

SEDIMENT TOXICITY PILOT STUDY FOR THE SAN DIEGO OCEAN OUTFALL MONITORING REGIONS, 2016 – 2018 FINAL PROJECT REPORT

Point Loma Wastewater Treatment Plant, Point Loma Ocean Outfall (Order No. R9-2017-0007, NPDES No. CA0107409)

South Bay Water Reclamation Plant, South Bay Ocean Outfall (Order No. R9-2013-0006 as amended; NPDES No. CA0109045)

South Bay International Wastewater Treatment Plant, South Bay Ocean Outfall (Order No. R9-2014-0009 as amended, NPDES No. CA0108928)

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INTRODUCTION

The requirement for toxicity testing of coastal offshore marine sediments for the Point Loma Ocean Outfall (PLOO) and South Bay Ocean Outfall (SBOO) monitoring regions off San Diego, California was added to the receiving waters monitoring and reporting programs for three different treatment facilities in 2017 through new or amended orders and permits issued to the City of San Diego (City) and the U.S. Section of the International Boundary and Water Commission (USIBWC). These included (1) Order R9-2017-0007, NPDES CA0107409 for the City's Point Loma Wastewater Treatment Plant, (2) Order R9-2013-0006 as amended by Orders R9-2014-0071 and R9-2017-0023 for the City's South Bay Water Reclamation Plant, and (3) Order R9-2014-0009 as amended by Orders R9-2014-0094 and R9-2017-0024 for the USIBWC's South Bay International Wastewater Treatment Plant. All three of the above Orders required that the two dischargers (City and USIBWC) implement the joint Sediment Toxicity Monitoring Plan for the South Bay Ocean Outfall and Point Loma Ocean Outfall Monitoring Regions, San Diego, California (City of San Diego, 2015), which had been developed in consultation between staff of the California Regional Water Quality Control Board, San Diego Region (San Diego Water Board), U.S. Environmental Protection Agency (USEPA), Region IX, Southern California Coastal Water Research Project (SCCWRP), City, and USIBWC.

The sediment toxicity plan for the San Diego ocean outfall regions was designed as a three (3) year pilot study with the goal of answering the following three primary questions:

- 1. What is the extent and magnitude of sediment toxicity in offshore marine sediments in the Point Loma and South Bay outfall monitoring regions?
- 2. How does the extent and magnitude of sediment toxicity off San Diego compare among different continental shelf strata (e.g., inner, mid, and outer shelf)?
- 3. How does the extent and magnitude of sediment toxicity off San Diego compare to results from the Southern California Bight regional monitoring surveys?

This final project report summarizes the results and conclusions of all sediment toxicity testing conducted for the San Diego ocean outfall areas during the summers of 2016, 2017 and 2018. Based on the results of this 3-year pilot study and additional considerations, recommendations are made for future toxicity testing of offshore marine sediments as part of the combined ocean monitoring program for the Point Loma and South Bay ocean outfalls. Raw data for the 2016-2017 surveys are available in Nautilus Environmental (2016, 2017), while raw data for the 2018 survey are available upon request.

MATERIALS & METHODS

Study Design

The sediment toxicity plan for the San Diego outfall regions was designed to ensure that the benthic stations sampled were spatially representative of the different types of sediments present within the areas of interest. These included stations drawn from a combination of "core" (permanent, fixed

location) and "random array" sites that are presently monitored each year. Details of core outfall stations (e.g., latitude and longitude, depth) that are monitored twice per year (Winter, Summer), as well as the requirements for an additional array of 40 randomly selected stations sampled just once per year and that range from near the City of Del Mar south to the USA/Mexico border, are available in the above orders and most recent biennial monitoring and assessment report (City of San Diego 2018).

The final sampling plan used a hybrid sampling design of both permanent (fixed) and random stations as described below for the 3-year pilot study. All field sampling was conducted by City of San Diego staff during July of each year following standard benthic sampling procedures for collecting sediment toxicity samples (e.g., see Bight'18 Toxicology Committee 2018, City of San Diego 2018). A map of the stations sampled and tested is shown in Figure 1.

