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# Trophic transfer of CuO nanoparticles from brine shrimp (*Artemia salina*) nauplii to convict cichlid (*Amatitlania nigrofasciata*) larvae: uptake, accumulation and elimination

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## Abstract

We investigated the trophic transfer potential of CuO-NPs from *Artemia salina* to *Amatitlania nigrofasciata*. The Cu uptake was investigated by exposure of the instar II nauplii to 0, 1, 10, and 100 mg/L CuO-NPs for 4 h. Dietborne exposure of fish larvae to CuO-NPs was done for 21 days through feeding with pre-exposed nauplii. Thereafter, all survived fish were fed for 21 more days with non-contaminated nauplii. The results showed that NPs could be taken up by nauplii in a concentration-dependent manner. The highest uptake of Cu by nauplii was found to be  $50.5 \pm 1.4$  mg/g dry weight at 100 mg/L. The copper accumulation in fish larvae increased significantly with increasing Cu content in pre-exposed nauplii to different concentrations of CuO-NPs ( $p < 0.05$ ). At the end of the depuration phase, although the Cu elimination was significantly higher in fish that were fed with more contaminated nauplii, but the survival rate, average final weight, and length of those larvae was still significantly less than the control group ( $p < 0.05$ ). The accumulated Cu after the depuration phase in cichlid larvae was  $25.4 \pm 0.5$ ,  $29 \pm 8.0$ ,  $33.9 \pm 9.7$ , and  $42.3 \pm 4.0$   $\mu\text{g/g}$  dry weight at 0, 1, 10, and 100 mg/L of CuO-NPs-treated *Artemia*. The current findings indicated the ability of manufactured CuO-NPs to be transferred from one trophic level to the next as assessed in the simple food chain consisting of pre-exposed *A. salina* and *A. nigrofasciata*.

**Keywords** Artemia · Bioaccumulation · Copper oxide nanoparticles · Dietary exposure · Food chain · *Amatitlania nigrofasciata*

## Introduction

Over the past two decades, the production and application of manufactured nanomaterials (MNMs) in different fields including electronics, medicine, remediation, engineering, and food industry has been raised (Vance et al. 2015). Metal and metal oxide nanoparticles exhibit specific physiochemical properties including its surface, optical, thermal, and electrical

properties that differentiate them from their native bulk compounds (Rastogi et al. 2017).

Copper oxide nanoparticles (CuO-NPs) due to excellent optical, electrical, physical, and magnetic properties are used as essential component in the nano-devices (Phiwdang et al. 2013). These nanoparticles because of their antimicrobial and biocide properties are also used in many biomedical applications (Katwal et al. 2015). Engineered nanomaterials may inevitably discharge into aquatic environment from its manufacturing waste, nanoproducts and its byproducts (Moore 2006; Navarro et al. 2008). It has been shown that aquatic animals such as fish can be exposed to nanoparticles via their digestive system with subsequent negative effects at cellular and molecular levels (Chupani et al. 2018a, b; Chupani et al. 2017). The predicted environmental concentration (PECs) of Cu-NPs in aquatic environments is 0.06 mg Cu/L (Chio et al. 2012). Although copper (Cu) is one of the essential trace elements for living organisms, but the copper ions released from NPs at high concentration could cause cellular damage (Gottschalk et al. 2009). Toxicity of CuO-

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NPs to fish is mainly related to release of copper ions (Black et al. 2015) and nanoparticles which cause oxidative stress mediated by reactive oxygen species (ROS) generation at the NP surface (Melegari et al. 2013), bioaccumulation in tissues, inducement of liver necrosis, and alteration of sinusoidal spaces in the gills (Al-Bairuty et al. 2013). The uptake of Cu in tissues of common carp (*Cyprinus carpio*) exposed to CuO-NPs (2.5 and 5 mg/L) for 20 days was in order of liver > gill > muscle > intestine (Mansouri et al. 2016). Song et al. (2015) found that aqueous exposure of rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*) fish to copper nanoparticles (50 nm) resulted in damage to gill filaments and gill pavement cells.

Trophic transfer of aqueous metals in the aquatic food chain has been widely recognized (Rainbow et al. 2006; Mathews and Fisher 2008) but studies into the potential trophic transfer of engineered nanoparticles (ENPs) are scarce (Rahmani et al. 2016; Tangaa et al. 2016). A few studies reported the food chain transport of ENPs in freshwater (Dalai et al. 2014; Mattsson et al. 2014) and marine (Wang et al. 2016; Conway et al. 2014) ecosystems. Ates et al. (2014) demonstrated CuO-NPs transfer between trophic levels from *Artemia salina* to goldfish (*Carassius auratus*). The transfer of TiO<sub>2</sub>-NPs from clamworms (*Perinereis aibuhitensis*) to juvenile turbot (*Scophthalmus maximus*) along a marine benthic food chain was also reported (Wang et al. 2016). Accumulated ENPs may cause pathological damages of target organisms and nutritional compositional changes of higher trophic level organisms (Wang et al. 2016). The bioaccumulation and trophic transfer of ENPs may be affected by waterborne exposure (direct route) and dietborne exposure (indirect route) (Wang et al. 2016). Wu et al. (2017) expressed that the uptake and trophic transfer of CuO-NPs from the algae *Chlorella vulgaris* to the crustacean *Daphnia magna* is dependent on the type of exposure. According to findings, dietary exposure resulted in lower toxicity and Cu accumulation compared to direct exposure.

