

**Term Contract for Provision of Sampling and Analyzing of
Wastewater and Sludge Samples for Various Sewage Treatment
Facilities and Marine Water Samples in Urban Area, Lantau and
Outlying Islands to the Drainage Services Department**

Whole Effluent Toxicity Test (WETT) at SCISTW

Report for the Month of July 2023

Contract No. : DE/2020/02

Applicant : SEWAGE TREATMENT DIVISION 2
ELECTRICAL AND MECHANICAL BRANCH
DRAINAGE SERVICES DEPARTMENT

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For and on behalf of
CMA Industrial Development Foundation Limited

Authorized Signature :



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1. Introduction

1.1. Background

The whole effluent toxicity tests (WETT) were carried out under the requirements of Drainage Services Department (DSD).

1.2. Testing laboratory and investigator

Name of Laboratory: Research Center for Ocean and Human Health, City University of Hong Kong

Principle investigator: Prof. Wen-Xiong WANG

1.3. Sample

A 24-hour flow-weighted composite effluent sample was collected from Stonecutters Island Sewage Treatment Works (SCISTW) on July 28, 2023. Effluent sample was shipped immediately to the testing laboratory on the same day of collection. Toxicity testings started immediately after the arrival of samples.

1.4. Test species

The following test species were included in the WETT:

- Amphipod (*Melita longidactyla*)
- Fish (*Lutjanus malabaricus*)
- Barnacle larvae (*Balanus amphitrite*)
- Diatom (*Skeletonema costatum*)
- Shrimp (*Metapenaeus ensis*)

1.5. Test protocols

The WETT testing methods and procedures follow those documented in “Consultancy Study on Fisheries and Marine Ecological Criteria for Impact Assessment-Final Report” commissioned by Agriculture, Fisheries and Conservation Department (AFCD), as indicated in tender addendum No. 1 by Drainage Services Department (DSD).

2. Report on Amphipod Acute Toxicity Test

Test report

2.1. Samples storage and pretreatment

Effluent sample was thoroughly mixed and passed through 2-mm mesh to remove the large debris. Effluent was added with ocean salt in order to raise the salinity to the required level (30‰) and then aerated moderately such that the dissolved oxygen (DO) reached saturation prior to use. Salinity control was set up to monitor if there was adverse effect on the test organisms.

2.2. Test organism

Species	Amphipod (<i>Melita longidactyla</i>).
Source:	Collected from local coastal waters from Sai Kung
Size/ age:	0.5-0.7 cm
Acclimatization:	Acclimatized in fully aerated seawater (temperature: 22±1°C, salinity: 30‰) at least 48 hours in the laboratory prior to test. Fed with green algae <i>Ulva lactuca</i> .

2.3. Summary of test conditions

Type of test:	Static
Duration:	48 h, 28/7/2023-30/7/2023
Dilution seawater source:	Seawater collected from a pristine site in Clear Water Bay, Sai Kung, Hong Kong
Dilution seawater pretreatment:	Filtered through 0.22 µm membrane
Testing temperature:	22±1 °C
Lighting:	Continuous
Salinity:	30‰
Testing chamber:	Pre-cleaned 150 mL glass flask
Feeding:	None
Number of organisms per replicate:	10
Replicate number:	4
Volume of test medium:	100 mL
Aeration:	Moderate, around 100 bubbles/min
Reference toxicant:	CdCl ₂
Positive control:	48-h acute toxicity test
Salinity control:	Prepared with ocean salt adding into de-ionized water, salinity: 30‰

2.4. Test results

Table 1. Survival of amphipods after 48 hours.

Treatment	Effluent concentration (%)	Number of living amphipods after 48 hour (individuals)					
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD
Negative control	0	10	9	8	10	9.25	0.96
Salinity control	0	10	10	8	9	9.25	0.96
Concentration 1	6.5	8	8	8	7	7.75	0.50
Concentration 2	12.5	6	7	8	8	7.25	0.96
Concentration 3	25	6	4	4	5	4.75	0.96
Concentration 4	50	5	4	4	3	4.00	0.82
Concentration 5	100	2	0	1	2	1.25	0.96

Table 2. Survival percentage of amphipods after 48 hours.

Treatment	Effluent concentration (%)	Percentage of living amphipods after 48 hour (%)					
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD
Negative control	0	100	90	80	100	92.5	9.60
Salinity control	0	100	100	80	90	92.5	9.60
Concentration 1	6.5	80	80	80	70	77.5	5.00
Concentration 2	12.5	60	70	80	80	72.5	9.57
Concentration 3	25	60	40	40	50	47.5	9.57
Concentration 4	50	50	40	40	30	40.0	8.16
Concentration 5	100	20	0	10	20	12.5	9.57

2.5. Summary of water quality parameters monitoring during test

Table 3. Summary of water quality parameters during amphipod acute toxicity test.

