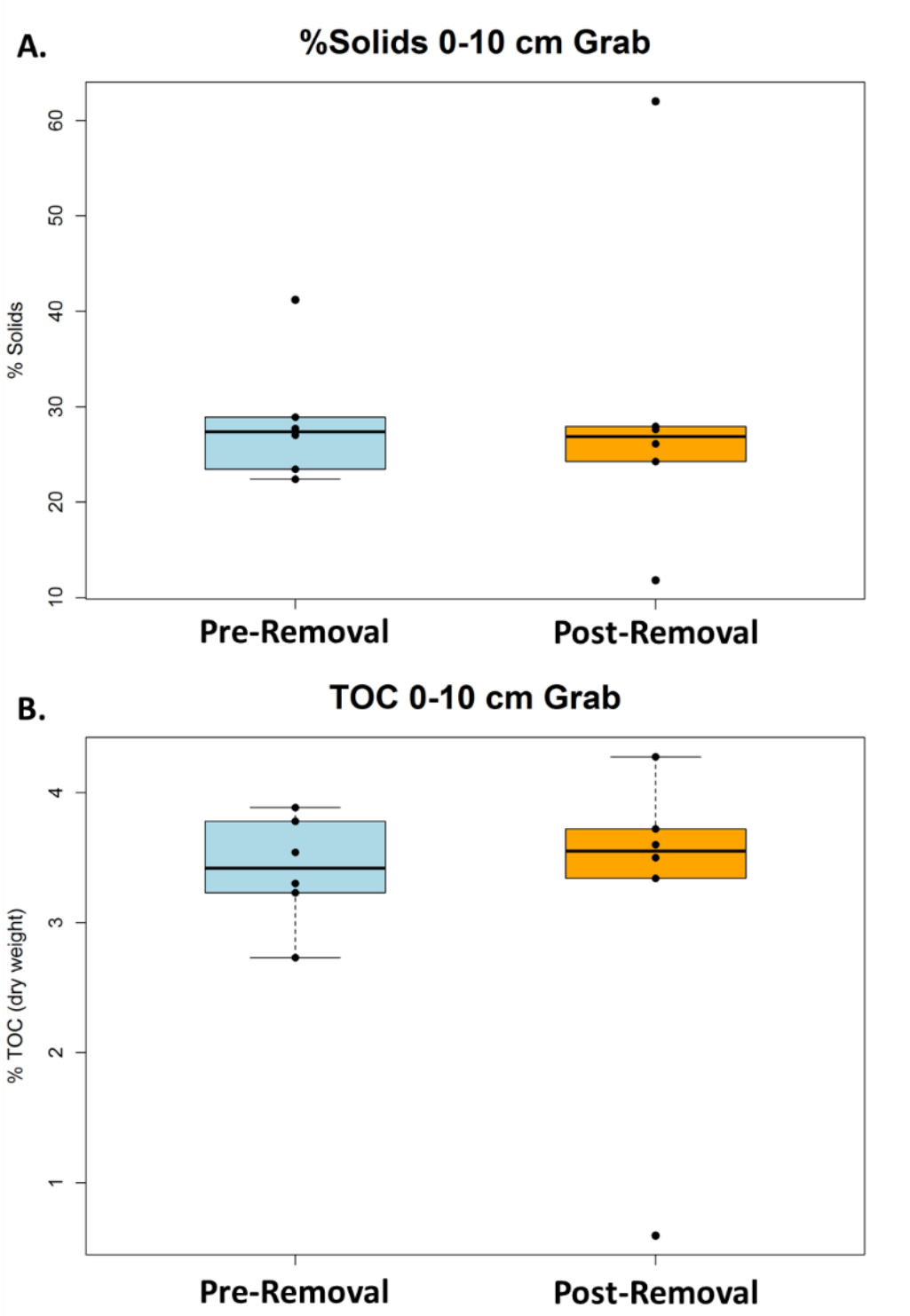

Figure 10. Box and whisker plot for percent solids (A) and TOC (B) measured in in Pre- and Post-Removal 0–10 cm surface sediment samples collected at each station location. The data (black points) are also shown. See Figure 6 for description of box and whisker statistical parameters.

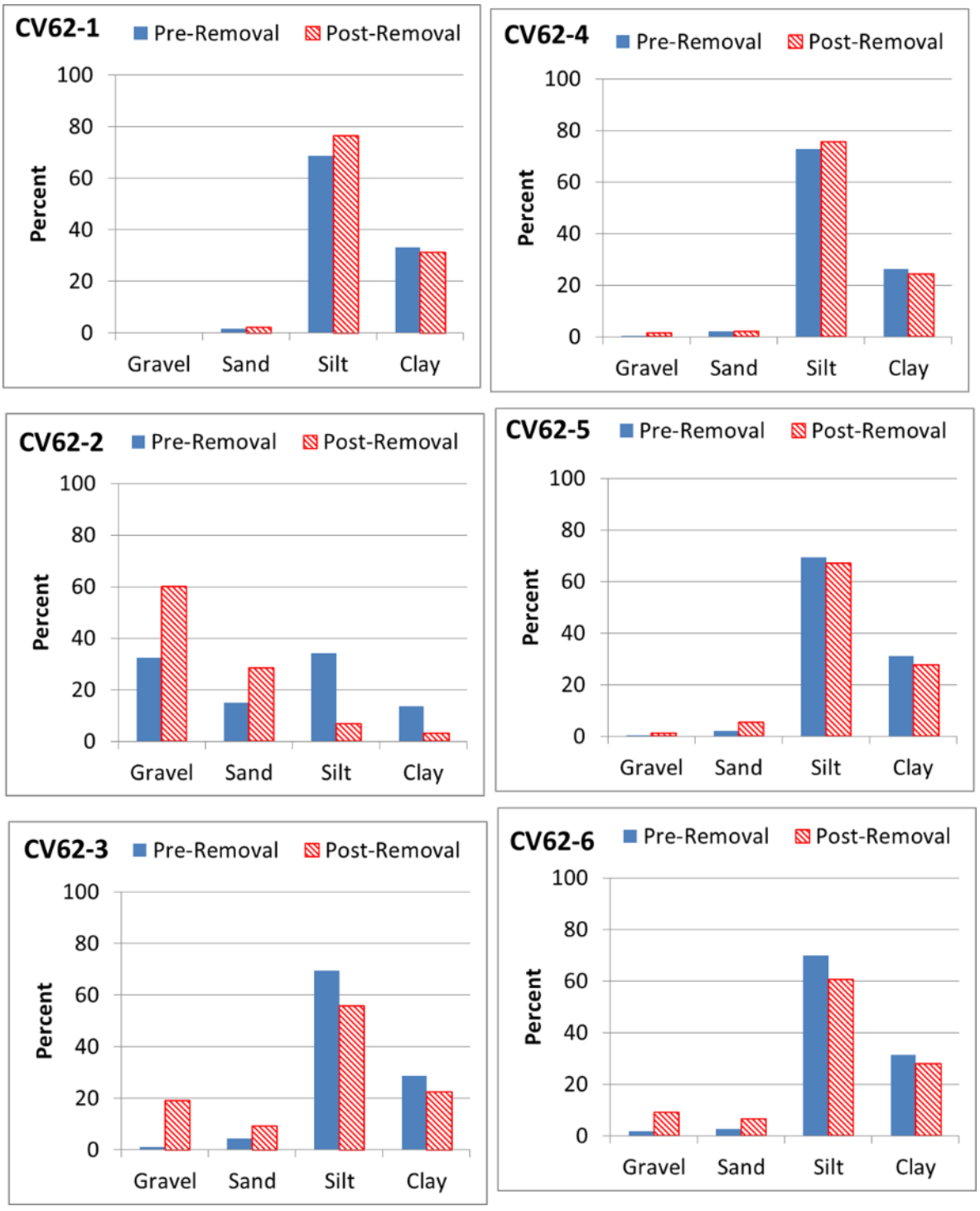

Figure 11. The percent of gravel, sand, silt, and clay measured in Pre- and Post-Removal 0–10 cm surface sediment samples collected at each station location.

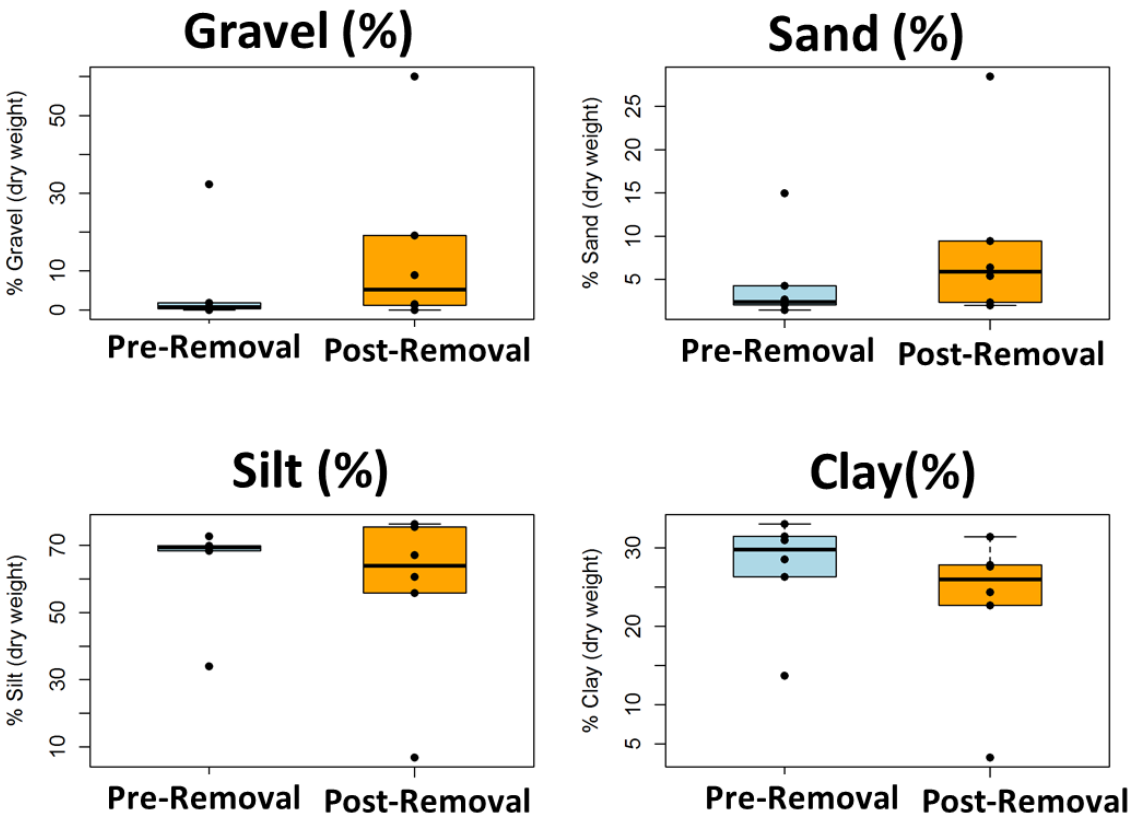

Figure 12. Box and whisker plot for percent gravel, sand, silt, and clay measured in Pre- and Post-Removal 0–10 cm surface sediment samples collected at each station location. The data (black points) are also shown. See Figure 6 for description of box and whisker statistical parameters.

The variability measured in the Post-Removal samples was much higher for total Cu and Zn, TOC, and solids, and to a lesser extent percent sand and percent fines than the Pre-Removal samples (Table 13). The RPD for field duplicates from the Post-Removal was also higher than Pre-Removal for Cu, Zn, TOC, solids and percent fines indicating that field variability had increased between sampling events (Table 13).

3.3 AVS SEDIMENT CORES

The AVS sediment cores were collected to characterize sediment conditions in the top 0–25 cm (0–10 inches) of sediment located under the former location of CV 62's hull, evaluate metal bioavailability, and assess the potential for metal exposure to cause adverse effects to benthic organisms. The AVS cores that were sectioned following collection were observed to be similar to the grab samples in that the material was soft, unconsolidated, black muck, with a strong sulfide-odor and no evidence of oxidation or bioturbation. The cores were interspersed with fragments of shells, rocks, and other debris that made it difficult to collect evenly sized segments. The results from chemical analysis of the individual core samples and the averages obtained for the 0–10 cm and >10 cm depth intervals for of AVS, SEM, TOC, solids, and total Cu and Zn are shown In Table 14. The chemical parameters measured were detected in all the samples except that SEM_Hg was only

detected in two samples and AVS was not detected in 2 core sections sampled from station CV62-4 (Table 14)

The concentration of AVS ranged from not detected to 46 µmol/g dry weight. The abundance of SEM metals was: SEM_Zn > SEM_Cu > SEM_Pb >> SEM_Ni >> SEM_Cd >> SEM_Hg. The ΣSEM ranged from 2.12–29.07 µmol/g dry weight. Most samples had higher AVS than SEM, however, there were notable exceptions (see below) resulting in ΣSEM – AVS > 0 for 8 out of the 23 samples (35%) analyzed (Table 14).

The TOC and Solids content in the core sections ranged from 0.5–4.5% and 18.5–83.9%, respectively (Table 14). A trend of lower TOC for higher Solids was observed for the core sections that was similar to the trend observed for the grab samples (Figure 13A). The TOC in the grab samples increased with percent fines (Figure 13B), so it is reasonable to assume a similar relationship between TOC and Fines in the core sections. Low TOC below 2% was measured in 3 of 23 (13%) of the core sections, while high TOC > 3.5% was present in 8 of 23 (35%) of the samples. The Solids content was also quite variable, about half of the samples had Solids < 25% indicating that most of the samples were very watery and loosely consolidated. There was not a strong relationship between TOC and AVS, however, AVS > 20 µmol/g dry weight only occurred when TOC > 2.5% Figure 14A). There was not a similar relationship between TOC and ΣSEM, as ΣSEM appeared to be independent of TOC (Figure 14B).

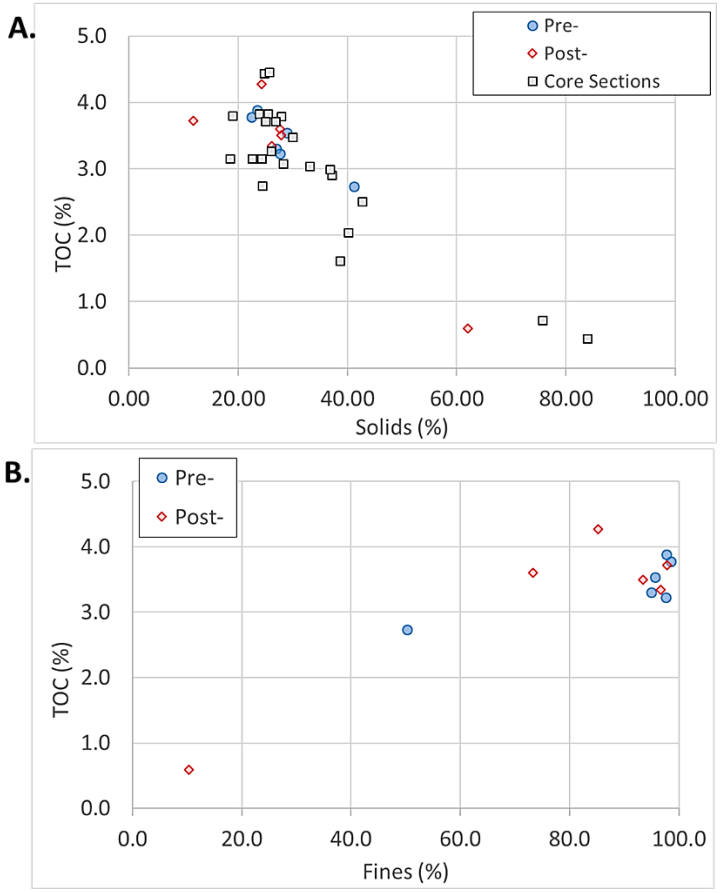

Figure 13. The relationship between TOC and solids in Pre- and Post- surface sediment samples and core sections (A) and TOC and Fines in the Pre- and Post- surface sediment samples (B).

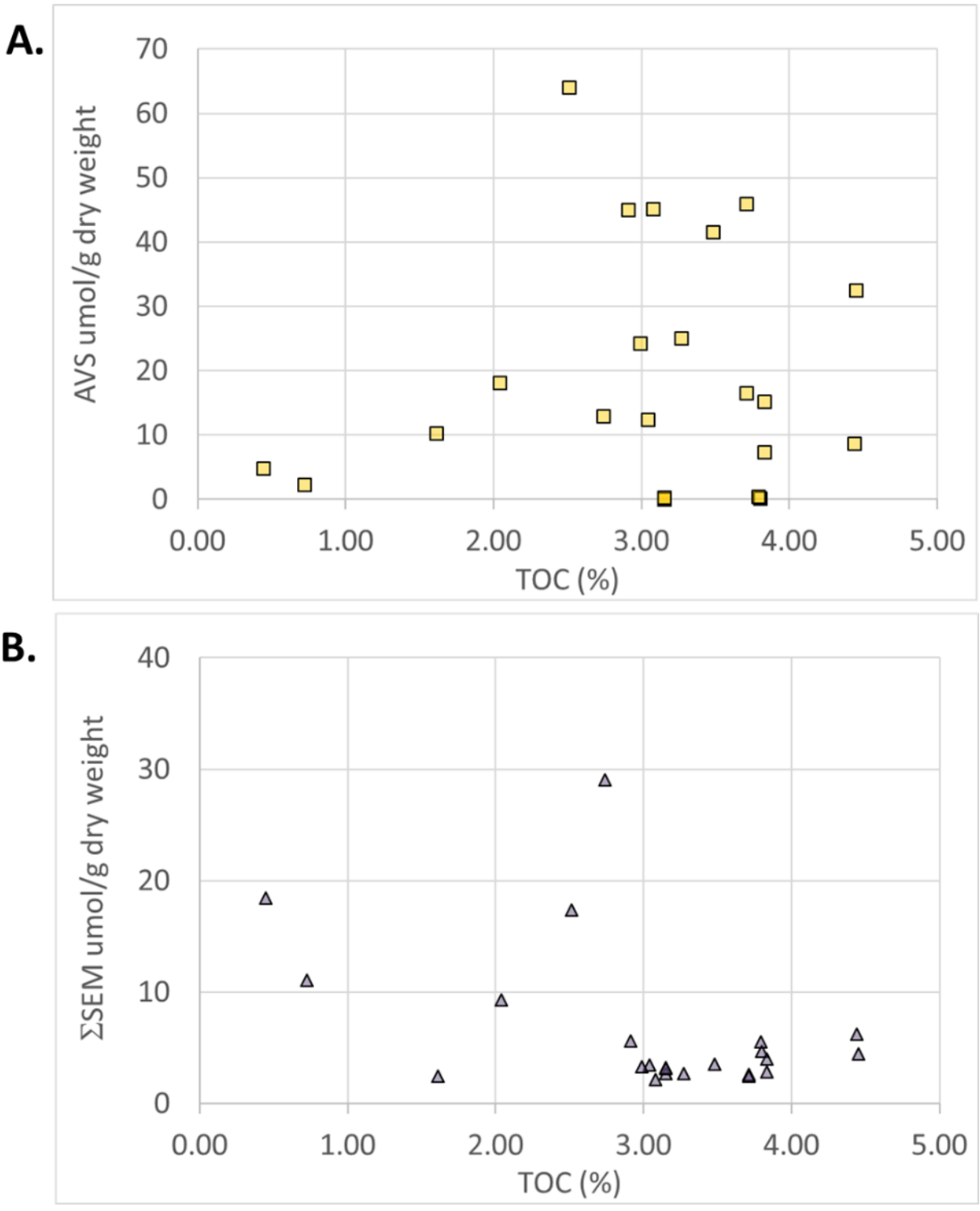

Figure 14. The relationship between TOC and AVS (A) and TOC and ΣSEM (B) measured in core sections.

