Term Contract for Provision of Sampling and Analyzing of Samples for Various Sewage Treatment Facilities in Urban Area, Lantau and Outlying Islands to the Drainage Services Department (2023-2026)

Whole Effluent Toxicity Test (WETT) at SCISTW

Report for the Month of January 2024

Contract No.	:	DE/2022/15
Applicant	:	SEWAGE TREATMENT DIVISION 2 ELECTRICAL AND MECHANICAL BRANCH DRAINAGE SERVICES DEPARTMENT
Address	:	STONECUTTERS ISLAND SEWAGE TREATMENT WORKS, NGONG SHUEN CHAU, KOWLOON, HONG KONG
Application No.	:	L0032375(9)
Report No.	:	A00056779(1)
Report Issued Date	:	23 Feb 2024

For and on behalf of
CMA Industrial Development Foundation Limited

Authorized Signature :

Lau Yan Kin
Senior Manager
Environmental Division

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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 the Oceans and Human Health, City University of Hong Kong
Principle investigator: Prof. Wen-Xiong WANG
Address: City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong

1.3. Sample

A 24-hour flow-weighted composite effluent sample was collected from Stonecutters Island Sewage Treatment Works (SCISTW) on January 27, 2024. 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)

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2. Report on Amphipod Acute Toxicity Test

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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 (*Melita longidactyla*).
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 *Ulva lactuca*.

2.3. Summary of test conditions

Type of test:	Static
Duration:	48 h, 27/1/2024-29/1/2024
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‰

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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	10	10	9.75	0.50
Salinity control	0	10	10	8	9	9.25	0.96
Concentration 1	6.5	8	10	8	8	8.50	1.00
Concentration 2	12.5	8	6	8	6	7.00	1.15
Concentration 3	25	7	7	6	6	6.50	0.58
Concentration 4	50	4	6	5	6	5.25	0.96
Concentration 5	100	0	0	0	2	0.50	1.00

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	100	100	97.50	5.00
Salinity control	0	100	100	80	90	92.50	9.57
Concentration 1	6.5	80	100	80	80	85.00	10.00
Concentration 2	12.5	80	60	80	60	70.00	11.55
Concentration 3	25	70	70	60	60	65.00	5.77
Concentration 4	50	40	60	50	60	52.50	9.57
Concentration 5	100	0	0	0	20	5.00	10.00

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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 (^{o}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})	0.96/< 0.01	0.71/< 0.01	1.79/0.42	1.94/1.11	2.42/2.08	2.99/2.99	3.95/3.01
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 (mg L^{-1})	13/4	11/7	15/14	21/12	35/27	47/31	61/32

2.6. LC_{50} for the amphipod *Melita longidactyla* and test acceptability

Table 4. LC_{50} for the amphipods and test acceptability.

Parameter	Value	Control limit
Calculated LC_{50}	43.6%	NA
Negative control survival	97.5%	≥90%
Reference toxicant 48-h acute test	1.27 mg L^{-1}	1.25±0.15 mg L^{-1}
95% of confidence range of reference toxicant test	1.08-1.52 mg L^{-1}	NA
Daily temperature variation	<0.5 ^{o}C	Average daily temperature variation: ±1 ^{o}C
Dissolved oxygen concentration	>6.8 mg L^{-1}	>4 mg L^{-1}

NA: Not applicable

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3. Report on Fish Acute Toxicity Test

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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 (*Lutjanus malabaricus*)
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, 27/1/2024-29/1/2024
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‰

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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	20	20	20	20.00	0.00
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	20	18	20	19	19.25	0.96

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	100	100	100	100.00	0.00
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	100	90	100	95	96.25	4.79

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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	30	30	30	30	30	30
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 (^{o}C)	22	22	22	22	22	22	22
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})	0.60/< 0.01	0.40/<0.01	2.50/<0.01	3.80/1.14	5.86/2.14	10.6/3.00	20.1/14.0
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 (mg L^{-1})	< 2	< 2	7/4	9/5	11/6	16/12	51/14

3.6. LC_{50} for the fish *Lutjanus malabaricus* and test acceptability

Table 4. LC_{50} 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	14.7 mg L^{-1}	14.6±1.78 mg L^{-1}
95% of confidence range of reference toxicant test	13.5-18.0 mg L^{-1}	NA
Daily temperature variation	<0.5 ^{o}C	Average daily temperature variation: ± 1 ^{o}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 LC_{50} cannot be calculated.
NA: Not applicable

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4. Report on Barnacle Larvae Acute Toxicity Test

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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 (*Balanus amphitrite*).
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 *Chaetoceros gracilis*.