Water quality parameters	Effluent concentration (%)						
	Negative control	Salinity control	6.5	12.5	25	50	100
Salinity (‰)	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Dissolved oxygen (mg L ⁻¹)	6.8-7.2	6.8-7.1	6.9-7.1	6.9-7.1	6.8-7.1	6.9-7.0	6.8-7.0
Temperature (°C)	22.0	22.0	22.0	22.0	22.0	22.0	22.0
pH	7.9-8.1	7.9-8.1	7.9-8.1	7.9-8.1	7.9-8.0	7.8-8.1	7.8-7.9
Ammonia-N (start/end, mg L ⁻¹)	0.20/0.16	0.16/0.10	3.89/3.65	4.73/3.81	9.91/9.40	12.8/12.3	23.0/21.5
Total sulphide (start/end, mg L ⁻¹)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.25/< 0.1	6.14/< 0.1
Total residual chlorine (start/end, mg L ⁻¹)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Total suspended solids (start/end, mg L ⁻¹)	2/<2	12/<2	12/6	20/11	24/19	30/24	47/28

2.6. LC₅₀ for the amphipod *Melita longidactyla* and test acceptability

Table 4. LC₅₀ for the amphipods and test acceptability.

Parameter	Value	Control limit
Calculated LC ₅₀	29.2%	NA
Negative control survival	92.5%	≥90%
Reference toxicant 48-h acute test	0.96 mg L ⁻¹	1.25±0.15 mg L ⁻¹
95% of confidence range of reference toxicant test	0.78-1.25 mg L ⁻¹	NA
Daily temperature variation	<0.5 °C	Average daily temperature variation: ±1 °C
Dissolved oxygen concentration	>6.8 mg L ⁻¹	>4 mg L ⁻¹

NA: Not applicable

3. Report on Fish Acute Toxicity Test

Test report

3.1. Samples storage and pretreatment

Effluent sample was thoroughly mixed and passed through 2-mm mesh to remove large debris. Effluent was added with ocean salt in order to raise the salinity to the required level (30‰) and then aerated moderately such that the dissolved oxygen (DO) reached saturation prior to use. Salinity control was set up to monitor if there was adverse effect on the test organisms.

3.2. Test organism

Species	Fish (<i>Lutjanus malabaricus</i>)
Source:	Purchased from contracted fish farm
Size/age:	2-3 cm
Acclimatization:	Acclimatized in fully aerated seawater (temperature: 22±1°C, salinity: 30‰) at least 48 hours in laboratory prior to test. Fed with fresh shrimp purchased from local market.

3.3. Summary of test conditions

Type of test:	Static
Duration:	48 h, 28/7/2023-30/7/2023
Dilution seawater source:	Seawater collected from a pristine site in Clear Water Bay, Sai Kung, Hong Kong
Dilution seawater pretreatment:	Filtered through 5 µm filtration bag
Testing temperature:	22±1 °C
Lighting:	Continuous
Salinity:	30‰
Testing chamber:	Pre-cleaned 20 L tank
Feeding:	None
Number of organisms per replicate:	20
Replicate number:	4
Volume of test medium:	20 L
Aeration:	Moderate, with air stone
Reference toxicant:	CdCl ₂
Positive control:	48 h acute toxicity test
Salinity control:	Prepared with ocean salt adding into de-ionized water, salinity: 30‰

3.4. Test results

Table 1. Survival of fish after 48 hours.

Treatment	Effluent concentration (%)	Number of living fish after 48 hour (individuals)					
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD
Negative control	0	20	20	20	20	20.00	0.00
Salinity control	0	20	20	20	20	20.00	0.00
Concentration 1	6.5	20	20	20	20	20.00	0.00
Concentration 2	12.5	20	19	20	20	19.75	0.50
Concentration 3	25	20	20	20	20	20.00	0.00
Concentration 4	50	20	20	20	20	20.00	0.00
Concentration 5	100	15	18	20	20	18.25	2.36

Table 2. Survival percentage of fish after 48 hours.

Treatment	Effluent concentration (%)	Percentage of living fish after 48 hour (%)					
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD
Negative control	0	100	100	100	100	100.00	0.00
Salinity control	0	100	100	100	100	100.00	0.00
Concentration 1	6.5	100	100	100	100	100.00	0.00
Concentration 2	12.5	100	95	100	100	98.75	2.50
Concentration 3	25	100	100	100	100	100.00	0.00
Concentration 4	50	100	100	100	100	100.00	0.00
Concentration 5	100	75	90	100	100	91.25	11.80

3.5. Summary of water quality parameters monitoring during test

Table 3. Summary of water quality parameters during fish acute toxicity test.

Water quality parameters	Effluent concentration (%)						
	Negative control	Salinity control	6.5	12.5	25	50	100
Salinity (‰)	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Dissolved oxygen (mg L ⁻¹)	6.8-7.0	6.9-7.1	6.9-7.1	6.8-7.1	6.8-7.0	6.8-7.1	6.7-7.0
Temperature (°C)	22.0	22.0	22.0	22.0	22.0	22.0	22.0
pH	7.9-8.0	7.8-8.0	7.9-8.0	7.7-8.1	7.8-8.1	7.8-8.0	7.9-8.0
Ammonia-N (start/end, mg L ⁻¹)	0.18/0.17	0.60/0.22	3.58/3.23	4.06/3.48	10.0/7.51	12.2/12.1	21.9/20.9
Total sulphide (start/end, mg L ⁻¹)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.31/< 0.1	1.30/< 0.1
Total residual chlorine (start/end, mg L ⁻¹)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Total suspended solids (mg L ⁻¹)	< 2	5/< 2	8/5	8/5	18/9	22/13	31/18

3.6. LC₅₀ for the fish *Lutjanus malabaricus* and test acceptability

Table 4. LC₅₀ for the fish and test acceptability.

