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Acute and Chronic Effects of Metal Nanoparticles on Daphnia Magna

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Abstract

With the increasing use of nanomaterials, their release into the environment is inevitable, which has resulted in an increasing concern regarding their potential environmental risks. In the present study, the acute and chronic effects of copper nanoparticle (nCu), chromium nanoparticle (nCr) and their mixtures on *Daphnia magna* were investigated. The median lethal concentrations were 0.63 mg/L for nCu and 1.57 mg/L for nCr after 48 h of exposure. The endpoints "days to first pregnancy", "days to first brood", "number of offspring per female in the first brood", "number of offspring per brood per female" and "intrinsic rate of natural increase" were measured during the 21 d testing period. nCu (≥0.002 mg/L), nCr (≥0.01 mg/L) and their mixtures (≥0.002 mg/L) significantly suppressed the growth and reproduction of D. magna and reduced their feeding rates, and obvious concentration-response relationships were observed. The results showed nCu was more toxic than nCr, and nCu combined with nCr may induce synergistic effects in D. *magna.*

Keywords: metal nanoparticles, acute toxicity, chronic toxicity, *Daphnia magna*

1 Introduction

Nanoparticles (NPs) are widely applied in many commercial industrial and consumer products such as semiconductors, cosmetics, textiles, and pigments (Gottschalk *et al.* 2009). Given these materials broad use, it is inevitable for the increasing quantities of NPs to be released directly or through discharges of municipal wastewater into the aquatic environment (Nowack & Bucheli 2007). At the same time, there is an increasing concern regarding the risks of nanoparticles to human and ecosystem health (Colvin 2003; Nel *et al.* 2006; Wiesner *et al.* 2006).

Until recently, most of the studies on the potential toxicity of nanoparticles have focused on metal oxide NPs such as $nTiO_2$, nZnO, nCuO, $nCeO_2$, nAl_2O_3 (Alok & Vyom 2010; Blinova *et al.* 2010; Heinlaan *et al.* 2008), carbon NPs (Cheng *et al.* 2009; Kang *et al.* 2009; Lovern & Klaper 2006; Murray *et al.* 2009) and quantum dots (Kloepfer *et al.* 2005). In comparison, studies on the ecotoxicity of metal NPs are limited to a few reports on gold NPs and silver NPs (Bar-Ilan *et al.* 2009; Farkas *et al.* 2010; Griffitt *et al.* 2012). Information is lacking on the toxicity of nanoscale copper (nCu) and chromium (nCr) in water, especially their joint effects on aquatic orgamisms.

However, the environmental effects of copper NPs and chromium NPs are still poorly known. Yoon et al. (2007) reported that Cu NPs can inhibit the activities of bacteria, including *Escherichia coli* and *Bacillus subtilis*. In fact, except for the mortality, the effects of oxidative stress, gill injury, heart rate, hatching rate, edema and malformations were also addressed as bio-endpoints (Gomes et al. 2012; Griffitt et al. 2008; Griffitt et al. 2009; Susana et al. 2012). Since contaminated aquatic ecosystems are often polluted with not only a kind of pollutant (Lu *et al.* 2007; Su *et al.* 2008), mixtures of chemicals to ecotoxicology constitute a prevalent issue.

Daphnia magna as a common zooplankton found in freshwater lakes and ponds, is one of the most sensitive organisms used in toxicity tests. So we investigated the toxicity effects of nCu, nCr and their mixtures on *D. magna* by a 48-h acute toxicity test as well as a 21-d reproductive and growth test. The objectives of the present study were threefold: (1) to find out acute toxicity of nCu and nCr on *D. magna* by observing immobilization and mortality; (2) to detect chronic cumulative effects through observing the growth and reproduction of *D. magna* such as the days to first pregnancy, the body length and the intrinsic rate of population growth; (3) to explore whether high bioaccumulation of nCu and nCr could interfere with food intake and ultimately cause toxicity on *D. magna.*