Overall, a total of 65 sediment samples from 49 different stations were tested during the pilot study as follows:

- 1. Year 1, July 2016 (n = 28) = combination of fixed and randomized stations
 - a. Eight (8) near-ZID stations located within 1,000 m of the PLOO or SBOO discharge sites were sampled and tested. These are predicted to be the sites most likely to be affected by wastewater discharge, and include PLOO stations E11, E14, E17 and E15, and SBOO stations I12, I14, I15 and I16.
 - b. Twenty (20) randomly selected regional stations were also tested. These stations represented half of the 40 random stations designated for the regular 2016 regional survey off San Diego, with the sediment toxicity subset being approved in consultation between City, USIBWC, USEPA, and San Diego Water Board staff.
- 2. <u>Year 2</u>, July 2017 (n = 8) = repeat sampling of fixed near-ZID stations only a. Repeat sampling of same 8 near-ZID stations as tested in 2016.
- 3. <u>Year 3, July 2018 (n = 29) = repeat of Year 1 design</u>
 a. Repeat sampling of same 8 near-ZID stations as tested in 2016 and 2017.
 - b. Twenty-one (21) stations selected from Bight'18 regional program station draw.

Toxicity Testing

All sediment toxicity testing was conducted on marine sediments using the marine amphipod *Eohaustorius* estuarius. These tests were conducted by either Nautilus Environmental (Nautilus) for the 2016 and 2017 surveys or the City of San Diego Toxicology Laboratory (CSDTL) for the 2018 survey. Both laboratories are certified by the California State Water Resources Control Board Environmental Laboratory Accreditation Program (Nautilus = ELAP Certificate No. 1802; CSDTL = ELAP Certificate No. 1989) and follow similar, comparable procedures for sediment bioassays. Specific details for the methods and analyses conducted by the CSDTL in 2018 are described below and in City of San Diego (2017). In contrast, additional information for the 2016-2017 surveys are available in Nautilus Environmental (2016, 2017).

Amphipod Bioassays

The 10-day amphipod sediment toxicity tests were conducted by the CSDTL in accordance with EPA 600/R-94/0925 (USEPA 1994) and the procedures approved for Southern California Bight 2018



Figure 1

Distribution of benthic stations selected for sediment toxicity testing during the 2016-2018 pilot study off San Diego. Near-ZID outfall stations sampled each year are shown as blue circles for the Point Loma Ocean Outfall (PLOO) and pink circles for the South Bay Ocean Outfall (SBOO); randomized regional stations sampled in 2016 are shown as green triangles; randomized Bight'18 stations sampled in 2018 are shown as orange triangles.

Regional Monitoring Program (Bight'18 Toxicology Committee 2018). Juvenile *E. estuarius*, were exposed for 10 days to both test and control sediments. Response criteria included amphipod mortality, emergence from sediment during exposure, and if considered a measurement of interest, ability of amphipods to rebury in clean sediment at the end of the bioassay. In addition, a reference toxicant test (using seawater only) was conducted concurrently and under identical environmental conditions as the sediment toxicity tests to determine test organism sensitivity.

Preparation of Test Organisms

Juvenile amphipods between 3-5 mm in length were purchased from Northwestern Aquatic Sciences (Newport, OR). These amphipods were collected from uncontaminated sites with large endemic populations and shipped overnight to the CSDTL. The organisms were shipped within control sediment that was collected from the same reference sites and sieved through a 500-micron screen. Upon receipt, temperature and salinity of the shipping sediment were measured and recorded. The condition of the test animals was observed for mortality and only amphipods deemed healthy and acceptable were used for testing. The test amphipods were then transferred from within their holding containers into larger aquaria, and held at $15 \pm 1^{\circ}$ C. The amphipods were left undisturbed in the home sediment and submerged within an overlying layer of filtered seawater (see Dilution Water below). All test animals were acclimated for between 2 and 10 days prior to test initiation.