There was also high variability in the total Cu and Zn measured in the core sections (Table 14). Four of the core section samples exceeded the MCC for Cu, one sample exceeded the MCC for Zn and one other sample exceeded the SQC for Zn.

The core profiles for TOC, Solids, total Cu and Zn, AVS and ΣSEM, and the individual SEM metals are plotted for each station in Figure 15–Figure 20. The core profiles for Station CV 62-1 (bow of ship) showed uniform distribution of TOC, Solids, total Cu, and total Zn with core depth (Figure 15). The AVS was low at the surface of the core (0–3 cm, 0.1 µmol/g dry weight), increased to about 10 µmol/g dry weight at 3–6 cm depth, decreased to 0.4 µmol/g dry weight for the 6–10 cm section and then remained above 12 µmol/g dry weight for the remainder of the core. The ΣSEM concentrations were relatively constant down core with the highest concentrations of SEM_Zn and SEM_Cu measured in the 6–10 cm section Figure 15.

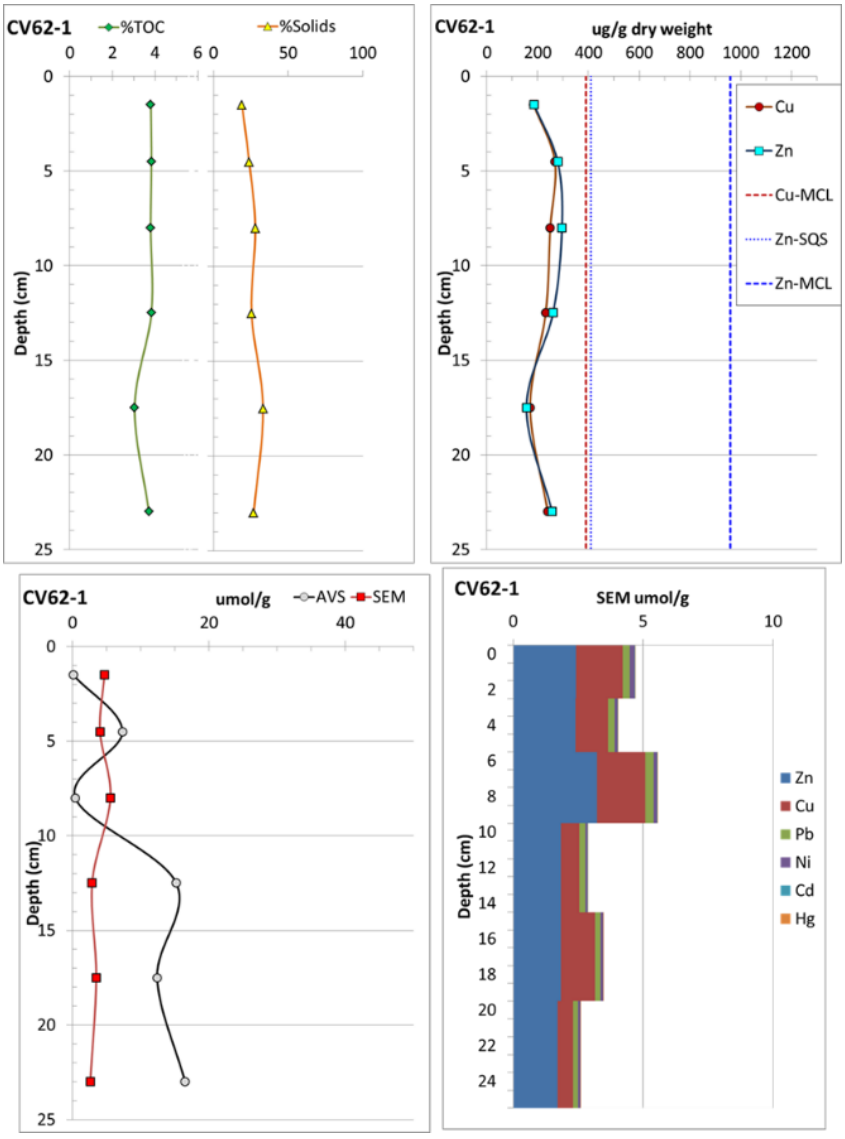

Figure 15. Core profiles for station CV62-1 showing the percent TOC and solids content (upper left panel), total Cu and Zn concentrations (upper right panel), AVS and ΣSEM concentrations (lower left panel), and the SEM metals (lower right panel).

The core collected from Station CV62-2 only penetrated 10 cm into the bottom due to the hard substrate present at the site (Figure 16). The divers reported the presence of boulders, rip rap, and other debris at this location which made it difficult to collect longer core samples. At this station TOC and AVS were much higher and Solids were much lower in the surface section (0–5 cm), while the lower section (5–10 cm) had much higher Solids content and lower TOC and AVS ((Figure 16). The SEM was also highest in the lower core section where the SEM was dominated by SEM_Zn and SEM_Pb ((Figure 16).

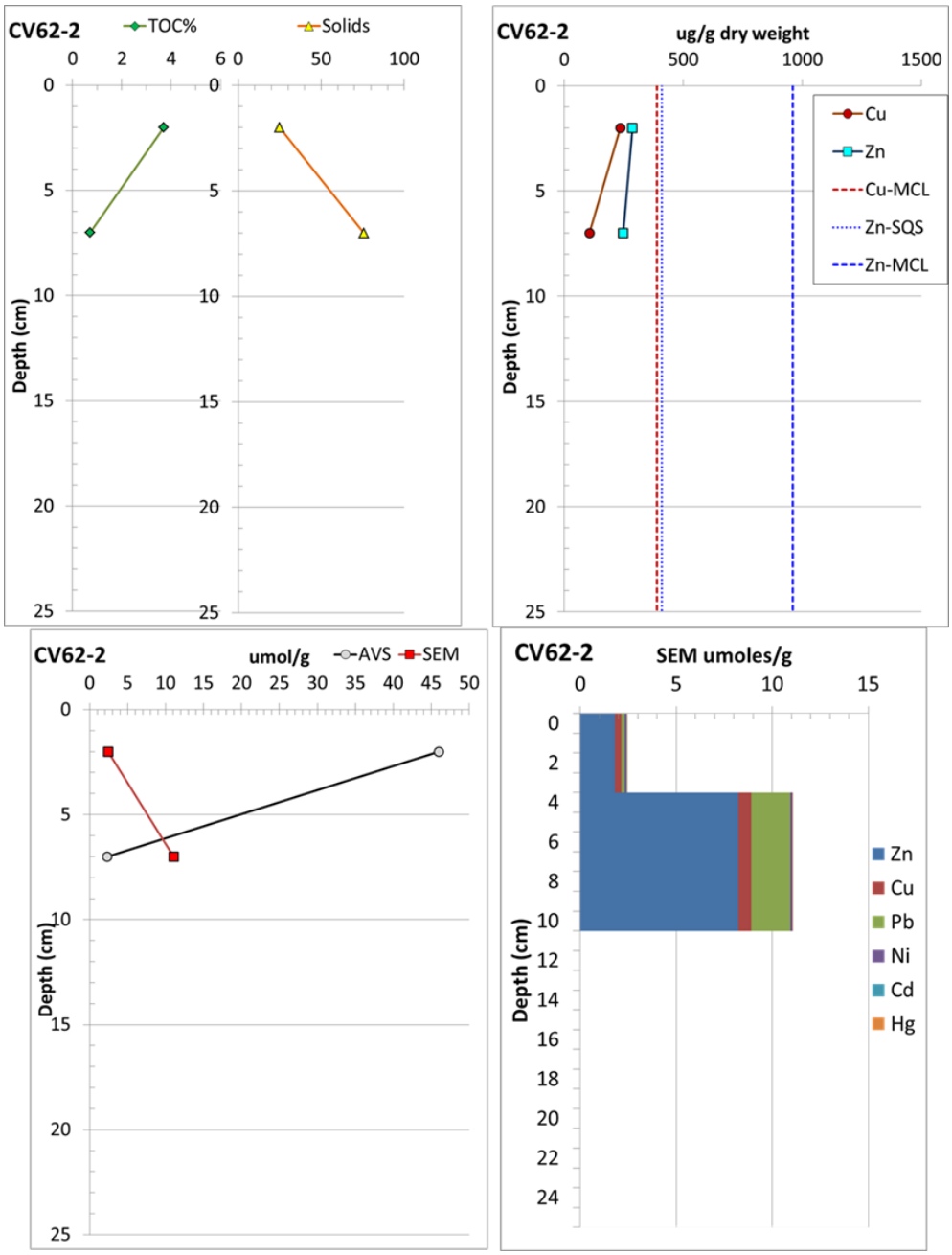

Figure 16. Core profiles for station CV62-2 showing the percent TOC and solids content (upper left panel), total Cu and Zn concentrations (upper right panel), AVS and ΣSEM concentrations (lower left panel), and the SEM metals (lower right panel).

The core profile from station CV62-3 was also limited in depth (0–14 cm, Figure 17). The TOC (0.45%) was very low in the 0–5 cm surface section while Solids was high (84%). The 5–10 cm section was very high for total Zn (7,400 µg/g dry weight) and Cu (23,400 µg/g dry weight) which was reduced in the 10–14 cm section (Figure 17). The AVS was lower than the SEM metal in the 0–5 cm and 5–10 cm sections, but AVS increased to 45 µmol/g dry weight, which was much higher than ΣSEM (5.6 µmol/g dry weight). The SEM metals were dominated by high concentrations of SEM_Zn that reached 17 and 21 µmol/g dry weight in the top two core sections, respectively, while SEM_Cu peaked at 7.3 µmol/g dry weight in the 5–10 cm section (Figure 17).

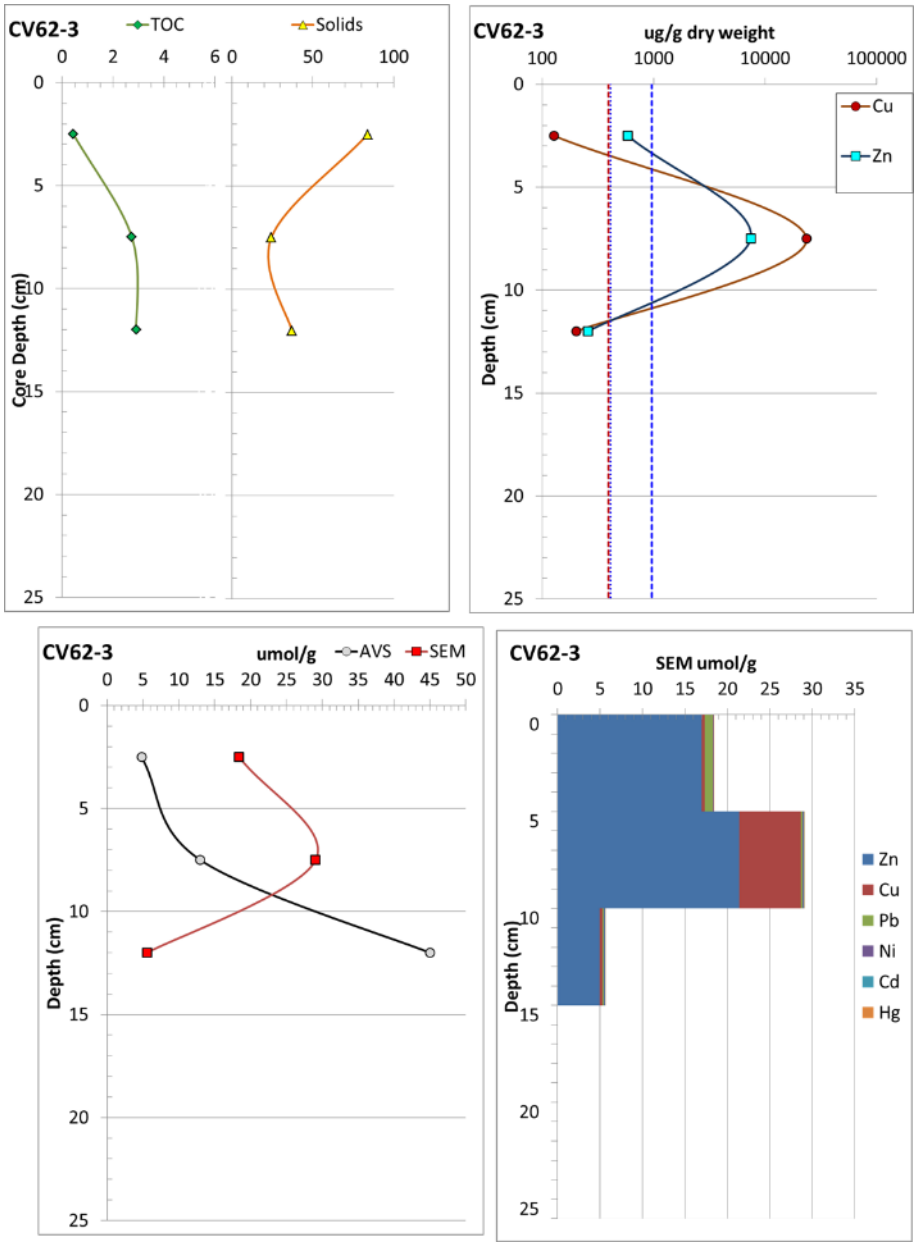

Figure 17. Core profiles for station CV62-3 showing the percent TOC and solids content (upper left panel), total Cu and Zn concentrations (upper right panel), AVS and ΣSEM concentrations (lower left panel), and the SEM metals (lower right panel).

For Station CV62-4 (stern of the vessel) the surface TOC was about 2% and then remained at 3.2% for the remainder of the core. Solids were higher at the surface (40%) but remained lower (18–26%) for the rest of the core (Figure 18). Total Cu and Zn were below the sediment quality benchmarks, while SEM metals, specifically SEM_Zn and SEM_Cu were elevated in the surface (0–3 cm) but were relatively constant for the remainder of the core (Figure 18). The AVS was high at the surface and bottom of the core, but was absent from the mid-core sections (Figure 18). The odd-looking AVS profile may be an artifact if AVS were lost during sample processing (Figure 18).

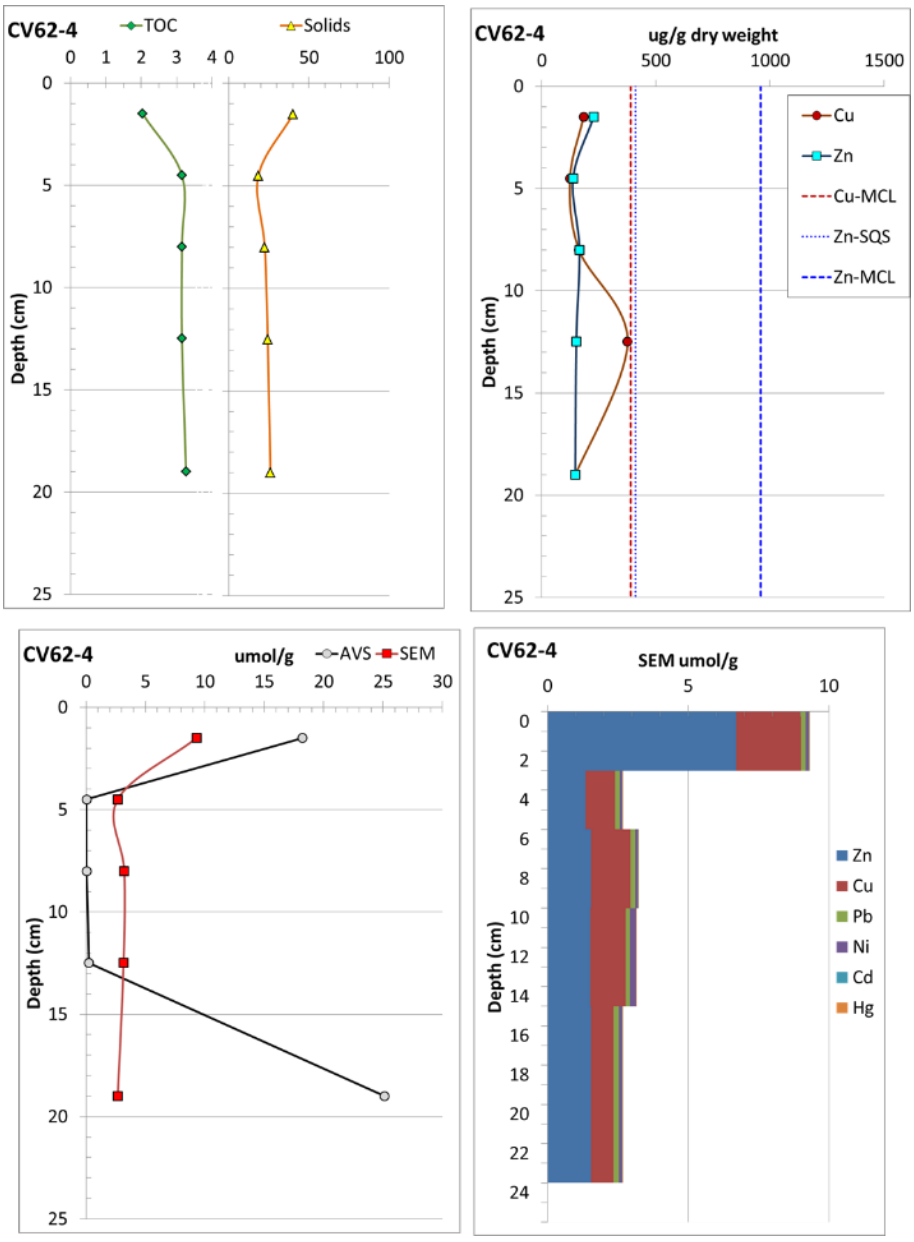

Figure 18 Core profiles for station CV62-4 showing the percent TOC and solids content (upper left panel), total Cu and Zn concentrations (upper right panel), AVS and ΣSEM concentrations (lower left panel), and the SEM metals (lower right panel).

