Original Article

Developing the Acute Bioassay on Suppression of *Daphnia magna* Filtration by Fluorescence Measurement

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ABSTRACT

The results of acute test on suppression of *Daphnia magna* filtration activity for one toxicant ($K_2Cr_2O_7$, $ZnSO_4$ and $MnSO_4$) were applied to carry out acute binary tests for 3 binary solutions: “Cr-Mn”, “Cr-Zn” and “Zn-Mn”, where the daphnids were exposed for 1, 2, 3, 4 and 5 hs. While the level of toxicity manifested by the “Cr-Mn” binary systems was lower than “additive”, the results that were observed in the binary solution of “Cr-Zn”, especially for the shorter exposure time of 1–3 hours, showed toxicity above “additive”. A nearly “additive effect” was observed in the binary solution of “Zn-Mn”. We supposed that the theory of metals competing for binding to biotic ligands or any yet unidentified physiological interactions of the metals could explain the toxicity effect on filtration activity of *D. magna* exposed in three binary solutions. So, the more-than-additive effect for the “Cr-Zn” binary system may indicate that Zn (II) demonstrated its high ability building up protein complexes due to competition with Cr (VI). The less-than-additive toxicity for the “Cr-Mn” solution likely revealed the fact that bioavailability of Cr (VI) to *D. magna* was reduced in the presence of Mn (II).

Keywords: metal toxicity, binary solution, fluorescent microbeads, filtration rate

INTRODUCTION

The *Daphnia magna* bioassays are used for environmental monitoring around the world. The list of physiological sub-lethal endpoints may be seriously extended if the additional information on the test object is used based on its functional efficiency, including behavioral responses [1]. An acute immobilization test using *D. magna* which is one of the daphnids used to evaluate toxicity of chemicals in water environment. However, the test is more effective for the high toxicity samples, because the response on the method indicates lethal damage to daphnids, furthermore this acute test requires long exposure time (24–48 hs). In order to evaluate toxicity more efficiently and more easily, it is needed the acute method which can assess not only lethality but also various physiological responses of *D. magna*. Some of *D. magna* test designs based on feeding activity of daphnids were reported,

such as counting method of labeled yeast cell taken and image analyzing method of fluorescent beads taken [2–4]. It is known that, the rate of filtration measured either as ingestion rate or as the rate of movement of thoracic appendages above incipient limiting concentration was no longer proportional to concentration but remained constant [3]. It was reported that toxicity test based on measurement of fluorescence of ingested chlorella by daphnids had high sensitivity, which was fifty times as high as a visible spectrophotometry [5]. The possibility of short-term impacts on feeding by metals and pesticides causing severe affects at the individual and population level (*i.e.* growing and reproduction rates) was shown for *D. magna* [6–10].

Multiple metals present in waste water cause toxicity that currently is difficult to predict due to non-additive mixture toxicity. Some study showed the toxicity of copper (Cu), lead (Pb), cadmium (Cd), nickel (Ni), and zinc (Zn) mixture

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Table 1 Concentration used for the acute exposure of ZnSO₄ and Mg SO₄ in both suppression of filtration rate and lethality tests.

Exposure time in toxicant	Concentrations (mg/L)	
	ZnSO ₄	MgSO ₄
1 h	5.6-80	65-650
2 h	5.6-80	65-650
3 h	7.0-70	60-590
4 h	6.6-60	53-530
5 h	6.6-60	45-500
24 h	5.0-50	94-219

exposure effect and that the combinations of the contaminant at lower concentrations may elicit “additive” and “non-additive” effects on aquatic organisms [11,12]. The additive toxicity does not come out to occur in most metal mixtures. Norwood et al. the authors [13] in their meta-analyses study specified that only 20–25% of metal mixtures tests resulted in additive toxicity, whereas about 30% of mixture solutions resulted in more-than-additive toxicity, and approximately 45–50% resulted in less-than-additive toxicity. Though several geochemical processes can contribute to non-additivity of metal-mixture toxicity (*i.e.* more-than-additive toxicity can result from competition for binding to dissolved organic matter and inorganic ligands), a more realistic indicator of risk is provided by calculations based on the bioavailable form: the extent to which a substance can be absorbed by a living organism and can cause an adverse physiological or toxicological response [14].