Dilution Water

Dilution water for the sediment and reference toxicant tests consisted of natural seawater obtained from the Scripps Institution of Oceanography (San Diego, CA). Dilution water was collected within 96 hours of first use, and transported to the CSDTL. The seawater was first filtered with an in-line system containing 1.0- μ m and 0.2- μ m polypropylene filters, then collected and held in 20-L carboys at 15 ± 1°C.

Test chambers

The test chambers consisted of standard 1-L glass jars, with five test replicates per sediment sample plus a sixth replicate used for pore water extraction and water quality measurements. On the day before test initiation, 175 mL of pre-sieved (1.0 mm mesh screen) test sediment was added to the bottom of each replicate jar to create a 2-cm deep layer, after which the jar was filled with seawater, covered, and placed in a 15°C temperature controlled room. Water in the test containers was gently aerated to promote constant circulation without disturbing the sediment surface. On the following day (Day 0), amphipods were sieved from the holding sediment through a 0.5 mm mesh screen and transferred with large bore plastic pipettes to transfer dishes containing approximately 50 mL of seawater until each container contained either 20 amphipods for the sediment tests or 10 amphipods for the reference toxicant test.

Sediment toxicity tests

For the acute sediment toxicity tests, juvenile amphipods were distributed into the test chambers in a randomized manner and with minimal disturbance to the test sediment. The amphipods were initially given 5 to 10 minutes to bury into the test sediments. Injured or stressed animals that remain emerged (not buried) were removed and replaced with healthy amphipods from the same sieved population.

A photoperiod of 16h light:8h dark was used for the amphipod testing. Light intensity was maintained between 50-100 ft candles in all areas of the environmental chamber throughout the test period. The

number of emergent (swimming) and surface-trapped amphipods were counted and recorded daily. Any amphipods trapped at the air-water interface were gently pushed down into the water with a widebore plastic pipette. The test was terminated after 10 days of exposure.

At completion of the 10-day test period, sediments were sieved through a 0.5 mm screen, and the number of live, dead, or missing amphipods recorded. Missing animals were assumed to have died and decomposed during the 10-day test; these missing animals were subsequently counted as dead when calculating percent survival for each replicate. A dead amphipod was considered any individual that did not exhibit any evidence of movement (e.g., neuromuscular twitch of pleopods or antennae) upon gentle prodding with a probe.

Reference toxicant tests

Reference toxicant tests were conducted in glass containers under constant darkness. Test concentrations for these tests were 0, 15.6, 31.2, 62.5, 125, and 250 mg/L total ammonia. Four 800-mL replicates of each concentration were tested for 96 hours at $15 \pm 2^{\circ}$ C. The reference toxicant test was terminated after 96 hours of exposure unless the un-ionized pore water ammonia concentration in the any of the sediment samples was ≥ 0.8 mg/L, in which case the ammonia reference toxicant test was extended from 4 days to 10 days for better comparison to the 10-day test sample results.

Data analysis procedures

All data were analyzed in accordance with procedures outlined in Sections 12 and 13 of EPA 600/R-94/0925 using the acceptability criterion of \geq 90% mean control survival at test termination. Additional information and the standard operation procedures for sediment toxicity testing are provided in Appendix B of the CSDTL's Quality Assurance Manual (City of San Diego 2017: SOP-TX003).

RESULTS & CONCLUSIONS

Toxicity testing using the amphipod *Eohaustorius estuarius* was successfully completed for 65 marine sediment samples collected from 49 stations for the Point Loma and South Bay outfall monitoring programs during the summers of 2016, 2017 and 2018 (see Figure 1). Eight of these stations represent near-ZID stations located within 1000 m of the discharge sites for the two outfalls, all of which were sampled during each of the three summer surveys. The other 41 stations included 20 randomly selected sites tested in 2016 and a different set of 21 randomized sites tested in 2018.

The above monitoring stations represented a diverse array of offshore soft-bottom benthic habitats ranging in depth from 5 to 350 m and with coarse to fine sediments of 1-80% fine silts and clays combined (see Table 1). These included: a) 24 inner shelf samples collected from depths of 5-28 m where sediments were relatively coarse averaging about 11% fines; b) 32 mid-shelf samples collected from depths of 30-116 m where sediments were typically finer with an average of about 35% fines; c) six outer shelf samples collected from depths of 130-195 m that averaged even finer sediments of about 50% fines; d) three upper slope samples collected at depths of 240-350 m that had the finest sediments averaging about 72% fines.