The core profiles for Station CV62-5 showed a total Cu spike of 1500 µg/g dry weight in the 5–10 cm section (Figure 19). The AVS was higher than the ΣSEM in all the cores sections, but AVS was reduced to 8.7 µmol/g dry weight in the 5–10 cm section (Figure 19). The 5–10 cm section was also relatively elevated with SEM_Zn and SEM_Cu (3.4 and 2.5 µmol/g dry weight, respectively, (Figure 19).

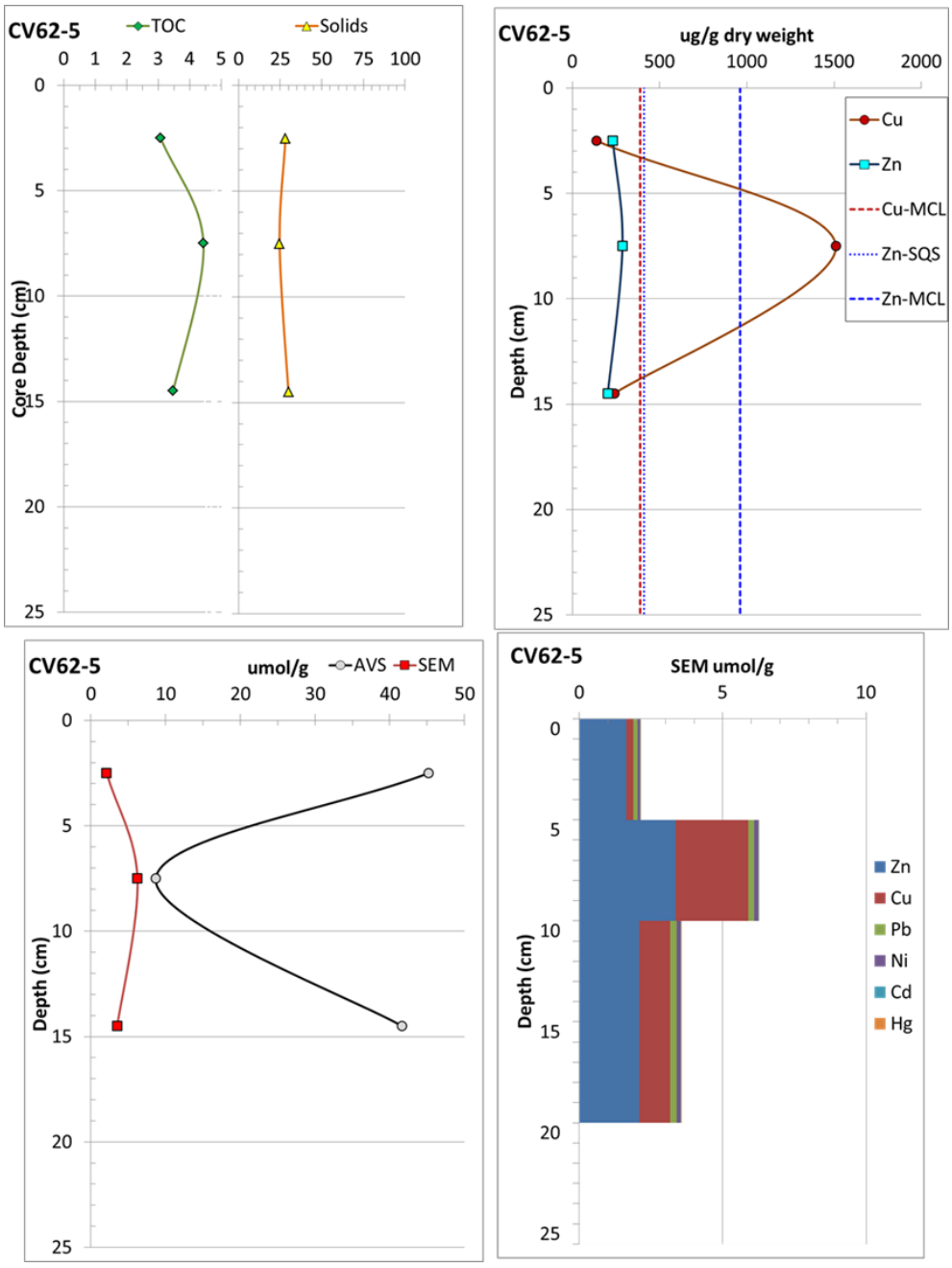

Figure 19. Core profiles for station CV62-5 showing the percent TOC and solids content (upper left panel), total Cu and Zn concentrations (upper right panel), AVS and ΣSEM concentrations (lower left panel), and the SEM metals (lower right panel).

The core profile for Station CV62-6 (portside, forward midship) showed high total Cu (1,810 µg/g dry weight) and high SEM-Zn (16.3 µmol/g dry weight) in the surface 0–5 cm section, but the AVS was greater than ΣSEM for all depth intervals (Figure 20). The SEM metals were dominated by SEM_Zn which was highest in the surface segment, but decreased to 1.3 to 2.4 µmol/g dry weight down the core (Figure 20).

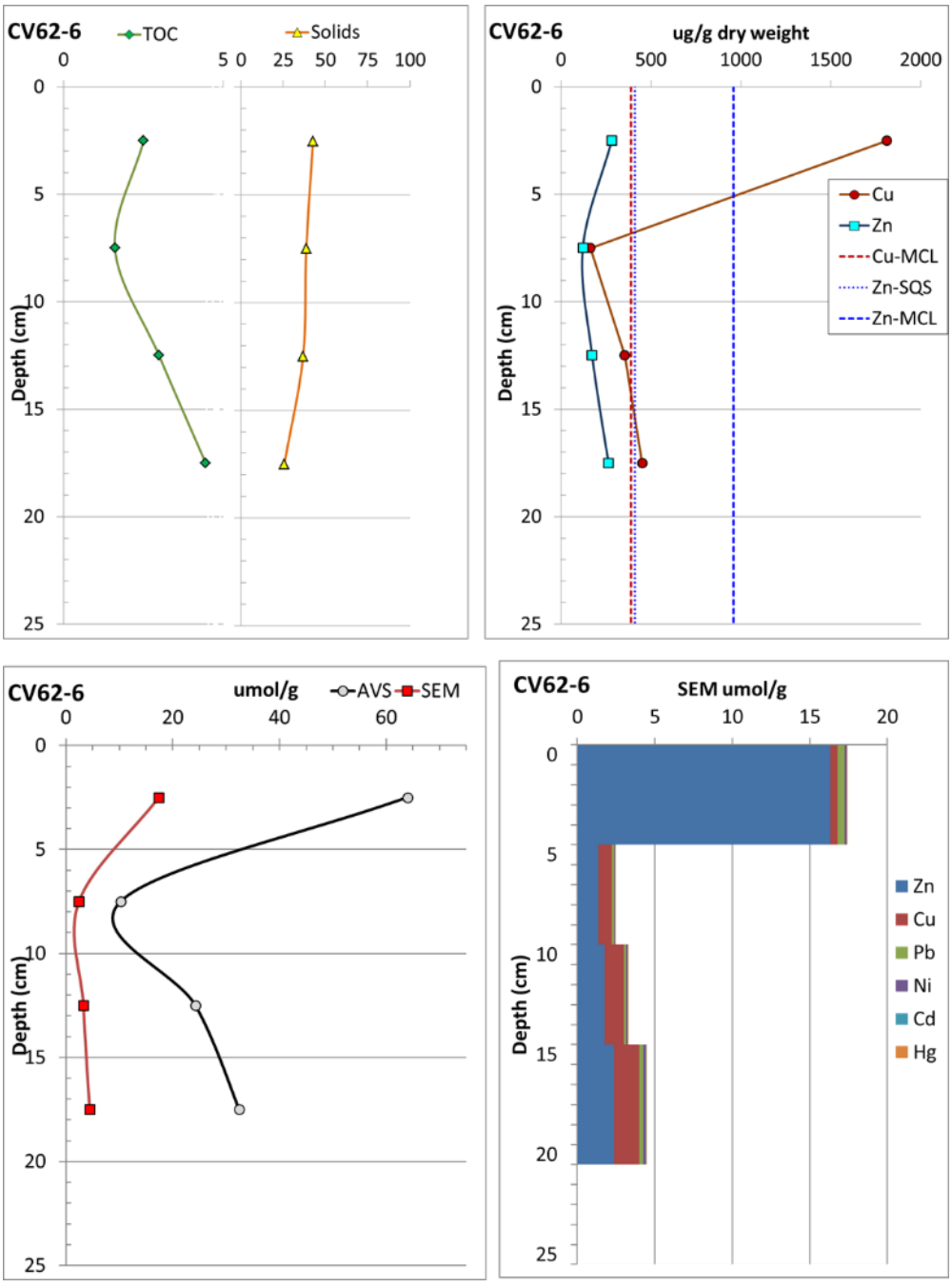

Figure 20. Core profiles for station CV62-6 showing the percent TOC and solids content (upper left panel), total Cu and Zn concentrations (upper right panel), AVS and ΣSEM concentrations (lower left panel), and the SEM metals (lower right panel).

In general, there was a weak relationship between total Cu and Zn and SEM_Cu and SEM_Zn that was driven by a few samples with very high concentrations of total metals that also had relatively high SEM (Figure 21). On average the SEM_Cu accounted for about 30% (±20%) of the total Cu, however, there were five samples in which the SEM_Zn was >> total Zn (Figure 21). This result is unexpected because the total digestion should yield more metal than the AVS procedure. This may be caused by inhomogeneity in the sample aliquots that were processed separately for the analysis. The laboratory reported high inhomogeneity in duplicate samples from the AVS core sections (see Appendix A-3. If the suspect samples with SEM_Zn >> total Zn are omitted, then SEM_Zn accounted for about 30% (± 27%) of the total Zn present (Figure 21).

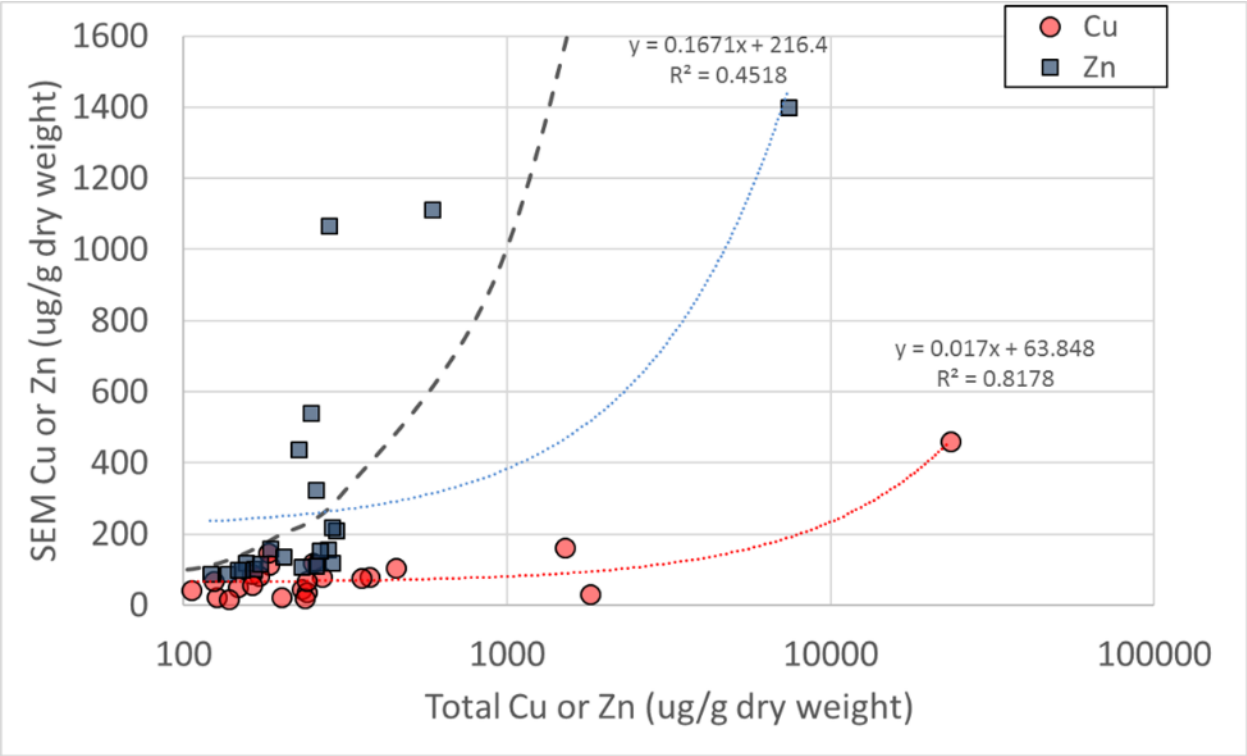

Figure 21. The relationship between total Cu and Zn (plotted on log scale) and the amount of SEM_Cu and SEM_Zn (in μg/g dry weight) measured in core sections collected Post-Removal. The regression lines for Cu (red) and Zn (blue) and the 1:1 ratio between SEM : Total Metal (dashed line) are also shown.

The sediment core data were pooled by depth to calculate the average concentrations of AVS in the 0–10 cm and >10 cm intervals Table 14B). Based on the average concentrations, only Station CV62-3 had ΣSEM concentrations significantly higher than AVS concentrations in the top 0–10 cm (Figure 22A) and all stations had AVS concentrations that were significantly higher than ΣSEM concentrations for depths > 10 cm (Figure 22B). When all the data are pooled by depth the average AVS concentration exceeded ΣSEM by about 5 and 25 μmol/g dry weight for the 0–10 cm and > 10 cm depth intervals, respectively (Table 14B, Figure 23A). When total Cu and Zn were averaged by depth, much higher concentrations of Cu and Zn were measured in the 0–10 cm surface layer (2,356 and 859 μg/g dry weight for total Cu and Zn, respectively) than in the deeper > 10 cm layer (264 and 210 μg/g dry weight for total Cu and Zn, respectively; Table 14B, Figure 23B).

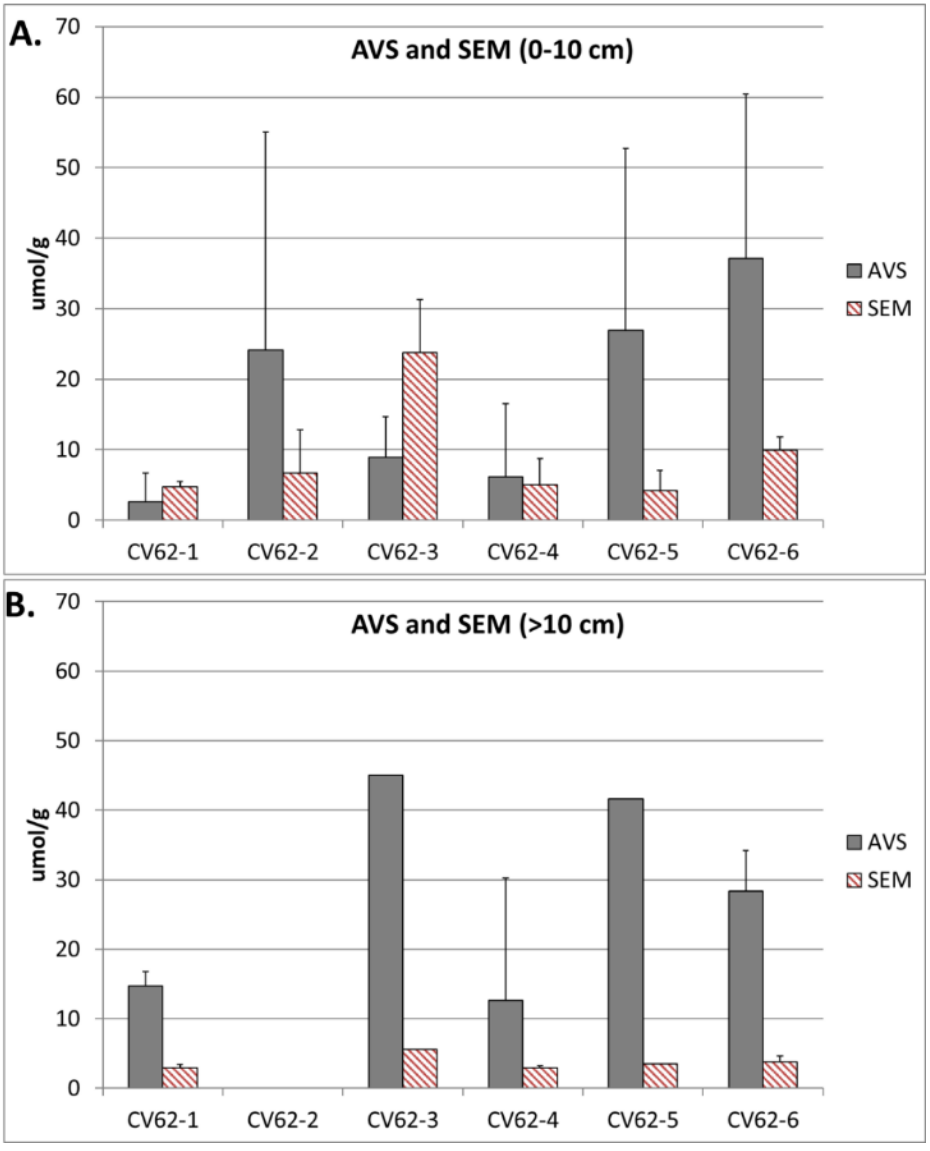

Figure 22. The average AVS and ΣSEM (error bars show standard deviation) measured in core sections from the top 0–10 cm (A) and >10 cm core depths (B) for each station.