Parameter	Value	Control limit
Calculated LC ₅₀	Cannot be calculated ^a	NA
Negative survival	100%	>90%
Reference toxicant 48-h acute test	16.7 mg L ⁻¹	14.6±1.78 mg L ⁻¹
95% of confidence range of reference toxicant test	13.8-19.5 mg L ⁻¹	NA
Daily temperature variation	<0.5 °C	Average daily temperature variation: ± 1 °C
Dissolved oxygen concentration	>6.6 mg L ⁻¹	>4 mg L ⁻¹

a: The mortalities in all concentration groups were less than 50% and thus LC₅₀ cannot be calculated.

NA: Not applicable

4. Report on Barnacle Larvae Acute Toxicity Test

Test report

4.1. Samples storage and pretreatment

Effluent sample was thoroughly mixed and passed through 5 µm membrane filter to remove large debris. Effluent was added with ocean salt in order to raise the salinity to the required level (30‰) and then aerated moderately to dissolved oxygen (DO) saturation prior to use. Salinity control was set up to monitor if there was adverse effect on the test organisms.

4.2. Test organism

Species	Barnacle larvae (<i>Balanus amphitrite</i>).
Source:	Introduced from adult barnacles collected from Sai Kung
Size/age:	Stage II
Acclimatization:	Acclimatized in fully aerated seawater held in 500 mL glass beaker (temperature: 22±1°C, salinity: 30‰) for at least 24 hours in laboratory prior to test. Fed with diatom <i>Chaetoceros gracilis</i> .

4.3. Summary of test conditions

Type of test:	Static
Duration:	48 h, 28/7/2023-30/7/2023
Dilution seawater source:	Seawater collected from a pristine site in Clear Water Bay, Sai Kung, Hong Kong
Dilution seawater pretreatment:	Filtered through 0.22 µm membrane
Testing temperature:	22±1 °C
Lighting:	Continuous
Salinity:	30‰
Testing chamber:	Pre-cleaned 50 mL glass beaker
Feeding:	None
Number of organisms per replicate:	20
Replicate number:	4
Volume of test medium:	20 mL
Aeration:	Moderate, around 100 bubbles/min
Reference toxicant:	CdCl ₂
Positive control:	48 h acute toxicity test
Salinity control:	Prepared with ocean salt adding into de-ionized water, salinity: 30‰

4.4. Test results

Table 1. Survival of barnacle larvae after 48 hours.

Treatment	Effluent concentration (%)	Number of living barnacle larvae after 48 hour (individuals)					
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD
Negative control	0	20	20	20	20	20.00	0.00
Salinity control	0	20	20	20	20	20.00	0.00
Concentration 1	6.5	17	18	18	18	17.75	0.43
Concentration 2	12.5	19	19	17	16	17.75	1.30
Concentration 3	25	19	17	16	16	17.00	1.22
Concentration 4	50	7	10	10	8	8.75	1.30
Concentration 5	100	2	1	1	1	1.25	0.43

Table 2. Survival percentage of barnacle larvae after 48 hours.

Treatment	Effluent concentration (%)	Percentage of living barnacle larvae after 48 hour (%)					
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD
Negative control	0	100	100	100	100	100.00	0.00
Salinity control	0	100	100	100	100	100.00	0.00
Concentration 1	6.5	85	90	90	90	88.75	2.17
Concentration 2	12.5	95	95	85	80	88.75	6.50
Concentration 3	25	95	85	80	80	85.00	6.12
Concentration 4	50	35	50	50	40	43.75	6.50
Concentration 5	100	10	5	5	5	6.25	2.17

4.5. Summary of water quality parameters monitoring during test

Table 3. Summary of water quality parameters during barnacle larvae acute toxicity test

Water quality parameters	Effluent concentration (%)						
	Negative control	Salinity control	6.5	12.5	25	50	100
Salinity (‰)	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Dissolved oxygen (mg L ⁻¹)	6.8-6.9	6.8-7.0	6.8-7.0	6.8-6.9	6.8-7.0	6.8-7.1	6.8-6.9
Temperature (°C)	22.0	22.0	22.0	22.0	22.0	22.0	22.0
pH	7.8-8.0	7.8-8.0	7.9-8.0	7.9-8.0	7.8-7.9	7.7-7.9	7.7-7.8
Ammonia-N (start/end, mg L ⁻¹)	0.16/0.11	0.16/0.14	1.08/0.57	3.83/2.10	5.87/4.24	9.88/9.60	18.8/12.8
Total sulphide (start/end, mg L ⁻¹)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.12/< 0.1	0.27/< 0.1
Total residual chlorine (start/end, mg L ⁻¹)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Total suspended solids (mg L ⁻¹)	< 2	< 2	2/< 2	4/3	5/5	21/7	23/12