4.3. Summary of test conditions

Type of test:	Static
Duration:	48 h, 27/1/2024-29/1/2024
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‰

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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	20	20	20	20	20.00	0.00
Concentration 2	12.5	19	20	20	19	19.50	0.50
Concentration 3	25	16	20	18	18	18.00	1.41
Concentration 4	50	16	14	16	16	15.50	0.87
Concentration 5	100	0	0	0	0	0.00	0.00

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	100	100	100	100	100.00	0.00
Concentration 2	12.5	95	100	100	95	97.50	2.50
Concentration 3	25	80	100	90	90	90.00	7.07
Concentration 4	50	80	70	80	80	77.50	4.33
Concentration 5	100	0	0	0	0	0.00	0.00

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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 (^oC)	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})	0.24/0.02	0.33/0.13	0.71/0.31	1.38/0.98	2.62/2.24	5.86/1.37	3.86/3.29
Total Sulphide (start/end, mg L^{-1})	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.14/< 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 (mg L^{-1})	5/<2	4/3	6/4	9/9	16/10	27/12	52/20

4.6. LC_{50} for the barnacle larvae *Balanus amphitrite* and test acceptability

Table 4. LC_{50} for the barnacle larvae and test acceptability

Parameter	Value	Control limit
Calculated LC_{50}	57.6%	NA
Negative survival	100%	>90%
Reference toxicant 48-h acute test	1.09 mg L^{-1}	1.04±0.11 mg L^{-1}
95% of confidence range of reference toxicant test:	0.97-1.25 mg L^{-1}	NA
Daily temperature variation	<0.5 ^oC	Average daily temperature variation: ± 1 ^oC
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)

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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 (*Skeletonema costatum*)
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, 27/1/2024-3/2/2024
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:	$(5.0\pm0.4)\times10^4$ 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‰

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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±0.4)×10^4 cell mL^{-1}.

Treatment	Effluent concentration (%)	Cell density after 7-day growth (×10^6 cell mL^{-1})					
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean	SD
Negative control	0	0.93	1.10	0.98	1.15	1.04	0.09
Salinity control	0	1.13	1.23	0.90	0.98	1.06	0.13
Concentration 1	2.5	1.90	1.65	1.78	2.00	1.83	0.13
Concentration 2	5	2.88	2.98	2.55	2.40	2.70	0.23
Concentration 3	10	1.43	1.25	0.98	1.13	1.19	0.17
Concentration 4	25	0.60	0.68	0.45	0.48	0.55	0.09
Concentration 5	50	0.20	0.28	0.13	0.18	0.19	0.05
Concentration 6	100	0.05	0.18	0.00	0.13	0.09	0.07

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	0.42	0.44	0.42	0.45	0.43	0.01
Salinity control	0	0.44	0.46	0.41	0.42	0.43	0.02
Concentration 1	2.5	0.52	0.50	0.51	0.53	0.51	0.01
Concentration 2	5	0.58	0.58	0.56	0.55	0.57	0.01
Concentration 3	10	0.48	0.46	0.42	0.44	0.45	0.02
Concentration 4	25	0.35	0.37	0.31	0.32	0.34	0.03
Concentration 5	50	0.20	0.24	0.13	0.18	0.19	0.05
Concentration 6	100	0.00	0.18	0.00	0.13	0.08	0.09

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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 (^{o}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})	0.43/< 0.01	0.39/< 0.01	0.61/< 0.01	1.08/ 0.03	2.44/ 0.03	2.81/0.05	4.19/3.98	56/41
Total Sulphide (start/end, mg L^{-1})	< 0.1	< 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	< 0.02
Total Suspended Solids (mg L^{-1})	21/3	20/4	32/4	35/6	45/9	56/35	56/38	59/41