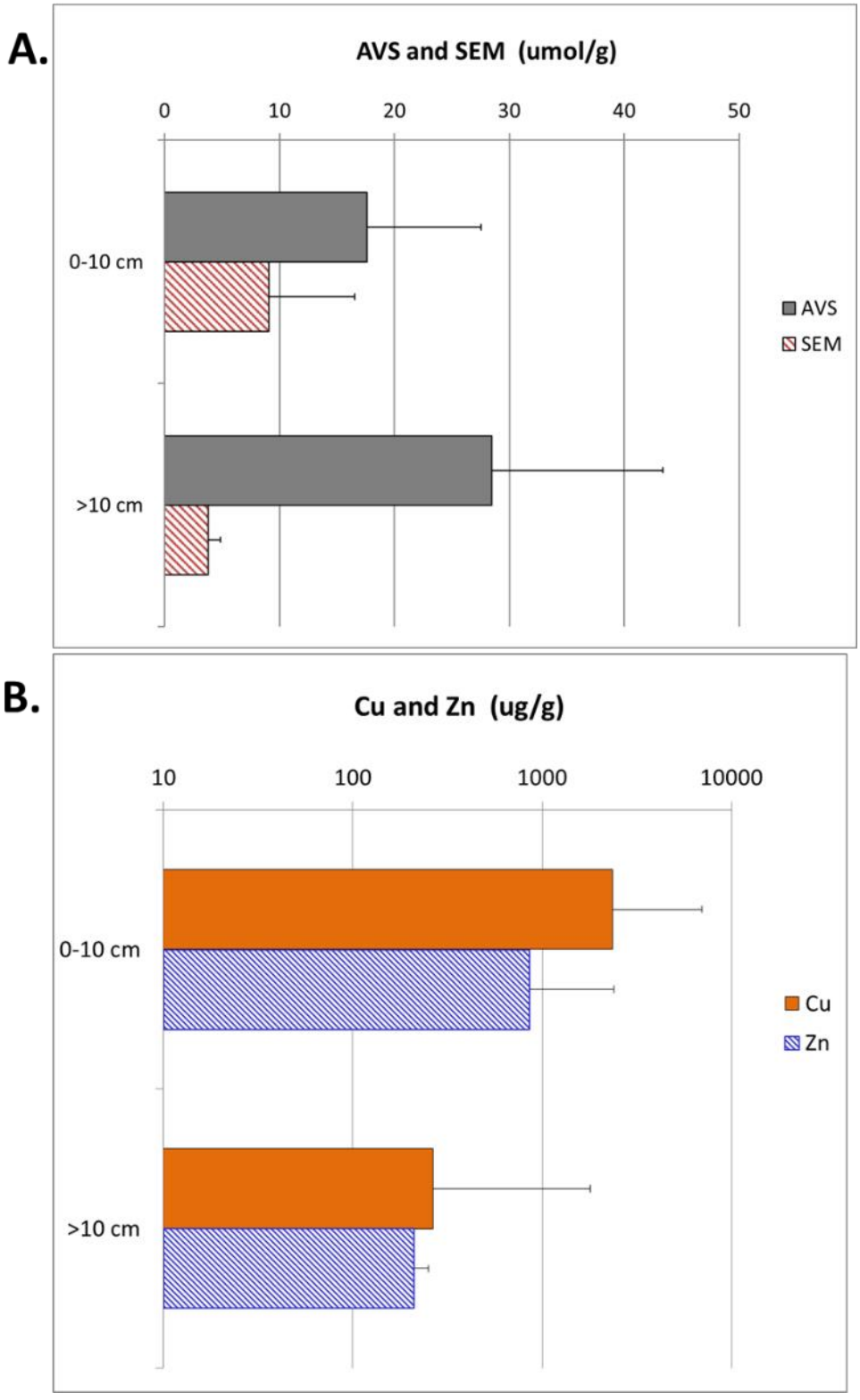

Figure 23. The average (standard deviation) of AVS and SEM (A) and total Cu and Zn (B) concentrations measured by core depth.

The sediment quality benchmarks for the protection of benthic organisms from metal exposure takes into account the ΣSEM – AVS normalized to the fraction of organic carbon (f_{OC} = TOC/100) present in the sample (US EPA, 2005). The distribution of (ΣSEM – AVS)/f_{OC} measured in all core samples as a function of depth is shown in Figure 24. Only one sample (4%) fell into the region of adverse biological effects expected (≥ 3,000 µmol/g OC, Equation 3), 3 samples (13%) were in the region that may cause biological effects (Equation 2), and most of the data (83%) were < 130 µmol/g OC (Equation 1) which therefore indicates low risk of adverse biological effects to benthic organisms (Figure 24).

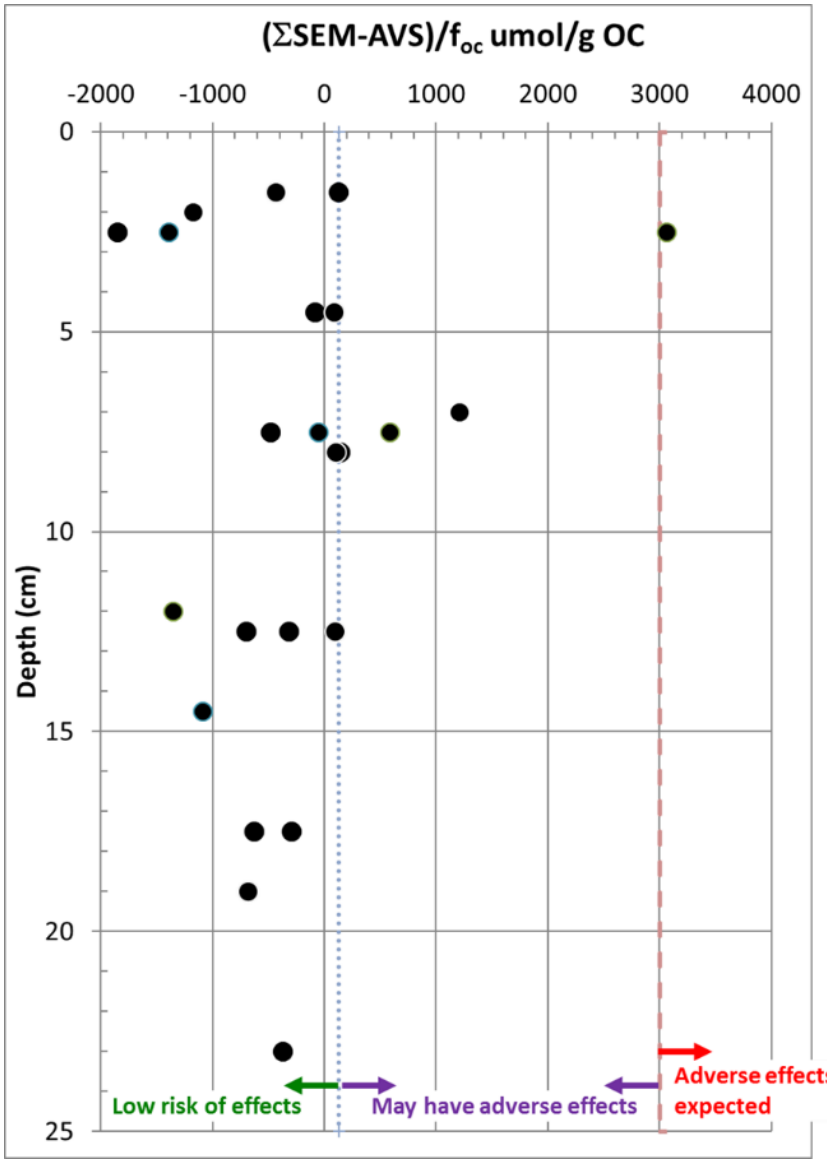

Figure 24. The distribution of the (ΣSEM – AVS)/f_{OC} measured in all the core sections as a function of core depth. Sediment quality benchmarks for the protection of benthic organisms from metal exposure (US EPA 2005) are also shown that delimit the regions of low risk of adverse biological effects, may have adverse biological effects, and adverse biological effects expected.

The probability of exceeding sediment quality benchmarks for the protection of benthic organisms was calculated by pooling the sediment core data by depth, calculating the mean (μ) and standard deviation (σ) for Y = (AVS-ΣSEM)/f_{oc}, and estimating the probability of exceeding the benchmarks using Equation 7 assuming a normal distribution and Equation 8 assuming a lognormal distribution (Table 16). The probability of exceeding sediment quality benchmarks is shown graphically for the 0-5 cm depth interval in Figure 25. The probability density function was used to calculate the probability of exceeding sediment quality benchmarks for the protection of benthic organisms from metal exposure (US EPA, 2005) assuming the data fit a normal (Figure 25A) and lognormal (Figure 25B) distribution. The data points are also shown and the lognormal probability density function is plotted on x-axis with back-transformed units (Figure 25C) to better visualize the data distribution (although the units of probability density function are undefined for the back transformed illustration). In the illustrations, the probability of exceeding the benchmark is the area under the curve to the right of the benchmark (Figure 25) which was calculated directly from the cumulative distribution functions in Equation 7 and Equation 8.

Based on average concentrations of metals and AVS measured in the 0–10 cm core sections and using the most conservative assumptions, the analysis showed that there was a low (8.9%) chance of possible impact, a medium chance (27.1%) of potential impact, and high chance (64%) of negligible impact to the benthic community from metal toxicity (Table 16A). The calculations using the lognormal distribution were slightly more conservative; there was a 9.5% chance for adverse effects expected, a 21% chance for may have adverse effects, and a 70% chance for low risk of adverse effects (Table 16B). The probability of adverse effects occurring decreased for the core segments deeper in the core (Table 16). For the 0–10 cm surface layer, there was a 8.9% chance for adverse effects expected, a 27% chance for may have adverse effects, and a 64% chance for low risk from metal exposure at the site (Table 16B).

Table 16. The probability of (ΣSEM – AVS)/f_{OC} exceeding sediment quality benchmarks for the protection of benthic organisms from metal exposure (US EPA, 2005) for core data pooled by core depth and calculated assuming data conformed to a normal (A) and lognormal distribution (B). The benchmarks define low risk of adverse biological effects (≤ 130 μmol/g OC), may have adverse biological effects (> 130 μmol/g OC), and adverse biological effects expected (> 3000 μmol/g OC).

		Probability of				Probability of
Core Depth	B_X	Low Risk	May have Adverse Effects	B_X		Adverse Effects Expected
cm	umol/g OC	$P(B_{130})$	$1-P(B_{130})-[1-P(B_{3000})]$	umol/g OC	$P(B_{3000})$	$1-P(B_{3000})$
A. Normal Distribution						
0-5 cm	130	0.5894	0.3938	3000	0.9832	0.0168
5-10 cm	130	0.4200	0.5800	3000	1.0000	0.0000
10-15 cm	130	0.9173	0.0827	3000	1.0000	0.0000
15-25 cm	130	0.9995	0.0005	3000	1.0000	0.0000
0-10 cm	130	0.5483	0.4460	3000	0.9943	0.0057
> 10 cm	130	0.9515	0.0485	3000	1.0000	0.0000
B. Lognormal Distribution						
0-5 cm	3.3284	0.6988	0.2065	3.6990	0.9053	0.0947
5-10 cm	3.3284	0.4588	0.5406	3.6990	0.9994	0.0006
10-15 cm	3.3284	0.8825	0.1161	3.6990	0.9986	0.0014
15-25 cm	3.3284	0.9973	0.0027	3.6990	1.0000	0.0000
0-10 cm	3.3284	0.6404	0.2711	3.6990	0.9115	0.0885
> 10 cm	3.3284	0.9039	0.0960	3.6990	0.9999	0.0001

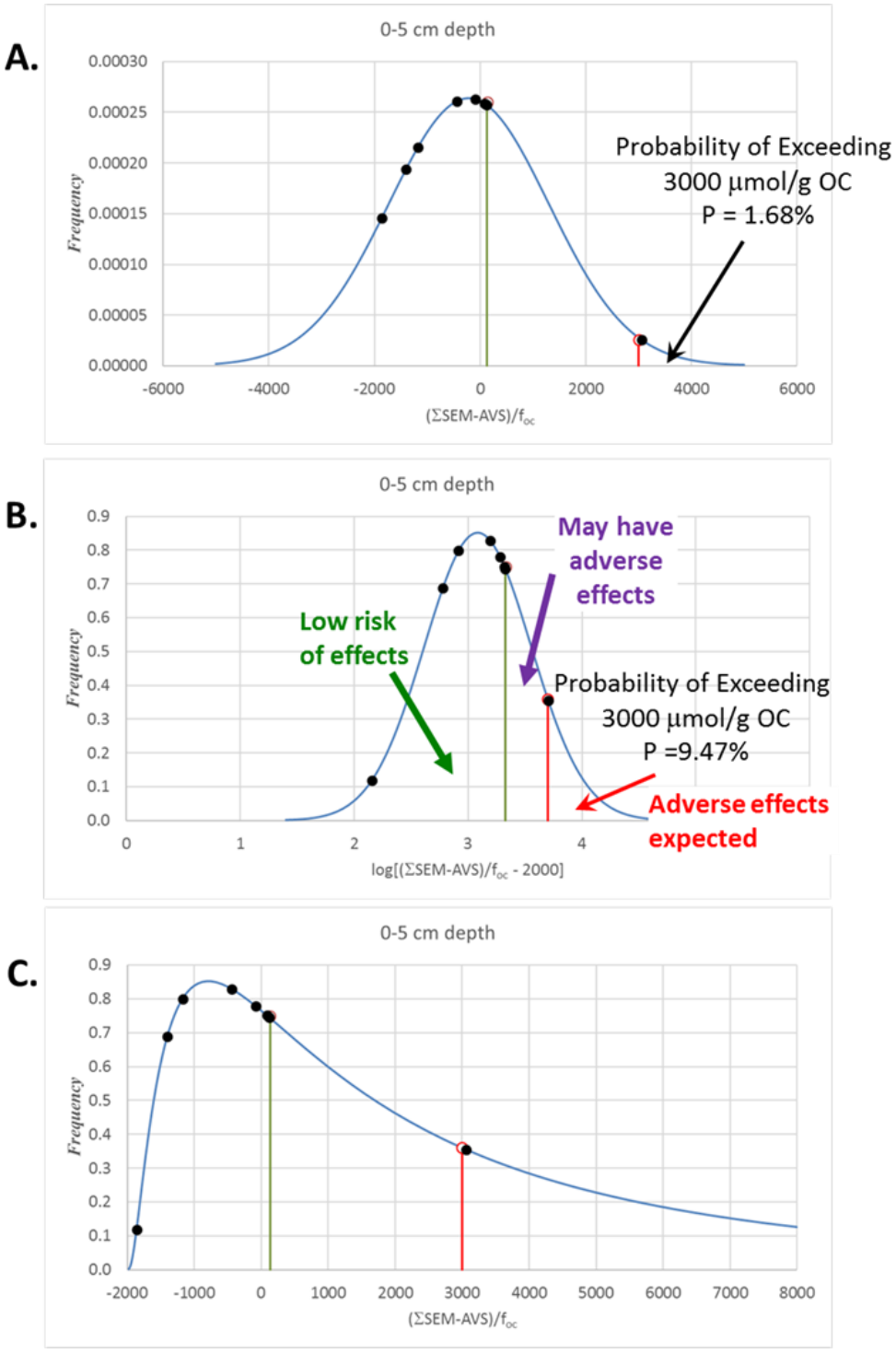

Figure 25. Probability density function (smooth line) used to calculate the probability of exceeding sediment quality benchmarks (vertical lines) for the protection of benthic organisms from metal exposure (US EPA, 2005) for the 0–5 cm depth interval plotted assuming the data fit a normal (A) and lognormal (B) distribution. Data are shown (black dots) and the lognormal probability density function is plotted on x-axis with back-transformed units (C).

3.4 MUSSEL TISSUES

Mussel tissues were sampled and analyzed for total Cu, total Zn, moisture, and lipid content to provide a source term estimate of the Cu and Zn present in the biomass living on the hull prior to biofouling removal. The data from the mussel tissue analysis are presented in Table 17 and plotted in Figure 26. The concentration of Cu ranged from 19–30 µg/g dry weight with a mean of 20 µg/g dry weight (CoefV* = 35%) and the concentration of Zn ranged from 129 -195 µg/g dry weight with a mean of 150 µg/g dry weight (CoefV* = 19%, Table 17). The RPD for the field duplicates was less than 10% for the metals on a dry weight basis (Table 17). The lipid and solid content were fairly consistent among the samples with a CoefV* of 12% and 9% for lipid and solid content, respectively (Table 17, Figure 26). A summary of benthic infauna measured in surface grabs collected near CV 62 prior to biofouling removal is shown in Table 18.

Table 17. Summary of total Cu and Zn, lipid, and solids measured in mussel (*Mytilus* spp.) tissue samples collected from the hull of CV 62 prior to biofouling removal.

			Copper	Zinc	Copper	Zinc	Lipids	Total Solids
Location ID	Sample_ID	Comment	ug/g wet weight		ug/g dry weight		% wet weight	
CV62-Transect1	CV62MUS-01	11 specimens	3.27	24.80	19.24	145.88	2.1	17.0
CV62-Transect2	CV62MUS-02	12 specimens	3.16	26.30	15.49	128.92	2.1	20.4
CV62-Transect3	CV62MUS-03	average of Field Dups	5.25	34.05	28.97	188.30	1.7	18.1
CV62-Transect4&5	CV62MUS-05	13 specimens	2.59	23.80	14.97	137.57	1.9	17.3
		average	3.57	27.24	19.67	150.17	1.94	18.20
		standard deviation	1.16	4.66	6.49	26.35	0.21	1.54
		CV*	34.6%	18.2%	35.0%	18.6%	11.7%	9.0%
CV62-Transect3	CV62MUS-03	7 specimens	5.63	33.8	30.27	181.72	1.8	18.6
	CV62MUS-04	7 specimens, Field Dup	4.87	34.3	27.67	194.89	1.5	17.6
		RPD of Field Dup	14.5%	1.5%	9.0%	7.0%	18.2%	5.5%

Table 18. Summary of benthic infauna measured in surface grabs collected near CV 62 prior to biofouling removal.