Brine shrimp, *Artemia* sp., as an invertebrate zooplankton involve in the energy flow of the food chain in many of salt-water lake ecosystems (Ates et al. 2013). *Artemia* sp. is a species of the non-selective filter feeders which can directly ingest particles smaller than 50 µm (Hund-Rinke and Simon 2006). Therefore, they are prime candidates for uptake of NPs from environmental discharges (Ates et al. 2014). International Organization for Standardization (ISO) has also recently issued a test specification (ISO TS 20787, International Organization for Standardization 2017) on the use of *Artemia* sp. nauplii in the toxicity assessment of MNMs (Johari et al. 2018). This zooplankton is also widely used as a live food for fish nutrition in aquaculture. Therefore, feeding of fish with ENPs-treated *Artemia* could be helpful for understanding the transfer and fate of ENPs by dietary exposure from lower to higher trophic levels. In this study, brine

shrimp nauplii (*Artemia salina*) and convict cichlid larvae (*Amatitlania nigrofasciata*) were selected as a non-selective filter feeder and a carnivorous fish to investigate the trophic transfer potential of CuO-NPs by dietary exposure and the ability of larvae to eliminate ingested NPs during depuration phase.

The objective of this study was to investigate the uptake of CuO-NPs by *Artemia* through waterborne exposure, as well as copper bioaccumulation and elimination rates in convict cichlid larvae after dietary exposure and depuration phases, respectively. The effects of pre-exposed *Artemia* to CuO-NPs on survival and growth performance of larvae were also determined.

## Materials and methods

### CuO nanoparticles and characterization

CuO-NPs were purchased from US Research Nanomaterials, Inc. (3302 Twig Leaf Lane, Houston, TX77084) in dry powder form. Imaging and elemental analysis of the CuO-NPs powder were done using field emission scanning electron microscopy (FE-SEM; MIRA3 TESCAN, Brno, Czech Republic) coupled with energy dispersive X-ray spectroscopy (EDS). The diameters of 200 individual nanoparticles or their aggregates/agglomerates were measured randomly on SEM images to estimate the mean size distribution of particles by AxioVision digital image processing software (Release 4.8.2.0, Carl Zeiss Micro Imaging GmbH, Germany).

To prepare a stock suspension of 1000 mg/L, 0.1 g dry powder of CuO-NPs (US Research Nanomaterials, Inc., 3302 Twig Leaf Lane, Houston, TX77084) was dispersed in 100 ml double distilled water by 60 min ultrasonication in a bath-type sonicator (SOLTEC 2200 MH-SD). This suspension stored in dark bottle at room temperature in the laboratory until utilization. Also, the hydrodynamic diameter and zeta potential of particles in 10 mg/L well-dispersed suspension of CuO-NPs were determined by Zetasizer Nano (ZS instrument) ZEN3600 Malvern Instruments Ltd., Worcestershire, UK).

### Test organisms

The cysts of *Artemia salina* were obtained from Tierarzt Company (Thailand) and stored at 4 °C. Artificial seawater were (ASW) used for all experiments which were prepared by dissolving 300 g of synthetic seawater salt (Delta Marine®, Inc., Iran) to 10 L of deionized water followed by continuous aeration for 24 h. For hatching, approximately 1 g of the dry cysts was incubated in 1 L artificial seawater (30 g/l) in a transparent “V”-bottomed glass incubator at 30 ± 1 °C. *Artemia* hatched within a period of 24 h under conditions of

continuous light illumination of 1500 lx provided by a fluorescent lamp and constant aeration from the bottom of the hatching incubators. The newly hatched nauplii (instar I) were maintained in the hatching incubators for another 24 h to turn to instar II.

Ten healthy and mature convict cichlid (*Amatitlania nigrofasciata*) breeders were purchased from a local aquarium shop. In order to allow the pairing to occur naturally, the fish were kept in mixed-sex in 150-L glass aquariums at  $26 \pm 1$  °C, pH 7.3 and fed on commercial pellets (BioMar®, France) twice a day. Following pair-bond formation, each pair of fish were transferred to 75-L spawning glass aquariums equipped with clay pots as spawning substrate and air stones for aeration. After spawning and hatching the eggs, the newly born larvae were kept until their yolk sacs were absorbed. Then, the larvae were transferred to 50-L glass aquariums for trophic transfer experiments.

