Toxic Effects on *Dunaliella salina*, *Mysidopsis bahia*, and *Mugilogobius chulae* from Ship Exhaust Gas Closed-loop Scrubber Wash Water

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Abstract

To meet the strict requirements of reducing sulfur emission, more and more commercial ships have installed exhaust gas cleaning systems (EGCSs). However, wash water produced during cleaning process is discharged back to the marine environment. We investigated effects of the closed-loop scrubber (sodium-alkali method) wash water on three trophic species. Severe toxic effects were found when *Dunaliella salina*, *Mysidopsis bahia* and *Mugilogobius chulae* were exposed to 0.63-6.25, 0.63-10 and 1.25-20% concentrations of wash water, respectively. EC₅₀-96 h of *D. salina* was 2.48%, corresponding to total polycyclic aromatic hydrocarbons (PAHs) or heavy metals were 22.81, 23.67 $\mu\text{g L}^{-1}$. LC₅₀-7 d of *M. bahia* and *M. chulae* were 3.57 and 20.50%. The LOEC of *M. bahia* and *M. chulae* were 1.25% and 2.5%, corresponding to total PAHs and heavy metals were 11.50, 11.93 and 22.99, 23.86 $\mu\text{g L}^{-1}$. *M. bahia*'s body weight was negatively correlated with concentrations of wash water. Although wash water contained high concentrations of PAHs (e.g., naphthalene) and heavy metals (e.g., Cr, Ni, Cu and Zn), none of the single pollutant could explain the high toxicity of wash water. The measured toxicity might arise from synergistic effects among several contaminants or from other more toxic contaminants we know very little about. We highly recommend that wash water should be treated prior to being discharged to the marine environment.

1 Introduction

Maritime trade has developed rapidly, accounting for nearly 90% of all international trade^[1]. At present, diesel engines that are the main power of marine ships generally use heavy fuel oil and marine diesel oil with high sulfur and ash contents^[2]. Among the exhaust gas emitted from its combustion, sulfur dioxide (SO₂) is the primary pollutants^[3], which has caused a serious air pollution problem and posed serious threats to human health. Therefore, the International Maritime Organization (IMO) has issued regulations to limit SO₂ emissions content^[4], and the regulations have become more and more stricter in recent years^[5]. In order to meet the IMO requirement, desulfurization of the ship's exhaust gas has become a necessary measure. Methodologies including low-sulfur fuels, alternative fuels (e.g., biodiesel, methanol, liquefied natural gas)^[6], and/or exhaust gas cleaning system (EGCS)^[7]. Among these methods, EGCS is often more cost efficient in large ships^[8]. However, EGCS will discharge wash water that is produced from desulfurization scrubbers using liquid solution (e.g., sodium hydroxide, magnesium hydroxide, seawater). Wash water contains many pollutants including suspended solids (SS), sulfite (SO₃²⁻), toxic hydrocarbons and heavy metals^[9-11], and is most frequently released to marine environment without treatment, which may introduce excessive unwanted environmental consequences^[12].

Nearshore waters provide important habitat and ecosystem protection services for a large number of organisms^[13]. Flora and fauna living near the sea surface will experience the large impact. Satomi et al.^[14] found that excessive SO₃²⁻ adversely affects photosynthetic activities and the growth of phytoplankton, exposure of *Synechococcus elongatus* PCC 7942 to 10 mM SO₃²⁻ for 24 h would hindered cell growth

and inhibited more than 90% of photosynthesis. Polycyclic aromatic hydrocarbons (PAHs) have high persistence, bioaccumulation, and have carcinogenic effects^[15, 16]. PAHs also can reduce zooplankton grazing rates and phytoplankton growth rates^[17, 18]. Heavy metals are a group of such compounds, which can accumulate in fish leading to developmental abnormalities, reduced growth, and increased rates of mortality^[19]. As the pH value of the wash water decreases, the solubility and toxicity of heavy metals in seawater increase^[20]. Considering the potential risk of wash water to the marine environment, the IMO has established strict standards for desulfurization wash water, which clearly specified the limitation of indicators including pH (>6.5), PAHs (<2250 $\mu\text{g L}^{-1}$, 0-1 t/MWh flow rate), turbidity (≤ 25 FNU/NTU) and nitrate (≤ 60 mg L^{-1})^[21]. However, the comprehensive toxicity effects of wash water from EGCS have not been considered, which is more important than limited level of single indicator^[22]. Current researches mainly have focused on the impact of open-loop scrubber wash water on algae or copepod^[23, 24], only one study on the toxicity of closed-loop scrubber wash water to the planktonic copepod^[25]. At present, there is a lack of toxicity research on closed-loop scrubber wash water, especially in terms of toxicity test species and trophic levels^[26].

