SALTON SEA ECOSYSTEM RESTORATION PROGRAM

Final

Bioaccumulation of Selenium in Laboratory Bioassays

January 2006

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BIOACCUMULATION OF SELENIUM IN LABORATORY BIOASSAYS

This technical memorandum presents results from laboratory bioassays using two kinds of annelid worms, the polychaete *Nereis virens* and the oligochaete *Lumbriculus variegates*, to evaluate bioaccumulation of selenium during laboratory exposures to surface sediments collected from the Salton Sea. The main objectives of the bioaccumulation study were to 1) produce biota-sediment accumulation factors (BSAFs) for use in the ecological risk assessment, and 2) determine if bioaccumulation of selenium by Salton Sea infauna might be affected by future, altered overlying water salinities. Salinities of 2, 20, and 35 parts-per-thousand (ppt) were tested to represent conditions under consideration for future restoration alternatives. Nereis was chosen as an appropriate test species to represent resident pileworms (*Neanthes succinea*) of the Salton Sea in estuarine and seawater salinities (i.e., 20 and 35 ppt, respectively). Additionally, Nereis is a common test species used in standard laboratory bioaccumulation studies of marine sediments (USEPA/USACE 1991). Lumbriculus was chosen to represent benthic macrofauna species under freshwater (i.e., 2 ppt salinity) conditions, because Nereis is not a suitable freshwater test species.

Selenium bioaccumulation is a significant concern for future Salton Sea restoration alternatives, and pileworms have historically provided an abundant food source for fish and birds. Elevated levels of selenium have been found in fish and in tissues or eggs of various species of birds that forage in the Salton Sea or its tributary streams (including rivers, creeks, and agricultural drains). Elevated levels of selenium in fish tissue are a concern at the Salton Sea and have resulted in a Public Health Advisory on fish consumption posted in the California Department of Fish and Game's 2004 Sport Fishing Regulations. An additional warning for the New River was published and posted by the Imperial County Health Department for people to avoid physical contact with the waters of the New River and to avoid eating fish of any variety taken from the river.

Field Methods

Sediment for bioaccumulation testing was collected from two broad areas of the lake as two separate composites, each of which comprised three stations (Figure 1). All sediments were collected from approximately 8 m of water depth. Initially, combined north lake sediment was used to test Nereis and combined south lake sediment was used for Lumbriculus. A laboratory error invalidated the Lumbriculus test, and it was re-run using north lake sediment remaining from the 20 ppt Nereis test, as there was not sufficient south lake sediment available. The re-used north lake sediment was conditioned in freshwater adjusted to 2 ppt salinity using Salton Sea water for 35 days before initiating the second Lumbriculus test.

Bioaccumulation of selenium in Nereis was tested using 20 and 35 ppt overlying water as a test of potential future restoration alternatives for the north lake area that is likely to include stabilized salinity at levels that are lower than current conditions. Bioaccumulation of selenium in Lumbriculus was re-tested with north lake sediment using 2 ppt water as a test of potential future restoration alternatives for the south lake area that may include freshwater wetlands constructed over historical Salton Sea sediments.

South lake sediments were collected on April 26, 2005, and north lake sediments were collected on April 27. The sediments were collected as surface grab samples of approximately the top 15 cm of sediment using a mini-Ponar sampler. The required laboratory sediment volumes were collected as equal parts from each of the three sites at both the north and south locations.

The sediments were placed from the field sampling equipment directly into clean, lined, 20-L plastic tubs. The tubs were sealed, kept chilled with ice, and driven to the bioassay laboratory on April 28, 2005.

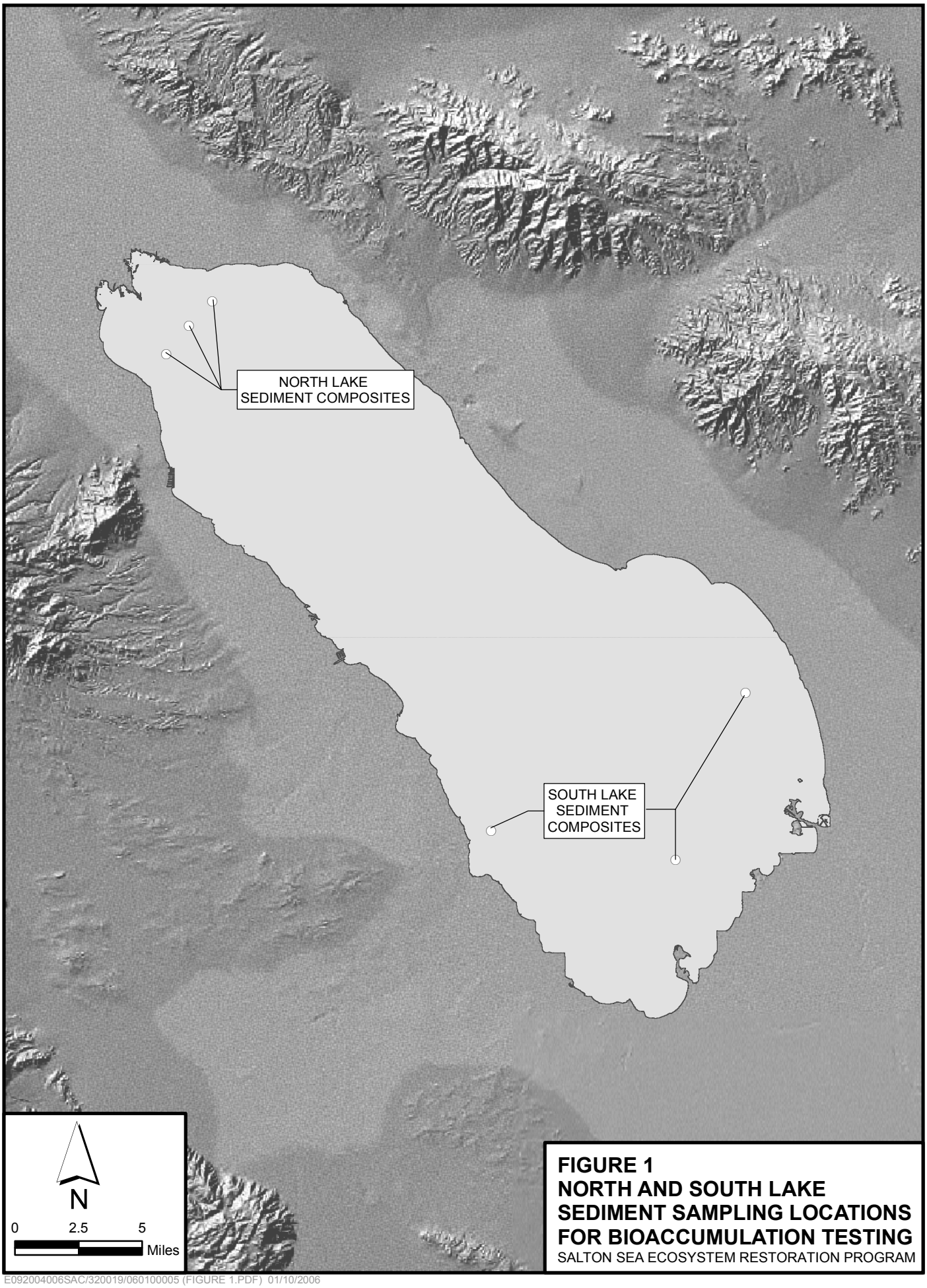

FIGURE 1
NORTH AND SOUTH LAKE SEDIMENT SAMPLING LOCATIONS FOR BIOACCUMULATION TESTING
SALTON SEA ECOSYSTEM RESTORATION PROGRAM