### Exposure of *A. salina* to CuO-NPs

After conducting a series of pre-tests (data not shown), concentrations of 0 (control), 1, 10, and 100 mg/L of copper oxide nanoparticles (CuO-NPs) were selected as exposure concentrations. A stock suspension of CuO-NPs (1000 mg/L) was diluted to each glass vessels (1 L) filled with 500 ml of artificial seawater (Table 1) to achieved the final exposure concentrations. At the same time, approximately 2000 *Artemia* nauplius (instar II) were transferred into each exposure vessels which contained CuO-NPs (0, 1, 10, and 100 mg/L) under continuous light and aeration from the bottom in triplicate. After 4-h exposure, nauplii were collected on 100- $\mu$ m sieve, thoroughly rinsed with deionized water, then filtered on 0.45-mm Whatman® filter paper and later used to measure copper accumulation.

### Exposure of cichlids to CuO-NPs pre-exposed *A. salina*

Dietary exposure included uptake (21 days) and depuration (21 days) phases. The treatments consisted of larvae fed with instar II nauplii of *A. salina* pre-exposed for 4 h to CuO-NPs

(1, 10, and 100 mg/L) or controls (fed on untreated *Artemia*, cultured in clean water) in triplicate. Each replicate consisted of 20 convict cichlid larvae (initial body weight was  $28.4 \pm 1.7$  mg) that were placed in a glass aquariums filled with 50 L dechlorinated tap water (Table 1) and equipped with sponge filter. Fish were fed with freshly treated *A. salina* once a day at density of about 2000 nauplii per aquarium (100 nauplii per fish). In order to prevent the effects of unconsumed brine shrimps and fish feces on the quality of water, 1 h after each meal, all the fish of each aquarium were transferred to new aquariums containing fresh water. The photoperiod was adjusted to 16:8 h dark/light by a fluorescent lamps and water temperature was adjusted to  $26 \pm 1.0$  °C using aquarium heaters. At the end of uptake phase (end of the 21st day), all the fishes of each aquarium were anesthetized using 50 mg/L tricaine methanesulfonate (MS222) and their weights and lengths were recorded. After biometry, ten larvae from each replicate were sampled for bioaccumulation study and the remaining larvae were transferred to fresh water in separate aquariums for depuration study and fed on non-contaminated brine shrimp at density of 100 nauplii/fish/day for 21 more days. At the end of the depuration phase (end of the 42nd day), all the remaining fish were euthanized using 250 mg/L MS222 and after biometry, these were used to measure copper body burden.

### Measurement of copper concentration

The sampled nauplii as well as sampled fish larvae of uptake and depuration phases were thoroughly rinsed with deionized water and dried for 24 h using a freeze-dryer (Dena Vacuum, FD-5005-BT). Dried samples were carefully weighted and then digested by adding 1–3 ml concentrated nitric acid (Suprapur® grade, Merck, Germany) and heating at 100 °C for 2 h on a Bain-Marie bath (Mettler, WNB 45 model). Once completely dissolved, the contents were diluted to 10 ml with double deionized water (ZOLALAN ZU101 model, Iran). The Cu concentrations were measured using a graphite furnace atomic absorption spectrophotometer (wavelength range 190–900 nm; sensitivity Cu 5  $\mu$ g/ml; GFAAS, Phoenix-986, Biotech, USA) in triplicate. The device was first calibrated with standard copper solution (TraceCERT®, 1000 mg/L Cu in nitric acid, Sigma-Aldrich).

To calculate the eliminated copper at the end of depuration phase, in each treatment, the amount of Cu body burden after 21 days of dietary exposure to contaminated nauplii was subtracted from the Cu body burden after 21 days of feeding with non-contaminated nauplii during depuration phase. Also, the percentage of elimination rates of copper were calculated using following equation (Sarkheil et al. 2018); where E is elimination rate at the end of depuration phase, A is the accumulated copper following 21 days of dietary exposure to

**Table 1** The properties of artificial seawater and tap water used in exposure experiments

Parameters	Unit	Concentration	
		Artificial seawater	Tap water
Salinity	g/L	30	–
Electrical conductivity (EC)	$\mu$ S/cm	$29.0 \pm 0.1$	–
Hardness (CaCO <sub>3</sub> )	mg/L	$5360 \pm 91$	$270 \pm 36$
Dissolved oxygen (DO)	mg/L	$6.4 \pm 0.1$	$8.1 \pm 0.2$
pH	–	$8.1 \pm 0.05$	$7.9 \pm 0.2$

CuO-NPs, and R is remained Cu in the body at the end of deuration phase.