Besides lack of knowledge on the toxicity of the effluents from EGCS, a wide range of standard biological species used in experimental aquatic toxicology, such as daphnia and zebra fish, are mostly freshwater organisms, whereas toxicity data for marine species are scarcer, increasing uncertainty of the protection for marine ecosystems^[27]. Bioaccumulation and biomagnification of PAHs and heavy metals have been detected in marine organisms^[28–30]. However, the impacts of wash water on populations from different trophic levels have never been evaluated. In fact, in order to fully assess the potential toxicity of pollutants, it is necessary to use organism representative of different trophic levels or habitats and with different sensitivity^[31]. Test species selection is a very important step. Algae is a natural food for aquatic organisms, and the impact of pollutants on algae may further threaten the life of higher trophic organisms^[32]. Mysids, as predators of algae and preferential prey of large organisms^[33], are widely used in ecotoxicological tests. The relationship between fish and other organisms is complex in the marine food web (i.e., decimation of fish populations also impacts the entire marine community)^[34] and fish growth can forecast physiological course of action in evaluating the impacts of pollutants. Consequently, it is vital to elucidate effects of the wash water on algae, mysid and fish.

This study aimed to investigate the closed-loop scrubber wash water toxicity to three marine species from distinct trophic levels, which including algae (*Dunaliella salina*), mysid (*Mysidopsis bahia*) and fish (*Mugilogobius chulae*). Study the composition and concentration of pollutants and potential synergistic effects, as well as to compare different species sensitivity to wash water. We conducted the following experiments under different concentrations of wash water exposure: 1) algae growth inhibition test; 2) mysid and fish chronic toxicity test.

2 Materials And Methods

2.1 Test water

Wash water samples were collected in acetone-washed 5 L glass bottles from the outlet from a closed-loop scrubber on board. Heavy fuel oils with a sulfur content of approximately 3% were employed by this ship. Wash water was collected from a recirculation system releasing from a sodium hydroxide desulfurization scrubber (vessel cruising speed was approximately 75% engine load). Samples were stored cold (4 °C) and dark prior to experiments (within 96 h). Chemical analysis of test water is shown in Table S1.

2.2 Test organisms

D. salina obtained from Shanghai Guangyu Biological Technology Co., Ltd. was selected to perform algae growth inhibition due to its unique and remarkable ability to survive in extreme conditions^[35]. *M. bahia* obtained from Shanghai Guanggang Colorful Base was selected because it was commonly used for marine whole effluent toxicity tests and toxicity identification evaluation studies^[36]. *M. chulae* purchased from Guangdong Provincial Laboratory Animal Monitoring Institute was selected due to it is a potential marine experimental fish, there have been many studies in the field of aquatic ecological toxicology evaluation^[37].

2.3 Aquatic ecotoxicity test

For algae growth inhibition test, before use, *D. salina* were cultured in an artificial climate incubator at 22 ± 2 °C under cool continuous white fluorescent lights (2000 lx) and 14/10 light/dark cycle, and manually shaken twice a day to prevent the sedimentation of algae^[38, 39]. When *D. salina* were cultured to the log phase growth (i.e., initial algal density was around 5×10⁴ cells/mL), they were exposed to wash water with concentrations ranging from 0 to 25% (dilution ratio was 2) in 250-mL conical flasks for 96 h. The dilution water (DW) consisted of 0.45 µm - filtered and sterilized seawater (made from sea salt purchased from Guangzhou Yier BE Co., Ltd) and f/2 medium (Tables S2 and S3) in a ratio of 1000 mL: 1 mL. For each treatment, cell density was measured by hemacytometer every 24 h under microscope with 400 magnification times. pH, temperature and dissolved oxygen were measured in each replicate at the beginning of the test and every 24 h thereafter (Table S4). Six replicates were set up for each concentration (Tables S5). The average specific growth rate (ASGR, μ) and inhibition rate (%*I_p*) were calculated from the cell density^[40]. The calculation equations are as follows:

$$\mu_{i-j} = \frac{\ln X_j - \ln X_i}{t_j - t_i} \text{ ——— [1]}$$

where:

μ_{i-j} is the average specific growth rate from time i to j, (d⁻¹);

X_i is the cell density at time i, (d);

X_j is the cell density at time j , (d).

$$\%I_r = \frac{\mu_C - \mu_T}{\mu_C} \times 100 \text{ ——— [2]}$$

where:

$\%I_r$ is the percent inhibition of growth rate for each treatment replicate;

μ_C is mean value for average specific growth rate (μ) in the control group (d^{-1});

μ_T is average specific growth rate for the treatment replicate (d^{-1}).

A semi-static renewal system was employed for chronic toxicity test (Supporting information, Figure S1). *M. bahia* (5-6 days old) and *M. chulae* (7 days old juvenile fish, body length = 30 ± 5 mm, body weight = 1.8 ± 0.2 g) were used during 7-day test. Forty *M. bahia* per concentration (i.e., 8 replicates per concentration, 5 test organisms for each replicate) were exposed to five concentrations (ranging from 0 to 10%) of wash water in a geometric series in which the ratio was 2 at 23 ± 2 °C in 1-L test chambers. Similar to *M. bahia*, forty *M. chulae* (i.e., 4 replicates per concentration, 10 test organisms for each replicate) were exposed to concentrations ranging from 0 to 20%. For food, live *Artemia* spp. (48-h-old nauplii) was fed daily to *M. bahia* during testing, and 0.1 g (wet weight) newly hatched *Artemia* nauplii per replicate on days 0-2 was fed to *M. chulae*, 0.15 g (w.w) per replicate on days 3-6, and no feeding on day 7 (Table S6). The test chambers were siphoned daily to remove food leftovers, excretion, and fecal matter. pH, temperature and dissolved oxygen were measured in each replicate at the beginning of the test and every 24 h thereafter (Table S7).

2.4 Chemical analysis

The pH, temperature and dissolved oxygen were determined with a pH electrode, thermometer and dissolved oxygen analyzer. Total suspended solids (TSS) were determined by gravimetric method (EPA method 160.2)^[41]. Turbidity was determined using a nephelometer detection and expressed in nephelometric turbidity units (NTU). Sulfite, sulfate, nitrite and nitrate were analyzed on an ion Chromatography (Thermo Scientific, Dionex)^[42]. PAHs were determined by Gas Chromatography/Mass Spectrometry (GC/MS) (EPA 8270 D)^[43]. For the analysis of eight metals (i.e., V, Cr, Ni, Cu, Zn, As, Cd, Pb), wash water was digested with HCl and HNO₃ and analyzed with Inductively Coupled Plasma- Mass Spectrometry (ICP-MS)^[44].

2.5 Endpoints and statistical analysis

Algal growth response (as percent of inhibition or stimulation in the test solutions compared to the controls) was calculated at the end of the test. Mortality observations were recorded every 24 h, and any abnormal behavior (e.g., body stiff, white and/or sank to the bottom) was recorded. The number of dead *M. bahia*, cumulative young per female, and body lengths and weight of male and female *M. bahia*,

survival and growth (length and weight) of *M. chulae* were recorded during the test. Fifty percent effective concentration (EC₅₀) was the concentration that resulted in an 50% reduction in growth of *D. salina* within a stated exposure period of 96 h, and it was calculated by probit regression from inhibition rate. Fifty percent lethal concentration (LC₅₀) was calculated by probit regression from mortality data of *M. bahia* and *M. chulae*. Lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC) were calculated from one-way analysis of variance (ANOVA) - Post Hoc-Dunnett^[45] (SPSS 23). Through one-sample T-test, significant differences between each exposed concentration and control concentration (at $p < 0.05$) were tested. Data were expressed as mean $\pm$ standard deviation (SD) in this study.