Test results for each lab control and individual station sample are shown in Table 1, while the average amphipod survival for the laboratory controls, near-ZID (outfall) sites, and randomly selected (regional)

Survev	Site/Sample	Station Type	Depth Stratum	Station Depth	Percent Fines	Sample Date	Test Initiation	% Survival
								(Mean ± SD)
	Lab Control	I	I	I	I	I	7/15/16	99 ± 2.2
	112	SBOO Near-ZID	Inner Shelf	28 m	6%	7/7/16	7/15/16	100
	114	SBOO Near-ZID	Inner Shelf	28 m	18%	7/7/16	7/15/16	99 ± 2.2
	115	SBOO Near-ZID	Inner Shelf	28 m	3%	7/7/16	7/15/16	98 ± 2.7
	116	SBOO Near-ZID	Inner Shelf	28 m	6%	7/7/16	7/15/16	100
	8501	SD Regional	Inner Shelf	17 m	22%	7/7/16	7/15/16	100
	8503	SD Regional	Middle Shelf	92 m	34%	7/7/16	7/15/16	100
	8507	SD Regional	Middle Shelf	91 m	38%	7/7/16	7/15/16	99 ± 2.2
	Lab Control						7/15/16	100
	E11	PLOO Near-ZID	Middle Shelf	98 m	35%	7/11/16	7/15/16	98 ± 2.7
	E14	PLOO Near-ZID	Middle Shelf	98 m	27%	7/11/16	7/15/16	98 ± 4.5
	E15	PLOO Near-ZID	Middle Shelf	116 m	40%	7/11/16	7/15/16	97 ± 4.5
	8502	SD Regional	Middle Shelf	35 m	28%	7/12/16	7/15/16	99 ± 2.2
	8505	SD Regional	Inner Shelf	26 m	17%	7/12/16	7/15/16	100
	8513	SD Regional	Inner Shelf	5 m	2%	7/12/16	7/15/16	100
YEAR 1	8515	SD Regional	Inner Shelf	20 m	3%	7/12/16	7/15/16	98 ± 2.7
(Summer 2016)	Lab Control		Ι		I		7/29/16	99 ± 2.2
	8520	SD Regional	Outer Shelf	138 m	47%	7/19/16	7/29/16	99 ± 2.2
	8522	SD Regional	Inner Shelf	22 m	2%	7/19/16	7/29/16	96 ± 4.2
	8523	SD Regional	Middle Shelf	81 m	29%	7/19/16	7/29/16	96 ± 4.2
	8526	SD Regional	Middle Shelf	101 m	50%	7/19/16	7/29/16	100
	8529	SD Regional	Middle Shelf	45 m	28%	7/19/16	7/29/16	100
	8533	SD Regional	Middle Shelf	36 m	2%	7/19/16	7/29/16	100
	8536	SD Regional	Outer Shelf	135 m	32%	7/20/16	7/29/16	99 ± 2.2
	8539	SD Regional	Middle Shelf	112 m	38%	7/20/16	7/29/16	99 ± 2.2
	Lab Control	I	I	I	I		8/5/16	99 ± 2.2
	E17	PLOO Near-ZID	Middle Shelf	98 m	34%	7/27/16	8/5/16	100
	8510	SD Regional	Outer Shelf	195 m	68%	7/27/16	8/5/16	98 ± 2.7
	8512	SD Regional	Upper Slope	240 m	76%	7/27/16	8/5/16	100
	8517	SD Regional	Middle Shelf	57 m	34%	7/28/16	8/5/16	100
	8521	SD Regional	Upper Slope	340 m	80%	7/27/16	8/5/16	99 ± 2.2
	8527	SD Regional	Upper Slope	350 m	61%	7/27/16	8/5/16	100
	Lab Control						7/21/17	97 ± 2.7
YEAR 2	112	SBOO Near-ZID	Inner Shelf	28 m	3%	7/10/17	7/21/17	96 ± 4.2
	114	SBOO Near-ZID	Inner Shelf	28 m	17%	7/10/17	7/21/17	98 ± 4.5
(Summer 2017)	115	SBOO Near-ZID	Inner Shelf	28 m	2%	7/10/17	7/21/17	100
	116	SBOO Near-ZID	Inner Shelf	28 m	1%	7/10/17	7/21/17	100