$$E = (A-R)/A \times 100$$

### Statistical analysis

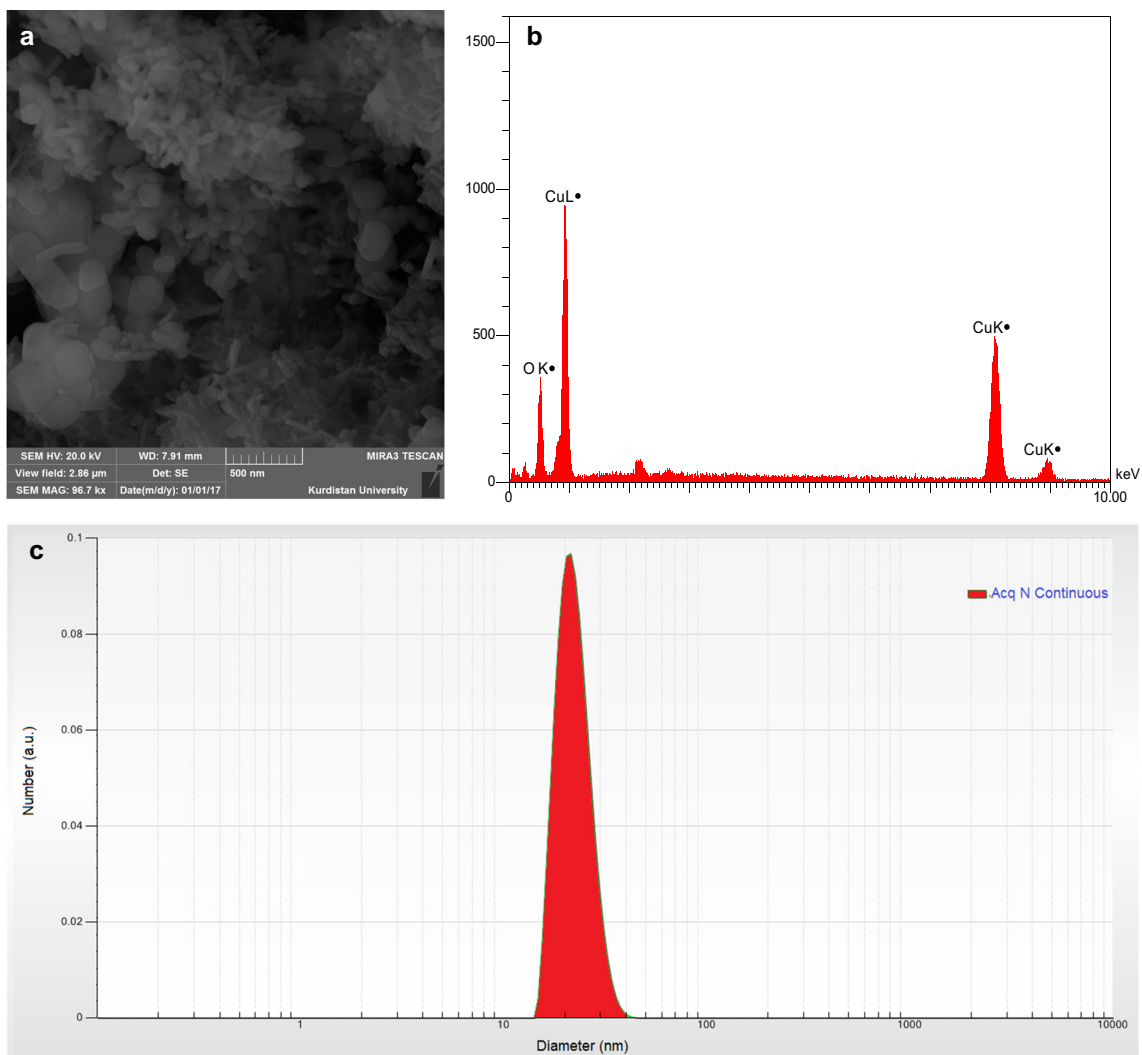
The percentage data were transformed using the arcsine square root. Normality assumption of data was determined using the Kolmogrov-Smirnov test. One-way analysis of variance (ANOVA) followed by a Duncan multiple range test were used to detect significant differences among groups. Statistical significance was accepted at the level of  $p < 0.05$ . All data were recorded as a mean value with standard deviation (mean  $\pm$  SD). All statistical analyses were performed

using SPSS software (Version, 19, IBM SPSS, Armonk, NY, USA).