LOCATION ID	LAB_ID	TAXON_NAME	PHYLUM	SUBPHYLUM	CLASS	ORDER	FAMILY	COMMON NAME	COUNT	Abundance/ m^2
CV62-1	7762.01-1	None							0	0
CV62-2	7762.01-2	*Aphelochaeta* sp.	Annelida	Aclitellata	Polychaeta	Canalipalpata	Cirratulidae	polychaete worm	1	70
		Balanus crenatus	Arthropoda	Crustacea	Maxillopoda	Sessilia	Balanidae	acorn barnacle	1	
		Bipalponephtys cornuta	Annelida	Aclitellata	Polychaeta	Aciculata	Nephtyidae	polychaete worm	2	
		Macoma sp.	Mollusca		Bivalvia	Veneroida	Tellinidae	bent-nosed clam	1	
		Nassarius mendicus	Mollusca		Gastropoda	Neogastropoda	Nassariidae	lean dog whelk	1	
		Scoletoma sp.	Annelida	Aclitellata	Polychaeta	Aciculata	Lumbrineridae	polychaete worm	1	
CV62-3	7762.01-3	None							0	0
CV62-4	7762.01-4	Cossura candida	Annelida	Aclitellata	Polychaeta		Cossuridae	polychaete worm	1	10
CV62-5	7762.01-5	None							0	0
CV62-6	7762.01-6	None							0	0
								Total Organisms	8	
								Number Taxon	7	
								Averge Abundance		13.3

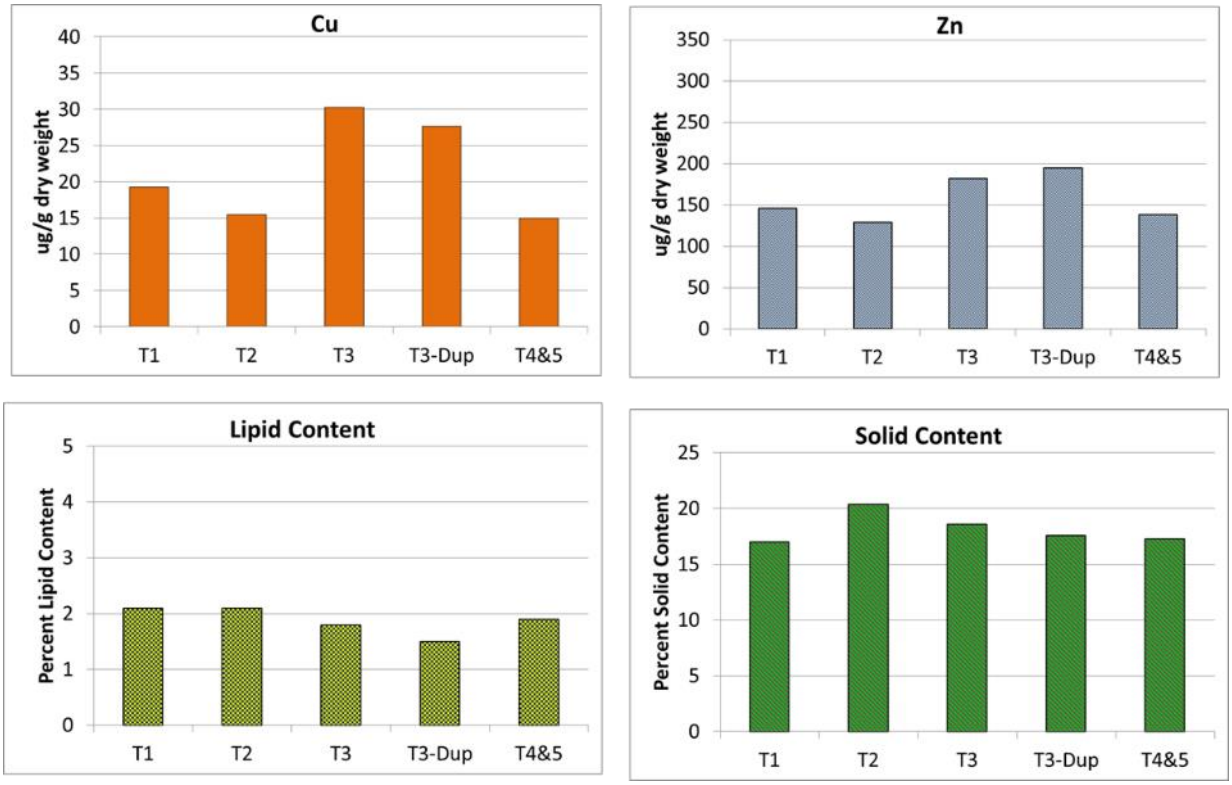

Figure 26. The concentration of total Cu (upper left panel), total Zn (upper right panel), percent lipid content (lower left panel), and percent solid content (lower right panel) measured in mussel tissues of specimens collected from Transect Belts during the biological survey of CV 62.

3.5 BENTHIC INFAUNA

Benthic infauna samples were collected to characterize the benthic community present at the site prior to biofouling removal. The benthic census showed that very limited macro-invertebrates were present at the site (Table 22). The extremely low abundance was expected given the highly anoxic conditions of the sediment. No evidence of macro-invertebrate activity or bioturbation was observed in the jelly-like muck collected with the Ponar sampler. The only biological activity observed in the sample was the abundance of anoxic microbial organisms whose presence was attested by the strong hydrogen sulfide odor and lack of visible oxidation in the samples.

3.6 HULL AND SEA FLOOR VIDEO SURVEY

The video taken by the PSNS&IMF Divers on March 30, 2017 was compared to the hull survey videos of portions of the hull and bottom of CV 62 recorded by Seaward Marine Inc. prior to hull cleaning in November 2016. The comparison showed that many organisms living on the hull had survived the hull cleaning were still alive and living on sea floor below the location where CV 62 was berthed (Appendix C-1: Video Clips). Dense assemblages of barnacles (*Balanus* spp.), mussels (*Mytilus* spp.), polychaete feather duster tube worms (Sabellidae and Serpulidae), large sea anemones (*Metridium senile*) and many other invertebrates were documented growing on CV62's hull (Earley et al., 2017). The video of the sea floor taken about 8 weeks after biofouling removal showed that the sea floor was covered with masses of tube worms, sea anemones, mussels, and other organisms from the vessel that were still alive and functioning on the sea floor. In addition, many crabs (*Cancer* spp.)

and other opportunistic bottom feeders were observed foraging and feeding on the biological material. Since the bottom consisted of soft mud with little substrate for epibenthic organisms the material from the hull appeared to be providing substrate and habitat increasing the biodiversity and abundance of the bottom community on the sea floor below CV62's former berth (Appendix C-1: Video Clips).

4. DISCUSSION

4.1 ANALYTICAL ACCURACY

The DQOs developed for this study (Table 1) were achieved by collecting sediment samples in the zone of influence under CV 62 prior to biofouling removal and resampling the same area after removal and towing of the vessel was complete. Additionally, sediment cores were collected and analyzed to evaluate the potential metal bioavailability and toxicity at the site. The analytical parameters selected for the study met the performance-based QA/QC for acceptability and usability, discrepancies were documented in the lab reports, and validated data with data qualifiers, if applicable, were used for the analysis.

The greatest source of error associated with the analytical results was the variation in the field samples and inhomogeneity of samples collected at the same time and location (Field Duplicates and Lab Duplicates). High variation in sediment samples collected near piers, pilings, and docking areas have been reported by other studies within the BNC (US Navy, 2012; Wang et al., 2016; Kirtay et al., 2017). This means that increasing the number of samples may not necessarily reduce the variability, it would however, increase the degrees of freedom in statistical tests.

The analytical methods required to determine AVS from the sediment core sections are not generally “routine” methods run by the contract lab, therefore, the laboratory was requested to run additional LCS. The six repeated measurements of the same sample resulted in a CV* of 41% which bounds the uncertainty that can be placed on the AVS results. In comparison to AVS, the results for total and SEM metals were within the expected accuracy of 70–130% for all the results reported.

4.2 COMPARABLE STUDIES

Comparable studies were identified that reported similar parameters from locations in the vicinity of Mooring G. The total Cu and Zn, grain size parameters, TOC, and Solids from this study (Table 19) were compared to data from the Washington State Department of Ecology's Urban Watershed Initiative (UWI) that collected samples throughout the Bainbridge Basin in May 2009 including 6 stations within inner Sinclair Inlet (Figure 27; Weakland et al., 2013), Charleston Beach monitoring conducted in May 2015 (Figure 28, URS Group 2016b), and LTM conducted for OUB Marine (Figure 29; US Navy, 2012; URS Group, 2015 & 2016a). The LTM monitoring consists of periodically collecting a composite of three 0-10 cm grabs from each 500 ft grid within OUB Marine (Figure 4) and 1500 ft grid in outer Sinclair Inlet (Figure 30). During LTM sampling conducted in October 2003 (Kohn, Miller, Brandenberger, and Johnston, 2004, 2008) and May 2010 (Brandenberger et al., in prep), the composite samples were split with the ENVVEST Monitoring Program to obtain additional data on metals in the sediments of Sinclair Inlet (Table 29). There was a general trend of increasing total Cu and Zn with increasing Fines and TOC in the samples (Figure 31). The Pre-Removal grab samples fell along the trend line, but three of the Post-Removal samples for Cu and one of the Post-Removal samples for Zn were enriched and were well above the trend line (Figure 31) indicating a source of metal other than that expected a function of grain size or TOC. In comparison to the available studies, the highest total Cu and Zn concentrations were measured in samples collected at Mooring G following biofouling removal (Post-Removal).

Table 19. Summary of sediment data collected near CV 62 for sampling conducted Pre- (A) and Post-Removal (B) of biofouling and data from comparable studies performed near the area reported from Ecology's Urban Waters Initiative (C), Charleston Beach monitoring (D), and long term monitoring for Operable Unit B conducted in 2003 (E and F) and 2010 (G and H).

				Copper	Zinc	Gravel	Sand	Silt	Clay	Fines	Solids	TOC
StationID	Depth	Type	Date	ug/g		Percent						
A. CV62 Pre-Removal												
CV62-1	0-10 cm	grab	12/13/2016	200	229	0.0	1.5	68.4	33.0	101.4	22.4	3.8
CV62-2	0-10 cm	grab	12/13/2016	170	420	32.3	15.0	34.0	13.7	47.7	41.2	2.7
CV62-3	0-10 cm	grab	12/13/2016	180	203	1.0	4.3	69.4	28.5	97.9	27.0	3.3
CV62-4	0-10 cm	grab	12/13/2016	146	163	0.4	2.1	72.6	26.3	98.9	27.7	3.2
CV62-5	0-10 cm	grab	12/13/2016	242	175	0.2	1.7	70.5	32.2	102.8	22.8	3.9
CV62-6	0-10 cm	grab	12/13/2016	206	215	1.8	2.7	69.9	31.4	101.3	28.9	3.5
CV62-Mean	0-10 cm	grab	12/13/2016	191	234	5.9	4.5	64.1	27.5	91.7	28.3	3.4
B. CV62 Post-Removal												
CV62-1	0-10 cm	grab	3/30/2017	240	208	0.0	2.4	76.3	31.4	100.0	3.7	11.8
CV62-2	0-10 cm	grab	3/30/2017	82	245	60.1	28.5	6.9	3.3	10.2	0.6	62.0
CV62-3	0-10 cm	grab	3/30/2017	778	314	19.1	9.5	55.7	22.7	78.4	3.6	27.6
CV62-4	0-10 cm	grab	3/30/2017	169	1200	1.4	2.0	75.4	24.3	99.7	3.3	26.1
CV62-5	0-10 cm	grab	3/30/2017	432	242	1.2	5.4	67.1	27.6	94.7	3.5	27.9
CV62-6	0-10 cm	grab	3/30/2017	678	284	9.7	4.2	58.7	27.1	85.8	4.1	25.3
CV62-Mean	0-10 cm	grab	3/30/2017	397	416	15.3	8.7	56.7	22.7	79.4	30.1	3.1
CV62-Mean	0-3 cm	core	3/30/2017	446	300						39.8	2.6
CV62-Mean	0-10 cm	core	3/30/2017	2356	859						37.0	2.7
C. Urban Waters Initiative (Weakland et al. 2013)												
UWI-SI-160	0-3 cm	grab	May-2009	120	168	2.0	10.6	53.7	33.7	87.4	29.0	3.9
UWI-SI-161	0-3 cm	grab	May-2009	90	144	4.0	13.0	51.9	31.2	83.1	26.8	3.2
UWI-SI-162	0-3 cm	grab	May-2009	90	140	1.3	13.7	55.5	29.4	84.9	29.8	2.8
UWI-SI-163	0-3 cm	grab	May-2009	105	155	0.2	4.2	60.0	35.7	95.7	26.6	3.0
UWI-SI-164	0-3 cm	grab	May-2009	86	122	0.3	9.9	66.7	23.4	90.0	35.2	2.3
UWI-SI-165	0-3 cm	grab	May-2009	95	134	1.1	14.6	57.8	26.5	84.3	33.8	2.5
D. Charleston Beach Monitoring (URS 2016)												
CB-1	0-10 cm	grab	May-2015	36	57					8.2		1.7
CB-2	0-10 cm	grab	May-2015	25	56					3.0		0.3
CB-3	0-10 cm	grab	May-2015	29	60					2.4		0.3
CB-4	0-10 cm	grab	May-2015	22	42					3.0		0.3
CB-4 (FD)	0-10 cm	grab	May-2015	22	37					3.2		0.3
CB-5	0-10 cm	grab	May-2015	33	36					2.3		0.2
CB-6***	0-10 cm	grab	Sep-2014	160 J	280 J					31.0		2.7
E. Outer OUB 1500 ft Grids (Kohn et al. 2004)												
OOUB-S13	0-10 cm	composite	10/30/2003	116	174					91.0		3.0
OOUB-S17	0-10 cm	composite	10/30/2003	103	161					90.0		3.2
OOUB-S20	0-10 cm	composite	10/30/2003	86	143					86.0		2.9
F. OUB Marine 500 ft Grids (Kohn et al. 2004)												
OUBM-G21	0-10 cm	composite	10/27/2003	108	145					65.0		1.7
OUBM-G22	0-10 cm	composite	10/27/2003	110	169					98.0		2.8
OUBM-G23	0-10 cm	composite	10/27/2003	128	168					85.0		2.7
OUBM-G24	0-10 cm	composite	10/28/2003	161	207					92.0		2.7
OUBM-G25	0-10 cm	composite	10/27/2003	191	273					82.0		2.8
OUBM-G28	0-10 cm	composite	10/27/2003	173	211					56.0		2.0
G. Outer OUB 1500 ft Grids (Brandenbeger et al. in prep)												
OOUB-S13	0-10 cm	composite	May-2010	104	161					88.0		3.1
OOUB-S17	0-10 cm	composite	May-2010	129	108					92.0		3.4
OOUB-S20	0-10 cm	composite	May-2010	125	136					84.0		2.9
H. OUB Marine 500 ft Grids (Brandenberger et al. in prep)												
OUBM-G21	0-10 cm	composite	May-2010	90	97					49.0		1.6
OUBM-G22	0-10 cm	composite	May-2010	136	123					96.0		3.3
OUBM-G23	0-10 cm	composite	May-2010	198	215					84.0		3.0
OUBM-G24	0-10 cm	composite	May-2010	83	103					78.0		3.4
OUBM-G25	0-10 cm	composite	May-2010	170	178					100.0		7.2
OUBM-G28	0-10 cm	composite	May-2010	215	178					94.0		3.7

*** Elevated detection limit likely caused by matrix interference. Data usability impacted for regulatory comparisons (see URS 2016b).

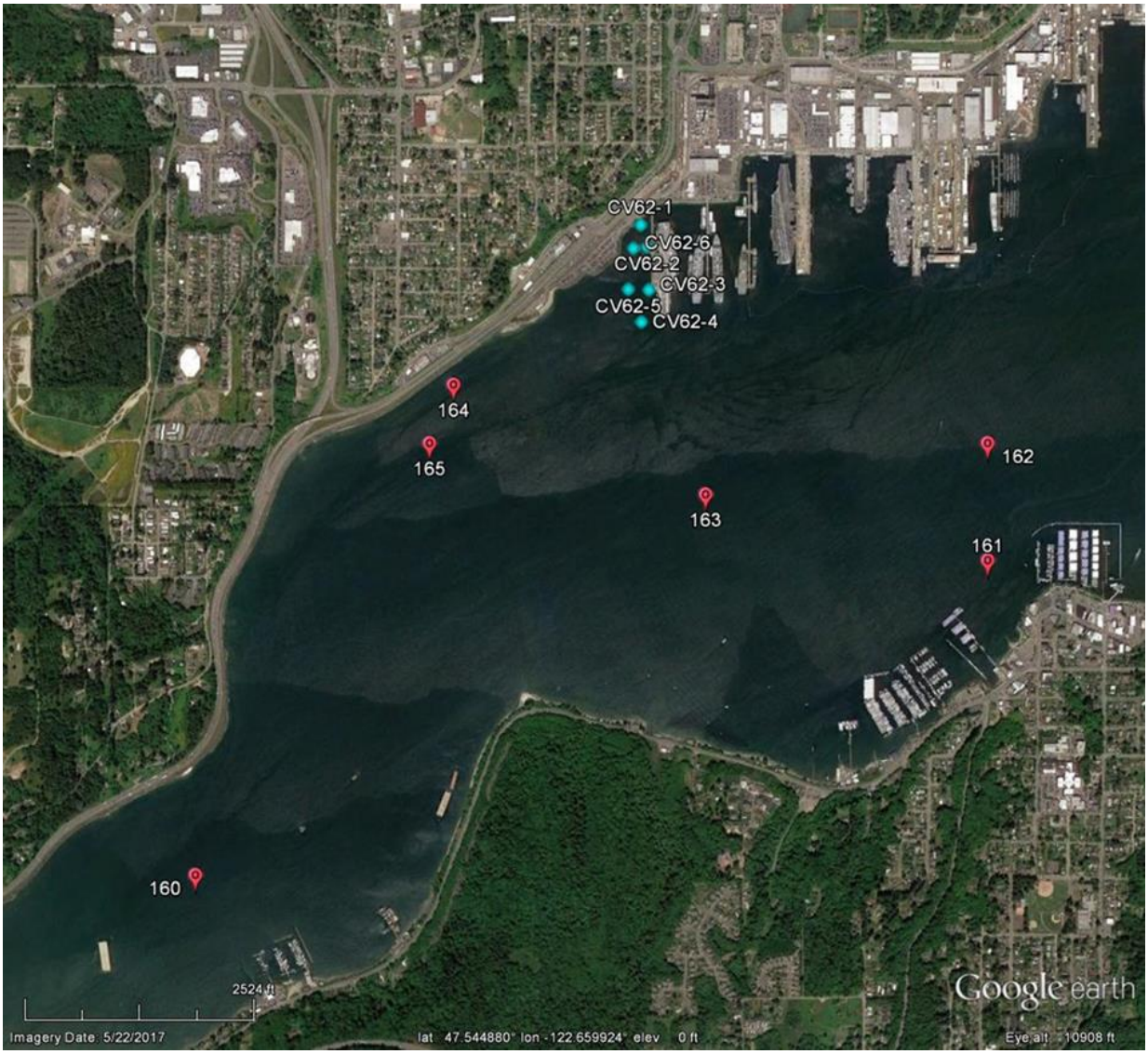

Figure 27. Location of stations in Sinclair Inlet sampled by Ecology's UWI project (Weakland et al., 2013) in May 2009 (red pins) and this study at Mooring G where CV 62 was formerly berthed (light blue spheres).