4.6. LC₅₀ for the barnacle larvae *Balanus amphitrite* and test acceptability

Table 4. LC₅₀ for the barnacle larvae and test acceptability

Parameter	Value	Control limit
Calculated LC ₅₀	46.2%	NA
Negative survival	100%	>90%
Reference toxicant 48-h acute test	1.17 mg L ⁻¹	1.04±0.11 mg L ⁻¹
95% of confidence range of reference toxicant test:	1.05-1.28 mg L ⁻¹	NA
Daily temperature variation	<0.5 °C	Average daily temperature variation: ± 1 °C
Dissolved oxygen concentration	>6.7 mg L ⁻¹	>4 mg L ⁻¹

NA: Not applicable

5. Report on Diatom Growth Inhibition Test (Chronic toxicity test)

Test report

5.1. Samples storage and pretreatment

Effluent sample was thoroughly mixed and passed through 5 µm membrane filter to remove large debris. Effluent was added with ocean salt in order to raise the salinity to the required level (30‰) and then aerated moderately to dissolved oxygen (DO) saturation prior to use. Salinity control was set up to monitor if there was adverse effect on the test organisms.

5.2. Test organism

Species	Diatom (<i>Skeletonema costatum</i>)
Source:	Grown from laboratory culture obtained from Coastal Marine Lab, Hong Kong University of Science and Technology
Size/age:	Log growth phase
Acclimatization:	Grown in 250 mL glass flask (temperature: 22±1°C, salinity: 30‰, 3000 lux) for at least two weeks prior to test.

5.3. Summary of test conditions

Type of test:	Static
Duration:	7 days, 28/7/2023-4/8/2023
Dilution seawater source:	Seawater collected from a pristine site in Clear Water Bay, Sai Kung, Hong Kong
Dilution seawater pretreatment:	Filtered through 0.22 µm membrane
Testing temperature:	22±1 °C
Lighting:	12 h light/12 h dark cycle, 3000±500 lux
Salinity:	30‰
Testing chamber:	Pre-cleaned 100 mL glass beaker
Initial cell density:	(5.0±0.4)×10 ⁴ cell mL ⁻¹
Replicate number:	4
Volume of test medium:	25 mL
Aeration:	None
Reference toxicant:	CdCl ₂
Positive control:	7-day IC ₅₀ toxicity test
Salinity control:	Prepared with ocean salt adding into de-ionized water, salinity: 30‰

5.4. Test results

Table 1. Cell density of diatom *Skeletonema costatum* at the beginning and end of growth inhibition test. Initial cell density: $(5.0 \pm 0.4) \times 10^4$ cell mL⁻¹.

Treatment	Effluent concentration (%)	Cell density after 7-day growth ($\times 10^6$ cell mL ⁻¹)					
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD
Negative control	0	1.67	1.62	1.95	1.85	1.77	0.13
Salinity control	0	1.93	1.65	1.83	1.90	1.83	0.11
Concentration 1	2.5	2.33	2.15	2.23	2.18	2.22	0.07
Concentration 2	5	3.08	2.93	3.63	3.20	3.21	0.26
Concentration 3	10	1.93	2.05	2.00	1.90	1.97	0.06
Concentration 4	25	1.75	1.93	1.65	1.80	1.78	0.10
Concentration 5	50	1.30	1.15	1.00	1.10	1.14	0.11
Concentration 6	100	0.83	1.17	0.82	0.77	0.90	0.16

Table 2. Growth rate of *Skeletonema costatum* within 7 days.

Treatment	Effluent concentration (%)	7-day average growth rate (d ⁻¹)					
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD
Negative control	0	0.50	0.50	0.52	0.52	0.51	0.01
Salinity control	0	0.52	0.50	0.51	0.52	0.51	0.01
Concentration 1	2.5	0.55	0.54	0.54	0.54	0.54	0.01
Concentration 2	5	0.59	0.58	0.61	0.59	0.59	0.01
Concentration 3	10	0.52	0.53	0.53	0.52	0.52	0.00
Concentration 4	25	0.51	0.52	0.50	0.51	0.51	0.01
Concentration 5	50	0.47	0.45	0.43	0.44	0.45	0.02
Concentration 6	100	0.40	0.45	0.40	0.39	0.41	0.03

5.5. Summary of water quality parameters monitoring during test

Table 3. Summary of water quality parameters during diatom growth inhibition test

Water quality parameters	Effluent concentration (%)							
	Negative control	Salinity control	2.5	5.0	10	25	50	100
Salinity (‰)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Dissolved oxygen (mg L ⁻¹)	6.8-8.2	6.8-8.2	6.8-8.2	6.8-8.2	6.8-8.3	6.8-8.2	6.8-7.9	6.8-7.3
Temperature (°C)	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
pH	7.9-8.1	7.9-8.1	7.9-8.1	7.9-8.1	7.9-8.1	7.9-8.1	7.9-8.1	7.9-8.0
Ammonia-N (start/end, mg L ⁻¹)	0.16/0.10	0.16/0.11	2.50/0.16	3.48/0.16	4.76/1.57	6.67/1.61	12.3/2.93	23.5/14.9
Total sulphide (start/end, mg L ⁻¹)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.25/< 0.1	7.5/< 0.1
Total residual chlorine (start/end, mg L ⁻¹)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Total suspended solids (mg L ⁻¹)	< 2	3/<2	59/9	71/15	74/16	83/27	89/33	94/42