2 Materials and Methods

2.1 Nanoparticles: Preparation and Characterization

The aqueous dispersion of nCu (grain size 50 nm, surface area 80 m^2/g) and nCr

(grain size 800 nm, surface area 10 m^2/g) were purchased from Beijing Nachen S&T Ltd. (Beijing, China), which the degree of purity is 20% (W/W). The stock solution was prepared by dispersing the NPs in ultrapure water (Millipore, Billerica, MA, USA) with ultrasonication (50-60 kHz) 30 min before dosing each day. The test solution were prepared immediately prior to use by diluting the stock solution with daphnia culture medium (consisting of 64.75 mg/L $NaHCO_3$, 5.75 mg/L KCl, 123.25 mg/L $MgSO{\cdot}7H_2O$, and 294 mg/L $CaCl_2{\cdot}2H_2O$) reconstituted according to standard OECD guideline (OECD, 2004). The pH of the culture medium is 7.8±0.2. For this experiment, the aqueous suspensions of nCu and nCr (both 80 mg/L) have been characterized by transmission electron microscopy (TEM) in ultrapure water (Fig. 1). Particle size distributions of the studied metal oxides (at 80 mg/L) in *D. magna* test medium were determined by Malvern Mastersizer 2000 and the pH of the aqueous suspensions is 7.7.

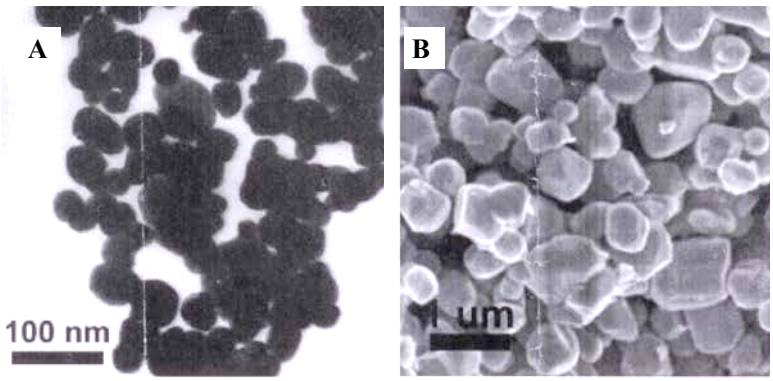


Fig. 1 TEM images of nCu (A) and nCr (B)

2.2 Test Organism

Daphnia Magna were obtained from Chinese Center for Disease Control and Prevention (Beijing, China). They were maintained in laboratory culture under controlled conditions, *e.g.* photoperiod 16h:8h light:dark and a constant temperature of 20±1°C. The culture medium was renewed two times a week and the daphnids were fed daily with the green algae *Scenedesmus obliquus*, which was supplied by Wuhan Institute of Hydrobiology, Chinese Academy of Science (Wuhan, China).

2.3 Acute Toxicity Test

The 48-h acute toxicity tests of nCu and nCr were conducted using the modified OECD standard procedure (OECD, 2004). *D. Magna* were exposed to seven concentrations (0.01, 0.05, 0.1, 0.5, 1.0, 5.0 and 10 mg/L of nCu and 0.05, 0.1, 0.5, 1.0, 5.0, 10 and 25 mg/L of nCr) plus a blank control. Ten neonates (6-24 h old) from a designated brood were exposed in a 50 mL glass beaker containing 35 mL test solution with three replicates per each concentration and control. The test solution was renewed daily to maintain the same concentration of exposure. *D. magna* were not fed during the testing period. At test end, the immobilization and mortality for the individuals in each container were recorded. The animals that are unable to swim within 15 sec of gentle agitation of the test container are considered immobile. Those animals whose heartbeats have stopped are considered dead. The heartbeats were watched under a stereoscopic

microscope (4×magnification). The dissolved oxygen content and pH of the test media were measured at the beginning and at the end of the test.

2.4 Chronic Toxicity Test

The chronic bioassay was conducted under static daily renewal condition for 21 days following a procedure adapted from OECD (1998). Based on the results of acute toxicity, neonates (6-24 h old) were exposed 21d to a series concentrations of nCu (0, 0.0004, 0.002, 0.01, 0.05 and 0.1 mg/L) and nCr (0, 0.002, 0.01, 0.05, 0.25 and 0.5 mg/L). Binary mixtures were also tested according to an equiconcentration (W/V) ratio of 1:1 and the total exposure concentrations were 0.0004, 0.002, 0.01, 0.05 and 0.1 mg/L. One single neonate was placed in a 100 mL glass beaker containing 50 mL test suspension. Twenty replicatess were completed for each concentration and control group. Danhnids were fed daily with algae at a concentration of 1×10^6 cells mL^{-1}. Each offspring (if present) was carefully separated from beaker and the test solutions were renewed every day. The criteria used to evaluate the reproduction of *D. magna* included days to the first pregnancy, days to the first brood, number of first brood per female and average offspring in each brood. At the endpoint the survival of adults in each treatment was documented to measure the body length under a stereoscopic microscope.