Historical data for total Cu, total Zn, AVS, and SEM is reported for a station sampled near Mooring G (3a, Figure 29) are reported in Johnston (1993) and Chadwick et al. (1993) (Table 20), and core profiles from age-dated cores from inner Sinclair Inlet are reported for Cu and Zn (Crecelius et al., 2003; Brandenberger et al., 2008; Figure 32). These data show that there are relatively lower metal concentrations near the surface of core that represents the most recent deposits. The down-core concentrations of Cu and Zn peak during the latter half of the 20th Century, which corresponds to core depths of about 15–30 cm (Brandenberger et al., 2008). The core profile representative of Sinclair Inlet near Mooring G (S2, Figure 32) yielded a long-term average deposition rate of 0.35 ± 0.05 cm/yr and a surface mixing depth of 2.5 cm (Table 21, Brandenberger et al., 2008). At this deposition rate it would take 28.5 (25–33) years to deposit 10 cm of sediment in this area of Sinclair Inlet. This estimate is the long-term estimate for the area (S2, Figure 32) as a whole and neglects near field inputs such as the raining of biogenic material from the hull, local runoff of particulates, and disturbances from propeller wash and ship movements in the area. These data imply that since CV 62

was berthed at Mooring G in September 1998 about 6.5 (5.7–7.4) cm of sediment accumulated below the vessel. Table 21 shows a summary of sedimentation and sediment accumulation rates determined for sediment cores collected from Sinclair (S) and Dyes (D) Inlets.

Table 20. Summary of total Cu and Zn and AVS and SEM metals measured in grab and core samples collected from station 3a, located between Moorings G and F reported in Johnston (1993).

				Cu	Zn		
StationID	Depth	Type	Date	ug/g			
Station 3a	Surface	grab	Fall 1989	210.97	267.40		
Station 3a	Surface	grab	July 1991	357.76	387.70		
				AVS	SEM_Cu	SEM_Zn	SEM_Pb
StationID	Depth	Type	Date	umol/g			
Station 3a	0-10 cm	core	July 1991	3.46	3.35	4.47	0.83
Station 3a	0-2 cm	core	July 1991	69.32	5.66	7.66	1.19

Table 21. Summary of sedimentation and sediment accumulation rates determined for sediment cores collected from Sinclair (S) and Dyes (D) Inlets using two independent methods and reported accumulation rates (from Brandenberger et al., 2008). Core S2 was collected within inner Sinclair Inlet and is applicable to the area near Mooring G.

Core ID	Mixing Depth (cm)	Sed. Rate (cm/yr)[a]	Sediment Accum. Rate from ^{210}Pb (g/cm^2/yr)[a]	Depth of First Excess Pb, Hg 1895 (cm)	Total Dry Accum. Post 1895 (g/cm^2)	Sediment Accum. Rates from Pb and Hg (g/cm^2/yr)[b]	Sediment Accum. Rates Applied to Data (g/cm^2/yr)
D1	2.5	0.50 ± 0.1	0.21 ± 0.01	45	22.5	0.21	0.21 ± 0.01
D2	2.5	0.20 ± 0.05	0.14 ± 0.01	25	15.5	0.15	0.14 ± 0.01
D3	5.0	0.37 ± 0.06	0.17 ± 0.01	40	19.7	0.18	0.17 ± 0.01
D4	2.5	0.18 ± 0.1	0.10 ± 0.01	35	18.2	0.17	0.10 ± 0.01
S1	2.5	0.16 ± 0.1	0.072 ± 0.01	25	10.2	0.095	0.072 ± 0.01
S2	2.5	0.35 ± 0.05	0.15 ± 0.01	45	20.1	0.19	0.15 ± 0.01
S3	5.0	0.34 ± 0.06	0.14 ± 0.02	40	17.7	0.17	0.14 ± 0.02
S4 [c]	5.0	0.31 ± 0.04	0.17 ± 0.03	52.5	29.4	0.27	0.17 ± 0.03
S5	5.0	0.20 ± 0.03	0.11 ± 0.01	40	26.8	0.25	0.18 ± 0.04

Footnotes:

a. Sedimentation and sediment accumulation rates determined using the ^{210}Pb data and ^{137}Cs confirmation.

b. Sediment accumulation rates derived from contaminant peaks (Pb and Hg) = total dry accumulation post 1895 (g/cm^2) divided by elapsed time (107 years). Sediment cores were collected in 2002 and shipyard activities began in 1895 for an elapsed time of 107 years.

c. Upper portion of this core was disturbed by dredging activities in 2001.

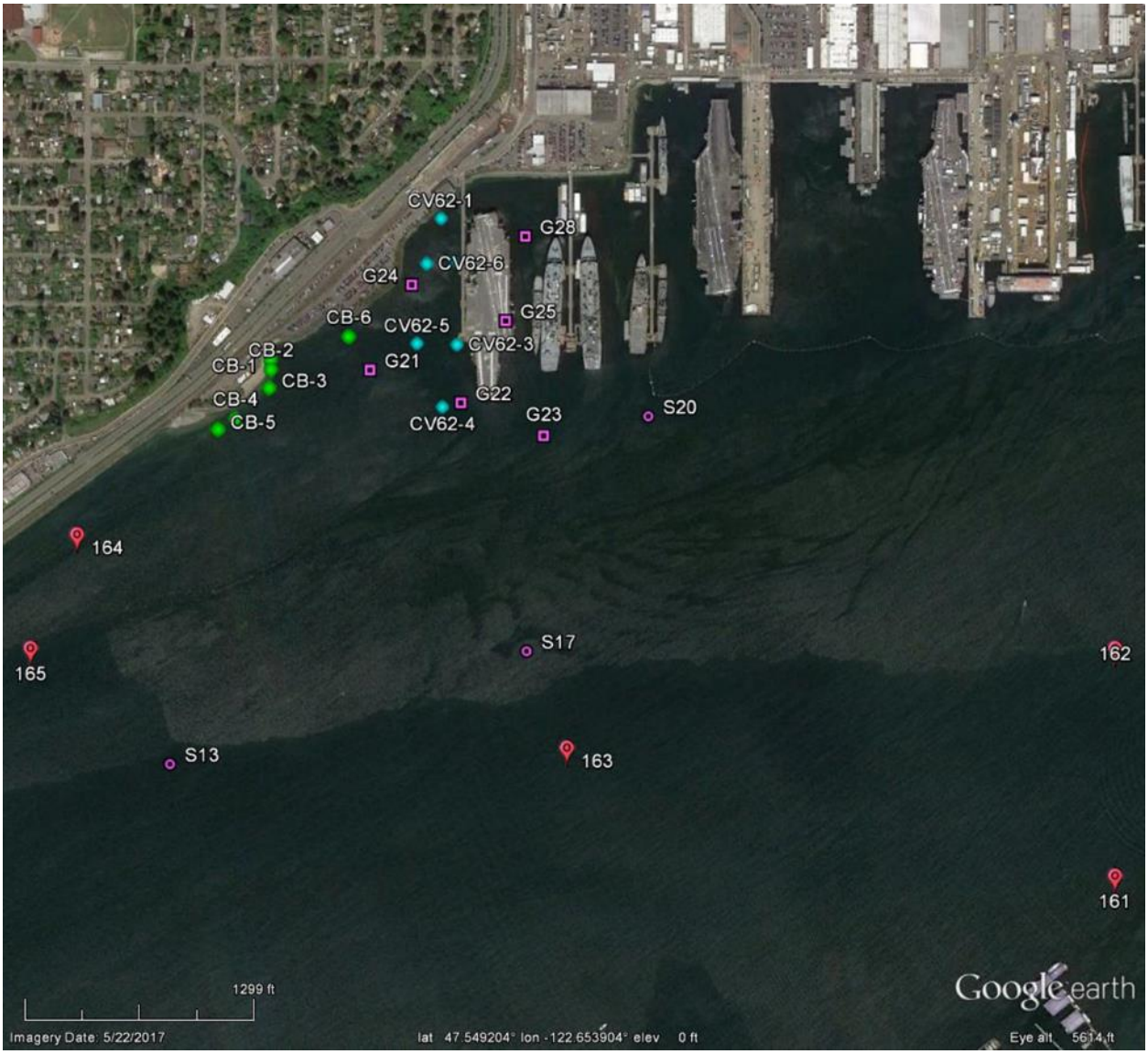

Figure 28. Location of stations in Sinclair Inlet sampled by Ecology's UWI project (red pins), OUB Marine LTM monitoring grids (purple hollow squares), Sinclair Inlet LTM monitoring grids (purple hollow circles), Charleston Beach sampling locations (green spheres), and this study at Mooring G where CV 62 was formerly berthed (light blue spheres).

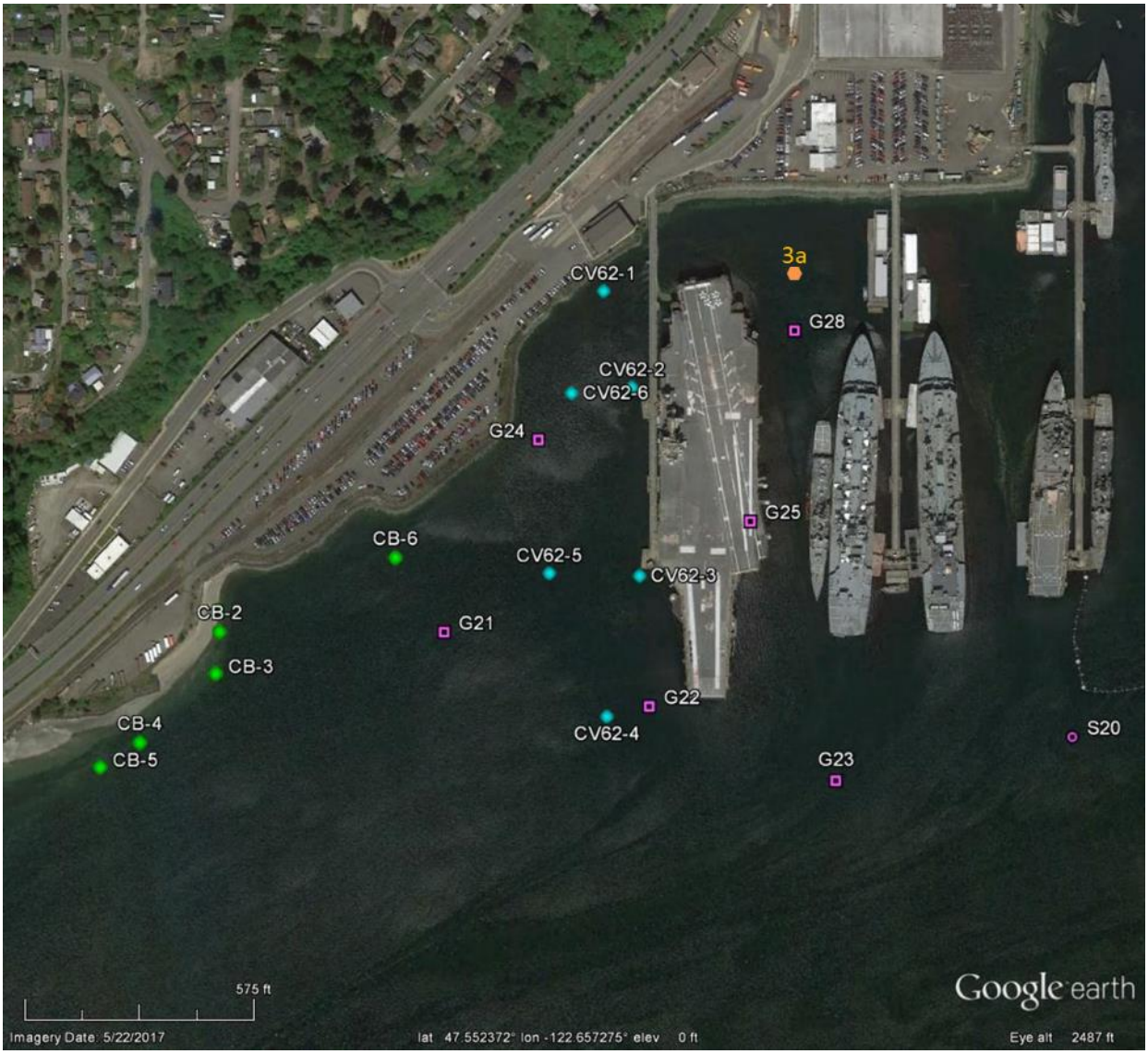

Figure 29. Location of stations near Mooring G sampled by Marine LTM monitoring grids (purple hollow squares), Charleston Beach sampling locations (green spheres), AVS-SEM sediment cores (orange hexagon [3a]), and this study where CV 62 was formerly berthed (light blue spheres).

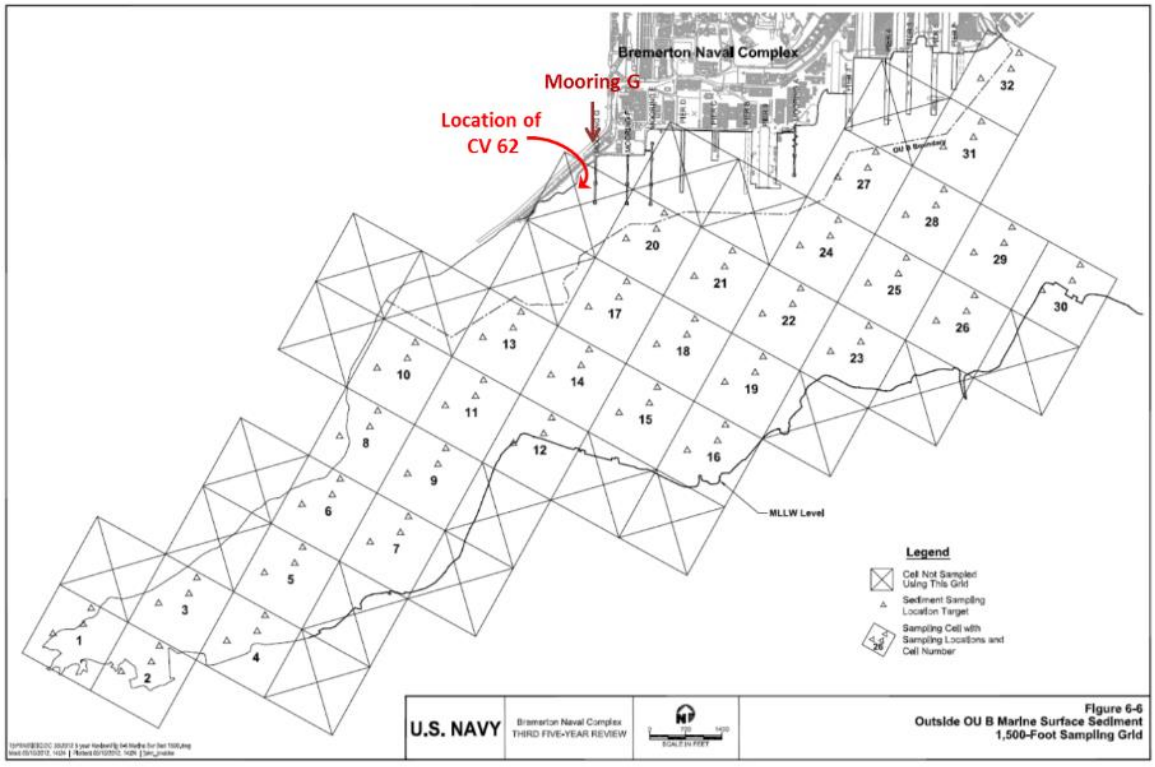

Figure 30. Location of Mooring G and the 1500-ft sampling grids in outer Sinclair Inlet sampled as part of the LTM for OUB Marine (figure from US Navy 2012).

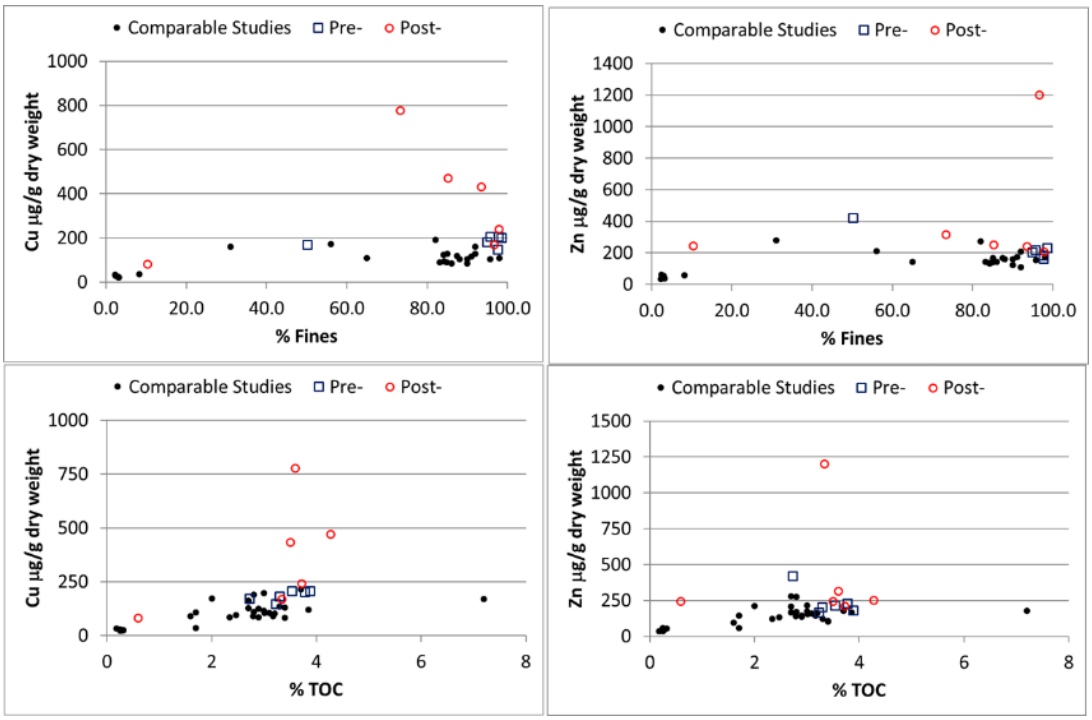

Figure 31. Relationship of Total Cu and Total Zn to percent fines (upper panels) and percent TOC measured in surface sediment samples from Pre- (blue squares) and Post-Removal (red circles) and comparable studies (black points) in Sinclair Inlet.