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							% Survival
ð	Station Type	Depth Stratum	Station Depth	Percent Fines	Sample Date	Test Initiation	(Mean ± SD)
	PLOO Near-ZID	Middle Shelf	98 m	33%	7/11/17	7/21/17	99 ± 2.2
	PLOO Near-ZID	Middle Shelf	98 m	21%	7/11/17	7/21/17	100
	PLOO Near-ZID	Middle Shelf	116 m	30%	7/11/17	7/21/17	97 ± 2.7
	PLOO Near-ZID	Middle Shelf	98 m	34%	7/11/17	7/21/17	98 ± 2.7
_	I	I	I	I	I	7/13/2018	98 ± 2.7
	Bight'18 Regional	Inner Shelf	23 m	10%	7/11/18	7/13/2018	98 ± 2.7
	Bight'18 Regional	Middle Shelf	34 m	27%	7/11/18	7/13/2018	99 ± 2.2
	Bight'18 Regional	Middle Shelf	57 m	20%	7/11/18	7/13/2018	98 ± 2.7
	Bight'18 Regional	Inner Shelf	11 m	21%	7/11/18	7/13/2018	100
	Bight'18 Regional	Middle Shelf	50 m	40%	7/10/18	7/13/2018	97 ± 6.7
	Bight'18 Regional	Inner-Mid Shelf	30 m	11%	7/10/18	7/13/2018	97 ± 2.7
	Bight'18 Regional	Middle Shelf	74 m	61%	7/10/18	7/13/2018	98 ± 2.7
-	I	I	I	I	I	7/20/2018	98 ± 2.7
	Bight'18 Regional	Outer Shelf	182 m	53%	7/11/18	7/20/2018	99 ± 2.2
	Bight'18 Regional	Inner Shelf	13 m	48%	7/11/18	7/20/2018	97 ± 4.5
	Bight'18 Regional	Middle Shelf	77 m	57%	7/11/18	7/20/2018	99 ± 2.2
	Bight'18 Regional	Middle Shelf	49 m	37%	7/11/18	7/20/2018	100
	Bight'18 Regional	Outer Shelf	185 m	61%	7/12/18	7/20/2018	99 ± 2.2
	Bight'18 Regional	Middle Shelf	76 m	63%	7/12/18	7/20/2018	98 ± 2.7
	Bight'18 Regional	Middle Shelf	70 m	41%	7/12/18	7/20/2018	100
0	I	Ι	I	I	I	7/31/2018	100
	SBOO Near-ZID	Inner Shelf	28 m	1%	7/24/18	7/31/2018	99 ± 2.2
	SBOO Near-ZID	Inner Shelf	28 m	20%	7/24/18	7/31/2018	99 ± 2.2
	SBOO Near-ZID	Inner Shelf	28 m	3%	7/24/18	7/31/2018	98 ± 2.7
	SBOO Near-ZID	Inner Shelf	28 m	5%	7/24/18	7/31/2018	99 ± 2.2
	Bight'18 Regional	Inner Shelf	20 m	20%	7/24/18	7/31/2018	97 ± 2.7
	Bight'18 Regional	Inner Shelf	27 m	6%	7/26/18	7/31/2018	99 ± 2.2
	Bight'18 Regional	Inner Shelf	24 m	24%	7/26/18	7/31/2018	99 ± 2.2
<u>o</u>	I	I	I	I	I	8/7/2018	96 ± 4.2
	PLOO Near-ZID	Middle Shelf	98 m	31%	7/31/18	8/7/2018	99 ± 2.2
	PLOO Near-ZID	Middle Shelf	98 m	24%	7/31/18	8/7/2018	94 ± 8.2
	PLOO Near-ZID	Middle Shelf	116 m	29%	7/31/18	8/7/2018	98 ± 2.7
	PLOO Near-ZID	Middle Shelf	98 m	36%	7/31/18	8/7/2018	92 ± 5.7
	Bight'18 Regional	Middle Shelf	47 m	19%	7/31/18	8/7/2018	95 ± 3.5
	Bight'18 Regional	Middle Shelf	87 m	51%	7/31/18	8/7/2018	95 ± 6.1
	Bight'18 Regional	Outer Shelf	130 m	39%	7/31/18	8/7/2018	99 ± 2.2
	Bight'18 Regional	Inner Shelf	13 m	2%	7/31/18	8/7/2018	100