3 Results And Discussion

3.1 Algae Growth inhibition

Wash water was significantly toxic to *D. salina*. We found exposure during 96-h test period to even lowest concentrations (e.g., 0.63%) of wash water had adverse effects on *D. salina*. Cell densities of *D. salina* increased in a slower rate in treatment group within 96 h compared to control group (e.g., from 5.0 to 7.9 $\times 10^4$ cells/mL in 6.25% concentration of wash water, whereas from 5.0 to 84.8 $\times 10^4$ cells/mL in control group), and a continuous decreased pattern followed by when exposed to more than 6.25% concentration of wash water, instead there was a continuous increased pattern in cell densities of control group (Table S8). For control group, the mean coefficient of variation for section-by-section growth rates (i.e., 0-24, 24-48, 48-72, 72-96 h) were less than 35%, and the coefficient of variation of ASGR during 96-h test period was less than 7%, indicating the test was valid.

On the other hand, ASGR was negatively correlated with the concentration of wash water, higher the concentration of wash water, more inhibited the growth of *D. salina*, indicating detrimental effects on *D. salina* became more and more serious with increasing wash water concentrations and prolong exposure time. Interestingly, compared with other exposure periods (i.e., 24-48, 48-72, 72-96 h), the strongest inhibiting effect occurred at exposure period of 24-48 h (green column in Figure 1(a)), and algae had a certain adaptability to toxic substances in the following exposure time. In comparison, the growth of *Rhodomonas sp.* was reduced to zero in 100% open-loop scrubber wash water^[24], while the growth of *D. salina* in this study was reduced to zero in 12.5% wash water. It can be explained that wash water that was obtained from a close-loop scrubber accumulated higher concentration of heavy metals and PAHs, which was more toxic than open-loop one^[46].

Complete inhibition of *D. salina* occurred at 12.5% wash water (Figure 1(b)). The calculated EC₅₀-96 h was 2.48%, while previous research was shown that the EC₅₀-6 d of *Nodularia spumigena* and *Melosira cf. arctica* after exposure to open-loop scrubber wash water was 38.9% and 18.3%, respectively^[23]. This difference can be explained by pollutant concentration in closed-loop scrubber wash water is much higher than open-loop one. We suspected that naphthalene (NAP), Cr, Ni, Cu and Zn played a vital role for

the inhibition effect on *D. salina*. It has been reported that there was significant inhibition for *Landoltia punctata* at NAP concentration low to $10 \mu\text{g L}^{-1}$ ^[47], hence, the concentration of NAP was $898.02 \mu\text{g L}^{-1}$ in our study, which was 90 times higher than that. In addition, Zaki et al.^[48] investigated the single toxicity (i.e., EC₅₀) of heavy metals on the growth of *Pseudokirchneriella subcapitata* were $200 \mu\text{g L}^{-1}$ for Cr, $55 \mu\text{g L}^{-1}$ for Ni, $48 \mu\text{g L}^{-1}$ for Cu, and $80 \mu\text{g L}^{-1}$ for Zn, respectively. In comparison, the EC₅₀-96 h of Cr, Ni, Cu and Zn were 8.05, 12.5, 0.39, and $2.38 \mu\text{g L}^{-1}$ in our study. The discrepancy may be due to the combined toxicity of heavy metals is stronger than that of single heavy metals. One previous study has shown that combined toxicity of Ni-Cr mixtures was additive effect, however, Ni-Zn mixtures presented additive effect and/or synergistic effect^[49]. We inferred that Ni-Zn mixture in wash water might have a great inhibitory effect on *D. salina*, since Ni and Zn were dominant heavy metals detected in wash water (Table S1).

3.2 Mortality

We found that the lowest concentration of wash water (i.e., 0.63%) had no lethal effect on *M. bahia*. However, the mortality rates of *M. bahia* and *M. chulae* gradually increased with increasing concentration of wash water (Figure 2). We also observed that *M. bahia* exposed to 1.25-10% wash water showed strange phenomenon – restlessness, slow swimming after 6 hours, some body distortions in 5 and 10%, reduced feeding and yellow excrement, it can be explained that pollutants affect the digestive system of *M. bahia*. Moreover, it can be seen that longer the exposure time, higher the toxic effect of wash water on *M. bahia* and *M. chulae*. In contrast, no individuals died in *M. bahia*'s control group. Survival rate in the control of *M. chulae* was more than 80% during 7-day experiment, which met the test standards (Table S6).