Most of the published binary metal studies are limited to acute effects [15–19], whereas chronic effects have rarely been considered yet [20–22]. The research by Nys *et al.* [23] showed that the threshold level of toxicity above which independent action and concentration addition synergistic effects on reactive toxicity to *D. magna* occur in Ni and Zn mixtures was situated around the EC₂₀ and EC₄₀ of the individual metals, respectively.

In this study we tested a hypothesis if an acute bioassay, in which only the extension of suppression of *D. magna*'s filtration activity was measured, could be used not only to measure the toxicity of one metal, but also to specify the toxicity of a solution of two metals. The experimentation design used in this study is different from the design we used earlier [24]. In our previous studies we expressed filtration suppression by measuring the fluorescence of microbeads filtered by *D. magna*, which remained in the latter's intestine. In this design we measured the fluorescence of microbeads that had not been filtrated and remained in the solution after the

exposition of daphnids. The new design used in the study is simpler than that we have developed in the laboratory before, since it does not require operations such as washing small organisms after exposition, transferring them to a clean water solution and, finally homogenizing them.

MATERIAL AND METHODS

Daphnia magna culture

Daphnis magna was cultured according to the standard procedure of Japanese Industrial Standards (JIS) [25] at 21 ± 1°C, using ElenDt M4 culture water. *D. magna* neonates not older than 24 h (used below as daphnids) were used for all acute tests; neonates were separated from mother culture 12 hs before the experiment.

Chemicals

K₂Cr₂O₇, (Kanto Chemical Co., Ltd. Special grade M.W.: 294.19 g/mol, Tokyo, Japan); ZnSO₄ (Kanto Chemical Co., Ltd. M.W.: 161.45 g/mol); MnSO₄ (Junsei Chemical Co., Ltd. Reagent class M.W.: 241.08 g/mol, Tokyo, Japan). ELGA Lab water was used for control treatment. 20 ml glass beakers were used for each experiment. **Table 1** shows the range of concentration used for each acute test (suppression of filtration rate and lethality) of ZnSO₄ and MnSO₄, mg/L.

Daphnia magna lethal test endpoint

Procedure and calculation of LC₅₀ (the concentration of a chemical that will kill 50 % of the sample population) using the conventional 24 h bioassay with lethality as an endpoint were carried out according to JIS [25].

Measuring of *Daphnia magna* suppression filtration rate through fluorescent intensity

For this study we used 0.2 µm fluorescent beads (F8805, Thermo Fisher Scientific, 4.55×10¹² microbeads/ml; ex-

Table 2 The $\frac{1}{2}$ EC₅₀ concentrations (mg/L) of 3 toxicants for each time of exposure used in binary solutions.

Expo	Exposure time	K ₂ Cr ₂ O ₇	ZnSO ₄	MnSO ₄
1 h		0.96	9.80	107.00
2 h		1.05	11.55	120.50
3 h		1.03	11.50	113.00
4 h		0.73	6.70	83.00
5 h		0.48	4.90	85.00

citation: 365 nm, emission: 415 nm). The amount of the microbeads in solution was measured by RF-5300PC spectrofluorometer (Shimadzu Co., Kyoto, Japan) and expressed as direct fluorescence intensity units. One control treatment (FI_{contr} ; 100% of filtration rate) and a series of 5 concentrations of toxicants (FI_{tox} ; x% of filtration rate) were used to calculate the suppression of the filtration activity of daphnids (*i.e.* the filtration rate expressed through fluorescence intensity unit – FI);

The relative filtration rate (%) was expressed as eq. (1):

$$(FI_{\text{tox}}) / (FI_{\text{contr}}) \times 100 \quad (1)$$

FI_{tox} : Fluorescence of toxicant treatment

FI_{contr} : fluorescence of control treatment.

Both the FI_{tox} and the FI_{contr} are the mathematical difference between the measurements of fluorescence intensity of blank solution before (B) and solution after exposure with 10 daphnids (E); eq. (2):

$$FI_{\text{tox}} = B_T - E_T; FI_{\text{contr}} = B_C - E_C \quad (2)$$

B_T and B_C : measurement of FI before exposure of daphnids in toxicant and control samples;

E_T and E_C : measurement of FI after exposure in toxicant and control samples.

The measurements of B - and E - samples were made for 1 control sample and 5 series of toxicant; the time of measurement of all samples was 3–5 minutes.