Laboratory Methods

Bioaccumulation tests were conducted by Weston Solutions, Inc. at their Carlsbad, California laboratory. Bioassay laboratory methods, water quality results, and survival results are presented in the report, "Salton Sea, California, Bioaccumulation Test Data Report, October 2005," prepared by Weston Solutions and included as Attachment 1 of this technical memorandum. Tissues were analyzed for selenium by Laboratory and Environmental Testing, Inc. (LET) in Columbia, Missouri. Sediments were analyzed by Columbia Analytical Services, Inc. (CAS) in Redding, California.

Total selenium was measured in a single water sample collected from the Salton Sea during sediment sampling to determine whether overlying water would provide a significant secondary exposure route for selenium. Homogenized sub-samples from test and control sediments were analyzed for total organic carbon (TOC) and particle size prior to initiation of the bioaccumulation tests by Weston Solutions.

Bioaccumulation Tests: Bioaccumulation tests were conducted using modifications of procedures outlined in USEPA/USACE (1998) and the Ocean Testing Manual (USEPA/USACE 1991) for Nereis and USEPA (2000) for Lumbriculus. Test species were supplied by Aquatic Research Organisms of Hampton, New Hampshire. Bioassay procedures and organism data for the bioaccumulation studies of the test sediment with Nereis and Lumbriculus are summarized in Tables 1 and 2, respectively.

Table 1
Test Conditions for the Bioaccumulation Study Using Nereis

Parameter	Data	
Test sediment	North Lake Salton Sea sediment acclimated to 20 ppt North Lake Salton Sea sediment acclimated to 35 ppt	
Date sampled	April 27, 2005	
Date received	April 29, 2005	
Approximate volume received	60L	
Sample storage conditions	4°C, dark, minimal head space	
Test Species	*Nereis virens*	
Supplier	Aquatic Research Organisms, Hampton, NH	
Date acquired	May 25, 2005	
Acclimation/holding time	6 days	
Age class	Adults	
Test Procedures	Modified USEPA/USACE (1998) and USEPA/USACE (1991)	
Test location	Carlsbad Lab, Room 3	
Test type/duration	Flow-through / 14 days	
Test dates	May 30 to June 13, 2005	
Control water	Scripps Institution of Oceanography seawater; 3-µm filtered, UV-sterilized, diluted with reconstituted moderately hard freshwater to a salinity of 20 or 35 ppt	
Exposure water	Salton Sea water diluted with reconstituted moderately hard freshwater to a salinity of 20 or 35 ppt	
Temperature (20 ppt test)	Recommended: 20° ± 2°C	Actual: 17.9-21.0°C
Temperature (35 ppt test)	Recommended: 20° ± 2°C	Actual: 17.8-21.3°C
Salinity (20 ppt test)	Target: 20 ± 2 ppt	Actual: 18.8-22.1 ppt
Salinity (35 ppt test)	Target: 35 ± 2 ppt	Actual: 32.0-36.0 ppt
Dissolved oxygen (20 ppt test)	Recommended: > 5.0 mg/L	Actual: 2.8-7.3 mg/L
Dissolved oxygen (35 ppt test)	Recommended: > 5.0 mg/L	Actual: 3.1-6.9 mg/L
pH (20 ppt test)	Recommended: 8 ± 0.5	Actual: 7.4-8.2

Table 1
Test Conditions for the Bioaccumulation Study Using Nereis

Parameter	Data	
pH (35 ppt test)	Recommended: 8 ± 0.5	Actual: 7.6-8.2
Photoperiod	16-hour light:8-hour dark	
Test chamber	22-L fiberglass trays with non-contaminating covers	
Replicates per treatment	5	
Organisms per replicate	15	
Exposure volume	4 cm sediment (5 L), 10 L water	
Feeding	None	
Water renewal	Flow-through ~21 mL per minute	
Modifications/deviations from Test Protocol	Test duration was 14 days instead of 28 days. Test pH and DO values fell slightly below recommended limits.	

Table 2
Test Conditions for the Bioaccumulation Study Using Lumbriculus

Parameter	Data	
Test sediment	North Lake Salton Sea sediment acclimated to 2 ppt	
Date sampled	April 26, 2005	
Date received	April 29, 2005	
Approximate volume received	20L	
Sample storage conditions	4°C, dark, minimal head space	
Test Species	*Lumbriculus variegatus*	
Supplier	Aquatic Research Organisms, Hampton, NH	
Date acquired	July 29, 2005	
Acclimation/holding time	0 days	
Age class	Adults	
Test Procedures	Modified USEPA (2000)	
Test location	Carlsbad Lab, Room 5	
Test type/duration	Twice Daily Static Renewal / 14 days	
Test dates	July 29 to August 12, 2005	
Control water	Scripps Institution of Oceanography seawater; 3-µm filtered, UV-sterilized, diluted with reconstituted moderately hard freshwater to a salinity of 2 ppt	
Exposure water	Salton Sea water diluted with reconstituted moderately hard freshwater to a salinity of 2 ppt	
Test temperature	Recommended: 23° ± 2°C	Actual: 22.3-25.8°C
Test salinity	Recommended: 2 ± 2 ppt	Actual: 2.0-2.6 ppt
Test dissolved oxygen	Recommended: > 2.5 mg/L	Actual: 5.0-8.0 mg/L
Test pH	Recommended: 7.8-8.2 (for laboratory renewal water)	Actual: 7.5-8.3 (within test chambers)
Test photoperiod	16-hour light:8-hour dark	
Test chamber	10-L plastic trays with non-contaminating covers	
Replicates/treatment	5	
Organisms/replicate	3 grams	

Table 2
Test Conditions for the Bioaccumulation Study Using Lumbriculus

Parameter	Data
Exposure volume	3 cm sediment (3 L), 3 L water
Feeding	None
Water renewal	Twice Daily Static Renewal
Modifications/deviations from Test Protocol	Test duration was 14 days instead of 28 days. Test temperatures and pH values were slightly out of recommended limits.

