

Joint toxicity of cadmium and SDBS on *Daphnia magna* and *Danio rerio*

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Abstract Information on joint toxicity is limited. To clarify the joint toxicity and the interactions among toxicants on different aquatic organisms, we investigated the acute toxicity of cadmium and sodium dodecyl benzene sulfonate, two chemicals with high concerns in Chinese waters, on the immobilization of Daphnia magna (D. magna) and the swimming behavior of Danio rerio (D. rerio). Our results illustrated that cadmium and sodium dodecyl benzene sulfonate expressed a synergistic effect on the immobilization of D. magna; and an antagonistic effect on the swimming speed D. rerio, but a synergistic effect on its vertical position in the water column. Based on the observed data, we found the independent action model was more appropriate than the concentration addition model in the prediction of their joint toxicity. Our results gave an example of the joint toxicity investigation, and aided to comprehensive the toxicity action mode of chemical mixtures.

Keywords Cadmium (Cd) · Sodium dodecyl benzene sulfonate (SDBS) · Behavior toxicity · Action mode, Independent action (IA) model · Concentration addition (CA) model

Introduction

Cadmium (Cd) is one of the most widely used metals, accounting up to 7600 tons annual output in China (Brown

⊠ Ying Zhang yzhang@dlut.edu.cn et al. 2016). With the release of Cd into the environment, Cd has been detected in the surface water in China, e.g., Xiangjiang river $(1.34 \pm 0.79 \,\mu\text{g/L})$ (Zeng et al. 2015), Yangtze river $(4.7 \pm 0.91 \,\mu\text{g/L})$ (Wu et al. 2009), and Jiehe river $(10.5 \pm 11.7 \,\mu\text{g/L})$ (Zhang et al. 2014). Sodium dodecyl benzene sulfonate (SDBS), is a widely used anionic surfactant accounting for 90 % of total production of synthetic detergents (Wang et al. 2006) in China, and has been detected in the surface waters of the Changjiang river (0.84 mg/L–1.43 mg/L) (Wu et al. 2007). Therefore, the impact of these chemicals on aquatic organisms merits further investigation.

Previous studies have demonstrated that individually Cd and SDBS posed toxicity to aquatic organisms. Cd posed acute toxicity to rainbow trout (Oncorhynchus mykiss), chronic toxicity to Ceriodaphnia dubia (Naddy et al. 2015), and caused adverse effects to the digestive organ of Daphnia magna (Poynton et al. 2007). SDBS was illustrated to present acute toxicity to D. magna (Oleszczuk et al. 2015; Wang et al. 2009) and affected the swimming behavior of freshwater fish (Zhang et al. 2015). As we know, aquatic organisms in the aquatic environment are always exposed to a mixture of chemicals or contaminants. Unfortunately, the joint toxicity of these chemical mixtures to aquatic organisms is not understood comprehensively. The reports on the joint toxicity of Cd and SDBS are very limited. For instance, the 96 h-LC50 of linear alkybenzene sulfonate to Pengze crucian carp decreased when copper was added (Zeng 2008); the presence of SDBS could induce a higher Cd concentration in liver and gills of Carassius auratus (Jin et al. 2010). Until now, the joint toxicity of these chemical mixtures, especially the mixture of metals and organics, was not comprehensively clarified.

The immobilization test of *D. magna* has become a standardized toxicity test, commonly used to characterize

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the biotoxicity of chemicals. The immobilization test of D. magna was applied to the toxicity of insecticide (Guadipyr) (Qi et al. 2013), pharmaceutical (cimetidine) (Lee et al. 2015), and carbon nanomaterials (Sanchis et al. 2016). Recently, behavior monitoring of fish gained increasingly application in toxicology field, due to the provision of more detailed information and the feasible early warning of chemical pollution (Needleman 1995; Zhou et al. 2015). It was reported that the toxicity of potassium cyanide (KCN) and phenol (Fukuda et al. 2010), quinaldine sulfate (Barry 2012), and polystyrene nanoparticles (Mattsson et al. 2014) can be reflected by monitoring the fish swimming behavior. As we know, the biotoxicity test with different aquatic organisms could provide more information on the biotoxicity of chemicals, thus some researchers conducted more than one biotoxicity test to reflect the toxicities of chemicals concerned (Lee et al. 2015; Naddy et al. 2015).