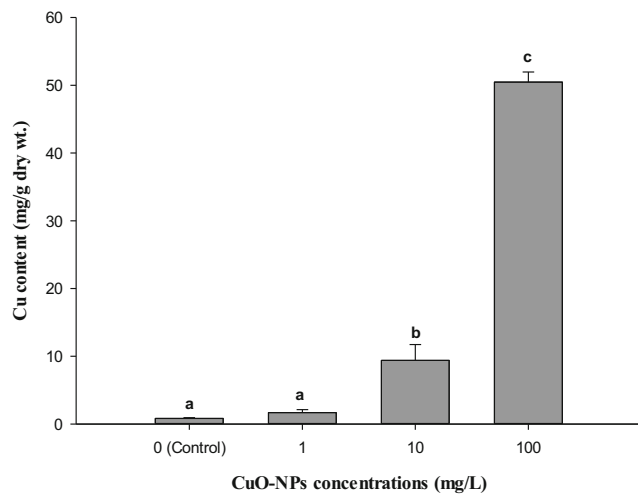
## Results

### Characterization of CuO-NPs

The results of FESEM and EDS analyses (Fig. 1a, b) proved nanoscale sizes and presence of copper and oxygen as the main elemental composition of CuO nanoparticles. Based on FESEM images part of the nanoparticles were aggregated/agglomerated in the dry powder and a mean diameter of  $83.2 \pm 73.2$  nm and a size distribution that ranged from 3.7 to 564.6 nm were determined. The results of Zetasizer instrument showed that the



**Fig. 1** FESEM image (a) and EDS analysis (b) of dry powder of CuO-NPs as well as hydrodynamic size distribution (c) of 100 mg/L well-dispersed CuO-NPs suspension

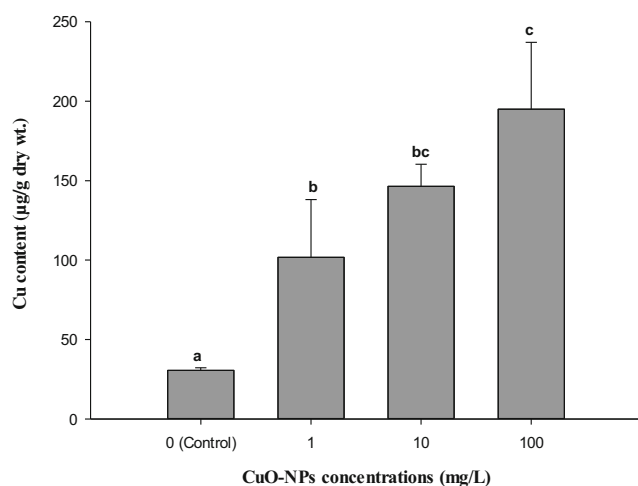


**Fig. 2** Cu content in *Artemia salina* (mg/g dry weight) after exposure to CuO-NPs (0, 1, 10, and 100 mg/L) for 4 h. The data with different letters are significantly different (mean  $\pm$  SD, ANOVA,  $p < 0.05$ )

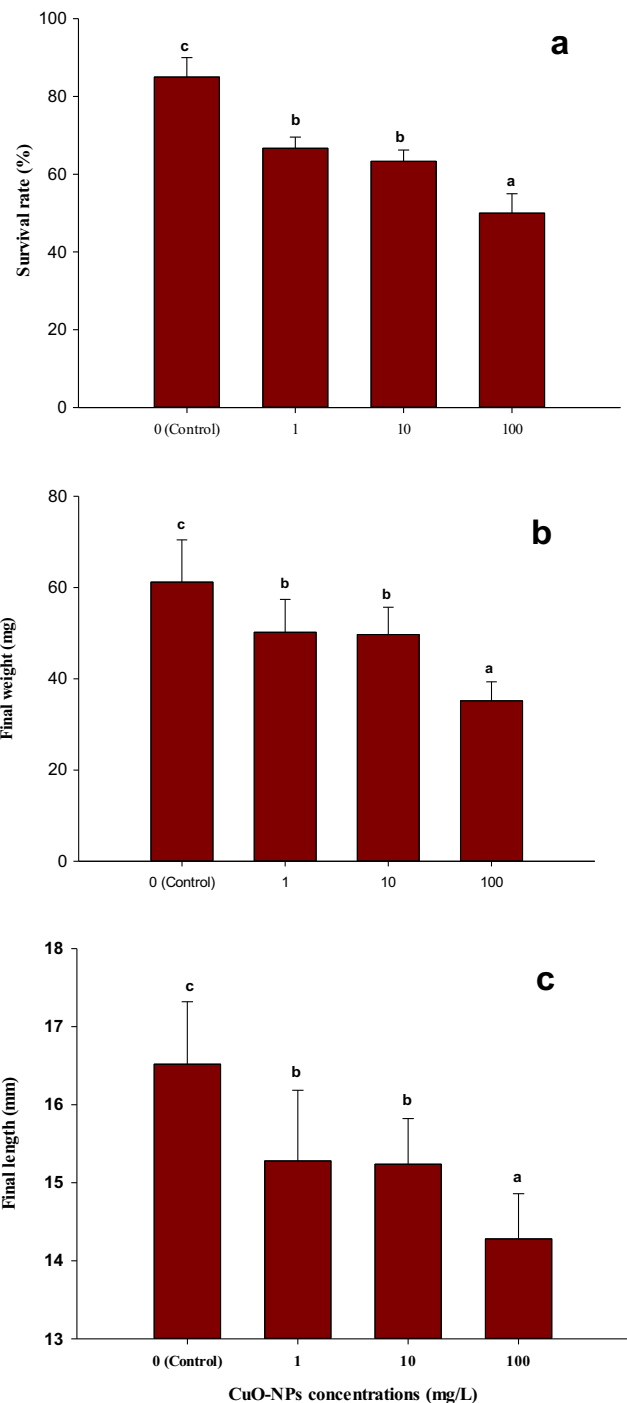
ultrasonication method well-dispersed aggregated/agglomerated NPs in distilled water and mean hydrodynamic diameter of CuO-NPs was  $30.8 \pm 4.2$  nm (Fig. 1c) and its zeta potential was  $-3.2 \pm 1.8$  mV.