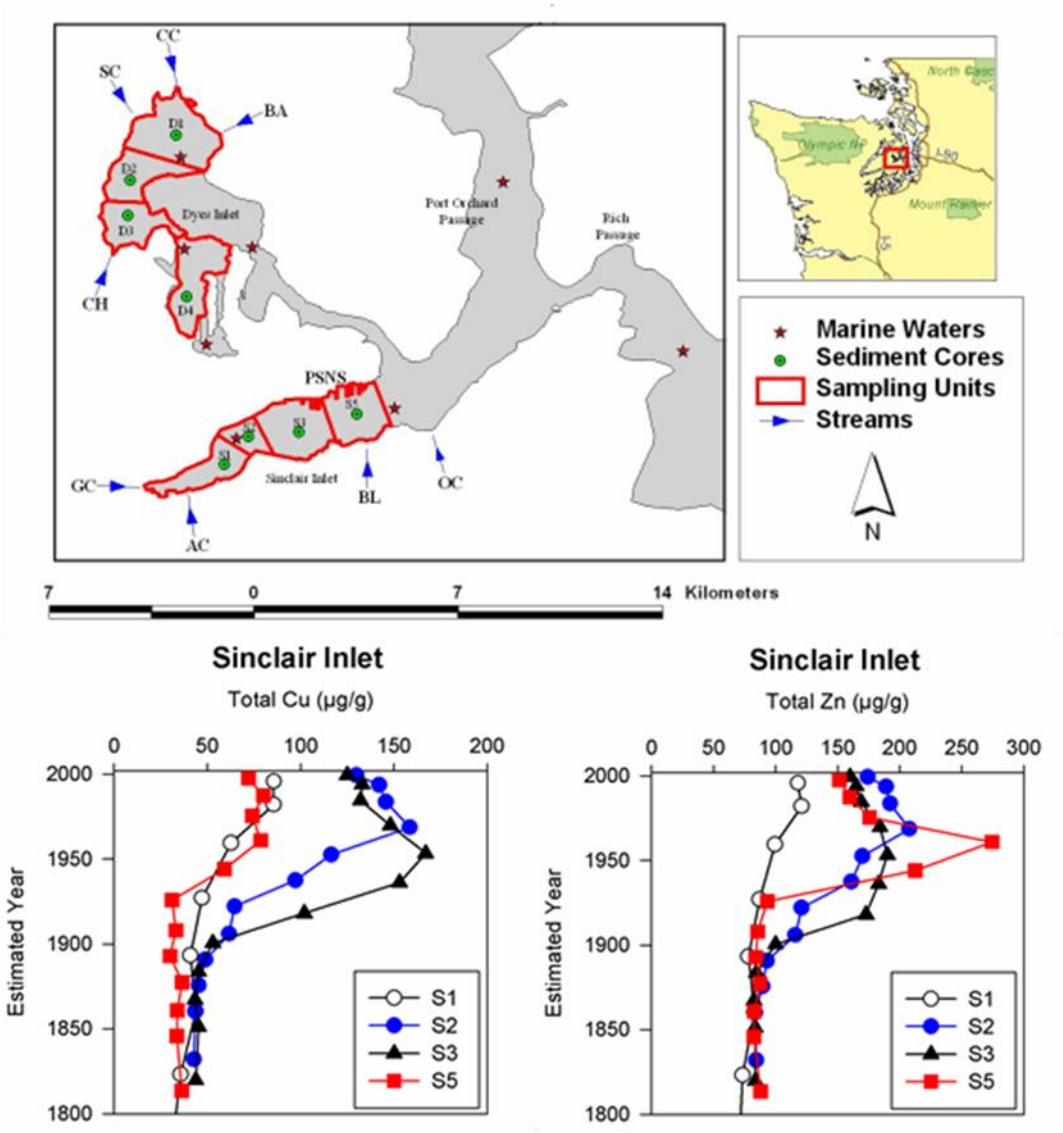

Figure 32. Age dated core profiles for total Cu and Zn measured from stations in Sinclair Inlet including station S2 located near Mooring G (Brandenberger et al., 2008).

The mussel tissue residues sampled from the hull of CV 62 were compared to the ENVVEST Mussel Watch data collected from a network of stations within Sinclair and Dyes Inlets and the passages connecting to the central basin of Puget Sound (Figure 33, and Figure 34; Johnston, 2017). The ENVVEST mussel watch stations have been sampled semi-annually from 2010-2016; the sampling includes a sample obtained from a local seafood market containing mussels harvested from Penn Cove, WA. The mussels sampled from CV 62 were within the range of Cu and Zn concentrations reported from other stations within Sinclair and Dyes Inlets (Figure 35) including station PS01 at the base of Mooring G and stations SIGST and SIRP located within inner Sinclair Inlet. The mussel tissue Cu concentrations from CV 62 were higher than the stations closest to Mooring G, while the Zn concentrations from CV 62 were lower than the nearest stations. The mussel tissue Cu and Zn concentrations were within the medium range of concentrations reported for the National Mussel Watch Program (Kimbrough et al., 2008). On average, the concentrations of Cu and Zn measured in mussels collected from the hull of CV 62 were at or below the Critical Body

Residue (CBR, (Figure 35) benchmark, below which effects from metal exposure to mussels are not expected (Johnston et al., 2007; Applied Biomonitoring, 2009). In comparison to seafood samples harvested from Penn Cove, WA (PKPLPC, Figure 35), mussel tissue concentrations from CV 62 were about twice as high for Cu and within the range of Zn concentrations measured in seafood samples harvested from Penn Cove (Figure 35).

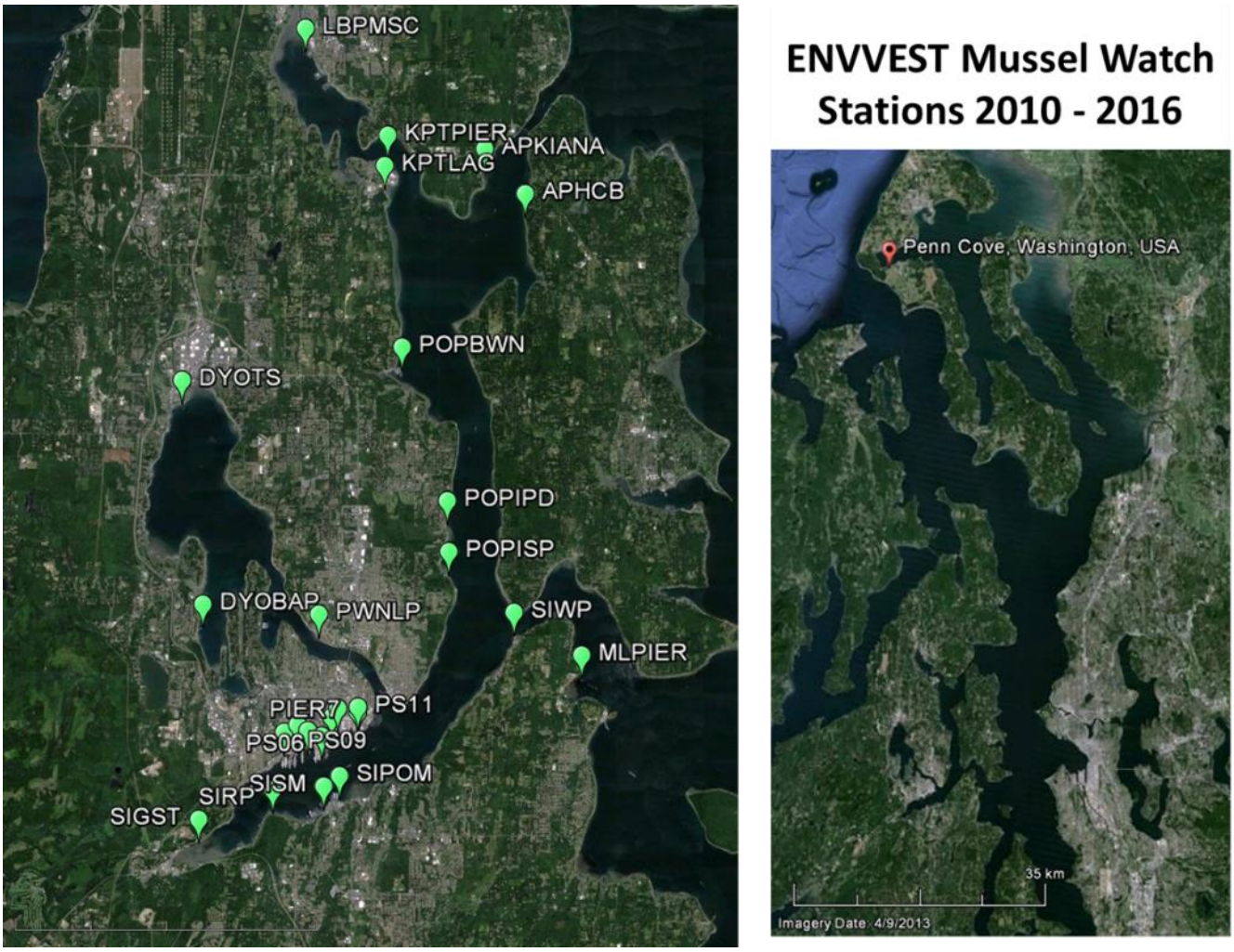

Figure 33. Location of ENVVEST mussel watch sampling stations sampled semiannually from 2010–2016 and the location of Penn Cove, WA where the seafood market samples were harvested.

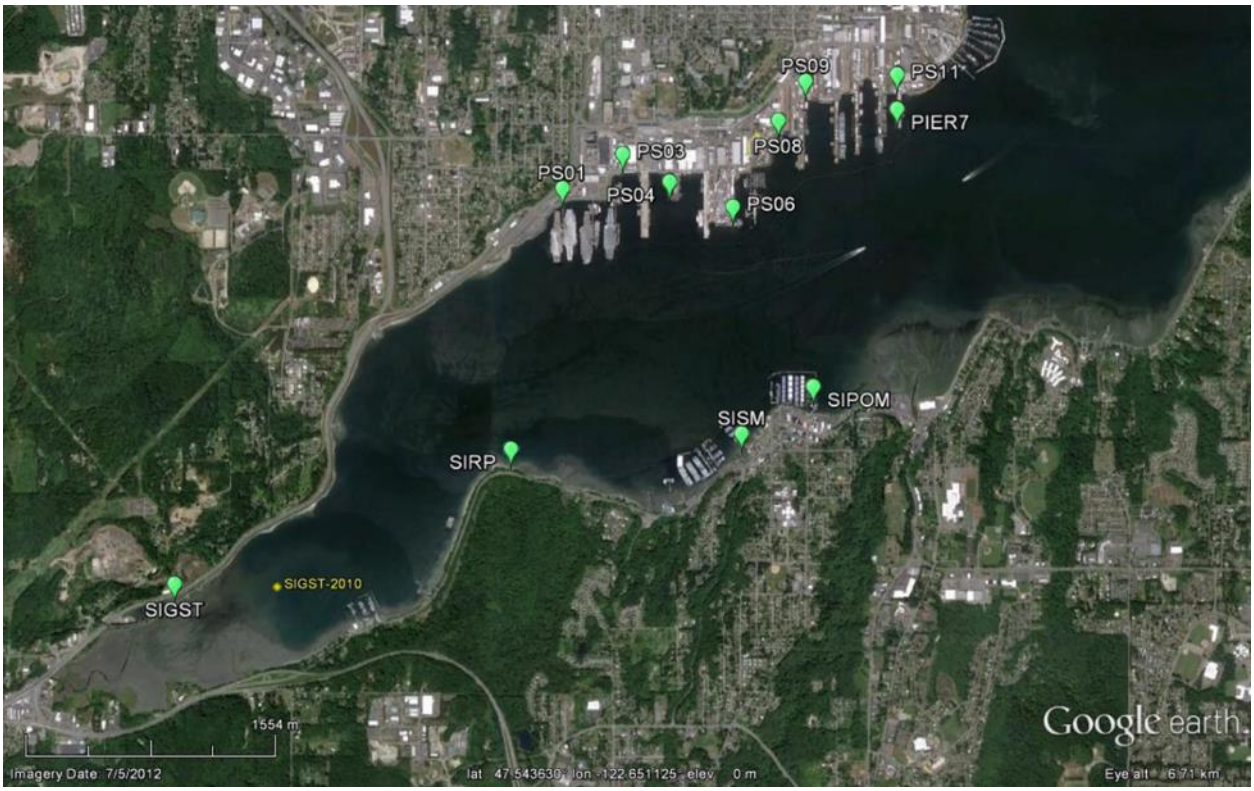

Figure 34. Location of ENVVEST mussel watch sampling stations in Sinclair Inlet sampled semiannually from 2010–2016.

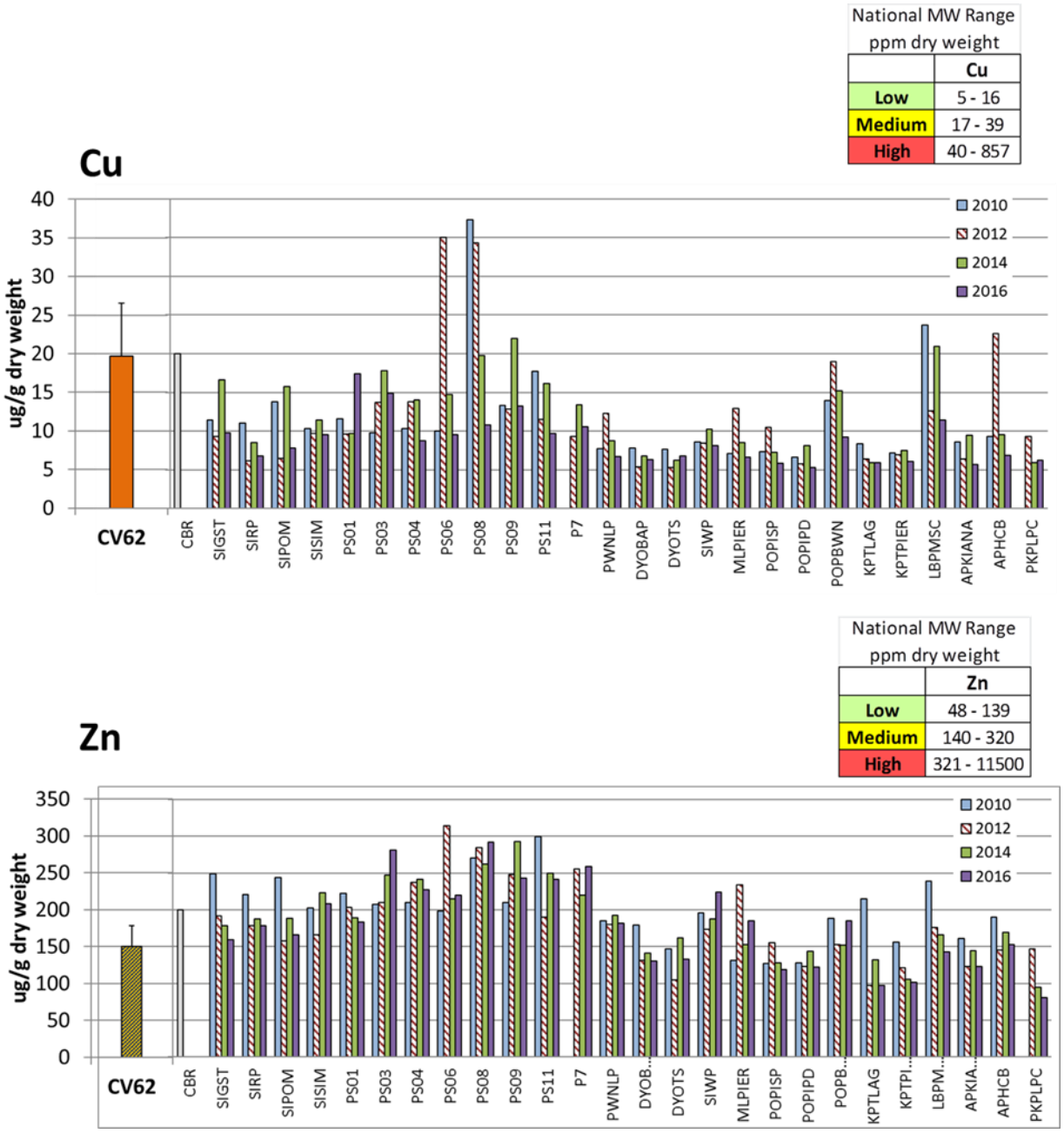

Figure 35. Comparison of the average (CV*) mussel tissue concentration of total Cu (upper panel) and Zn (lower panel) sampled from the hull of CV62, the ENVVEST mussel watch tissue concentrations sampled semi-annually from 2010–2016, and the range of tissue concentrations reported from the National Mussel Watch (MW) Program (Kimbrough, 2008).

The benthic community data collected during this study were also compared to other infaunal studies conducted in Sinclair Inlet (Table 22; Weakland et al., 2013; Kirtay et. al., 2017). The infaunal counts from Mooring G (stations CV62-1 to CV62-6) were extremely low compared to abundance and number of taxa reported from the UWI study and Pier 7 activated carbon demonstration project (Table 22). The samples from Mooring G were collected during the winter, which would have much lower abundance than samples collected in spring and summer when

temperature and primary productivity levels are much higher. The lack of benthic diversity and abundance in the sediment was contrasted by the very high abundance and diversity of the epibenthic fouling community present on the hull of CV 62 as well as the nearby docks and pilings. The biofouling removal essentially transferred the epibenthic community from the hull of CV 62 to the sea floor, which greatly increased the abundance and diversity present. Prior to biofouling removal, the biological material from the hull would rain down upon the bottom, as evidenced by the abundance of shells and other debris present in the Pre-Removal samples. This process would occur slowly, however the hull cleaning resulted in a mass transfer of organisms and substrate from the hull to the sea floor where, it appears, the organism were able to re-establish themselves on the bottom creating a new frontier for colonization (see Appendix C-1 Video Clips).

Table 22. Summary of sediment infauna data collected near CV 62 (A) prior to biofouling removal and sediment infauna data from comparable studies performed near the area reported from Ecology's Urban Waters Initiative (B) and the Pier 7 Activated Carbon Demo Project (C).