The Nereis bioaccumulation study was conducted in 70 x 20 x 18 cm (20-L) fiberglass tanks with a continuous flow (21 mL/min) of the test salinities of Salton Sea water (diluted to either 20 or 35 ppt). Test organisms collected from Boothbay Harbor, Maine, were placed in homogenized test (north lake Salton Sea) and home (control) sediments that were shipped with the animals. Control water was Scripps Institution of Oceanography seawater that was 3-µm filtered, UV-sterilized, and diluted with reconstituted moderately hard freshwater to a salinity of 20 or 35 ppt. Prior to test initiation, 10 to 20 worms were selected at random for analysis of pre-exposure (zero-time) selenium concentrations.

Test and control sediments were acclimated to 20 or 35 ppt salinity over several weeks, representing two individual treatments with five replicates each. Exposures were conducted for 14 days under a 16-hour, 8-hour light:dark photoperiod, and animals were not fed during testing. Tanks were stocked at densities of 15 organisms in one liter of sediment per replicate. All surviving worms were counted at test termination and placed in sediment-free containers under test conditions of water salinity for a period of 24 hours in order for the organisms to evacuate (depurate) material from their guts. Total selenium was measured in homogenized worms for each test and control replicate following depuration.

The Lumbriculus test was run twice due to a laboratory error in the initial test with south lake Salton Sea sediment, in which 20 ppt salinity water was used in the final exchange instead of 2 ppt salinity water as required. The first test was terminated at 12 days, after the error was discovered. The second test was conducted for 14 days with north lake Salton Sea sediment, as there was insufficient south lake sediment to re-run the test. Results from the second test using north lake sediment are presented in this technical memorandum. Prior to test initiation, approximately 5 grams of worms were selected at random for analysis of pre-exposure (zero-time) selenium concentrations.

The Lumbriculus bioaccumulation study was conducted in 10-liter plastic tanks with twice-daily static renewals of Salton Sea water diluted to 2 ppt salinity using reconstituted moderately hard freshwater. Test organisms were laboratory-reared at Aquatic Research Organisms' Hampton facility. Organisms were placed in 3 liters of composited north lake test sediments that had been acclimated in the diluent for 35 days to produce porewater with < 4 ppt salinity and < 1 mg/L ammonia. Control tests were run under identical conditions using freshwater sediments collected from Sandia Creek, Fallbrook, California. Control water was Scripps Institution of Oceanography seawater that was 3-µm filtered, UV-sterilized, and diluted with reconstituted moderately hard freshwater to a salinity of 2 ppt. Five replicate test chambers were stocked at densities of 3 grams of animals each. Water quality measurements, including salinity, pH, dissolved oxygen, and temperature, were monitored in all replicates on Day 0 and daily in one replicate per treatment for the remainder of each test. Interstitial and overlying ammonia were measured on Day 0 and at test termination. After 14 days, worms were separated (wet-sieved) from sediments and depurated for 24 hours.

Following depuration, worms of each test species from each treatment replicate were placed in clean glass jars with Teflon-lined lids, frozen, packaged with blue ice in sealed coolers and sent overnight under chain-of-custody to the LET analytical laboratory for analysis of selenium and moisture content.

Chemical Analysis: Tissues were analyzed for selenium by LET laboratory using atomic absorption with hydride generation following nitric acid digestion. Sediment samples were analyzed using inductively coupled plasma with mass detector (EPA Method 6020) following nitric acid digestion. Methods were selected to achieve low detection limits in tissue and sediment matrices. All tissue and sediment results were reported in dry weight. Quality control samples that included a procedural blank, a matrix spike, and a laboratory duplicate were analyzed with each analytical batch (i.e., ≤20 samples). Tissue analyses included a standard reference material (e.g., a tissue sample of known selenium concentration) with each analytical batch.

Total organic carbon (TOC) analyses were performed by CAS using a combustion technique followed by detection of CO_2 with an infra-red detector (EPA Method 415.1). Sediment grain size was determined by Weston Solutions using a sieve and pipette method (Plumb 1981).

Data Analysis Methods

Tissue concentrations were associated with corresponding sediment concentrations through the use of BSAFs to evaluate the bioaccumulation potential of selenium in the aquatic food web. BSAFs (which are unitless) were calculated for each test replicate by dividing the dry-weight tissue selenium concentration by the corresponding dry-weight sediment selenium concentration.

Evaluation of selenium bioaccumulation relied primarily on statistical comparisons of results using analysis of variance (ANOVA) grouped by sediment type and salinity, using tissue and sediment concentrations from five laboratory replicates per experimental condition (treatment). Tissue selenium concentrations and BSAF values showing significant differences between treatments at the $p \leq 0.05$ level were further examined using a Tukey HSD multiple range test (Sokal and Rohlf 1995). The Tukey HSD test is a result-guided test that compares the treatment (effects) means, while controlling the comparison-wise error rate, and was used to differentiate significant differences ($p \leq 0.05$) between treatment groups (e.g., grouped by salinity and sediment type). Non-detect selenium results were treated as one-half of the sample detection limit in all statistical analyses.

RESULTS

Water Quality

Summary results for water quality are presented in Tables 1 and 2 for the Nereis and Lumbriculus tests, respectively. Dissolved oxygen levels fell below protocol limits on Day 3 for the 20 ppt Nereis test control and Salton Sea treatments. Aeration was increased in all chambers and dissolved oxygen concentrations remained within limits for the remainder of the test.

Dissolved oxygen levels within the 35 ppt Nereis test fell slightly below protocol limits on Days 5, 8, and 11. These deviations were corrected by increasing flow and aeration to maintain dissolved oxygen levels. All other water quality results were within acceptable limits.

Temperature slightly exceeded the recommended upper limit of 25oC on Day 0 in the 2 ppt north lake Lumbriculus test; pH was up to 0.3 units out of the recommended range of 7.8 to 8.2 several times. All other measured water quality parameters were within recommended limits for the Lumbriculus test.

The sample of Salton Sea water used to formulate exposure water for the 20 and 35 ppt Nereis tests had a selenium concentration of 0.8 µg/L. Ambient Salton Sea water at 46 ppt salinity was diluted 2.3:1 and 1.3:1 to produce exposure water with salinities of 20 and 35 ppt, respectively. Resulting selenium concentrations in exposure water would have been < 0.4 µg/L for all tests, assuming negligible selenium in the laboratory diluent. Therefore, overlying water would not have provided a significant secondary exposure route for selenium to test organisms.