Here, we conduct a study aiming to clarify the joint toxicity of metals and organics, and to assess the interactions among toxicants on different organisms. To evaluate the joint toxicity of Cd and SDBS, we applied the acute immobilization test on D. magna and behavior monitoring on D. rerio. A combination index (CI) was calculated to determine the joint toxicity type: synergistic, antagonistic, or addictive. Independent action (IA) and concentration addition (CA) models, differed from the chemicals' action mode on the organisms (Gonzalez-Pleiter et al. 2013; Le and Peijnenburg 2013; Tollefsen et al. 2012; Watanabe et al. 2015), were applied to predict the joint toxicity based on individual component toxicity. Our results give the examples of a joint toxicity investigation and an illustration of whether the prediction models (IA and CA) could predict the joint toxicity of metals and organics.

Materials and methods

Chemicals

Cadmium chloride (analytical pure, >99% purity) was obtained from Kemiou Chemical Reagent Co., Ltd (Tianjin, China). SDBS (SDBS, >90% purity) was imported from Germany and retailed in China. The test solutions were prepared directly in dechlorinated tap water.

Toxicity test of D. magna

D. magna was obtained from Dalian Ocean University (Dalian, China) and cultured at 30~40 animals/L in a climate incubator at 20 ± 1 °C with a 16 h light/8 h dark cycle. Green alga (*Scenedesmus obliquus*) was served as food at a concentration of 5×10^8 cells/mL, and the culturing medium

 Table 1
 Concentrations set for individual and joint chemicals (units: mg/L)

	D. magna				D. rerio					
Individual chemi	cal									
Cd	0.063	0.125	0.250	0.5	1	2	4	8	16	32
SDBS	9.87	14.8	22.2	33.3	50	2.5	5	10	20	40
Chemicals mixtu	re									
Cd	0.063	0.125	0.250	0.5	1	2	4	8	16	32
SDBS	9.87	14.8	22.2	33.3	50	2.5	5	10	20	40

(dechlorinated tap water with aeration) was renewed three times a week.

The acute immobilization test was carried out following OECD guideline 202 (OECD 2004). All tests were performed under the same temperature and light conditions as the culture maintenance. Test concentrations of chemicals used for exposure in this study were shown in Table 1, which were assigned according to the results of our preexperiment. Each toxicity test was conducted with four replicates for each test concentration. The immobilization of *D. magna* neonates was checked at 24 and 48 h after exposure.

Toxicity test of D. rerio

D. rerio (length 35 ± 5 mm, weight 0.25 ± 0.1 g), were purchased from a professional fish shop at Yule pet market (Dalian, China), were kept at 22 ± 1 °C under a photoperiod of 14 h light/10 h dark and fed with threadworm (Dandong, China) twice daily.

The behavior monitoring test was conducted with a biomonitor, as described in Zhang et al. (2015). It was supplied by Seiko Electric Co., Ltd. (Fukuoka, Japan) and its schematic was shown as Fig. 1. Simply, the exposure tests were carried out in a flow-through test chamber $(10 \times 10 \times 15 \text{ cm}^3)$ with 1.5 L test solution. The test solution or dechlorinated tap water was delivered to the test chamber by a roller (peristaltic) pump (Longerpump BT300-2J, Baoding, China) with a continuous flow (flow rate 400 mm³/s). The movement tracks of the test fish were monitored and recorded with the three dimensional (3D) data, x, y, z coordinates three times per second.