The exposure time into individual metal solutions was established as 1, 2, 3, 4, and 5 hs. In each experiment 20 min before the end of the toxicant exposure, a solution of fluorescent microbeads 0.2 μm in the optimal concentration [24] of 5×10^7 particles/ml was added to 10 daphnids transferred in 25 ml g. However, for the construction of bioavailability-based model is required the knowledge of the toxicity mechanisms of the different binary-metal systems and of the nature of their interactions too. Glass beakers filled with 10 ml of toxicant solutions.

EC₅₀s (the concentration that will suppress 50% of filtration rate of sample population) was calculated through the

linear regression of a log of $(FI_{\text{contr}} - FI_{\text{tox}}) / FI_{\text{tox}}$ and log of toxicant concentration (C); the EC₅₀s were obtained at $(FI_{\text{contr}} - FI_{\text{tox}}) / FI_{\text{tox}} = 0$.

For each experiment was done three replicate series ($n = 3$).

Test on suppression of filtration rate in binary solution

The following combinations of mixing 2 metal salts were used: K₂Cr₂O₇ and ZnSO₄ (“Cr-Zn”); ZnSO₄ and MnSO₄ (“Cr-Mn”); K₂Cr₂O₇ and MnSO₄ (“Cr-Zn”) (Table 2 [26]);. 5 ml of each toxic substance were mixed for every test; then, 10 daphnids were exposed into 10 ml of the binary solution and 20 min before the expiration of the exposure time 0.2 μm fluorescent beads (5×10^9 beads/ml) were added in the binary solution with exposed daphnids. The relative daphnids filtration rate in the solution was established by the eq. (1). For each exposure time (1, 2, 3, 4, and 5 hs) both components – the most toxic compound and another metal in binary solution were added at nearly 1/2 of their expected EC₅₀ concentrations. ;

To predict the toxicity of the binary solution we used the TUs (toxic units) concept based on the concentration addition (CA) model [21], which is based on metal concentration. According to this concept, to achieve a 2 metal system, their proportions are added equal to 1/2 of the values of their effective concentrations (EC₅₀; the concentration that will affect 50% of damage of a physiological function of organism). It is believed that, if the result is the expected 50% response of the organism to the binary system, the TUs total is around 1, and the toxicity (of the binary system) is additive.

In toxicology, synergism refers to the effect caused when exposure to two or more toxicants at a time effects that are greater than the sum of the effects of the individual chemicals. Antagonism is a biological response to exposure to multiple substances that is less than would be expected if the known effects of the individual substances were added together.

Table 3 Suppression of filtration rate and lethality (24 h) endpoints for acute exposure into ZnSO₄ and Mg SO₄.

Exposure in toxicant	EC ₅₀ (mg/L)			
	ZnSO ₄		MgSO ₄	
	variables	mean±SD	variables	mean±SD
1 h	19.58; 24.76; 22.33	22.22±2.59	218.00; 167.84; 227.89	204.58±32.20
2 h	23.12; 20.28; 34.80	26.07±7.70	241.63; 261.75; 176.49	226.62±44.57
3 h	26.14; 29.49; 22.55	26.06±3.47	226.68; 179.68; 129.47	178.61±48.61
4h	15.07; 18.82; 20.33	18.07±2.71	165.80; 126.29; 159.32	150.49±21.20
5 h	12.27; 22.48; 12.16	15.64±5.93	169.92; 156.06; 191.74	172.57±17.98
24 h	18.06; 18.07; 17.65	17.92±0.25	146.55; 146.53; 147.63	146.90±0.63

Examination of adsorption of the toxic substances on both: 0.2 µm fluorescent beads and daphnids (1 h test)

If toxic substances adsorb on fluorescent beads or daphnids, this may lead to misrepresentation of results. To perform the measurement of 3 absorbed metal ions Cr (VI), Zn (II), Mn (II) the ICP-AES ICPE-9000 (Shimadzu Co.) was used. For the experiments, the working solutions of K₂Cr₂O₇, ZnSO₄ and MnSO₄ were prepared with their concentrations (mg/L) close to the value of EC₅₀, *i.e.* obtained in the experiment for measuring filtration rate endpoint for 1 h of exposure. Binary mixtures were prepared from these working solutions mixed in the following proportions: 15 ml Cr (VI) and 15 ml Zn (II) for “Cr-Zn” solution; 15 ml Zn (II) and 15 ml Mn (II) for “Zn-Mn”; 15 ml Cr (VI) and 15 ml Mn (II) for “Cr-Mn” binary solution; their ion concentrations were used as reference measurements (**Table 3**). The second and third measurements of ion concentrations were made after adding to the binary mixtures either microbeads solutions (5×10⁷ particles/ml) or 10 daphnia. The exposure time for each experiment (n = 3) was 1 hour.