5.6. IC_{50} for the diatom *Skeletonema costatum* and test acceptability

Table 4. IC_{50}, none observed effect concentration (NOEC) for the diatom and test acceptability

Parameter	Value	Control limit
Calculated IC_{50}	40.1%	NA
None observed effect concentration (NOEC)	10%	-
Reference toxicant 7-day test:	0.12 mg L^{-1}	0.13±0.02 mg L^{-1}
95% of confidence range of reference toxicant test	0.07-0.14 mg L^{-1}	NA
Temperature variation	<0.5 ^{o}C	Average daily temperature variation: ± 1 ^{o}C

NA: Not applicable

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6. Report on Shrimp Acute Toxicity Test

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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 (*Metapenaeus ensis*)
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, 27/1/2024-29/1/2024
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‰

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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	10	9	9.75	0.50
Salinity control	0	8	8	9	8	8.25	0.50
Concentration 1	6.5	9	8	10	10	9.25	0.96
Concentration 2	12.5	9	9	10	10	9.50	0.58
Concentration 3	25	10	8	9	7	8.50	1.29
Concentration 4	50	8	10	9	9	9.00	0.82
Concentration 5	100	5	6	5	8	6.00	1.41

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	100	90	97.50	5.00
Salinity control	0	80	80	90	80	82.50	5.00
Concentration 1	6.5	90	80	100	100	92.50	9.57
Concentration 2	12.5	90	90	100	100	95.00	5.77
Concentration 3	25	100	80	90	70	85.00	12.91
Concentration 4	50	80	100	90	90	90.00	8.16
Concentration 5	100	50	60	50	80	60.00	14.14

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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 (^{o}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})	0.34/< 0.01	0.38/< 0.01	1.23/0.38	2.13/0.35	2.23/1.01	3.65/2.16	3.17/2.39
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 (mg L^{-1})	< 2	4/< 2	8/4	11/8	12/8	15/12	33/12

6.6. LC_{50} for the shrimp *Metapenaeus ensis* and test acceptability

Table 4. LC_{50} for the *Metapenaeus ensis* and test acceptability.

Parameter	Value	Control limit
Calculated LC_{50}	Cannot be calculated[a]	NA
Negative control survival	97.5%	>90%
Reference toxicant 48-h acute test	5.2 mg L^{-1}	5.19±0.51 mg L^{-1}
95% of confidence range of reference toxicant test	4.89-5.57 mg L^{-1}	NA
Daily temperature variation	<0.5 ^{o}C	Average daily temperature variation: ±1 ^{o}C
Dissolved oxygen concentration	>6.7 mg L^{-1}	>4 mg L^{-1}

a: The mortalities in all concentration groups were less than 50% and thus LC_{50} cannot be calculated.
NA: Not applicable

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7. Conclusion

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

Test species	Measured LC_{50}/IC_{50}/NOEC	Target toxicity level
Amphipod *Melita longidactyla*	43.6%	≥7.1%
Fish *Lutjanus malabaricus*	Cannot be calculated because animals displayed very high survival rate (>50%) at the highest tested effluent concentration (100%)	≥7.1%
Barnacle larvae *Balanus amphitrite*	57.6%	≥7.1%
Diatom *Skeletonema costatum*	40.1%	-
Diatom *Skeletonema costatum*	10% (NOEC)	≥0.51%
Shrimp *Metapenaeus ensis*	Cannot be calculated because animals displayed very high survival rate (>50%) at the highest tested effluent concentration (100%)	≥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 LC_{50} cannot be calculated.