Sediment Physical Characteristics

Total organic carbon and particle size results for composited sediment samples are shown in Table 3. Homogenized north lake Salton Sea sediment was sub-sampled prior to initiation of the Nereis 20 and 35 ppt tests. The procedure was repeated for north lake sediment and Sandia Creek control sediment that was used in the re-run Lumbriculus test. Total organic carbon concentrations and grain size for north lake sediments were fairly consistent between the Nereis and Lumbriculus tests. Sediments were predominately fine-grained (> 93 percent silt-clay fraction) and TOC concentrations were 6.6 percent and 7.24 percent, respectively. Total organic carbon concentrations in marine sediments typically range from < 1 to 4 percent, in the absence of organic loading from anthropogenic sources (e.g., sewage outfalls); however, land-locked lake sediment concentrations can be higher. Total organic carbon concentrations greater than 5 percent would result in significant biological oxygen demand at the sediment surface, especially in poorly circulated water bodies like the Salton Sea. High TOC concentrations would be expected to produce abiotic sediments independent of potential compounding deleterious effects from elevated salinity. Additionally, elevated TOC concentrations can reduce bioavailability of some contaminants.

Table 3
Zero-time Sediment Physical Results for Composite Sample

Parameter	%TOC	%Gravel	%Sand	%Fines (Silt + Clay)
Nereis North Lake - 20 & 35 ppt[a]	6.6	0	3.1	96.9
Nereis Control - 20 & 35 ppt[a]	NM	NM	NM	NM
Lumbriculus North Lake - 2 ppt[b]	7.24	2.37	4.50	93.13
Lumbriculus Control - 2 ppt[b]	0.063	0	98.764	1.236

Notes:
[a] particle size analyzed using ASTM D-422
[b] particle size analyzed using Plumb (1981)
NM = not measured

The significant differences between control and experimental sediment quality may be important in explaining contaminant bioaccumulation results. The Lumbriculus control sediments were essentially sand, versus Salton Sea sediments that were organically rich and fine-grained (Table 3).

Bioaccumulation

Selenium concentrations in worm tissues varied primarily as a function of species, and were not significantly correlated with exposure sediment concentrations (Table 4). Selenium was not appreciably concentrated in Nereis tissues, even though concentrations were elevated in Salton Sea sediments. Lumbriculus tissues exposed to north lake and control sediments were elevated (means of 4.34 and 3.04 mg/kg; Table 4); however, a composite sample of Lumbriculus tissue had a selenium concentration of 3.3 mg/kg prior to test initiation (zero-time). These animals were laboratory-reared and may have been exposed to elevated selenium concentrations in their food or water. For this reason, tissue results for Lumbriculus and Nereis were analyzed independently in the ANOVA tests (Table 4).

Table 4
ANOVA with Contrasts Results for Tissue and Sediment Selenium Concentrations and BSAFs

Treatment[a]	ANOVA Results[b]	Mean[c]	Range	Standard Deviation
Lumbriculus Tissue Results (mg/kg - dry weight)				
Lumbriculus North Lake - 2 ppt	A	4.34	4.3-4.6	1.35
Lumbriculus Control - 2 ppt	B	3.04	3.8-4.1	0.32
Nereis Tissue Results (mg/kg - dry weight)				
Nereis North Lake - 20 ppt	C	1.48	1.2-1.7	0.15
Nereis Control - 20 ppt	C	1.36	1.2-1.5	0.11
Nereis North Lake - 35 ppt	C	1.44	1.2-1.6	0.15
Nereis Control - 35 ppt	C	1.30	1.0-1.5	0.20
Sediment Results (mg/kg - dry weight)[c]				
Lumbriculus North Lake - 2 ppt	A	12.78	11.2-14.6	1.35
Lumbriculus Control - 2 ppt	B	< 1.46	all non-detect	0.043
Nereis North Lake - 20 ppt	C	6.88	5.53-9.83	1.79
Nereis Control - 20 ppt	D	1.87	1.77-2.06	0.12
Nereis North Lake - 35 ppt	C	6.00	4.90-7.20	0.97
Nereis Control - 35 ppt	D	1.83	1.78-1.93	0.06
Biota-Sediment Accumulation Factors (unitless)				
Lumbriculus Control - 2 ppt	NA	NA	NA	NA
Nereis Control - 20 ppt	A	0.73	0.63-0.85	0.08
Nereis Control - 35 ppt	A	0.71	0.52-0.82	0.12
Lumbriculus North Lake - 2 ppt	B	0.34	0.26-0.50	0.09
Nereis North Lake - 20 ppt	B	0.22	0.15-0.27	0.05
Nereis North Lake - 35 ppt	B	0.25	0.18-0.31	0.05

Notes:
[a] ANOVA results for tissue were analyzed by species.
[b] Treatments not sharing the same letter are significantly different at $p < 0.05$
[c] Non-detects treated as one-half the detection limit in all statistical tests
NA = not applicable

Sediment-biota accumulation factors (BSAFs) were below unity (1) in all tests, except for Lumbriculus exposed to control sediment, indicating that selenium may not significantly accumulate in resident infauna of the Salton Sea during a two-week exposure. A mean BSAF value of 4.17 was calculated for Lumbriculus exposed to control sediment; however, this test is not representative of bioaccumulation potential for Salton Sea sediment, as Lumbriculus used in these tests had elevated zero-time (initial) selenium tissue concentrations and the control sediments were all non-detect (mean = < 1.46 mg/kg). Test animals would not have bioaccumulated selenium under these conditions, and may have slightly depurated or reduced tissue selenium during the 14-day exposure. BSAF results for the Lumbriculus north lake treatment were similar to Nereis results due to higher selenium concentrations measured in the exposure sediment. Selenium concentrations in sediment for the Lumbriculus north lake treatment were roughly twice those reported for the 20-ppt Nereis treatment, even though (presumably) the same sediments were used. Poor homogenization of sediment by the bioassay laboratory seems to be the most likely reason for this difference.

Results from statistical comparisons of grouped laboratory replicates indicated significant differences ($p < 0.05$) between mean selenium tissue concentrations in Lumbriculus exposed to north lake sediment and Lumbriculus exposed to the control sediment (Table 4). Lumbriculus differences may be due to the high and variable zero-time selenium concentrations in their tissues or the differences in physical qualities of the test versus control sediments (Table 3). However, the Lumbriculus results also show decreasing concentrations in controls versus increasing concentrations in the experiment as compared to zero-time concentrations. Those trends suggest that longer-term bioaccumulation of selenium may occur in freshwater invertebrates exposed to Salton Sea sediments. As noted, the experiment duration may have been too short to show BSAFs greater than unity but the significant differences between Lumbriculus controls and experimental conditions suggest the trend towards uptake in the freshwater condition.