The intrinsic rate of natural increase r value was calculated using the formula of Lotka (Lotka et al. 1913), $\sum l_x m_x e^{-rx}=1$, where l_x is the proportion of individuals surviving to age x, m_x is the age-specific fecundity (number of neonates produced per surviving female at age x), and x in days. As r calculated in *D. magna* organisms after 21 days is indistinguishable from r estimated for the entire lifespan, due to the great importance of early reproduction (Leeuwen et al. 1985), all calculations were based on 21 d experiments.

2.5 Feeding Experiment

Filtration and ingestion rates were used as measures of the feeding behavior (Villarroel et al. 2003). The feeding experiment was run according to a method described by Zhu et al. (2010), and the exposure concentrations were the same as the chronic experiments. Each treatment consisted of three replicates. Ten neonates (6-24 h old) were placed in a 100 mL glass beaker containing 50 mL test solution. The tests were conducted at 20±1°C with a dark photoperiod for 5 h. During the exposed period the organisms were fed with 1×10^6 cell mL^{-1} of algae. Then final food concentration was measured using a hemocytometer under an electron microscope (400×magnification).

Filtration rate (F) is defined as the volume of medium swept clear by an animal per unit of time and the ingestion rate (I) as the number of cells consumed by an animal during a specific time interval. To calculate the average F (μL/(ind·h)) and I (cells/(ind·h)) the equations (1)-(3) were used (Gauld et al. 1951) :

$$F = \frac{\mathrm{V}}{n} \times \frac{\ln C_0 - \ln C_t}{t} - A \quad (1)$$

$$A = \frac{\ln C_0 - \ln C_t'}{t} \quad (2)$$

$$I = F \times \sqrt{C_0 \cdot C_t} \quad (3)$$

Where C_0 and C_t are initial and final food concentrations (cell/μL), t is time (duration of the experiment in hours), and n is the number of daphnias in volume V (μL); and A is a correction factor for changes in the control with final concentration C_t' after time t.

The expression $\sqrt{C_0 \cdot C_t}$ represents the geometric mean of food concentration during time t.

2.6 Statistical Analysis

Data were expressed as the mean with standard deviation (SD). Data from acute tests, chronic tests and feeding experiments were analyzed using an analysis of variance (ANOVA) to detect significant differences between different treated groups and control (p<0.05). All statistical analyses were performed using SPSS 17.0 (SPSS Co., Chicago, IL, USA).

3 Results and Discussion

In the acute test, the observation of exposure duration showed a concentration-response feature, and no mortality occurred in the control groups. In the groups exposed to 0.01 mg/L of nCu and 0.05 mg/L of nCr, the movement of *D. magna* was fast and smart, just like the daphnids in control groups. However, the increased concentrations of the metal NPs caused immobility and death of daphnids. The concentration-response curves of the NPs tested on *D. magna* are presented in Fig. 2. Through one variable linear regression analyses of the negative logarithm of NP concentrations and the immobilization or mortality rates as the relative toxic potency, the median effective concentration (EC_{50}) on immobilization and the median lethal concentration (LC_{50}) on *D. magna* were otained, which were 0.34 mg/L and 0.63 mg/L for nCu, and 0.96 mg/L and 1.57 mg/L for nCr, respectively. These results were consistent with those of previous studies. Griffitt et al. (2007) examined the acute toxicity of 80 nm copper nanoparticle suspensions on zebrafish, which demonstrated that nCu was acutely toxic to zebrafish, with a 48 h LC_{50} value of 1.5 mg/L. Zhen et al. (2006) assessed the *in vivo* toxicity of nCu to mice, and the median lethal dose (LD_{50}) via oral gavage was 413 mg/kg body weight. However, no acute toxicity data were provided for nCr up to now.