Any individual fish selected for toxicity test was first preexposed for 30 min under dechlorinated tap water, aiming to make the fish adapt to the narrow space in test chamber, and then exposed to test solution for 130 min. The toxicity test of each concentration of chemical was repeated 8 times, i.e., 8 individual fish used in parallel for each concentration. Test concentrations (Table 1) were set according to the discharge limits of Cd and SDBS in the integrated wastewater discharge standard (SEPA 1996).



Fig. 1 Schematic of fish swimming behavior monitoring

Quality control

Concentrations of Cd and SDBS were examined according to GB/T 7475-1987 (SEPA 1987b) and GB/T7494-1987 (SEPA 1987a), respectively. No significant differences were found between the nominal and measured concentrations for the two chemicals either in the presence or absence of *D. magna* and *D. rerio*. Therefore, throughout the whole study, their nominal concentrations were used for data analysis.

D. magna and *D. rerio* used in toxicity tests were healthy and consistent with the standards (ISO 1996; OECD 2004). Briefly, *D. magna* neonates (< 24 h) are the third generation cultured in our laboratory, and *D. rerio* was acclimated to our laboratory environment for at least 2 weeks before toxicity tests. The mortality of test aquatic organisms did not exceed 10 % during the culture, whose 24 h-EC₅₀ or LC₅₀ to reference substance was within the recommended limit. The water quality parameters of test solutions (e.g., the dissolved oxygen, temperature, and pH) were measured at the start and end of tests.

Data and Statistical analysis

For *D. magn*a, the EC₅₀ values at 24 and 48 h, and their 95 % confidence intervals were calculated using the probit analysis method within Ecotox-Statics software (Version 2.6, developed by Japanese Society of Environmental Toxicology, Japan) (JSET 2006).

For *D. rerio*, swimming speed and vertical position, recorded by 3D-biomonitor, were the two main parameters to analyze the toxicity effect of chemicals on fish swimming behavior. In this study, inhibition rate of swimming speed and vertical position were assigned by

Eq.
$$(1)$$
 and Eq. (2)

Inhibition of speed =
$$\frac{S_{under \ control} - S_{after \ exposure}}{S_{under \ control}}$$
 (1)

Inhibition of position =
$$\frac{V_{under \ control} - V_{after \ exposure}}{V_{under \ control}}$$
 (2)

where $S_{under \ control}$ is the mean value of speed in the 130 min in control, $S_{after \ exposure}$ is the mean value of speed in the exposure time, $V_{under \ control}$ is the mean value of vertical position in the 130 min in control, $V_{after \ exposure}$ is the mean value of vertical position in the exposure time. The mean values were calculated by descriptive statistics analysis in SPSS software (Version 20, IBM Corporation, USA).

All EC_x values for *D. rerio* were determined using the regression analysis, sigmoidal fitting, as it was the model that showed the best fit to the data, considering the coefficient of determination (R^2). All fitting tasks were performed with the Origin software (Version 8.0, OriginLab Corporation, USA). All of the available values of EC_x (x from 0 to 100) were used to feed the following IA and CA models.

The joint toxicity of Cd and SDBS were predicted using two classical models, IA model and CA model (Gonzalez-Pleiter et al. 2013; Le and Peijnenburg 2013; Tollefsen et al. 2012). IA model is based on the dissimilar mode of action (MOA) of chemicals or toxicants, and CA model is on that the similar MOA. The models of IA and CA are expressed with Eq. (3) and Eq. (4), respectively:

IA model :
$$E(c_{mix}) = 1 - \prod_{1}^{n} (1 - E(c_i))$$
 (3)

CA model :
$$ECx_{mix} = \left(\sum_{i=1}^{n} \frac{p_i}{EC_{xi}}\right)^{-1}$$
 (4)

where $E(c_{\text{mix}})$ is the total effect of the mixture, $E(c_i)$ is the effect of the *i*th component with the concentration c_i in the mixture, $\text{EC}x_{\text{mix}}$ is the effect concentration of the mixture provoking x% effect, EC_{xi} is the concentration of the *i*th component provoking the same effect (x%) as the mixture when applied individually, and p_i is the molar ratio of the *i*th component in the mixture.