Within-treatment variability was extremely low (coefficient of variation < 15 percent) for all tissue results, indicating that sediments were thoroughly homogenized (except for the Lumbriculus re-test, see above) and laboratory test controls were meeting or exceeding data quality objectives.

Nereis exposed to sediments acclimated to 20 and 35 ppt salinities accumulated an average of 1.48 and 1.44 mg/kg selenium, respectively, after 14 days exposure to north lake sediments. These concentrations were similar to concentrations in control tissues. Nereis BSAFs were less than unity (one) for test and control sediments, indicating that selenium may not readily bioaccumulate in this species or in similar species of the Salton Sea aquatic food web during short-term exposures. Factors potentially affecting bioavailability of selenium include 1) non-equilibrium in tissues due to insufficient test period; 2) sediment physical features (e.g., elevated TOC); 3) reduced uptake due to test animal stress; and/or 4) organisms' uptake of selenium achieved the maximum possible, but that limit was unexpectedly low. Factor 1 may have had the greatest influence, as the standard test period for Nereis is 28 days following test protocols published by USEPA and the Army Corps of Engineers (USEPA/USACE 1991). Tissue selenium concentrations in the only two samples of pileworms that could be collected from the Salton Sea in spring 2005 were 4.0 and 6.2 mg/kg, suggesting that the laboratory bioaccumulation test duration may not have been long enough to reach equilibrium. Nereis may have been sufficiently stressed under test conditions to inhibit selenium uptake (Factor 3), since they were exposed to sediments with much higher organic carbon content and different salinities compared with their native habitats. Test animals exposed to Salton Sea sediments were noted as "less active" as compared with control animals at the end of the 20 and 35 ppt salinity tests.

Although non-detected values, problems with sediment homogenization, and the short time period for bioaccumulation combined to limit our ability to detect significant differences, the results suggest a potential for bioaccumulation of selenium in the freshwater condition not revealed in the higher salinity tests. Nevertheless, field samples verify that pileworms accumulate selenium under hypersaline conditions in the Salton Sea. It is likely that worms in the future, under both saline and freshwater Salton Sea conditions, also will bioaccumulate selenium.

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ATTACHMENT 1

Bioaccumulation Test Data Report, Salton Sea, California

Salton Sea, California
Bioaccumulation Test Data Report

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WESTON SOLUTIONS

Salton Sea, California
Bioaccumulation Test Data Report

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APPENDICES

ACRONYMS AND ABBREVIATIONS

%	percent
BP	bioaccumulation potential
cm	centimeters
°C	degrees Celsius
DO	dissolved oxygen
L	liter
mg/L	milligram per liter
mL	milliliter
mL/min	milliliter per minute
mm	millimeter
mS/cm	millisiemens per centimeter
ppt	parts per thousand
SD	Standard deviation
µg/L	microgram per liter
USACE	United States Army Corp of Engineers
USEPA	United States Environmental Protection Agency

1. INTRODUCTION

The Salton Sea is the largest lake in California, located in the southeast desert region of Imperial County. This 376 square mile lake is an important wetland located on the Pacific Flyway. Ever increasing salinity levels and frequent reports of bird and fish mortalities have raised many questions about how to manage this water body. Of particular concern are the levels of the element selenium found in the lake sediment and water. Selenium is a naturally occurring element that comes from the Colorado River water and agricultural runoff which composes 90 percent of the input into the Salton Sea. The apparent salinity of the Salton Sea is a result of evaporation of this incoming water. The salts that are concentrated through this evaporative process are different than standard oceanic seawater conditions. Due to the difference in salt ions, the elevated concentrations of selenium, and the observation of adverse biological effects, a testing program has been developed to evaluate the bioaccumulation potential of selenium in aquatic organisms. The objective of this program is to determine the ideal "salinity" and required alterations that could be made to the lake as part of a management practice. *Neanthes succinia* is one of the few successful introduced species in the Salton Sea and forms the basis of this marine food web. This introduced Nereid polychaete worm has adapted to the high salinity of these abherent salts found in the Salton Sea. While this worm was not available to perform laboratory bioaccumulation studies, a similar marine polychaete worm, *Nereis virens*, was used as an analog to *N. succinia*. A freshwater oligochaete worm, *Lumbriculus variegatus*, was also used in this evaluation to determine its potential success in less saline environments. The results of the bioaccumulation studies on sediments collected from the Salton Sea are detailed below.

2. MATERIALS AND METHODS

2.1 BIOACCUMULATION POTENTIAL TESTING

Assessment of bioaccumulation potential (BP) was carried out using the marine polychaete worm *Nereis virens* and the freshwater oligochaete *Lumbriculus variegatus* over a 14-day test period. Both test species were supplied by Aquatic Research Organisms of Hampton, New Hampshire. Bioaccumulation tests were conducted in accordance with those procedures outlined in the United States Environmental Protection Agency (USEPA) and the United States Army Corp of Engineers (USACE) documents EPA-823-B-98-004 (Inland Testing Manual) and EPA 503/8-91/001 (Ocean Testing Manual) for the testing with *N. virens* and EPA/600/R-99/064 for the testing with *L. variegatus*. The test duration of both the *N. virens* and *L. variegatus* was modified from 28 to 14 days because of the uncertainty associated with survival of these test organisms in salinity regimes that are not normal for these species. A time period of 10 to 14 days has been recommended as sufficient time to reach steady state conditions with *L. variegatus* (ASTM 2005). USEPA/USACE guidance documents infer that shorter duration tests may be adequate for testing depending on the steady state nature of the contaminant of concern and the test organism (USEPA/USACE 1977, 1991). The accelerated nature of this testing program also influenced the decision to reduce the test duration.

The *N. virens* bioaccumulation study was conducted in 70 × 20 × 18 centimeter (20 liter [L]) capacity) fiberglass tanks with a continuous flow (21 milliliters per minute [mL/min]) of two salinities of Salton Sea water (20 and 35 parts per thousand [ppt])). Exposures

were conducted under a 16-hour light: 8-hour dark photoperiod and animals were not fed over the 14-day exposure. Test organisms were placed in 5 L of North Lake Salton Sea sediment (five replicates each) that was previously acclimated to the two salinity regimes. Tanks were stocked at densities of 15 animals per chamber. The *L. variegatus* bioaccumulation study was conducted in 10-L plastic tanks with twice daily static-renewals of Salton Sea water adjusted to 2 ppt. Exposures were conducted under a 16-hour light: 8-hour dark photoperiod and animals were not fed over the 14-day exposure. Test organisms were placed in 3 L of South Lake Salton Sea sediment (five replicates each) that was previously acclimated to 2 ppt. Tanks were stocked at densities of 3 grams (g) of animals per chamber. Additionally, the North Lake sample was also tested with *L. variegates* with sediment recovered from the initial assessment.