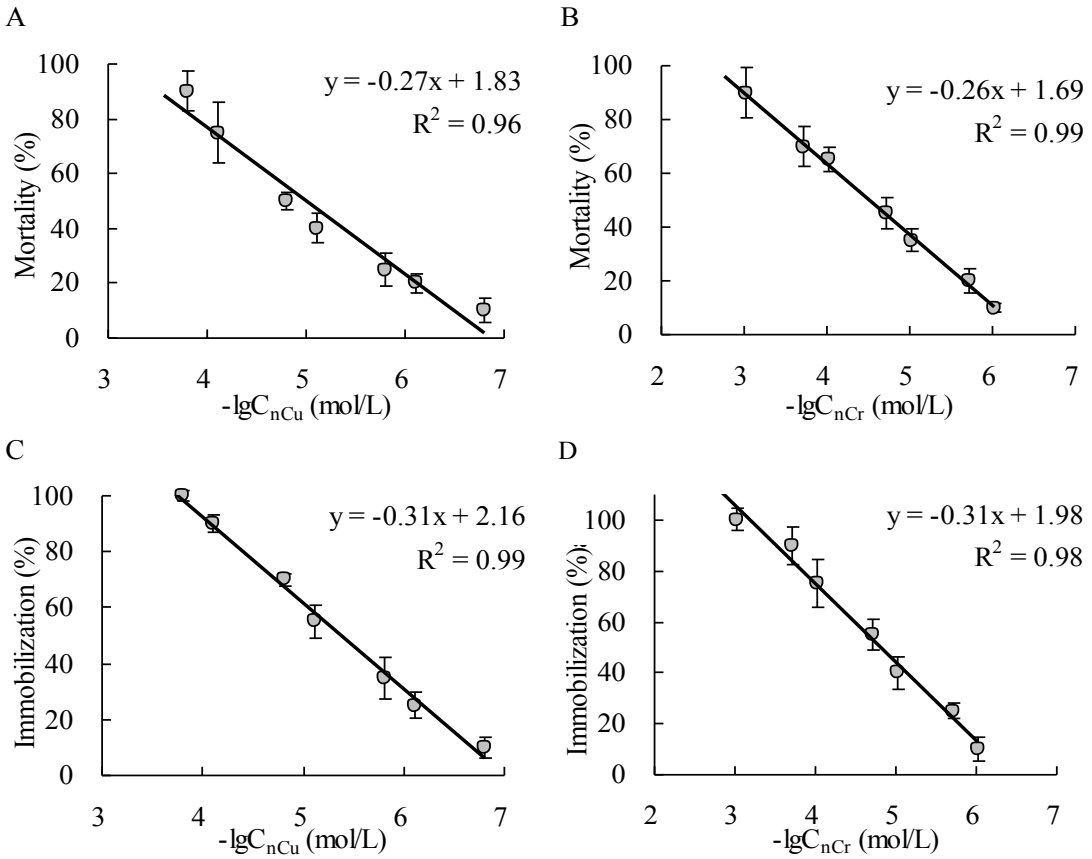
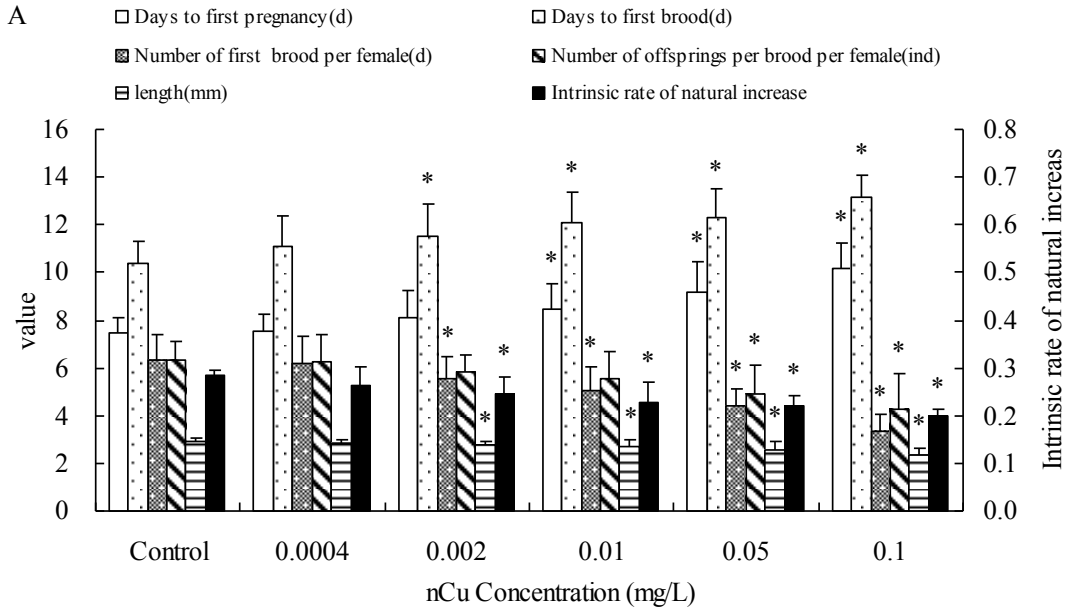
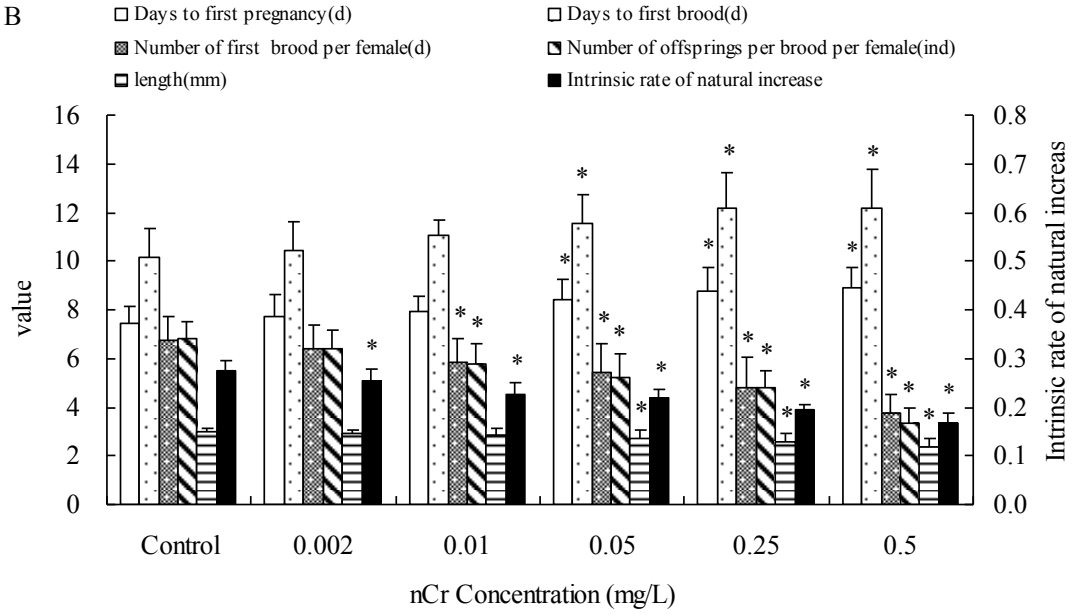