5.6. IC₅₀ for the diatom *Skeletonema costatum* and test acceptability

Table 4. IC₅₀, none observed effect concentration (NOEC) for the diatom and test acceptability

Parameter	Value	Control limit
Calculated IC ₅₀	Cannot be calculated ^a	NA
None observed effect concentration (NOEC)	25%	-
Reference toxicant 7-day test:	0.16 mg L ⁻¹	0.13±0.02 mg L ⁻¹
95% of confidence range of reference toxicant test	0.12-0.21 mg L ⁻¹	NA
Temperature variation	<0.5 °C	Average daily temperature variation: ± 1 °C

a: The growth inhibitions in all concentration groups were less than 50% and thus IC₅₀ cannot be calculated.

NA: Not applicable

6. Report on Shrimp Acute Toxicity Test

Test report

6.1. Samples storage and pretreatment

Effluent sample was thoroughly mixed and passed through 2-mm mesh to remove the large debris. Effluent was added with ocean salt in order to raise the salinity to 25‰ and then aerated moderately such that the dissolved oxygen (DO) reached saturation prior to use. Salinity control was set up to monitor if there was adverse effect on the test organisms.

6.2. Test organism

Species	Shrimp (<i>Metapenaeus ensis</i>)
Source:	Purchased from contracted fish dealer
Size/age:	5-7 cm
Acclimatization:	Acclimatized in fully aerated seawater (temperature: 22±1°C, salinity: 25‰) at least 48 hours in the laboratory prior to test. Fed with commercial shrimp feeds.

6.3. Summary of test conditions

Type of test:	Static
Duration:	48 h, 28/7/2023-30/7/2023
Dilution seawater source:	Seawater collected from a pristine site in Clear Water Bay, Sai Kung, Hong Kong
Dilution seawater pretreatment:	Filtered through 0.22 µm membrane
Testing temperature:	22±1 °C
Lighting:	Continuous
Salinity:	25‰
Testing chamber:	Pre-cleaned 20 L tank
Feeding:	None
Number of organisms per replicate:	10
Replicate number:	4
Volume of test medium:	10 L
Aeration:	Moderate, with air stone
Reference toxicant:	CdCl ₂
Positive control:	48 h acute toxicity test
Salinity control:	Prepared with ocean salt adding into de-ionized water, salinity: 25‰

6.4. Test results

Table 1. Survival of shrimps after 48 hours.

Treatment	Effluent concentration (%)	Number of living shrimps after 48 hour (individuals)					
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD
Negative control	0	10	10	8	8	9.00	1.15
Salinity control	0	9	8	9	10	9.00	0.82
Concentration 1	6.5	7	8	9	8	8.00	0.82
Concentration 2	12.5	9	8	10	10	9.25	0.96
Concentration 3	25	7	6	6	6	6.25	0.50
Concentration 4	50	5	3	3	5	4.00	1.15
Concentration 5	100	0	0	0	0	0.00	0.00

Table 2. Survival percentage of shrimps after 48 hours.

Treatment	Effluent concentration (%)	Percentage of living shrimps after 48 hour (%)					
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD
Negative control	0	100	100	80	80	90.00	11.55
Salinity control	0	90	80	90	100	90.00	8.16
Concentration 1	6.5	70	80	90	80	80.00	8.16
Concentration 2	12.5	90	80	100	100	92.50	9.57
Concentration 3	25	70	60	60	60	62.50	5.00
Concentration 4	50	50	30	30	50	40.00	11.55
Concentration 5	100	0	0	0	0	0.00	0.00

6.5. Summary of water quality parameters monitoring during test.

Table 3. Summary of water quality parameters during shrimp acute toxicity test.

Water quality parameters	Effluent concentration (%)						
	Negative control	Salinity control	6.5	12.5	25	50	100
Salinity (‰)	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Dissolved oxygen (mg L ⁻¹)	6.8-6.9	6.9-7.0	6.9-7.0	6.9-7.0	6.9-7.1	6.8-7.0	6.8-7.0
Temperature (°C)	22.0	22.0	22.0	22.0	22.0	22.0	22.0
pH	7.9-8.0	7.9-8.0	7.9-8.0	7.9-7.9	7.9-8.0	7.7-7.9	7.7-8.0
Ammonia-N (start/end, mg L ⁻¹)	0.57/0.16	0.14/0.11	7.33/3.46	7.97/4.71	8.95/6.63	13.5/12.3	23.5/21.9
Total sulphide (start/end, mg L ⁻¹)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total residual chlorine (start/end, mg L ⁻¹)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Total suspended solids (start/end, mg L ⁻¹)	2/<2	3/<2	10/8	10/9	11/10	20/11	33/22

6.6. LC₅₀ for the shrimp *Metapenaeus ensis* and test acceptability

Table 4. LC₅₀ for the *Metapenaeus ensis* and test acceptability.