Water quality measurements including salinity, pH, dissolved oxygen, and temperature were monitored in all replicates on day 0 and daily in one replicate per treatment for the remainder of the test. Interstitial and overlying ammonia was measured on day 0 and day 14.

Test organisms were recovered at exposure termination (14 days). All surviving worms were counted and placed in sediment-free containers under test conditions for a period of 24 hours in order for the organisms to purge their gut contents. Following gut purging, animal tissues for each test species from each treatment replicate were placed in clean glass jars with Teflon-lined lids, frozen, packaged with blue ice in sealed coolers and then sent overnight under chain-of-custody to the project analytical laboratory (Columbia Analytical Services, Inc.). Bioassay procedures and organism data for the bioaccumulation studies of the test sediment are summarized in Table 1 for *N. virens* and Tables 2 and 3 for *L. variegatus*.

Table 1: Bioassay Procedure and Organism Data for the 14-Day Bioaccumulation Study using *N. virens*

Parameter	Datum	
Sample Identification	**North Lake Salton Sea acclimated to 20ppt** **North Lake Salton Sea acclimated to 35ppt**	
Date sampled	April 26-27, 2005	
Date received at Weston	April 29, 2005	
Approximate volume received	60L	
Sample storage conditions	4°C, dark, minimal head space	
Test Species	***N. virens***	
Supplier	Aquatic Research Organisms, Hampton, NH	
Date acquired	May 25, 2005	
Acclimation/holding time	6 days	
Age class	Adults	
Test Procedures	**USEPA (1994); OTM (USEPA and USACE, 1991)**	
Test location	Carlsbad Lab, Room 3	
Test type/duration	Flow-through / 14 days	
Test dates	May 30 – June 13, 2005	
Control water	Scripps Institution of Oceanography seawater; 3 µm filtered, UV –sterilized Diluted with reconstituted moderately hard freshwater to a salinity of 20 and 35 ppt.	
Exposure water	Salton Sea water diluted with reconstituted moderately hard freshwater to a salinity of 20 and 35 ppt.	
Test temperature (20 ppt exposure)	Recommended: 20 ± 2°C	Actual: 17.9 – 21.0°C
Test temperature (35 ppt exposure)	Recommended: 20 ± 2°C	Actual: 17.8 – 21.3°C
Test salinity (20 ppt exposure)	Target: 20 ± 2 ppt	Actual: 18.7 – 22.1 ppt
Test salinity (35 ppt exposure)	Target: 35 ± 2 ppt	Actual: 32.0 – 36.0 ppt
Test dissolved oxygen (20 ppt exposure)	Recommended: > 5.0 mg/L	Actual: 2.8 – 7.3 mg/L
Test dissolved oxygen (35 ppt exposure)	Recommended: > 5.0 mg/L	Actual: 3.1 – 6.9 mg/L
Test pH (20 ppt exposure)	Recommended: 8 ± 0.5	Actual: 7.4 - 8.2
Test pH (35 ppt exposure)	Recommended: 8 ± 0.5	Actual: 7.6 - 8.2
Test photoperiod	16-hour light:8-hour dark	
Test chamber	22-L fiberglass trays with non-contaminating covers	
Replicates/treatment	5	
Organisms/replicate	15	
Exposure volume	4 cm sediment (5 L), 10 L water	
Feeding	None	
Water renewal	Flow through ~20 mL per minute	
Deviations from Test Protocol	Test pH and DO values fell slightly below the recommended limits.	

Table 2: Bioassay Procedure and Organism Data for the 14-Day Bioaccumulation Study using *L. variegatus* (South Lake)

Parameter	Datum	
Sample Identification	**South Lake Salton Sea acclimated to 2 ppt**	
Date sampled	April 26-27, 2005	
Date received at MEC - Tiburon	April 29, 2005	
Approximate volume received	20L	
Sample storage conditions	4°C, dark, minimal head space	
Test Species	***L. variegatus***	
Supplier	Aquatic Research Organisms, Hampton, NH	
Date acquired	May 26, 2003	
Acclimation/holding time	0 days	
Age class	Adults	
Test Procedures	**USEPA (2000)**	
Test location	Carlsbad Lab, Room 5	
Test type/duration	Twice Daily Static Renewal / 14 days	
Test dates	May 26 – June 7, 2005 (South Lake Sample) May 26 – June 9, 2005 (Control)	
Control water	Scripps Institution of Oceanography seawater; 3 µm filtered, UV –sterilized Diluted with reconstituted moderately hard freshwater to a salinity of 2 ppt.	
Exposure water	Salton Sea water diluted with reconstituted moderately hard freshwater to a salinity of 2 ppt.	
Test temperature	Recommended: 23° ± 2°C	Actual: 21.9°–24.4°C
Test salinity	Recommended: 2 ± 2 ppt	Actual: 2.0–16.5 ppt
Test dissolved oxygen	Recommended: > 2.5 mg/L	Actual: 2.7–8.3 mg/L
Test pH	Recommended: 7.8 – 8.2 (for laboratory renewal water)	Actual: 7.2–8.1 (within test chambers)
Test photoperiod	16-hour light:8-hour dark	
Test chamber	10-L plastic trays with non-contaminating covers	
Replicates/treatment	5	
Organisms/replicate	3 grams	
Exposure volume	3 cm sediment (3 L), 3 L water	
Feeding	None	
Water renewal	Twice Daily Static Renewal	
Deviations from Test Protocol	Test pH values fell outside the recommended limits. Test salinities were above the desired limits.	