Fig. 2 The concentration-response curves of nCu (A, C) and nCr (B, D) to *D. magna*.

The traditional 48 h acute toxicity test may not be sufficient for the toxicity assessment of NPs due to their low environmental concentrations. More attention should be paid to chronic or long-term exposure (Yi et al. 2010; Dao et al. 2010), which is a part of an integrated environmental monitoring and assessment strategy (Mendonça et al. 2011). This point was further substantiated by the 21 d chronic toxicity tests for daphnia reproduction in the present study, as reported by Wienh et al. (2009). We assessed the survival, reproduction, body length and population parameters of *D. magna* after 21 d of exposure and the results are shown in Fig.3. It showed that 0.002 mg/L of nCu and 0.01 mg/L of nCr could inhibit the reproduction of *D. magna* and even affected the population of *D. magna*. Moreover, the growth inhibition and mortality of *D. magna* were also observed at the concentration 0.002 mg/L of nCu and nCr mixture. Obvious concentration-response relationships were obtained for both individual and combined exposure. The parameters “days to first brood”, “number of offspring per female in the first brood” and “intrinsic rate of natural increase” were more sensitive to indicate the toxicity of metal NPs compared with the other endpoints. The inhibitory effects of binary mixtures on the growth and reproduction of *D. magna* were higher than that of the corresponding individual exposures, suggesting that nCu combined with nCr may play a synergistic effect.