Parameter	Value	Control limit
Calculated LC ₅₀	44.4%	NA
Negative control survival	90%	>90%
Reference toxicant 48-h acute test	5.7 mg L ⁻¹	5.19±0.51 mg L ⁻¹
95% of confidence range of reference toxicant test	4.8-6.8 mg L ⁻¹	NA
Daily temperature variation	<0.5 °C	Average daily temperature variation: ±1 °C
Dissolved oxygen concentration	>6.7 mg L ⁻¹	

NA: Not applicable

7. Conclusion

Table 1. Comparison of measured toxicity values with the target toxicity levels.

Test species	Measured LC ₅₀ /IC ₅₀ /NOEC	Target toxicity level
Amphipod <i>Melita longidactyla</i>	29.2%	≥7.1%
Fish <i>Lutjanus malabaricus</i>	Cannot be calculated because animals displayed very high survival rate (>50%) at the highest tested effluent concentration (100%)	≥7.1%
Barnacle larvae <i>Balanus amphitrite</i>	46.2%	≥7.1%
Diatom <i>Skeletonema costatum</i>	Cannot be calculated because diatoms displayed very high growth rate (>50%) at the highest tested effluent concentration (100%)	-
Diatom <i>Skeletonema costatum</i>	25% (NOEC)	≥0.51%
Shrimp <i>Metapenaeus ensis</i>	44.4%	≥7.1%

Conclusion: all the measured values met the target toxicity levels as indicated in the EM&A Manual.

Cannot be calculated: The mortalities in all concentration groups (even at the highest effluent concentration) were less than 50% and thus LC₅₀ cannot be calculated.

Appendix A

**Monitoring Data
for
Amphipod Acute Toxicity Test**

Table 1. Dissolved oxygen concentration and pH in each concentration treatment in amphipod acute toxicity test.

Concentration treatment (%)	Dissolved oxygen (mg L ⁻¹)					pH				
	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48h
Negative control	6.9	7.2	7.2	6.9	6.8	7.9	7.9	8.1	8.1	8.0
Salinity control	6.8	6.9	7.1	7.1	7.0	7.9	8.0	8.1	8.1	8.1
6.5	6.9	7.0	7.0	7.0	7.1	8.0	7.9	8.1	8.1	8.1
12.5	6.9	6.9	7.0	7.1	7.0	8.0	8.0	8.0	7.9	8.1
25	6.8	7.1	7.1	7.1	7.0	8.0	7.9	7.9	7.9	8.0
50	6.9	7.0	6.9	6.9	6.9	7.8	7.9	8.0	8.1	8.0
100	7.0	6.9	6.9	6.8	6.9	7.8	7.8	7.9	7.9	7.8

Table 2. Salinity and temperature in each concentration treatment in amphipod acute toxicity test.

Concentration treatment (%)	Salinity (‰)					Temperature (°C)				
	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48 h
Negative control	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
Salinity control	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
6.5	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
12.5	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
25	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
50	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
100	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0

Table 3. Ammonia-N, Total sulphide, Total suspended solids, Total residual chlorine concentration at the beginning and ending of the amphipod acute toxicity test.

Concentration treatment (%)	Ammonia-N (mg L ⁻¹)		Total sulphide (mg L ⁻¹)		Total suspended solids (mg L ⁻¹)		Total residual chlorine (mg L ⁻¹)	
	Initial	End	Initial	End	Initial	End	Initial	End
Negative control	0.20	0.16	< 0.1	< 0.1	2	< 2	< 0.02	< 0.02
Salinity control	0.16	0.10	< 0.1	< 0.1	12	< 2	< 0.02	< 0.02
6.5	3.89	3.65	< 0.1	< 0.1	12	6	< 0.02	< 0.02
12.5	4.73	3.81	< 0.1	< 0.1	20	11	< 0.02	< 0.02
25	9.91	9.40	< 0.1	< 0.1	24	19	< 0.02	< 0.02
50	12.8	12.3	0.25	< 0.1	30	24	< 0.02	< 0.02
100	23.0	21.5	6.14	< 0.1	47	28	< 0.02	< 0.02

Appendix B

Monitoring Data for Fish Acute Toxicity Test

Table 1. Dissolved oxygen concentration and pH in each concentration treatment in fish acute toxicity test.

Concentration treatment (%)	Dissolved oxygen (mg L ⁻¹)					pH				
	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48 h
Negative control	6.8	6.9	7.0	6.9	7.0	7.9	7.9	7.9	8.0	7.9
Salinity control	6.9	6.9	7.0	7.0	7.1	8.0	7.9	7.9	7.9	7.8
6.5	6.9	7.1	7.1	7.0	7.0	7.9	7.9	7.9	7.8	8.0
12.5	6.8	6.9	6.9	7.0	7.1	8.1	8.0	7.9	7.9	7.7
25	7.0	6.9	7.0	6.8	6.9	8.0	8.1	7.9	7.8	7.9
50	6.9	6.9	7.1	6.8	6.8	7.9	8.0	8.0	7.9	7.8
100	7.0	6.8	6.7	6.8	6.8	7.9	8.0	7.9	7.9	7.9

Table 2. Salinity and temperature in each concentration treatment in fish acute toxicity test.