In addition, CI was used to determine the joint toxicity type of Cd and SDBS on *D. magna* and *D. rerio* (Gonzalez-Pleiter et al. 2013). CI was calculated using EC_{50} values with Eq. (5):

$$CI = \sum_{i}^{n} \frac{EC_x \times p_i}{EC_{xi}}$$
(5)

where CI is the combination index for chemicals at x% inhibition; EC_x is the sum of the dose of chemicals that exerts x% inhibition in combination; p_i is the proportionality of the dose of each chemical that exerts x% inhibition in combination; EC_{xi} is the dose of *i*th component alone that

Table 2 EC_{50} of individual and joint Cd/SDBS on *D. magna* (units: mg/L)

			24 h	48 h
Individual chemical	Cd	EC ₅₀	0.71	0.26
		95 % CI	(0.43, 2.12)	(0.17, 0.57)
	SDBS	EC ₅₀	36.96	26.94
		95 % CI	(17.27, 46.07)	(24.68, 29.42)
Mixture of chemicals	Cd	EC ₅₀	0.074	0.019
		95 % CI	(0.034, 0.11)	(0.009, 2.08)
	SDBS	EC50	10.89	4.94
		95 % CI	(6.88, 13.52)	(3.17, 78.06)

95 % CI 95 % confidence interval

exerts x% inhibition. If the value of CI is 1, it means the toxicity of the mixture is simply additive effect; if CI > 1, it means antagonistic effect and CI < 1 means synergistic effect.

One-way analysis of variance (ANOVA) followed by Duncan's test within SPSS software (Version 20, IBM Corporation, USA) was used to determine the significance of differences (p < 0.05) between exposure tests and controls.

Results

Toxicity effect on D. magna

The EC₅₀ and 95 % confidence interval (CI) of individual Cd and SDBS to *D. magna* were summarized in Table 2. It can be seen that 48 h-EC₅₀ of individual Cd and SDBS were 0.26 and 26.94 mg/L, respectively (Table 2). The lower EC₅₀ of Cd than that of SDBS suggested that Cd had a more toxic effect on *D. magna*. However, the 48 h-EC₅₀ of Cd and SDBS decreased when *D. magna* were exposed to their mixture, which implied that the toxicity of Cd or SDBS became greater in mixture, compared with individual one.

The predicted results of joint toxicity of Cd and SDBS by IA and CA models are given in Fig. 2. IA model is closer to the observed data than CA model, although both of them overestimated the impact. It can be seen that IA model was more suitable in application to predict the joint toxicity, implying that Cd and SDBS may have dissimilar modes of action on *D. magna*, i.e., metal Cd and organic compound SDBS have dissimilar toxicity mechanism on *D. magna*.

Since the MOA of Cd and SDBS on *D. magna* appear to be dissimilar, the CI was used to examine their joint toxicity type and to confirm whether it was synergistic, antagonistic, or additive. According to Eq. (5), CI was calculated to be 0.21 and 0.11 (< 1) using the data of 24 h-EC₅₀ and 48 h-



Fig. 2 Concentration-response curve (data points and line of best fit) of *D. magna* after 48 h exposure to mixtures of Cd and SDBS The *dashed* and *dotted lines* are the concentration-response curves calculated by IA and CA models using nonlinear curve fitting (logistic function). The total concentration represents the total concentration of Cd and SDBS in mixtures

 EC_{50} given in Table 2. This indicates that the joint toxicity effect of Cd and SDBS on *D. magna* is synergistic or greater than additive toxicity.

Toxicity effect on swimming speed of D. rerio

The results of swimming speed and the time of speed plunging of fish exposed to individual Cd and SDBS were analyzed and given in Fig. 3a, d and Fig. 3b, e. In our study, the speed difference between the neighbor time periods under control is within10 mm/s, so when the speed difference between the neighbor time periods is more than 10 mm/s we called it "speed plunging"; the time of speed plunging means the time when speed plunging occurs. The joint toxicity of Cd and SDBS was investigated with the combination of varied concentrations of Cd and SDBS, given the results in Fig. 3c, f.