### Copper uptake by *Artemia salina*

The Cu content in *A. salina* exposed to different concentrations of CuO-NPs for 4 h are illustrated in Fig. 2. The Cu content in instar II nauplii exposed to 10 and 100 mg/L of CuO-NPs increased significantly compared to control ( $p < 0.05$ ). The uptake of Cu increased to  $50.4 \pm 1.4$  mg/g dry weight at 100 mg/L of CuO-NPs.



**Fig. 3** Cu content in whole body of convict cichlid (*Amatitlania nigrofasciata*) larvae ( $\mu\text{g/g}$  dry weight) after 21 days of dietary exposure to CuO-NPs through nauplii of *Artemia salina* pre-exposed to 0, 1, 10, and 100 mg/L NPs. The data with different letters are significantly different (mean  $\pm$  SD, ANOVA,  $p < 0.05$ )



**Fig. 4** Survival rate (%) (a), average final body weight (b), and length (c) of convict cichlid (*Amatitlania nigrofasciata*) larvae after 21 days of dietary exposure to CuO-NPs through nauplii of *Artemia salina* pre-exposed to 0, 1, 10, and 100 mg/L NPs. The data with different letters are significantly different (mean  $\pm$  SD, ANOVA,  $p < 0.05$ )

### Effects of dietary exposure to CuO-NPs on cichlid larvae

Figure 3 shows the Cu content in whole body of convict cichlid (*A. nigrofasciata*) larvae fed for 21 days with

**Table 2** Elimination and accumulation rates of copper in convict cichlid (*Amatitlania nigrofasciata*) larvae fed with CuO-NPs-treated *Artemia* after 21 days of depuration phase (mean  $\pm$  SD)

Exposure concentrations of CuO-NPs (mg/L)	Eliminated Cu ( $\mu\text{g/g}$ dry weight)	Elimination rate of Cu (%)	Accumulated Cu ( $\mu\text{g/g}$ dry weight)
0 (control)	5.1 $\pm$ 2.0 <sup>a</sup>	16.5	25.4 $\pm$ 0.5 <sup>a</sup>
1	72.6 $\pm$ 39.8 <sup>b</sup>	68.4	29.0 $\pm$ 8.0 <sup>a</sup>
10	112.4 $\pm$ 23.6 <sup>bc</sup>	76.2	33.9 $\pm$ 9.7 <sup>ab</sup>
100	152.6 $\pm$ 38.1 <sup>c</sup>	77.8	42.3 $\pm$ 4.0 <sup>b</sup>

Means with different letters in the same column are significantly different (ANOVA,  $p < 0.05$ )

*A. salina* pre-exposed to different concentrations of CuO-NPs. In the control group, the background Cu content in larvae was  $30.5 \pm 1.6 \mu\text{g/g}$  dry weight which increased significantly coincident with increasing the concentration of Cu in dietary exposure ( $p < 0.05$ ). The highest value of Cu content was observed in fish larvae following feeding with pre-exposed *Artemia* to 100 mg/L CuO-NPs ( $p < 0.05$ ).

The variations in the survival rate, average final weight, and length of fish larvae exposed to CuO-NPs-treated *Artemia* are shown in Fig. 4 (a–c). After 21 days of exposure, the survival rate, average final weight, and length of larvae inversely decreased coincident with increasing the concentration of CuO-NPs ( $p < 0.05$ ). The highest toxicity effect on larvae was observed following the dietary exposure with pre-exposed *Artemia* to 100 mg/L CuO-NPs ( $p < 0.05$ ).