Statistical analysis

An EC₅₀ for both the lethality bioassay and the feeding suppression bioassay were computed for each replicate series of dilutions, hence three EC₅₀s were computed for each sample. Each of EC₅₀th obtained from the filtration activity suppression assays was compared to the standard 24 h lethality LC₅₀ using unpaired *t*-test.

RESULTS

Test for measuring the filtration rate and lethal endpoints of *D. magna*

We experimented on the suppression of filtration rate of *D. magna* for 1, 2, 3, 4, 5 hs exposure time with the use of

ZnSO₄ and MgSO₄. Inversely correlation was observed between the concentration of toxicant and relative filtration rate (%) of daphnids (eq. (1); **Fig. 1**).

In the next experiment, we calculated the suppression of the filtration activity of daphnids as the endpoint of suppression 50% filtration rate relative to the reference sample for the ZnSO₄ and MnSO₄, via linear regression of a log of ($FI_{\text{contr}} - FI_{\text{tox}}$)/ FI_{tox} and log of toxicant concentration (C); (**Fig. 2**).

The results of ZnSO₄ and MnSO₄ acute test's endpoints: suppression of filtration rate (EC₅₀) for 1, 2, 3, 4, 5 hours and 24 hours (LC₅₀) lethality shown in **Table 3**. ANOVA and Tukey pairwise test showed no difference between each of the values of EC₅₀ in filtration test and EC₅₀ in 24 h lethality test. For ZnSO₄ *t*-test shown that the means of EC₅₀ of 4 h and 5 h tests (18.07 and 15.64 mg/l) were not significantly different ($p > 0.05$) from the mean of 24 h LC₅₀ lethality concentration (17.92 mg/l). For MgSO₄ lethality test endpoint – 146.90 mg/l was significantly different ($p < 0.05$) from 1 h and 2 h tests'EC₅₀: 204.58 and 226.62 mg/l). For both toxicants the Bland-Altman analysis based on the quantification of the agreement between two quantitative measurements (one of which was the 24 h lethality endpoint and the other the filtration rate suppression endpoint) showed the least mean differences and limit of agreements of 4 h EC₅₀ endpoints.

Suppression of filtration rate in binary solution

In this study expected additive effect in the binary solution should be caused daphnia activity is around 50% of filtration rate. Binary effects in each treatment were identified as “non-addictive” effect, if the filtration rate was observed less than 50% (TU > 1, *i.e.* “more-than-addictive” toxicity), and was more than 50% (TU < 1, *i.e.* “less-than-addictive” toxicity). The results of binary toxicity shown in **Fig. 3** for each binary mixture and each time of exposure as filtration

Fig. 1 Relative filtration rate of 10 daphnids exposed into 5 different of MnSO₄ (a) and ZnSO₄ (b).

Fig. 2 Calculation of EC₅₀s endpoints through the linear regressions for 4 hours exposure of 10 daphnids into MnSO₄ (a) and ZnSO₄ (b).

Fig. 3 Relative filtration rate of daphnids in binary solutions of Zn-Mn, Cr-Zn, Cr-Mn for exposure time 1, 2, 3, 4 and 5 hs.

Table 4 The metal ions concentration with ICP-AES instrument given as a reference mixture, mixture with added fluorescent beads and mixture with added 10 daphnids measurements.

Sample (30 ml)	Measured metal concentration (mg/L)		
	Cr	Zn	Mn
Cr-Mn	3.53	-	219
Cr- Mn + microbds	3.62	-	211
Cr- Mn + daphds	3.45	-	214
Mn-Zn	-	16.4	218
Mn-Zn + microbds	-	16.0	209
Mn-Zn + daphds	-	16.5	213
Zn-Cr	3.66	17.8	-
Zn-Cr + microbds	3.57	17.3	-
Zn-Cr + daphds	3.67	17.7	-

rate (eq. (1)) mean (treatment's percentage to filtration rates of daphnids into reference water).

When daphnids were exposed to the binary mixture of "Cr-Mn", the observed relative filtration rate was above 50% (less-than-addictive effect) for each exposure time. In the case of binary solution of the "Cr-Zn", the filtration rate was altered for each exposure time, but less than 50% for each treatment (more-than-addictive toxicity); thus, a tendency to a minor increase of filtration rate was observed from 1 h to 5 hs of exposure, but for exposure 1, 2 and 3 hs it was a little below 25%, and for 4 and 5 hs – well above 25%. For the treatment of "Zn-Mn" mixture the filtration rate was rising from 1 to 5 hs of exposure and comprised slightly less than 50% for 1, 2, 3 hs, and slightly more than 50% for 4 and 5 hs exposure time.