The swimming speed of fish changed with the concentrations of Cd was given in Fig. 3a. Under the concentration of 2 mg/L Cd, the swimming speed of fish was about 30 mm/s, slightly lower than that of 35 mm/s under control. When the concentration of Cd increased gradually from 4 mg/L to 32 mg/L, the swimming speed decreased sharply from 21 mm/s to 5 mm/s. On the other hand, when the concentration of Cd increased from 2 mg/L to 32 mg/L, the time of speed plunging was decreased from 60 min to 15 min (see Fig. 3d). In a word, the swimming speed, as well as the time of speed plunging, decreased with the increment of Cd concentration.



Fig. 3 Changes of swimming speed and the time of speed plunging for *D. rerio* under exposure to Cd/SDBS. **a b c** Changes of swimming speed; **d e f** changes of the time of speed plunging; **a d** exposure to individual Cd; **b e** exposure to individual SDBS; **c f** exposure to

From Fig. 3b, the swimming speed of fish was observed to be changed with the concentrations of SDBS. When the fish exposed to 2.5 mg/L SDBS, the speed was about 10 mm/s, significantly lower than that of 35 mm/s under control. When the concentration increased from 5 mg/L to 40 mg/L, the swimming speed decreased from 6 mm/s to 2 mm/s. It can be seen that the swimming speed decreased with the increment of SDBS concentration. In addition, when the concentration increased gradually from 2.5 mg/L to 40 mg/L, the time of speed plunging was decreased from 18 min to 5 min (see Fig. 3e). The time of speed plunging also decreased with the increment of SDBS concentration.

Comparing Fig. 3a with Fig. 3b, as well as Fig. 3d with Fig. 3e, it can be seen that the fitting lines' slopes for both swimming speed and the time of speed plunging exposed to Cd were larger than those under exposure to SDBS. It indicates that the concentration of Cd influenced the swimming speed of fish more significantly than that of SDBS.

The swimming speed changes of fish when it exposed to the mixture was depicted in Fig. 3c. The swimming speed was about 14 mm/s when the fish exposed to the mixture of 2 mg/L Cd and 2.5 mg/L SDBS, significantly lower than that of 35 mm/s under control. When the total concentration of Cd and SDBS increased gradually from 9 mg/L to 72 mg/ L, the swimming speed decreased from 10 mm/s to 4 mm/s. Therefore, the swimming speed decreased with the increment of concentration. Meanwhile, the time of speed

mixtures of Cd and SDBS. The columns with asterisk indicates significant differences (p < 0.05) between the test solutions and controls, and vice versus. The lines between the columns were the linear fitted lines of response and chemical concentration

plunging also decreased with the increment of total concentration (see Fig. 3f).

Comparing Fig. 3c with Fig. 3a, b, as well as Fig. 3f with Fig. 3d, e it can be seen that the concentration of mixture was not so significantly influenced, compared with that of individual Cd, the swimming speed, and the time of speed plunging, with the evidence of the fitting lines' slope for both swimming speed and the time of speed plunging.

Toxicity effect on vertical position of D. rerio

The vertical position and the time of surface behavior of fish exposed to the individual Cd and SDBS, and their mixture was also recorded and analyzed (see Fig. 4). The surface behavior means the fish stay at the surface of water, where its vertical position in water is less than 20 mm; the time of surface behavior means the time when the surface behavior occurs. Note the water used in toxicity test chamber is totally 150 mm in height.

The vertical position of fish changed with the concentrations of individual Cd and SDBS were given in Fig. 4a, b as well as the time of surface behavior in Fig. 4d, e. It can be seen that both the vertical position and the time of surface behavior decreased with the increment of individual chemical concentration. Similar results can be observed from Fig. 4c, f that both of them also decreased with the increment of chemicals concentration even in the mixture of Cd and SDBS.