Figure 2

Mean percent amphipod survival for 3-year San Diego sediment toxicity pilot study. Lab Control averages for each year are shown in blue. Randomized site averages for 2016 (San Diego regional stations) and 2018 (Bight'18 regional stations) are shown in green. Near-ZID (outfall) sites combined for the PLOO and SBOO are shown in orange.

sites, are summarized for each year in Figure 2. Mean survival of *E. estuarius* in the laboratory controls was 99-100% in 2016, 97% in 2017, and 96-100% in 2018, all of which satisfied the required protocol of 90% survival for this species. Mean amphipod survival among all sample sites tested ranged from 96 to 100% in 2016 and 2017, and from 92 to 100% in 2018.

Overall, no evidence of sediment toxicity was observed at any offshore station tested in the San Diego region during the 3-year pilot study regardless of depth, sediment type, or proximity to either outfall. These results are consistent with the findings from previous regional monitoring programs that have demonstrated very little sediment toxicity on the southern California continental shelf in contrast to offshore submarine canyons and local embayments (e.g., Bay et al. 2015, Bight'13 Contaminant Impact Assessment Planning Committee 2017).

RECOMMENDATIONS

Although no sediment toxicity was observed at any offshore monitoring site during the 3-year pilot study, the City in consultation with the USIBWC, San Diego Water Board, USEPA and SCCWRP recommend continuing annual sediment toxicity testing of the PLOO and SBOO regions for at least the next five years. Such testing off Point Loma is recommended to monitor whether or not there may be any effects caused by changes in PLOO discharge flows related to implementation of the City's

Pure Water program. Likewise, continued testing of South Bay outfall area sediments is recommended to monitoring possible effects due to changes in SBOO discharge flows that could occur if treatment processes change or because of other transboundary issues or sources of contaminants in that region. Based on the above, but considering the findings of the pilot study and the general low sediment toxicity expected along the southern California continental shelf, we recommend annual testing of a reduced number of samples alternating between permanent fixed monitoring sites and randomly selected sites as was done during the pilot study. Specifically, we propose the following sample design for surveys to be conducted during the summers of 2019 - 2023.

- <u>Year 1 (summer 2019)</u>: retest the 8 near-ZID PLOO and SBOO benthic stations that are monitored twice each year for sediment chemistry and benthic infauna (i.e., PLOO station E-11, E-14, E-15, E-17; SBOO stations I-12, I-14, I-15, I-16).
- <u>Year 2 (summer 2020)</u>: test subset of 8 of the 40 randomized stations that will be selected for study by the combined PLOO and SBOO monitoring programs. These stations may be targeted for specific areas of interest in consultation between the City, USIBWC, San Diego Water Board, USEPA, and SCCWRP,
- <u>Year 3 (summer 2021):</u> repeat Year 1 sample design.
- <u>Year 4 (summer 2022):</u> repeat Year 2 sample design.
- <u>Year 5 (summer 2023)</u>: test up to 20 randomly selected sites to be selected as part of the Bight'23 regional monitoring program. The final number of samples will be determined as part of the Bight'23 regulatory relief approval process.

Following completion and publication of the results and findings of Years 1-5, along with the full sediment toxicity findings for Bight'23, the City, USIBWC, San Diego Water Board, USEPA and SCCWRP will consult to determine the best course of action for subsequent sediment toxicity monitoring in the PLOO and SBOO regions.

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