Table 3: Bioassay Procedure and Organism Data for the 14-Day Bioaccumulation Study using *L. variegatus* (North Lake)

Parameter	Datum	
Sample Identification	**North Lake Salton Sea acclimated to 2 ppt**	
Date sampled	April 26-27, 2005	
Date received at MEC - Tiburon	April 29, 2005	
Approximate volume received	20L	
Sample storage conditions	4°C, dark, minimal head space	
Test Species	***L. variegatus***	
Supplier	Aquatic Research Organisms, Hampton, NH	
Date acquired	July 29, 2005	
Acclimation/holding time	0 days	
Age class	Adults	
Test Procedures	**USEPA (2000)**	
Test location	Carlsbad Lab, Room 5	
Test type/duration	Twice Daily Static Renewal / 14 days	
Test dates	July 29 – August 12, 2005	
Control water	Scripps Institution of Oceanography seawater; 3 µm filtered, UV –sterilized Diluted with reconstituted moderately hard freshwater to a salinity of 2 ppt.	
Exposure water	Salton Sea water diluted with reconstituted moderately hard freshwater to a salinity of 2 ppt.	
Test temperature	Recommended: 23° ± 2°C	Actual: 22.3°–25.8°C
Test salinity	Recommended: 2 ± 2 ppt	Actual: 2.0–2.6 ppt
Test dissolved oxygen	Recommended: > 2.5 mg/L	Actual: 5.0–8.0 mg/L
Test pH	Recommended: 7.8 – 8.2 (for laboratory renewal water)	Actual: 7.5–8.3 (within test chambers)
Test photoperiod	16-hour light:8-hour dark	
Test chamber	10-L plastic trays with non-contaminating covers	
Replicates/treatment	5	
Organisms/replicate	3 grams	
Exposure volume	3 cm sediment (3 L), 3 L water	
Feeding	None	
Water renewal	Twice Daily Static Renewal	
Deviations from Test Protocol	Test temperatures and pH values rose slightly above protocol limits.	

2.2 SEDIMENT ACCLIMATION

Project sediments represent two areas within the Salton Sea designated as North Lake and South Lake. The North Lake sample is a composite of three samples provided to the Weston Laboratory. Prior to testing, the project sediments were allowed to acclimate to the specific test conditions in each exposure. These experiments were designed to investigate the impact of a reduction in salinity of the Salton Sea on the bioaccumulation potential of selenium. The pore water salinity of the North and South Lake samples were 44 and 45 ppt, respectively. The North Lake sediments were acclimated to two salinities; 20 and 35 ppt, while the South Lake sample was acclimated to 2 ppt.

The sediment acclimation was required to allow the sediment pore water to come into equilibrium with the target salinity. These acclimations would also permit the growth of desired microbial communities suited to each salinity. This was especially important in the case of the South Lake sample which is testing a marine sediment (45 ppt) after being converted to a freshwater environment (2 ppt). The sediment salinity acclimation was accomplished by layering the sediment and overlying water for each test following those procedures outlined in Section 2.1 and Tables 1, 2, and 3. The addition of animals to initiate the tests was delayed until the acclimation period was completed. Overlying water renewals were performed as prescribed by each protocol with the appropriate water salinity. The *N. virens* test was conducted under flow-through conditions, while the *L. variegatus* was conducted as a twice daily static renewal. During acclimation, slight agitation of the sediment was conducted with a glass rod to encourage mixing of the overlying and interstitial water.

The pore water salinity was tracked over the course of the acclimation to determine when test initiation could commence. The North Lake samples tested with *N. virens* were allowed to acclimate for 14 days prior to test initiation. Pore water salinities of the 20 and 35 ppt exposures were at 27 and 42 ppt, respectively. The salinity difference (7 ppt in both cases) was felt to be an acceptable variance to initiate testing. Pore water salinities taken at test termination (28 days of acclimation) were 25 and 40 ppt for the 20 and 35 ppt exposures, respectively. This indicates that time to equilibrium would have taken much longer (months), which would have been outside the scope of the project. The South Lake sample tested with *L. variegatus* was allowed to acclimate for 13 days prior to test initiation. The pore water salinity of the 2 ppt exposure was at 7 ppt at test initiation. The method of static water renewals for these tests is slightly more disruptive to the sediment surface than the flow through exposures. This may have aided the acclimation of the 44 ppt South Lake sample to the low salinity. The presence of green algae that developed on the surface of the sediment indicated that the resident microfauna adapted well to the low salinity environment. An additional *L. variegatus* test was performed on North Lake sediment retained from the 20 ppt *N. virens* test. This material was acclimated to a salinity of 2 ppt for 35 days prior to test initiation.

2.3 SEAWATER FOR BIOASSAY TESTING

Salton Sea water was collected at Johnsons's Landing in Salton City. The point of collection was off of the northern boat launch jetty which was graded to allow vehicle access. Water was pumped from 2-3 feet below the surface into a 1500 gallon stainless steel tank outfitted onto a truck. This water was then transported to Weston's Carlsbad laboratory where it was transferred to polypropylene tanks for use in the bioaccumulation tests. Collections were made on May 8th and 18th and June 1st, 2005. Additional water collected was made on July 28th, 2005 using a peristaltic pump to collect 60 L in plastic cubitainers.

Seawater used in this study came from Scripps Institution of Oceanography for the control treatments. This control seawater source has been used successfully on similar bioassay testing programs by Weston. Extensive testing on a variety of test species and biannual chemical analysis of this seawater source has shown that there is no significant potential for toxicity or bioaccumulation from this water supply. Similarly, good survival of organisms in the control sediment utilized in this testing program has been achieved consistently in previous dredged material testing.

Reconstituted moderately hard freshwater was used to dilute the Salton Sea water and the Scripps seawater to the appropriate salinities. This was prepared be adding reagent grade chemical to de-ionized water following USEPA guidelines for moderately hard water preparation (USEPA 2002). As with the Seawater described above, testing on a variety of test species and biannual chemical analysis of this fresh water source has shown that there is no significant potential for toxicity or bioaccumulation from this water supply.

2.4 WATER QUALITY

Water quality was monitored daily as appropriate for each test, and data were recorded on data sheets. Dissolved oxygen and temperature was measured using Orion™ Model 840 oxygen meters and probes; pH was measured using Orion™ Model 230A pH meters and probes. Conductivity was measured with Orion™ Models 142 conductivity/salinity meters. Ammonia was analyzed using an Orion™ 720 digital ion analyzer with a three-point calibration curve (1, 10, and 100 mg/L). Hardness and alkalinity were measured utilizing LaMotte™ titration kits.

3. RESULTS

3.1 N. VIRENS – NORTH LAKE SAMPLE

The survival results of the 14-day *N. virens* BP test conducted on the North Lake Salton Sea material are included in Table 4. The mean survival of the laboratory control treatments were 100 and 89.3% for the 20 and 35 ppt test series, respectively. These high survival rates indicate that the organisms were healthy and that the laboratory conditions were adequate in supporting the life of the worms over the course of the tests. The mean survival of the 20 and 35 ppt North Lake treatments were 94.7 and 82.7%, respectively. Anecdotal observations of the worms at termination indicate that the worms recovered from the North Lake treatments, although alive, expressed a decrease in sinusoidal body motion than those recovered from the Controls. Potential contaminates or the differing salt ions contained in the Control versus the Salton Sea treatment may have contributed to these observed effects.