Previously, it was reported that adult female copepods (*Acartia tonsa*) mortality increased in 10% open-loop scrubber wash water, with close to all individuals being dead at 30%^[24]. Thor et al.^[25] studied all copepods died within 1 day when exposed to the 5% concentration of closed-loop scrubber wash water and within 8 days when exposed to the 40% concentration of open-loop scrubber wash water. In comparison, *M. bahia* and *M. chulae* mortality increased in 1.25% wash water, with close to all *M. bahia* being dead at 10% wash water and the mortality rate of *M. chulae* in 20% wash water was only 42.5% in our study. The discrepancy can be explained either by the difference in the chemical compositions of wash water from these three studies or by the difference in sensitivity of the test organisms – fish has a relatively high metabolic capacity for PAHs^[50], therefore, compared to copepods or mysid, fish might be decreased potential susceptibility to adverse effects to wash water. Hunt et al.^[51] observed marine mysid (*Mysidopsis intii*) was more sensitive to Ni than the marine fish (*Atherinops affinis*). David et al.^[52] also found marine fish (*Sheepshead minnow*, *Topsmelt silverside*) were less sensitive to Ni than mysid (*Americamysis bahia*). These conclusions further prove that fish is less sensitive than mysid. Additionally, LC₅₀-7 d of *M. bahia* and *M. chulae* calculated based on mortality rate were 3.57 and 20.50% (Table 1). These significant toxic effects highly depend on the type of interaction (metal-metal, metal-organic,

organic-organic) and on the concentration of each pollutant in the wash water, which can either trigger antagonistic, synergistic, or non-interactive toxicities^[53–56].

Table 1
LC₅₀ values of *M. bahia* and *M. chulae* exposed
to the wash water

Exposure time (d)	<i>M. bahia</i>	<i>M. chulae</i>
1	18.10	43.77
2	14.64	55.98
3	15.10	30.18
4	9.56	26.29
5	6.75	23.54
6	5.02	21.97
7	3.57	20.50

3.3 Growth, development and reproduction

For *M. bahia*, there was a significant difference in body weight between control and test groups ($p < 0.01$) (Table S9), and body weights were negatively correlated with the concentration of wash water, the minimum weight (4.2 ± 0.02 mg) observed in 10% wash water (Figures 3 (a) and (b)). This phenomenon can be explained that heavy metals disturb the growth and development of aquatic fauna^[57]. Moreover, *M. chulae*'s weight and length changed irregularly during 7-day experiment, with ranges from 1.87-2.21 g and 3.0-3.5 cm, respectively (Figures 3 (c) and (d), Table S10), which might be due to heavy metals can agitate the fish by physically stimulating the fish's external tissues and disrupting the metabolism^[58].

We found there was no reproduction in 10% wash water ($p < 0.05$), while that was 1.06 ± 0.38 in control group. When concentration of wash water was less than 5%, the higher the concentration of wash water, the lower the reproductive rate, but there was no significant difference compared with control group ($p > 0.05$) (Table 2), indicating that low concentrations had little effect on the reproductions of *M. bahia* and reproductive endpoints of mysid had been less sensitive in previous studies^[51]. As previous research has shown that environmental defense mechanisms of crustaceans depended principally on the innate immune system that was activated when a stressor factor was recognized by soluble or surface host cell proteins, such as lectins, and/or antimicrobial, clotting and pattern recognition proteins^[59]. Therefore, the cessation of reproductive behavior might be due to 10% concentration of wash water broke this defense mechanisms. In addition, crustaceans showed relatively lower levels of metabolic capacity of PAHs than fishes and polychaetes^[60, 61]. This means that marine crustaceans may have higher potential to

accumulate PAHs inside their body, especially lipid-rich cells and tissues. Lotufo^[62] and Charry et al.^[63] also proposed that this process was the main cause for the inhibitions of the development and reproduction in benthic copepods.