Fig. 4 Changes of vertical position and the time of surface behavior for *D. rerio* under exposure to Cd/SDBS. **a b c** Changes of vertical position; **d e f** changes of the time of surface behavior; **a d** exposure to individual Cd; **b e** exposure to individual SDBS; **c f** exposure to

From Fig. 4a, b it can be seen that there were no significant differences in the vertical position when exposed to individual Cd and SDBS. From Fig. 4d, e, it can be seen that the fish had more time swimming in water surface when exposed to SDBS, compared to that of Cd. Compared Fig. 4a with Fig. 4b, as well as Fig. 4d with Fig. 4e, it can be seen that the concentration of Cd influenced the vertical position more significantly than that that of SDBS, based on the slope of their relative fitting lines.

Comparing the results of mixture and individual of Cd and SDBS (Fig. 4), it can be concluded that the concentration of mixture influenced both vertical position and the time of surface behavior more significantly than that of individual SDBS, but as significantly as that of individual Cd, based on the slope of relative fitting lines.

Prediction of the joint toxicity effect of Cd and SDBS on *D. rerio*

The prediction using IA and CA models for the joint toxicity of Cd and SDBS on *D. rerio* were depicted in Fig. 5. It can be seen that the predicted swimming speeds with IA and CA models were very similar when the chemical concentration was lower than 20 mg/L, though both of them were higher than the observed (Fig. 5a). From the results of vertical position in Fig. 5b, it can be observed that the predicted toxicity with CA model was close to the measured data at lower concentration ($0\sim10$ mg/L), while the

mixtures of Cd and SDBS. The columns with asterisk indicates significant differences (p < 0.05) between the test solutions and controls, and vice versus. The lines between the columns were the linear fitted lines of response and chemical concentration

prediction with IA model was close to the measured one at higher concentration (30~70 mg/L). These results suggested that the joint toxicity of Cd and SDBS on *D. rerio* was very complex, and they may have dissimilar toxicity mode on *D. rerio* at different concentrations.

The joint toxicity of SDBS and Cd was assessed with CI according to Eq. (5). CI was calculated to be 79.24 and 0.26 for swimming speed and vertical position, respectively, with a conclusion that Cd and SDBS had an antagonistic effect on the swimming speed, while synergistic on the vertical position, of *D. rerio* swimming behavior. This difference may be attributed to the different toxic mechanisms in swimming speed and vertical position.

Discussion

Behavior is considered to be a window of the nervous system (Ramazani et al. 2007). In the field of toxicology, behavior can be used as an endpoint to indicate the toxicant impact on organisms, which was considered to be more sensitive than other toxicity endpoints (e.g., survival, growth, or reproduction) (Garaventa et al. 2010). Thus, behavioral monitoring can be applied to provide continuous information on environmental changes (e.g., water quality), as an effective, economical, and practical tool for accident precaution and risk assessment (Chon et al. 2009). In this study, we demonstrated the joint toxicity of Cd and SDBS





on aquatic organisms quantitatively, based on behavior monitoring of *D. rerio*. Our results provided the quantitative information for the joint toxicity of metals and organics, as well as further risk assessment.

As to the toxicity of individual Cd, the 48 h-EC_{50} on D. magna was 0.26 mg/L (with the 95 % confidence interval from 0.17 to 0.57 mg/L), which was in agreement with the outcome (0.01 mg/L~0.77 mg/L) of other studies (AIST 2013). In terms of D. rerio, the range of 96 h-LC₅₀ of Cd was found to be from 6.49 mg/L to 17.72 mg/L according to the conventional lethal test (AIST 2013; Wu et al. 2006; Wu and Li, 2002). In our study, Cd was observed to inhibit the swimming speed of D. rerio at the low concentration of 4 mg/L, while the concentration of 8 mg/L Cd would influence the vertical position of D. rerio (Fig. 3 and Fig. 4). This result suggested the swimming speed of D. rerio is more sensitive to the toxicity of Cd, compared with its vertical position. Furthermore, it also demonstrated that the behavior monitoring, especially the swimming speed, can detect the acute toxicity at lower concentration than the conventional lethal test. Based on the above results, it can be seen that the order of sensitivity to Cd was D. magna lethal test > swimming speed of *D. rerio* > vertical position of D. rerio. That is to say, D. magna was the most sensitive to the toxicity of Cd.