Adsorption of the toxic substances

In this experiment, we investigated the feasibility of the adsorption of these metal ions on both the 0.2 μm fluorescent beads and the daphnids during 1 h of exposure period. **Table 4** shows the result of three measurements of metal-ions concentration in binary solution through ICP-AES instrument: 1) a reference metal' measurement without microbeads or daphnids (Cr-Zn, Zn-Mn, Cr-Mn), 2) after adding either microbeads (+ ms), or 3) 10 daphnids (+ ds).

The ICP-AES measurements after 1 h of exposure with either added microbeads or added daphnids did not differ significantly (significance level $p \leq 0.05$) from reference measurements of metal-ion concentrations.

DISCUSSION

Comparison of suppression filtration rate and lethal endpoints

Sensitivity in bioassay testing is expressed by the value of toxicant concentration, which affects the lethality of an object or its sub-lethal physiological parameters. The lower is the value of effective concentration; the more sensitive is the biological object to this toxicant.

Lethality LC_{50} concentrations were used to compare the suppression filtration rate result with the standard endpoints. The highest suppression filtration endpoint EC_{50} in *D. magna* for two toxicants ($ZnSO_4$, $MgSO_4$) was observed during either 4 or 5 hs exposure time. The test results of filtration activity suppression tests shown the same or even higher sensitivity as that in 24 h LC_{50} lethality tests for the toxicants (respectively for 4 and 5 hs: 18.07 and 15.64 compare to 17.90 mg/l in lethality test for $ZnSO_4$ and 150.49 and 172.57 compare to 146.90 mg/l for $MgSO_4$). Smaller suppression of filtration rate was observed for the exposure periods of 1, 2 and 3 hs than for 4 and 5 hs exposures, and it was nonsense, but the sensitivity of the 1 h exposure test for both toxicants was slightly higher than for 2 and 3 hs exposure. Probably, this effect of 1 h exposure can be caused by the insertion of some compensatory mechanism by daphnids characteristic of the short period of toxicant exposure.

Suppression of filtration rate in binary solution

There are two models applied for mixture toxicity assessment: concentration addition (CA) and response addition (IA). Both models assume that there is no interaction among the substances in the mixture ("non-interaction", *i.e.* additive effect). In the CA model, if chemical substances interact with each other, the toxicity of a mixture can be higher or lower than expected in comparison to that of the individual substances in the mixture [11]. This model is supposed to be suitable when the toxicants have the same mechanism of toxic action (*e.g.* Cd and Zn both impair calcium (Ca) homeostasis [27];). The IA model is generally assumed to be appropriate when chemicals have different mechanism of actions (*e.g.* impairment by Cu of sodium/potassium (Na/K) homeostasis versus impairment of Ca homeostasis) [17,28]. So, the authors of chronic toxicity study with daphnids Muschel *et al.* [29] suggested a competitive effect of Zn on Ca uptake: disturbance of Ca balance was probably the first cause for Zn toxicity effects (*i.e.* suppression of filtration rate) in *D. magna* chronic test. While in the study by Cremazy *et al.* [7] authors considered the typical classification of metals

between Ca antagonists (Cd, Zn, and Pb) and Na antagonists (Cu and Ag) did not predict accurately these types of metal interaction, as may be expected given their mode of actions are most consistently observed in acute rather than chronic exposure.

Acute 24 h lethality test performed by Traudt *et al.*, 2017 [19] in with Ni, Cu, Zn and Cd resulted in a less-than-additive toxicity in their mixture metal study. The authors suggested the results were likely caused by processes occurring at the surface of or inside the organism rather than physical-chemical processes controlled by the bulk exposure-water chemistry. Those processes at the surface or inside the organism might include metal-metal competition for binding to the biotic ligand (s) and as-yet-unidentified physiological interactions of the metals. The metal-metal competition for binding to DOM (dissolved organic matters) and inorganic ligands in the exposure water could potentially increase the free-ion concentration of one or more of the metals in the mixture. It might be contributing to either more-than-additive, if the less-toxic metal is competing for binding to non-biotic ligands or less-than-additive mixture toxicity, if the more toxic metal is competitively binding to the non-biotic ligands [19].