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Appendix A

Monitoring Data
for
Amphipod Acute Toxicity Test

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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

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Table 3. Ammonia-N, 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	0.96	< 0.01	< 0.1	< 0.1	13	4	< 0.02	< 0.02
Salinity control	0.96	< 0.01	< 0.1	< 0.1	11	7	< 0.02	< 0.02
6.5	0.96	< 0.01	< 0.1	< 0.1	15	14	< 0.02	< 0.02
12.5	0.96	< 0.01	< 0.1	< 0.1	21	12	< 0.02	< 0.02
25	0.96	< 0.01	< 0.1	< 0.1	35	27	< 0.02	< 0.02
50	0.96	< 0.01	< 0.1	< 0.1	47	31	< 0.02	< 0.02
100	0.96	< 0.01	< 0.1	< 0.1	61	32	< 0.02	< 0.02

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Appendix B

Monitoring Data
for
Fish Acute Toxicity Test

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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 (^{o}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

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Table 3. Ammonia-N, 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	0.60	< 0.01	< 0.1	< 0.1	< 2	< 2	< 0.02	< 0.02
Salinity control	0.40	< 0.01	< 0.1	< 0.1	< 2	< 2	< 0.02	< 0.02
6.5	2.50	< 0.01	< 0.1	< 0.1	7	4	< 0.02	< 0.02
12.5	3.80	1.14	< 0.1	< 0.1	9	5	< 0.02	< 0.02
25	5.86	2.14	< 0.1	< 0.1	11	6	< 0.02	< 0.02
50	10.6	3.00	< 0.1	< 0.1	16	12	< 0.02	< 0.02
100	30.1	14.0	< 0.1	< 0.1	51	14	< 0.02	< 0.02

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Appendix C

Monitoring Data
for
Barnacle Larvae Acute Toxicity Test

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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 (^{o}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

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Table 3. Ammonia-N, 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	0.24	0.02	< 0.1	< 0.1	5	< 2	< 0.02	< 0.02
Salinity control	0.33	0.13	< 0.1	< 0.1	4	3	< 0.02	< 0.02
6.5	0.71	0.31	< 0.1	< 0.1	6	4	< 0.02	< 0.02
12.5	1.38	0.98	< 0.1	< 0.1	9	9	< 0.02	< 0.02
25	2.62	2.24	< 0.1	< 0.1	16	10	< 0.02	< 0.02
50	5.86	1.37	< 0.1	< 0.1	27	12	< 0.02	< 0.02
100	3.86	3.29	0.14	< 0.1	52	20	< 0.02	< 0.02

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Appendix D

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

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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	24h	48h	72h	96h	120h	144h	168h	0h	24h	48h	72h	96h	120h	144h	168h
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

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Table 3. Ammonia-N, 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	0.43	< 0.01	< 0.1	< 0.1	21	3	< 0.02	< 0.02
Salinity control	0.39	< 0.01	< 0.1	< 0.1	20	4	< 0.02	< 0.02
2.5	0.61	< 0.01	< 0.1	< 0.1	32	4	< 0.02	< 0.02
5	1.08	0.03	< 0.1	< 0.1	35	6	< 0.02	< 0.02
10	2.44	0.03	< 0.1	< 0.1	45	9	< 0.02	< 0.02
25	2.81	0.05	< 0.1	< 0.1	56	35	< 0.02	< 0.02
50	4.19	3.98	< 0.1	< 0.1	56	38	< 0.02	< 0.02
100	56.0	41.0	< 0.1	< 0.1	59	41	< 0.02	< 0.02

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Appendix E

Monitoring Data
for
Shrimp Acute Toxicity Test

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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

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Table 3. Ammonia-N, 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	0.34	< 0.01	< 0.1	< 0.1	< 2	< 2	< 0.02	< 0.02
Salinity control	0.38	< 0.01	< 0.1	< 0.1	4	< 2	< 0.02	< 0.02
6.5	1.23	< 0.01	< 0.1	< 0.1	8	4	< 0.02	< 0.02
12.5	2.13	< 0.01	< 0.1	< 0.1	11	8	< 0.02	< 0.02
25	2.23	< 0.01	< 0.1	< 0.1	12	8	< 0.02	< 0.02
50	3.65	< 0.01	< 0.1	< 0.1	15	12	< 0.02	< 0.02
100	3.17	< 0.01	< 0.1	< 0.1	33	12	< 0.02	< 0.02

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