Concentration treatment (%)	Salinity (‰)					Temperature (°C)				
	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48 h
Negative control	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
Salinity control	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
6.5	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
12.5	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
25	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
50	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
100	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0

Table 3. Ammonia-N, Total sulphide, Total suspended solids, Total residual chlorine concentration at the beginning and ending of the fish acute toxicity test.

Concentration treatment (%)	Ammonia-N (mg L ⁻¹)		Total sulphide (mg L ⁻¹)		Total suspended solids (mg L ⁻¹)		Total residual chlorine (mg L ⁻¹)	
	Initial	End	Initial	End	Initial	End	Initial	End
Negative control	0.18	0.17	< 0.1	< 0.1	< 2	< 2	< 0.02	< 0.02
Salinity control	0.60	0.22	< 0.1	< 0.1	5	< 2	< 0.02	< 0.02
6.5	3.58	3.23	< 0.1	< 0.1	8	5	< 0.02	< 0.02
12.5	4.06	3.48	< 0.1	< 0.1	8	5	< 0.02	< 0.02
25	10.0	7.51	< 0.1	< 0.1	18	9	< 0.02	< 0.02
50	12.2	12.1	0.31	< 0.1	22	13	< 0.02	< 0.02
100	21.9	20.9	1.30	< 0.1	31	18	< 0.02	< 0.02

Appendix C

Monitoring Data for Barnacle Larvae Acute Toxicity Test

Table 1. Dissolved oxygen concentration and pH in each concentration treatment in barnacle larvae acute toxicity test.

Concentration treatment (%)	Dissolved oxygen (mg L ⁻¹)					pH				
	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48 h
Negative control	6.8	6.9	6.9	6.9	6.8	7.9	8.0	8.0	7.9	7.8
Salinity control	6.9	6.9	7.0	7.0	6.8	7.9	8.0	8.0	7.8	7.8
6.5	6.9	7.0	6.8	6.9	6.8	8.0	7.9	7.9	7.9	8.0
12.5	6.9	6.9	6.9	6.8	6.8	7.9	7.9	7.9	7.9	8.0
25	6.9	6.9	6.8	6.9	7.0	7.9	7.8	7.8	7.8	7.8
50	6.8	6.9	6.9	6.9	7.1	7.9	7.9	7.9	7.8	7.7
100	6.8	6.8	6.8	6.9	6.8	7.8	7.8	7.8	7.8	7.7

Table 2. Salinity and temperature in each concentration treatment in barnacle larvae acute toxicity test.

Concentration treatment (%)	Salinity (‰)					Temperature (°C)				
	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48 h
Negative control	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
Salinity control	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
6.5	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
12.5	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
25	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
50	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0
100	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0

Table 3. Ammonia-N, Total sulphide, Total suspended solids, Total residual chlorine concentration at the beginning and ending of the barnacle larvae acute toxicity test.

Concentration treatment (%)	Ammonia-N (mg L ⁻¹)		Total sulphide (mg L ⁻¹)		Total suspended solids (mg L ⁻¹)		Total residual chlorine (mg L ⁻¹)	
	Initial	End	Initial	End	Initial	End	Initial	End
Negative control	0.16	0.11	< 0.1	< 0.1	< 2	< 2	< 0.02	< 0.02
Salinity control	0.16	0.14	< 0.1	< 0.1	< 2	< 2	< 0.02	< 0.02
6.5	1.08	0.57	< 0.1	< 0.1	2	< 2	< 0.02	< 0.02
12.5	3.83	2.10	< 0.1	< 0.1	4	3	< 0.02	< 0.02
25	5.87	4.24	< 0.1	< 0.1	5	5	< 0.02	< 0.02
50	9.88	9.60	0.12	< 0.1	21	7	< 0.02	< 0.02
100	18.8	12.8	0.27	< 0.1	23	12	< 0.02	< 0.02

Appendix D

**Monitoring Data
for
Diatom Growth Inhibition Test
(Chronic toxicity test)**

Table 1. Dissolved oxygen concentration and pH in each concentration treatment in diatom growth inhibition test.

Concentration treatment (%)	Dissolved oxygen (mg L ⁻¹)								pH							
	0 h	24 h	48 h	72 h	96 h	120 h	144 h	168 h	0 h	24 h	48 h	72 h	96 h	120 h	144 h	168 h
Negative control	6.8	6.9	7.1	7.2	7.5	7.7	8.0	8.2	8.0	7.9	8.0	8.0	7.9	8.1	8.1	8.1
Salinity control	6.9	6.8	7.0	7.2	7.4	7.8	7.9	8.2	7.9	7.9	8.1	8.0	8.0	8.1	8.0	8.1
2.5	6.9	6.9	7.2	7.2	7.6	7.8	8.1	8.2	8.0	7.9	7.9	8.0	8.1	8.1	8.1	8.0
5	6.8	6.8	7.1	7.1	7.6	7.9	8.0	8.2	8.0	8.0	8.1	8.0	7.9	7.9	8.0	8.1
10	6.8	6.9	7.1	7.3	7.4	7.6	7.9	8.3	7.9	7.9	8.0	8.1	8.0	8.0	8.0	8.1
25	6.9	6.8	7.0	7.1	7.2	7.6	7.8	8.2	7.9	8.0	8.0	8.0	8.1	8.0	7.9	8.0
50	6.8	6.9	7.0	7.2	7.4	7.4	7.6	7.8	7.9	7.9	7.9	8.0	8.0	8.0	8.1	8.0
100	6.9	6.8	6.9	6.9	6.9	7.1	7.2	7.3	7.9	7.8	7.9	7.9	7.9	7.9	8.0	8.0

Table 2. Salinity and temperature in each concentration treatment in diatom growth inhibition test.