### Accumulation and elimination rates of ingested copper in cichlid larvae

The values of accumulation and elimination of Cu in cichlid larvae after 21 days of depuration phase are shown in Table 2. The amount of eliminated Cu increased significantly with elevation of CuO-NPs concentration in dietary exposure compared to the control ( $p < 0.05$ ). The elimination rate of Cu ranged between 68.4 and 77.8% among different treatments. After 21 days of depuration phase, there was no significant difference in the Cu body burden of larvae which dietary exposed to pre-exposed *Artemia* to 1 and 10 mg/L CuO-NPs and the control ( $p > 0.05$ ), but this value was still significantly higher at 100 mg/L ( $p < 0.05$ ).

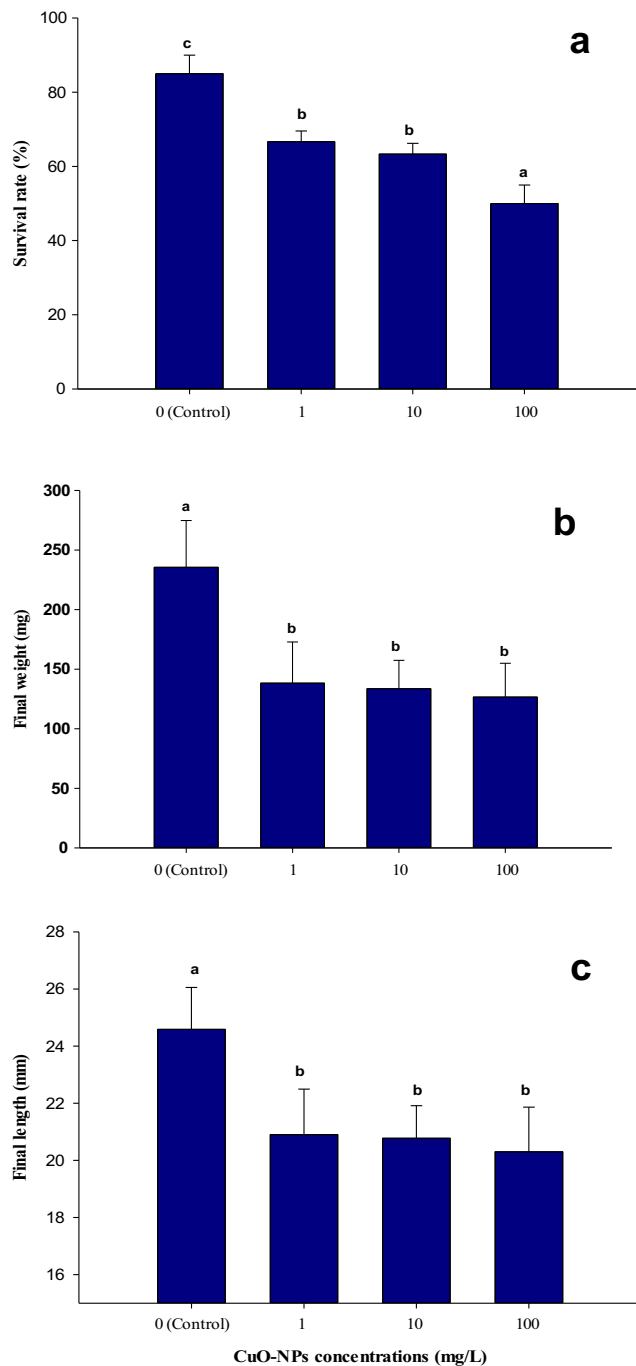
After 21 days of depuration phase, the survival rate, average final weight, and length of cichlid larvae exposed to different concentrations of CuO-NPs-treated *Artemia* were significantly lower than the control ( $p < 0.05$ ) (Fig. 5a–c). The lowest survival rate was recorded in larvae dietary exposed with pre-exposed *Artemia* to 100 mg/L CuO-NPs ( $p < 0.05$ ) (Fig. 5a).

### Discussion

The results of the present study revealed that the uptake of copper by larval stage (instar II) of *A. salina* from saltwater was in concentration-dependent manner. Cu body burden in the nauplii increased to  $50.4 \pm 1.4 \text{ mg/g}$  dry weight because of 4-h exposure to high concentration of CuO-NPs (100 mg/L). CuO-NPs aggregate more significantly in saltwater compared with the freshwater due to the increasing counter ions and positively charged cations that reduced the surface stabilization (Ates et al. 2014). Conway et al. (2014) reported that the CuO nanoparticles size reached 416 nm in natural seawater medium. Adam et al. (2015) also revealed that the most of CuO nanoparticles formed large aggregates during the exposure of *Daphnia magna* to sub-lethal concentrations of these nanoparticles. However, the nanoparticles that aggregate sizes are still in the size range that *Artemia* can assimilate (Bhuvaneshwari et al. 2016). The results of a study showed a concentration-dependent increase in the accumulation of CuO-NPs in different life stages of *A. salina* (Madhav et al. 2017). Sarkheil et al. (2018) also reported that the toxicity of nanoparticulate zinc oxide on the brine shrimp *A. franciscana* was dependent on concentration and exposure period.