				Individuals	Taxon	Abundance
StationID	**Depth**	**Type**	**Date**	**#**	**#**	**#/m^2**
A. CV62 Pre Removal						
CV62	0-10 cm	grab	12/13/2016	8	7	13
B. Urban Waters Initiative (Weakland et al. 2013)						
UWI-SI-160	0-3 cm	grab	May-2009	147	8	1470
UWI-SI-161	0-3 cm	grab	May-2009	87	8	870
UWI-SI-162	0-3 cm	grab	May-2009	392	31	3920
UWI-SI-163	0-3 cm	grab	May-2009	421	26	4210
UWI-SI-164	0-3 cm	grab	May-2009	560	39	5600
UWI-SI-165	0-3 cm	grab	May-2009	473	34	4730
C. Pier 7 Demo Project (Kirtay et al. 2017)						
Pier 7 Cap* (n=10)	0-15 cm	core	Aug-2012		13.9	6554.2
Pier 7 Reference (n=4)	0-15 cm	core	Aug-2012		5.0	1685.3
Pier 7 Cap (n=10)	0-15 cm	core	Aug-2013		12.8	8077.7
Pier 7 Reference (n=4)	0-15 cm	core	Aug-2013		9.3	2777.8
Pier 7 Cap (n=10)	0-15 cm	core	Jul-2014		13.4	5855.5
Pier 7 Reference (n=4)	0-15 cm	core	Jul-2014		11.5	5638.8
Pier 7 Cap (n=10)	0-15 cm	core	Jul-2015		9.5	4699.7
Pier 7 Reference (n=4)	0-15 cm	core	Jul-2015		8.3	4027.8
*Baseline samples collected prior to amendment cap placement						

4.3 METAL BIOAVAILABILITY AND TOXICITY

Metal bioavailability and the potential for metal exposure to cause adverse effects to benthic organisms were evaluated by collecting sediment cores from the site and conducting AVS and SEM measurements of core sections in the top 0–25 cm (0–10 inches) of sediment located under the former location of CV62's hull. When all the data are pooled by depth, there is enough AVS present to bind the SEM metals, reducing the risk of adverse effects (Figure 23) and the benchmark for adverse effects from metal exposure $(\Sigma SEM - AVS)/f_{OC} > 3{,}000$ μmol/g dry weight was only exceeded by one sample (1/23 samples = 4%) during the study (Figure 24). However, there is

uncertainty about this finding due to the variability and interferences associated with the method, including analytical inaccuracy, seasonal variation of AVS, sample inhomogeneity, and other sources of variation.

As discussed above, the analytical precision of the AVS measurement was about 41% of the "true value" and there was uncertainty about how effective the laboratory was in controlling for the loss of AVS in the samples by removing the oxidized layer prior to processing and preventing oxidation during extraction. Overall the AVS analytical results met acceptable QA/QC criteria, however, the AVS concentrations may have been under estimated because AVS was not detected in two samples, and there were large variations in field and lab duplicates (Table 14).

The concentrations of AVS are known to vary in marine and estuarine sediments with maximum values occurring during the warm summer months when sulfate reduction by marine bacteria are at the highest levels and lowest during the winter when water column dissolved oxygen levels are near saturation (Boothman and Helmsetter, 1992; Boothman et al., 2001; US EPA, 2005). Since the AVS samples for this study were collected late winter/early spring (March 30, 2017), it is very likely that the AVS concentrations were at or near minimum levels. The AVS concentrations during the summer months can be much higher (Table 20; Johnston, 1993) while seasonal SEM would remain relatively constant. Consequently, the additional AVS would afford more protection from metal exposure during the warmer months when marine organisms are most active.

Inhomogeneity and variability in field sampling is an inherent component of studies conducted within non-uniform environments such as active naval harbors with hardened shorelines, overwater structures, vessel berthing, ship traffic, propeller wash, and other disturbances that can affect the sedimentary environment (Johnston, 1993; Chadwick et al., 1993; Wang et al., 2016; Kirtay et al., 2017). Therefore, the sediment results reported for this study should be considered a snap shot of the heterogeneous environment present at the site.

There was a high amount of variability in the samples collected near Mooring G. The core profiles obtained for each sampling station varied considerably in terms of sediment texture, contaminant levels, and distribution of AVS and SEM (Table 13, Table 14, Figure 15–Figure 20). Concentrations of total Cu did not exceed the WA State Sediment Management Standards (SMS) Maximum Chemical Criteria (MCC) of 390 μg/g dry weight in any of the Pre-Removal 0–10 cm grabs, three (50%) of the Post-Removal 0–10 cm grabs exceeded Cu concentrations of 390 μg/g dry weight (Table 13), and 4 of 23 (17%) sediment core sections exceeded the Cu MCC benchmark (Table 14). For Zn, only two samples exceeded the Zn MCC of 960 μg/g dry weight, one sample from the Post-Removal grabs (station CV62-4, Table 13) and the 5-10 cm section from the core collected at station CV62-3 (Table 14). The SMS benchmarks are useful for determining the relative magnitude of contamination present (Ecology, 2013). However, they are not very useful in predicting ecological effects because the MCC values are based on apparent effects thresholds of a mixture of sediment contaminants (Ginn and Pastorok, 1992), and do not represent a dose-response threshold for individual sediment contaminants, as such as those that are available for water quality criteria thresholds, for example.

Metal toxicity in sediments is controlled by geochemical processes that affect metal bioavailability and toxicity, therefore, it is more appropriate to assess metal toxicity using the AVS benchmarks derived based on $(\Sigma SEM - AVS)/f_{OC}$ (US EPA, 2005). The AVS benchmarks only evaluate the potential for metal toxicity through pore water exposure to free metal, the AVS benchmarks do not address metal bioaccumulation or trophic transfer in the food web. Food-web biomagnification of metals is not likely a major concern as marine invertebrates are well adapted for mediating Cu and

Zn since these metals are both micro-nutrients and essential to life at low levels and toxic at higher levels, thus limiting biomagnification in the food web (Paquin et al., 2011).

The variation in concentrations of total Cu and Zn in the Pre-Removal samples was 18% and 42%, respectively and this variance increased to 74% and 97% for the Post-Removal event, respectively. Additionally, the variance in grain size parameters for Fines, TOC, and Solids increased by at least a factor of two between the two sampling events (Table 13). This indicates that the biofouling removal caused a disturbance in the sea floor conditions. However, it is unclear whether the disturbance also contributed contamination to the site or if the disturbance stirred up contamination that was already present. Because the bottom consists of soft muds, the material falling from the ship would not necessarily remain on the surface but would "splash" on the bottom sinking into the muck and disturbing the bottom conditions. Additionally, diving operations during the cleaning and other operations required to prepare the ship for towing may have also contributed to disturbance.

Under the anoxic conditions, the metal contaminants present would most likely be inert, bound up in insoluble metal sulfides. Whether the disturbance will contribute to the release of contamination depends on the rate of sediment reworking and oxidation. For example, if sediments are recolonized by benthic organisms, they might release metals and other contaminants that were previously sequestered at the site. However, if sulfides remain higher than the metals then any effect from metal exposure would be minimized. Even if the upper sediments are reworked and oxidized there will still remain a large pool of AVS that will serve as a sink (or source) of metals at the site (Johnston, 1993).

4.4 SEA FLOOR BOTTOM COMMUNITY

As seen in the video clips of the sea floor, the masses of tube worms, mussels, and anemones evidently increased the structure and diversity of the bottom community that attracted crabs and other bottom feeders. Prior to biofouling removal, the bottom below the ship was essentially a biological desert, except for the anoxic bacteria that dominated the benthic environment. In contrast, the biofouling community living on the hull was a highly abundant and diverse community (Earley et al., 2018a).

The shells and other calcareous material from the hull cleaning evolution served as substrate for attachment space which was quite limited on the pre-existing unconsolidated sediment. Some of the species in the community transferred from the hull to the sea floor were filter and suspension feeders that need substrate to remain above the mud. This community also consisted of invertebrate predators, grazers, omnivores and scavengers including an abundance of polychaetes that included both selective and non-selective deposit feeders. The latter produce volumes of rejected fecal matter such as feces, also known as castings. The castings contain unharvested organic matter and bacteria which are a rich food source for other consumers. Whether this community remains viable over time was outside the scope of this study, however, the video surveys clearly showed that many of the organisms remained viable for at least two months after cleaning and were still functioning on the sea floor where the hull cleaning occurred.

4.5 OUTCOME FOR DECISION MATRIX

The decision matrix was used to summarize the evaluation based on the difference between the Pre- and Post-Removal 0-10 cm sediment grabs and the potential for adverse biological effects from metal exposure estimated from the AVS core sections collected at the site (Table 23). There were no statistically significant differences ($p \leq 0.05$) detected for any of the sediment parameters measured Pre- and Post-Removal for both parametric (normal and lognormal) and non-parametric statistical tests (Table 15). The lack of statistical significance between the two sampling periods was due to the high variability for both sampling periods. There was a statistically significant difference for total Zn

at the $p \leq 0.10$ significance level, with Zn concentration about 1.8 times higher in the Post- samples. Based on metal bioavailability and toxicity estimated from the AVS cores there was a low (9%) chance of possible impact, a medium chance (27%) of potential impact, and high chance (64%) of negligible impact (Table 23).

Table 23. Outcome of the decision matrix based on the evaluation of sediment data collected Pre- and Post-Removal and the chance of metal bioavailability causing adverse biological effects determined from sediment cores after CV 62 was removed Sinclair Inlet.

				Metal Bioavailability In Sediment		
				$(\Sigma SEM\text{-}AVS)/f_{OC}$		
				≤ 130 umol/g OC	130 umol/g OC > and ≤3000 umol/g OC	> 3000 umol/g OC
		Magnitude of Difference		Low Risk of Adverse Biological Effects	May have Adverse Biological Effects	Adverse Biological Effects May be Expected
	Statistical Difference from Baseline	No Difference or Better than Pre-Removal	**None** **Cu, Gravel, Sand, Silt, Clay, TOC, Solids (p≥0.05)**			
		≤2x Pre-Removal	**Slightly Different** **Zn (p = 0.078)**	**High Chance of Negligible Impact** **64.0%**	**Medium Chance of Potential Impact** **27.1%**	**Low Chance of Probable Impact** **8.9%**
		>2x and <5x Pre-Removal				
		≥5x and <10x Pre-Removal				
		≥10x Pre-Removal				

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5. SUMMARY AND CONCLUSIONS

The data developed for this study provided a basis for (1) determining whether biofouling removal from the ex-INDEPENDENCE (CV62) impacted sediment quality at Mooring G and (2) assessing the nature and extent of any impact. The before and after total Cu and Zn data were evaluated to determine whether any increase chemical contamination could be attributed to biofouling removal. Furthermore, the potential bioavailability and toxicity of metals were evaluated by measuring AVS, SEM, moisture, and TOC from the core sections obtained from the site after CV 62 was removed from Sinclair Inlet.

Sediment chemistry sampling was performed at six locations within the zone of influence near the ship prior to the start of operations (Pre-Removal) and approximately one month after the ship was moved from Sinclair Inlet and about two months after hull cleaning was completed (Post-Removal). Surface sediment chemistry samples were collected by ponar grab for the Pre-Removal samples and with divers for Post-Removal samples. The surface sediment samples (0–10 cm) were composited and analyzed for total Cu, total Zn, grain size, Solids, and TOC. During the Post-Removal sampling event, sediment cores (0–25 cm) were also collected from each station to obtain samples for analysis of AVS and SEM, total Cu and Zn, Solids, and TOC to evaluate metal bioavailability and toxicity at the site.

During the Pre-Removal sampling event, benthic community samples were also collected by ponar grab at the six sediment sampling stations. The benthic community samples were sieved through a 1 mm sieve, preserved with formalin, and submitted for benthic community analysis.

Mussel tissues were sampled from the hull of CV 62 and analyzed for total Cu, total Zn, moisture, and lipid content to provide a source term estimate of the Cu and Zn present in the biomass living on the hull prior to biofouling removal.

Underwater video of the sea floor obtained during the post project sampling by the PSNS&IMF Divers on March 30, 2017 was compared to the hull survey videos of portions of the hull and bottom of CV 62 recorded by Seaward Marine Inc. prior to hull cleaning in November 2016. The comparison showed that many organisms living on the hull had survived the hull cleaning were still alive and living on the sea floor below the location where CV 62 was berthed (Appendix C-1 Video Clips).

Data from the sediment surface samples (0–10 cm) taken before and after biofouling removal of project show that there were no statistically significantly ($p \leq 0.05$) differences in concentrations of total Cu , grain size, and TOC. However, total Zn concentrations were significantly different at the $p \leq 0.10$ level, and were about twice as high as concentrations prior to biofouling removal. In addition, data from the sediment cores for AVS, SEM, and TOC were used to assess the bioavailability and potential toxic effects of metals by comparing the $(\Sigma SEM - AVS)/f_{OC}$ measured to benchmarks of adverse biological effects from metal toxicity to the benthic community developed by US EPA (2005). Based on average concentrations of metals and AVS measured in the 0–10 cm core sections and using the most conservative assumptions, the analysis showed that there was a low (8.9%) chance of possible impact, a medium chance (27.1%) of potential impact, and high chance (64%) of negligible impact to the benthic community from metal toxicity. This finding suggests that the potential impact on the benthic community from metal exposure associated with biofouling removal was low.

The concentrations of total Cu and Zn measured in mussel tissues sampled from the hull of CV 62 prior to cleaning were within the range of mussel tissue concentrations reported for mussel watch stations within Sinclair and Dyes Inlets. The benthic census showed very limited macro-invertebrates

were present at the site prior to cleaning, however the video of the sea floor taken about 8 weeks after biofouling removal showed that the sea floor was covered with masses of tube worms, sea anemones, mussels, and other organisms from the vessel that were still alive and functioning on the sea floor. In addition, many crabs and other opportunistic bottom feeders were observed foraging and feeding on the biological material.

The findings from this study showed that the potential impact of biofouling removal to the benthic community from the release of copper and zinc was low and that the benthic community in the area of the vessel was not adversely degraded. However, it is unclear whether the disturbance also contributed contamination to the site or if the disturbance stirred up contamination that was already present. As seen in the video clips of the sea floor, the masses of tube worms, mussels, and anemones evidently increased the structure and diversity of the bottom community that attracted crabs and other bottom feeders. Under the anoxic conditions, the metal contaminants present would most likely be inert, bound up in insoluble metal sulfides. Whether the disturbance will contribute to the release of contamination depends on the rate of sediment reworking and oxidation. The findings from this study showed that the potential impact of biofouling removal to the benthic community from the release of copper and zinc was low and that the benthic community in the area of the vessel was not adversely degraded.

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APPENDIX A
RAW DATA REPORTS FROM ALS GLOBAL, KELSO, WA

APPENDIX A.1 ALS GLOBAL DATA REPORT FOR SEDIMENT GRABS PRE-REMOVAL

Appendices\A1_ALS-1_K1705946_Sed_Grabs_Pre.pdf

APPENDIX A.2 ALS GLOBAL DATA REPORT FOR SEDIMENT GRABS POST-REMOVAL

Appendices\A2_ALS-2_K1705817_Sed_Grabs_Post.pdf

APPENDIX A.3 ALS GLOBAL DATA REPORT FOR AVS CORES

Appendices\A3_ALS-3_K1705808_AVS_Cores.pdf

APPENDIX A.4 ALS GLOBAL DATA REPORT FOR MUSSEL TISSUES

Appendices\A4_ALS-4_K1705945_Mussel_Tissues.pdf

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APPENDIX B
TAXONOMIC DATA REPORT FROM ECOANALYST, MOSCOW, ID

B.1 BENTHIC DATA REPORT

Appendices\B_Apdx_Benthic_Data_Report.pdf

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APPENDIX C
VIDEO SURVEY RESULTS

C.1 VIDEO CLIPS

Video Clips of CV 62 Hull and Keel Prior to Cleaning and Sea Floor Adjacent to Mooring G After CV 62 was Towed from Sinclair Inlet.

Appendices\C_^FOUO_CV62_Hull&Keel_SeaFloor_long_Annotated3.avi

Transcript of Video: Appendices\C_Video_Transcript.pdf

Note: For printed versions of the report there is a CD included that contains content shown here as hyperlinks on the CD.

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APPENDIX D
ELECTRONIC DATA DELIVERABLE (EED) FORMATTED DATA REQUIRED FOR SUBMISSION TO ECOLOGY'S ENVIRONMENTAL INFORMATION MANAGEMENT (EIM) SYSTEM

D.1 OVERVIEW

Appendix D contains three Excel files containing electronic data deliverable for study, location, and Results

Study: Appendices\AppendixD\AppendixD_StudyDetails.xlsx

Location: Appendices\AppendixD\AppendixD_LocationDetails.xlsx

Results: Appendices\AppendixD\AppendixD_Results.xlsx

Note: For printed versions of the report there is a CD included that contains content shown here as hyperlinks on the CD.

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Biofouling Removal from the ex-INDEPENDENCE (CV 62) Moored in Sinclair Inlet, Puget Sound, WA:
Sediment Monitoring Report

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5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER

5e. TASK NUMBER

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13. SUPPLEMENTARY NOTES

Water Body Number
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14. ABSTRACT

The ex-INDEPENDENCE (CV 62), which had been moored at Mooring G at Naval Base Kitsap in Bremerton, WA (NBK-BREM) since decommissioning in September 1998 was towed on March 11, 2017 to Brownsville, TX, where the ship arrived on June 1, 2017 for dismantling. Based on a consultation with National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service (NMFS), the Navy was required to clean the ship's hull prior to towing in order to mitigate the possibility of transferring invasive species to other regions and thereby harm endangered species and habitats. This report presents the sediment sampling and analysis results to assess potential impacts to sediment quality from biofouling removal from CV 62 while it was moored at NBK-BREM and the Puget Sound Naval Shipyard & Intermediate Maintenance Facility (PSNS&IMF) in Sinclair Inlet, Puget Sound, WA. The data developed for this study provided a basis for
1- determining whether biofouling removal from CV 62 impacted sediment quality at Mooring G and
2- assessing the nature and extent of any impact.

The findings from this study showed that the potential impact of biofouling removal to the benthic community from the release of copper and zinc was low and that the benthic community in the area of the vessel was not adversely degraded.

15. SUBJECT TERMS

Hull Cleaning; CV-62; USS Independence; Sediment analysis; biofouling removal; sediment impact assessment

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