According to the result of our standard acute lethality *D. magna* test the toxicity of three tested metals decreased in the following row: Cr (VI) > Zn (II) > Mn (II), consequently the lethality endpoints are 1.1 mg/L > 13.0 mg/L > 146.6 mg/L respectively. Because of this fact, in the binary solution "Cr-Mn" and "Cr-Zn" Cr (VI) may have expressed stronger individual toxicity than Zn (II) and Mn (II); in the binary solution "Zn-Mn" the "primary" toxicant was zinc, which had greater individual toxicity than manganese.

For the binary mixture of "Cr-Zn" the result showed a more-than-additive toxicity. This effect was particularly remarkable for 1–3 hs exposure time and might be explained as an interactive competing for binding to non-biotic ligands in the binary system of "Cr-Zn". It was known that Zn (II) in this mixture had a good ability to form a complex with carboxyl- and/or amino-groups, which led to increasing free metal ions and construction complexes of proteins. Chromium is considered to be an essential trace element for animals. Cr (VI) whose strong oxidizing properties are very toxic and the reactions between Cr (VI) and biological reductants like thiols and ascorbate result in the production of reactive oxygen species such as superoxide ion, hydrogen peroxide, and hydroxyl radical, ultimately leading to oxidative stress in the cell causing damage to DNA and proteins [30]. In the binary "Cr-Zn" system, Zn (II) is the less-toxic metal showed

ability for binding to organic ligands that caused more-than-additive effect.

For the binary system of “Cr-Mn” in this study the relative filtration rate was observed above 50% for 1, 2, 3, 4 and 5 hs exposure time. This may indicate a less-than-additive effect or the presence of a slightly antagonist effect due to some geochemical interaction resulting from the diminution toxicity of a binary system compared to the expected sum of the individual toxicity of two metals in the treatment. In the binary system Cr-Mn, Mn (II) may prevent Cr (VI) from binding to its biological receptors and hence decrease its strong oxidase properties to daphnids. Likely similar results were obtained for the study to determine the influence of reduction on the toxicity of chromium to aquatic organisms. The conducted toxicity test [31] indicated that the bioavailability of chromate to *D. magna* was reduced in the presence of Fe (II) and that it decreased further with increasing Fe (II) concentrations.

A nearly additive effect was observed for all the exposure time of “Zn-Mn” treatments. Manganese (II) is considered to have no oxidizing properties as Mn (VII) does, and has no competing ability for binding chemical ligand as Zn (II) does. The Zn (II) and Mn (II) are essential elements to living organisms where they are primarily involved as a co-factor, which is a non-protein chemical compound or metallic ion that is required for an enzyme’s activity as a catalyst; e.g. Mn- and Zn-SODs). The result observed in the binary system “Zn-Mn” as additive effect might display there was not any interaction between zinc and manganese in the mixture to binding neither to chemical nor to biological ligands.

Adsorption of toxic substances

The experimental result did likely not confirm the adsorption of toxic substances neither on fluorescent beads nor on daphnids. The results of the experiment on adsorption of metals indicated that the mechanisms of action of the tested toxicants were not directly related to metal accumulation in the surface of *D. magna* body, though the result was obtained only for the exposure time of 1 h. Though the toxicity interactive effects during exposure to metal mixtures may be reflected by the level of accumulation and depend on the time of exposure, authors Bianchini *et al.* [15] in their acute study showed that, instead, the toxic effect of silver depended on incorporating metals by daphnids rather than merely were adsorbed on the surface. In another study for measurement of incorporate zinc concentration, Wang and Guan [32] found that lethality in acute *D. magna* 48 h lethality test was coincident with the saturation of Zn accumulation level (up

to 40 µg/g wet weight), below which no obvious lethality occurred.

CONCLUSION

The results of *D. magna* bioassay on the suppression filtration activity for both toxicants ZnSO₄ and MgSO₄ (individual toxicity) showed that the most appropriate exposure time for this test design was 4–5 hours. As for the binary metal test using the suppression of filtration rate, we found that the toxicity of three metal binary systems was either more-than-additive, near-additive or less-than-additive. It is currently not well-understood how the interactions of binary metals and water chemistry parameters affect the toxicity of metal mixtures on aquatic organisms. However, for the construction of bioavailability-based model is required the knowledge of the toxicity mechanisms of the different binary-metal systems too as well as the nature of their interactions.

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