Recently, concerns about the adverse effects of NPs on the food chain have grown rapidly because of the fact that ingested nanoparticles at the lower trophic levels could be transferred to higher organisms (Ates et al. 2014). The transfer and bioaccumulation of metal oxide ENPs to fish by dietary exposure has been reported in literatures (Skjolding et al. 2014; Ates et al. 2014; Wang et al. 2016). In the present study, the accumulation of copper in convict cichlid larvae through dietary exposure (pre-exposed *A. salina* to CuO-NPs) was investigated. At the end of exposure phase, Cu content of larvae exposed to 1, 10, and 100 mg/L of CuO-NPs-treated *Artemia* reached the values of  $101.7 \pm 26.4$ ,  $146.4 \pm 13.8$ , and  $194.9 \pm 32.0 \mu\text{g/g}$  dry weight respectively. These results revealed that Cu body burden in convict cichlid larvae was affected by Cu content in contaminated *Artemia*, so that, the exposure of larvae to *Artemia* with a higher Cu content resulted to higher Cu body burden in larvae. Similarly, Ates et al. (2014) revealed that in a dietary exposure route including pre-





**Fig. 5** Survival rate (%) (a), average final body weight (b), and length (c) of convict cichlid (*Amatitlania nigrofasciata*) larvae after 21 days of depuration phase (the depuration phase followed 21 days of dietary exposure to CuO-NPs through nauplii of *Artemia salina* pre-exposed to 0, 1, 10, and 100 mg/L NPs). The data with different letters are significantly different (mean  $\pm$  SD, ANOVA,  $p < 0.05$ )

exposed *Artemia*, accumulation of Cu in different tissues of goldfish, *C. auratus*, increased with increasing CuO-NPs concentration. The results of the present study revealed that survival rate, average final weight, and length of convict cichlid larvae decreased inversely with increasing copper concentration in the diet (CuO-NPs contaminate nauplii). It is reported

that the growth of juvenile turbot (*S. maximus*) exposed to 50 and 100 mg/L of TiO<sub>2</sub> NPs-treated clamworms (*P. aibuhitensis*) gradually decreased with increasing Ti contents in clamworms after 20 days of dietary exposure (Wang et al. 2016).

This study included a 21-day depuration phase to assess the ability of nanoparticles to be eliminated from the body of fish. During this phase, Cu body burden in larvae decreased by about 16.5%, 68.4%, 76.2%, and 77.8% at 0, 1, 10, and 100 mg/L of CuO-NPs-treated *Artemia*, respectively. In fact, the higher elimination rate occurred in larvae exposed to higher concentrations of CuO-NPs-treated *Artemia* due to more Cu body burden. Lakani et al. (2016) reported that during recovery time for 7 days, accumulated Cu in tissues of Siberian sturgeon (*Acipenser baerii*) juvenile exposed to waterborne Cu-NPs (50, 100, and 200  $\mu\text{g/l}$ ) for 14 days significantly decreased but it was still higher than the control. The amount of accumulated Cu after the depuration phase in cichlid larvae that exposed to 0, 1, 10, and 100 mg/L of CuO-NPs-treated *Artemia* were found to be  $25.4 \pm 0.5$ ,  $29.0 \pm 8.0$ ,  $33.9 \pm 9.7$ , and  $42.38 \pm 4.01$   $\mu\text{g/g}$  dry weight, respectively. Despite the highest elimination rate of Cu in larvae exposed to 100 mg/L of CuO-NPs-treated *Artemia*, the 21-day depuration phase was not enough for complete elimination of excess copper from the body and the Cu body burden in this group was still significantly higher than control. As is clear, the convict cichlid larvae were not able to eliminate all of the ingested NPs, and part of these NPs accumulated inside their body. Although in comparison to the control group, at the end of depuration phase, there were no significant differences in the Cu body burden of larvae that dietary exposed to 1 and 10 mg/L CuO-NPs-treated *Artemia*, but even at low concentrations, the dietary exposure to CuO-NPs-treated *Artemia* showed their toxic effect on decline in growth performance of larvae. The survival rate, average final weight, and length of larvae exposed to 1, 10, and 100 mg/L of CuO-NPs-treated *Artemia* were significantly lower than the control group.

## Conclusion

It was found that the *A. salina* nauplii (instar II) were able to uptake CuO-NPs (and or copper compounds derived from them) from saltwater in a concentration-dependent manner. The results indicated the trophic transfer ability and bioaccumulation of manufactured CuO-NPs from brine shrimp nauplii to convict cichlid (*A. nigrofasciata*) larvae. The reductions of survival and growth performance of larvae were observed following dietary exposure, even after elimination of a large amount of Cu during the depuration phase.

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## Compliance with ethical standards

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

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