In our privious study, nCuO and nZnO (alone and on combination) were also found to significantly inhibit the growth and reproduction of *D. magna*, and the highest concentration of mixtures (0.25 mg/L) caused the death of *D. magna* **(**Zhao et al. 2012**)**.


A
Days to first pregnancy(d)
Days to first brood(d)
Number of first brood per female(d)
Number of offsprings per brood per female(ind)
length(mm)
Intrinsic rate of natural increase
value
Intrinsic rate of natural increas
0
2
4
6
8
10
12
14
16
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Control
0.0004
0.002
0.01
0.05
0.1
nCu Concentration (mg/L)


B
Days to first pregnancy(d)
Days to first brood(d)
Number of first brood per female(d)
Number of offsprings per brood per female(ind)
length(mm)
Intrinsic rate of natural increase
value
Intrinsic rate of natural increas
0
2
4
6
8
10
12
14
16
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Control
0.002
0.01
0.05
0.25
0.5
nCr Concentration (mg/L)

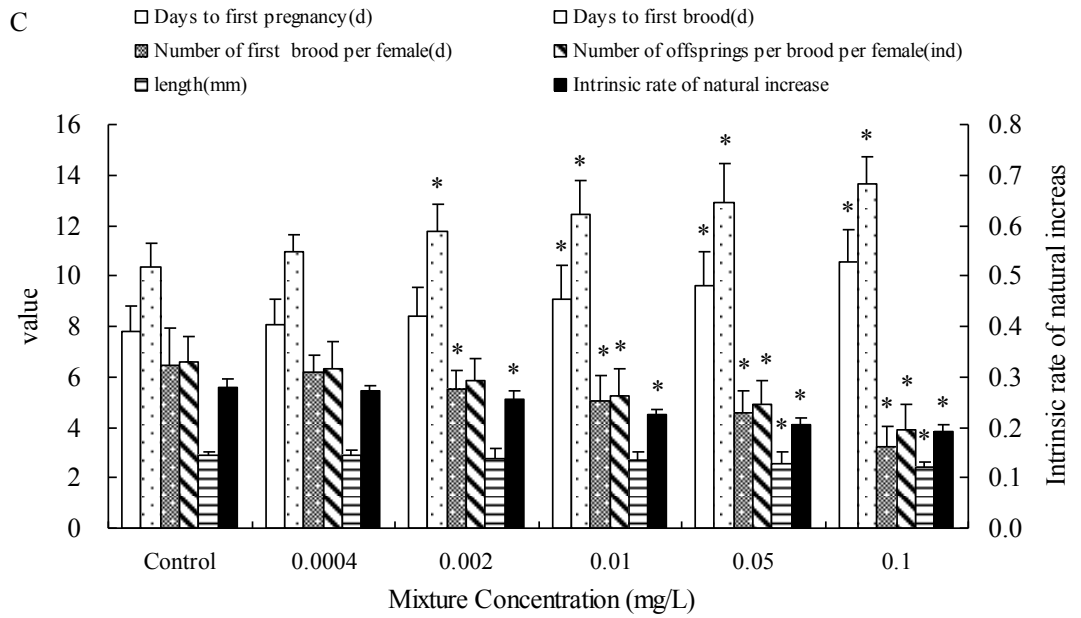


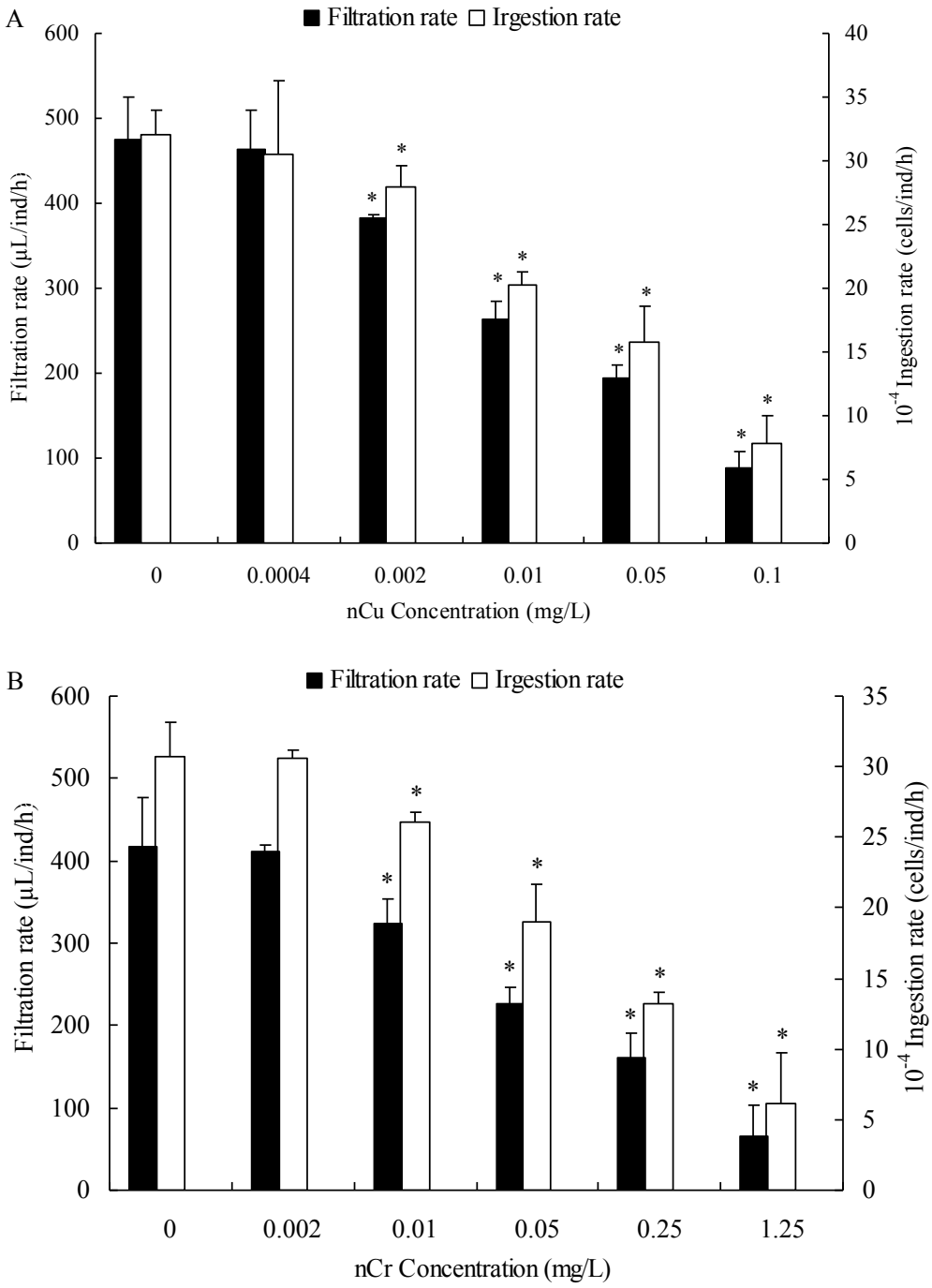
Fig. 3 Size, survival and fecundity of *D. magna* exposed to several concentrations of nCu(A), nCr(B) and the mixtures(C) in a 21 d life study

The intrinsic rate *r* was used to estimate chronic effects in *D. magna* and which has becoming a critical component of population level to evaluate ecological risk including cladocerans (Tanaka 2003). The toxicants which cause a decrease in the number of the first few broods of daphnias could cause *r* values to decrease significantly, while the later broods of daphnias have little effect on *r* values (Villarroel et al. 2003). Therefore, the lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC) were estimated based on intrinsic rate data in the present study. The maximum acceptable toxicant concentration (MATC) was represented as the geometric mean of NOEC and LOEC. The NOEC, LOEC and MATC values of nCu, nCr and their mixtures to *D. magna* were shown in Table 1. The results showed that nCu was more toxic than nCr, and the mixtures exhibited the toxicity similar to nCu, but higher than nCr.