Table 4: Test result summary for the 14-Day Bioaccumulation Study using *N. virens* (North Lake)

Treatment	Replicate	Number Initiated	Number Surviving	Number Dead or Missing	Percent Survival	Mean Percent Survival	SD
Native Control (20 ppt)	1	15	15	0	100	100.00	0.00
	2	15	15	0	100		
	3	15	15	0	100		
	4	15	15	0	100		
	5	15	15	0	100		
North Lake (20 ppt)	1	15	15	0	100	94.67	7.30
	2	15	15	0	100		
	3	15	15	0	100		
	4	15	13	2	87		
	5	15	13	2	87		
Native Control (32 ppt)	1	15	15	0	100	89.33	7.60
	2	15	14	1	93		
	3	15	13	2	87		
	4	15	13	2	87		
	5	15	12	3	80		
North Lake (35 ppt)	1	15	12	3	80	82.67	7.60
	2	15	13	2	87		
	3	15	14	1	93		
	4	15	12	3	80		
	5	15	11	4	73		

The Native Control treatment run in conjunction with the 35 ppt North Lake sample was conducted at a slightly lower salinity of 32 ppt. This reflects the highest achievable salinity of natural seawater used for the overlying water. Dissolved oxygen (DO) levels fell below the protocol limit of 5.0 milligrams per liter (mg/L) in the 20 ppt Native Control (Days 1 and 3), 35 ppt North Lake sample (Days 5, 8, and 11), and the 32 ppt Native Control (Day 1). Test chamber aeration was increased or restored in most cases in order to bring the dissolved oxygen levels above protocol limits. The drop in the dissolved oxygen level of the 35 ppt North Lake sample on day 5 was due to a temporary interruption of the renewal water flow into the chamber. In all cases, dissolved oxygen levels returned to above protocol limits on the days following the deviation. The test pH value of the 20 ppt Native Control was 7.4 on day 0, slightly below the recommended limit of 7.5. This pH value is still well within the tolerance range of N. virens (6-9) and does not reflect a significant deviation from protocol limits. All other water quality parameters were within protocol limits.

3.2 L. VARIEGATUS – SOUTH LAKE SAMPLE

The weight recovery results of the 14-day *L. variegatus* BP test conducted on the South Lake Salton Sea material are included in Table 5. Due to the small size of the test organisms (40-90 millimeter [mm] in length), *L. variegatus* are added to test chambers by wet weight (3.0 grams [g] per chamber). This weight equates to initial stocking density of worms in the hundreds (100+). The small size and large quantity of worms in each test chamber make enumeration of final counts and weights difficult. The standard practice at test termination is to obtain final wet weights. The mean recovery wet weight

of the laboratory control treatment was 3.4 g. The mean wet weight recovered from the South Lake sample was 2.7 g. While these weights are useful in determining if sufficient tissue mass is available for analysis, these weights are not commonly extrapolated into survival or growth determinations. Observations of test chambers during the course of test indicated that worms exposed to the South Lake sample tended to migrate towards the edge of the test chamber. This differs from the laboratory control treatment where worms were more evenly distributed throughout the sediment.

Table 5: Test result summary for the 14-Day Bioaccumulation Study using *L. variegatus* (South Lake)

Treatment	Replicate	Grams of Tissue at Initiation (Wet Weight)	Grams of Tissue Recovered at Termination (Wet Weight)	Change in Weight	Mean Wet Weight (grams)
Sandia Creek Control	1	3.0	3.8	0.8	3.44
	2	3.0	4.1	1.1	
	3	3.0	3.0	0	
	4	3.0	3.1	0.1	
	5	3.0	3.2	0.2	
South Lake	1	3.0	3.0	0.0	2.66
	2	3.0	1.5	-1.5	
	3	3.0	1.9	-1.1	
	4	3.0	3.4	0.4	
	5	3.0	3.5	0.5	

The test pH values (ranging from 7.2 – 8.1) for the North Lake *L. variegatus* test experienced a slight drift from the recommended pH of 7.8 to 8.2 units for the laboratory water. Test chambers were renewed twice daily with laboratory water within the pH range of 7.8 to 8.2. Since water quality measurements were taken immediately prior to the first renewal of each day, the test organisms were not exposed to those pH deviations for long durations. The pH drift represented a maximum of a 0.6 unit change from the desired range and were a result of the water interacting with the test material. This deviation is not expected to impact the significance of the test results. All other water quality parameters were within protocol limits.

On day 11, the overlying water of the North Lake sediment treatments was inadvertently renewed with 20 ppt Salton Sea water instead of the desired 2 ppt. This deviation was discovered on the morning of day 12 with a salinity of 16.4 ppt observed in the overlying water. Due to this deviation, the North Lake treatment was terminated on day 12 for fear of excess mortality due to the salinity change. Recovery of the test organisms indicated that excess mortality had not occurred and that sufficient tissue mass was available for analyses. Worms were allowed to depurate for 24 hours as required. The Control treatments which had not been exposed to the salinity change were allowed to continue to day 14 and treated as normal.

3.3 L. VARIEGATUS – NORTH LAKE SAMPLE

The weight recovery results of the 14-day *L. variegatus* BP test conducted on the North Lake Salton Sea material are included in Table 6. The mean recovery wet weight of the laboratory control treatment and the South Lake sample was 3.5 and 3.1 g, respectively.

Table 6: Test result summary for the 14-Day Bioaccumulation Study using *L. variegatus* (North Lake)

Treatment	Replicate	Grams of Tissue at Initiation (Wet Weight)	Grams of Tissue Recovered at Termination (Wet Weight)	Change in Weight	Mean Wet Weight (grams)
Sandia Creek Control	1	3.0	3.4	0.4	3.54
	2	3.0	3.5	0.5	
	3	3.0	3.6	0.6	
	4	3.0	4.1	1.1	
	5	3.0	3.1	0.1	
North Lake	1	3.0	2.8	-0.2	3.06
	2	3.0	2.9	-0.1	
	3	3.0	4.0	1.0	
	4	3.0	2.7	-0.3	
	5	3.0	2.9	-0.1	

The temperature of Control treatment was slightly above protocol limits on day 0. The test chamber was moved slightly away from lighting fixtures which may have been influencing the temperate. Test temperatures remained within protocol limits for the remainder of the test. The test pH values (ranging from 7.5 – 8.3) for the South Lake *L. variegatus* test experienced a slight drift from the recommended pH of 7.8 to 8.2 units for the laboratory water. Test chambers were renewed twice daily with laboratory water within the pH range of 7.8 to 8.2. Since water quality measurements were taken immediately prior to the first renewal of each day, the test organisms were not exposed to those pH deviations for long durations. The pH drift represented a maximum of a 0.1 unit change from the desired range and were a result of the water interacting with the test material. This deviation is not expected to impact the significance of the test results. All other water quality parameters were within protocol limits.

4. REFERENCES

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