As to the toxicity of individual SDBS, it was reported that 24 h-EC₅₀ of SDBS on *D. magna* was 6.3 mg/L, 7.5 mg/L or 9.5 mg/L (Wang et al. 2009) (Ecotox database, USEPA), but there was no data on *D. rerio*. In our study, the 48 h-EC₅₀ on *D. magna* was observed to be 26.94 mg/L (with the 95 % confidence interval from 24.68 to 29.42 mg/L), and the concentration of SDBS at 2.5 mg/L could inhibit the swimming speed, while the concentration of SDBS at 5 mg/L could inhibit the vertical position (Fig. 3). It can be seen that the order of sensitivity to SDBS was swimming

speed of *D. rerio* > vertical position of *D. rerio* > *D. magna* lethal test. Herein, swimming speed of *D. rerio* was the most sensitive endpoint to SDBS, which can be applied for the precaution of water quality changes.

Several studies have reported the toxicity mechanisms of individual Cd and SDBS at cellular level. The researchers considered that SDBS could induce the damage on cell membrane structure by ion transport over the cell membrane (Bjerregaard 2001; Cheng et al. 1992; Luo et al. 2015; Pareschi et al. 1997), while Cd can decrease the activity of antioxidant enzymes in the cells and alter the redox state of the cell (Pourahmad and O'Brien 2000; Stohs and Bagchi 1995). Based on the individual toxicity mechanisms, the synergistic effect of Cd and SDBS was speculated to be that SDBS first induced the damage on cell membrane and then enhanced the affinity of heavy metal to the cell membranes (Pourahmad and O'Brien 2000; Stohs and Bagchi 1995; Tong et al. 2015; Zou et al. 2012). Our results are consistent with other studies that trace metals (Cd, Cu) and organic chemicals (TC and phenols) demonstrated a synergistic effect (Luo et al. 2015; Pourahmad and O'Brien 2000; Stohs and Bagchi 1995; Tong et al. 2015). However, it cannot be concluded that the presence of all organics can increase the toxicity of metals on aquatic organisms, unless further studies were conducted. The joint toxicity mode may vary with the length of exposure time, e.g., the joint toxicity of sulfonamide and trimethoprim on photobacterium changed from antagonistic to synergistic as the exposure time prolonged (Zou et al. 2012). This merits further investigation to clarify the joint toxicity mechanisms of different kinds of toxicants (e.g., trace metals and organics).

In our study, the immobilization test on *D. magna* is based on the comprehensive effect on the behavior of aquatic organisms, while the swimming behavior test on *D. rerio* is monitored from two aspects: swimming speed and vertical position. Our results showed that Cd and SDBS had a synergistic effect on *D. magna* and synergistic on the vertical position of *D. rerio*, while antagonistic on the swimming speed of *D. rerio*. This may be attributed to their different toxicity mechanisms on the swimming speed and vertical position of *D. rerio*, which may trigger further exploration on the difference of toxicity mechanisms between them.

Conclusion

- (1) The joint toxicity of Cd and SDBS was illustrated to be synergistic on the immobilization of *D. magna*.
- (2) Cd and SDBS presented a synergistic effect on vertical position of *D. rerio*, but antagonistic on swimming speed of *D. rerio*.
- (3) Cd and SDBS expressed dissimilar toxicity action mode on aquatic organisms evidenced by *in-situ* behavior monitoring.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent Informed consent was obtained from all individual participants included in the study.

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