Table 1 NOEC, LOEC and MATC values of NPs to *D. magna*

NPs	NOEC (mg/L)	LOEC (mg/L)	MATC (mg/L)
nCu	0.0004	0.002	0.0009
nCr	0.002	0.001	0.0014
nCu/nCr	0.0004	0.002	0.0009

The effects of nCu, nCr and their mixtures on the feeding behavior of *D. magna* are shown in Fig. 4. *F* and *I* values significantly decreased in all treatments (alone and in combination) at all tested concentrations except for the lowest concentration, and concentration dependence was apparent. The maximal inhibition rates for filtration exceeded 80% at the highest exposure concentrations. Since NPs may accumulate in the *D. magna*, the poor food intake and malnutrition may further cause observed chronic toxicity (Mendonça et al. 2011).


A
Filtration rate
Irgestion rate
Filtration rate (μL/ind/h)
10-4 Ingestion rate (cells/ind/h)
0
100
200
300
400
500
600
0
5
10
15
20
25
30
35
40
0
0.0004
0.002
0.01
0.05
0.1
nCu Concentration (mg/L)
B
Filtration rate
Irgestion rate
Filtration rate (μL/ind/h)
10-4 Ingestion rate (cells/ind/h)
0
100
200
300
400
500
600
0
5
10
15
20
25
30
35
0
0.002
0.01
0.05
0.25
1.25
nCr Concentration (mg/L)

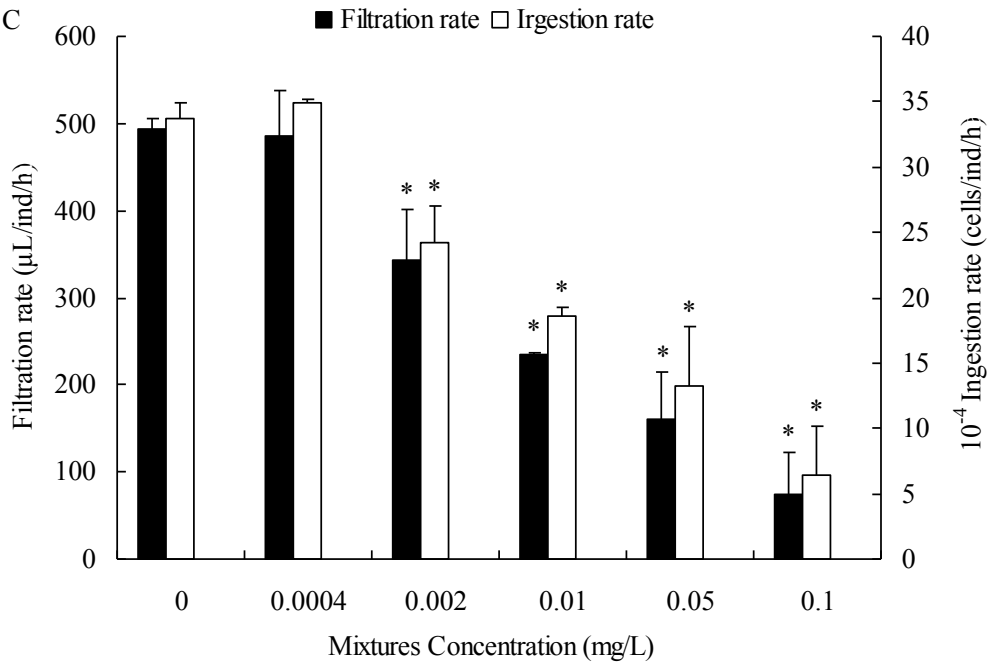


Fig. 4 Ingestion and filtration rates of *D. magna* after exposure to nCu (A), nCr (B) and the Mixtures (C) for 5 h.

4 Conclusions

This study demonstrated the acute and chronic effects of two metal NPs (alone and in combination) on *D. magna*, and concentration dependence was apparent in all cases. nCu was more toxic than nCr, and nCu combined with nCr may play synergistic effects on *D. magna*. The intrinsic rate of natural increase *r* is confirmed to be a sensitive parameter to NPs exposure. Our results also suggested that coexistent metal NPs might cause more adverse effects on aquatic ecosystem health when they were simultaneously or successively released into the aquatic environment.

Acknowledgments

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