Table 2
Number of females and reproductions of *M. bahia* during 7-day exposure period

Concentration (%)	Females		Reproductions	
	Mean ± standard deviation	Significant difference	Mean ± standard deviation	Significant difference
Control	2.00 ± 0.82	None	1.06 ± 0.38	None
0.63	2.00 ± 1.15	p = 1.000 > 0.05	0.84 ± 0.19	p = 0.338 > 0.05
1.25	3.25 ± 1.26	p = 0.148 > 0.05	0.58 ± 0.50	p = 0.177 > 0.05
2.5	1.75 ± 1.71	p = 0.800 > 0.05	0.44 ± 0.43	p = 0.072 > 0.05
5	2.50 ± 1.29	p = 0.537 > 0.05	0.38 ± 0.44	p = 0.056 > 0.05
10	1.75 ± 0.50	p = 0.621 > 0.05	0.00	0.01 < p = 0.001 < 0.05

Based on the chemical analysis of wash water (Table S1), we believed that not all pollutants were highly toxic to *M. bahia* and *M. chulae*, since there were large differences between the concentrations of some pollutants in wash water. The dominant pollutants were NAP, Cr, Ni, Cu and Zn. Alves et al.^[64] reported that the toxicity of effluents from gasoline stations oil-water separators to embryos of zebrafish was 302.67 $\mu\text{g L}^{-1}$ as NAP (LC₅₀-96 h value), however, NAP concentrations of *M. bahia* and *M. chulae* were 42.93 and 72.36 $\mu\text{g L}^{-1}$ in our study, indicating wash water was more toxic. Cr in aquatic environments is mainly found in the form of Cr (VI) and Cr (III)^[65] which can also be converted to Cr (VI) under certain conditions^[66], showing stronger levels of toxicity and carcinogenicity than Cr (III). Regrettably, we only analyzed the total concentration of Cr. It has reported that LC₅₀-96 h of *Penaeus monodon* exposed to Ni concentration up to 2490 $\mu\text{g L}^{-1}$ ^[67]. In our study, Ni concentration (EC₅₀-96 h value) of *M. bahia* and *M. chulae* were 24.09 and 40.61 $\mu\text{g L}^{-1}$, which further proved that wash water is extremely toxic. Juan et al.^[68] explored the effect of Cu on pacific white shrimp, *Litopenaeus vannamei*. It was found that the highest mean mortality was only 8.5 ± 0.1% after 96 h of exposure to 18650 $\mu\text{g L}^{-1}$ of Cu. Therefore, we speculate the impact of Cu in wash water to *M. bahia* and *M. chulae* can negligible. In addition, the toxic effects of Zn in wash water on *M. bahia* and *M. chulae* might be weakened, since Cu or Zn accumulation might be inhibited by each other^[69, 70]. We deduced that NAP, Cr and Ni might be decisive toxicants in wash water.

4 Conclusions

The present study clearly showed that wash water from desulfurization scrubber was highly toxic to marine algae, mysid and fish. Although wash water contained high concentrations of PAHs and heavy metals, none of the single PAH or heavy metal concentration could explain the high toxicity of wash water. Thus, the measured toxicity may arise from synergistic effects among several contaminants or from other more toxic contaminants we know very little about. Considering the potential risk of wash water to the marine environment, IMO clearly specified the limitation of indicators including pH, total PAHs, turbidity and nitrate, but other toxic pollutants and comprehensive toxic effects have not been considered. The results of this study suggest that wash water must be treated before discharging to marine environment to reduce its potentially adverse risk to marine species. In addition to the direct detrimental effects, some of the pollutants in wash water may have bioaccumulation effects in the food chain. Therefore, future work also needs to clarify other more toxic pollutants in wash water, as well as their distribution, migration, transformation of pollutants in organisms.

Declarations

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Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest.

Ethics approval

This research was conducted under the ethical standards of animal care in our institution.

Consent to participate Not applicable.

Consent for publication Not applicable.

Availability of data and material Not applicable.

Authors' contributions

All authors contributed to the study conception and design. Manuscript writing, data curation, review and editing were performed by Zhiyi Ji. Investigation (theoretical part) was written by Yunqi Yang. Part of original draft was written by Ying Zhu. Validation, review and editing were performed by Yun Ling. Investigation, visualization and supervision were performed by Dezhang Ren. Review, editing and supervision were performed by Zhibao Huo. Review, editing, supervision and funding acquisition were performed by Nahui Zhang. All authors read and approved the final manuscript.

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Figures

Figure 1

(a) Average specific growth rate; (b) Inhibition rate of *D. salina* exposed to different concentrations the wash water

Figure 2

Mortality rates of *M. bahia* (a) and *M. chulae* (b) exposed to the wash water.

Figure 3

Body weight (a) and length (b) of *M. bahia*; body weight (c) and length (d) of *M. chulae*.

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