Concentration treatment (%)	Salinity (‰)								Temperature (°C)							
	0 h	24h	48h	72h	96h	120h	144h	168h	0h	24h	48h	72h	96h	120h	144h	168h
Negative control	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
Salinity control	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
2.5	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
5	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
10	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
25	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
50	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
100	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0

Table 3. Ammonia-N, Total sulphide, Total suspended solids, Total residual chlorine concentration at the beginning and ending of the diatom growth inhibition toxicity test.

Concentration treatment (%)	Ammonia-N (mg L ⁻¹)		Total sulphide (mg L ⁻¹)		Total suspended solids (mg L ⁻¹)		Total residual chlorine (mg L ⁻¹)	
	Initial	End	Initial	End	Initial	End	Initial	End
Negative control	0.16	0.10	< 0.1	< 0.1	< 2	< 2	< 0.02	< 0.02
Salinity control	0.16	0.11	< 0.1	< 0.1	3	< 2	< 0.02	< 0.02
2.5	2.50	0.16	< 0.1	< 0.1	59	9	< 0.02	< 0.02
5	3.48	0.16	< 0.1	< 0.1	71	15	< 0.02	< 0.02
10	4.76	1.57	< 0.1	< 0.1	74	16	< 0.02	< 0.02
25	6.67	1.61	< 0.1	< 0.1	83	27	< 0.02	< 0.02
50	12.3	2.93	0.25	< 0.1	89	33	< 0.02	< 0.02
100	23.5	14.9	7.5	< 0.1	94	42	< 0.02	< 0.02

Appendix E

Monitoring Data
for
Shrimp Acute Toxicity Test

Table 1. Dissolved oxygen concentration and pH in each concentration treatment in shrimp acute toxicity test.

Concentration treatment (%)	Dissolved oxygen (mg L ⁻¹)					pH				
	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48 h
Negative control	6.8	6.9	6.9	6.9	6.8	7.9	7.9	8.0	8.0	8.0
Salinity control	6.9	6.9	6.9	7.0	7.0	8.0	7.9	8.0	7.9	8.0
6.5	6.9	6.9	6.9	7.0	7.0	7.9	7.9	8.0	8.0	7.9
12.5	6.9	6.9	6.9	7.0	6.9	7.9	7.9	7.9	7.9	7.9
25	6.9	7.1	7.0	7.0	7.0	8.0	8.0	7.9	8.0	7.9
50	6.9	7.0	7.0	6.9	6.8	7.9	7.9	7.9	7.7	7.7
100	7.0	7.0	6.9	6.8	6.8	7.9	8.0	7.9	7.8	7.7

Table 2. Salinity and temperature in each concentration treatment in shrimp acute toxicity test.

Concentration treatment (%)	Salinity (‰)					Temperature (°C)				
	0 h	12 h	24 h	36 h	48 h	0 h	12 h	24 h	36 h	48 h
Negative control	25.0	25.0	25.0	25.0	25.0	22.0	22.0	22.0	22.0	22.0
Salinity control	25.0	25.0	25.0	25.0	25.0	22.0	22.0	22.0	22.0	22.0
6.5	25.0	25.0	25.0	25.0	25.0	22.0	22.0	22.0	22.0	22.0
12.5	25.0	25.0	25.0	25.0	25.0	22.0	22.0	22.0	22.0	22.0
25	25.0	25.0	25.0	25.0	25.0	22.0	22.0	22.0	22.0	22.0
50	25.0	25.0	25.0	25.0	25.0	22.0	22.0	22.0	22.0	22.0
100	25.0	25.0	25.0	25.0	25.0	22.0	22.0	22.0	22.0	22.0

Table 3. Ammonia-N, Total sulphide, Total suspended solids, Total residual chlorine concentration at the beginning and ending of the shrimp acute toxicity test.

Concentration treatment (%)	Ammonia-N (mg L ⁻¹)		Total sulphide (mg L ⁻¹)		Total suspended solids (mg L ⁻¹)		Total residual chlorine (mg L ⁻¹)	
	Initial	End	Initial	End	Initial	End	Initial	End
Negative control	0.57	0.16	< 0.1	< 0.1	2	< 2	< 0.02	< 0.02
Salinity control	0.14	0.11	< 0.1	< 0.1	3	< 2	< 0.02	< 0.02
6.5	7.33	3.46	< 0.1	< 0.1	10	8	< 0.02	< 0.02
12.5	7.97	4.71	< 0.1	< 0.1	10	9	< 0.02	< 0.02
25	8.95	6.63	< 0.1	< 0.1	11	10	< 0.02	< 0.02
50	13.5	12.3	< 0.1	< 0.1	20	11	< 0.02	< 0.02
100	23.5	21.9	< 0.1	< 0.1	33	22	< 0.02	< 0.02