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Kinetics of Deposition, Acute Toxicity and Bioaccumulation of Copper in some Freshwater Organisms

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Abstract Experiments with environmentally relevant concentrations of Cu in glass aquaria revealed that Cu was quickly removed from water. Cubic regression of Cu concentration against time showed that maximum rate of removal was around 69.34–72.11 h irrespective of treatment. The 96 h LC_{50} value of Cu was respectively 0.18, 0.19 and 0.35 mg/L for fish *Cyprinus carpio*, crustacean *Diaptomus forbesi* and worm *Branchiura sowerbyi*. Normalizing the lethal values and plotting them against time it was observed that there was sharp differences in mortality over time between the organisms and 96 h lethal values could misrepresent susceptibility of the organisms to Cu. Treatment of 0.1 mg/L of Cu in water resulted in accumulation of 10.57, 4.38, 1.46 and 2.44 µg/g of Cu, respectively in sediment, worm, crustacean zooplankton and whole body of fish. But, Cu deposited in high concentrations in gut and liver of fish indicating that Cu was principally accumulated through food.

Keywords Copper · LC_{50} value · Bioaccumulation · Aquatic organisms · Cubic regression

Influx of metals from terrestrial sources into aquatic environment in India has tremendously increased in recent years due to rapid progress in industrialization and urbanization (Javed and Usmani 2013). Freshwater ponds used for culturing fish are also not spared from this influx (Raychaudhuri et al. 2008). Some of these metals are highly toxic, persistent in the aquatic ecosystem and tend to magnify through food chain. The main objective of the present study is to determine kinetics of deposition of Cu in water, acute toxicity of Cu to selected freshwater organisms and pattern of bioaccumulation of Cu in selected species of worms, crustacean zooplankton and fish through experiments conducted in laboratory.

Copper (Cu) is a common metallic pollutant of water and is highly toxic to fish (Kiyani et al. 2013) and aquatic invertebrates (Brown et al. 2004). In India, Cu is frequently detected in river water from trace (0.43 ppb) to high (1.4 mg/L) concentration (Singh et al. 2012; Ghorade et al. 2014). Areas influenced by mining activities (Pandey et al. 2007) and industrial discharges (Tewari et al. 2015) show higher level of Cu in water. Chemical controls of algal bloom in water also contribute to contamination of water by Cu (Kumar and Sinha 2014).

Adequate literatures are not available to relate toxicity of copper to aquatic organisms with the ambient concentration of the metal in water or to the concentration at which the metal is discharged into water from different sources. Attempts were made in this study to address these issues.

Materials and Methods

Three separate experiments were conducted in this study. These included kinetics of deposition, acute toxicity and bioaccumulation of Cu. Deep tube-well water stored in an overhead tank (dissolved oxygen 7.25 ± 0.24 mg/L; free CO_2 0.25 ± 0.01 mg/L, pH 7.12 ± 0.15, alkalinity 125 ± 12 mg/L as $CaCO_3$ and hardness 140 ± 8.0 mg/L as $CaCO_3$) was used

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as test medium for all the experiments. Copper(II) sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$; Purity ≥99.0%) salt procured from MERCK, Mumbai, India was used for Cu treatment. The salt was dissolved in double distilled water to make a stock solution of 1000 mg/L Cu. Necessary amount from this stock solution was added to the test water to achieve desired concentration of Cu.

Experiments to study kinetics of Cu deposition in water and acute toxicity bioassays for fish were made in the laboratory in 20 L glass aquaria (38 × 25 × 25.5 cm) each containing 10 L of water. Acute toxicity bioassays for crustacean and oligochaet worms were carried out in 300 mL beakers each holding 250 mL of water. Experiments on bioaccumulation of Cu were made in outdoor field (12:12 h dark-light photoperiod) in bowl shaped earthen vats, each with 0.098 m^3 space (surface radius = 0.33 m, maximum height = 0.36 m) and stocked with 60 L of water and 5 kg of uncontaminated soil (pH 7.2 ± 0.2, organic carbon 1.72 ± 0.10%, available N 12.23 ± 0.22 mg/100 g, available P 7.25 ± 0.05 mg/100 g). The test vials were arranged as per randomized block design so that there were three replicates for each of the concentrations of Cu and control tested.

Based on concentrations of Cu determined in natural freshwater bodies in India and assumptions of actual discharges from the sources, three concentrations of Cu (1.5, 2.5 and 3.5 mg/L) were used for experiments on deposition of Cu. The aquaria were filled with water and kept undisturbed for 24 h before making the above treatments and a control with appropriate replications as mentioned above. Water samples were collected by siphoning from mid depth of each aquarium after 1, 6, 12, 24, 48, 72 and 96 h of treatment for determination of Cu. The water samples were filtered and the filtrates were digested by strong nitric acid following the procedures of APHA (1995). The digested samples were diluted with double distilled water. A blank was prepared from double distilled water using the above method. Levels of Cu in the digested samples were determined in flame atomic absorption spectrophotometer (Spectra AA 240, Agilent Technologies) against the blank after calibration with Varian standards of known concentrations. The data generated were used to fit cubic regression using the method of least squares to determine Cu concentration against time for each treatment. The classical optimization technique was applied to determine the optimal rate of change of Cu concentration with respect to time and the time at which the rate was optimal.

The 96 h static bioassays were conducted following the procedure of APHA (1995) to determine LC_{50} values of Cu for the crustacean *Diaptomus forbesi*, oligochaet worms *Branchiura sowerbyi* and the teleost fish *Cyprinus carpio*. The crustaceans and the worms were collected from the local unpolluted water bodies and fingerlings of the fish *C. carpio* (L = 3.47 ± 0.28 cm; W = 525 ± 30 mg) were procured from a local fish farm. The test organisms were acclimatized in the laboratory conditions for 96 h and were stocked at ten individuals of crustacean or worms in 300 mL beaker and at five individuals of fish in 20 L aquarium. For control and each treatment of Cu, three replicates were maintained. After 1 h of treatment, water samples were collected from each test vial to determine actual concentration of Cu in water by flame atomic absorption spectrophotometer (AAS). Mortality of the organisms was recorded every 24 h and dead organisms were removed immediately. Lethal concentration at which 50 percent animals died (LC_{50}) was calculated by computer programme of probit analysis (EPA Ver. 1.5) based on probit analysis of Finney (1971). The mortality patterns of the test organisms were also evaluated by normalizing the lethal values and plotting them against time. Finally, the linear regression curves were calculated to get a comparative view about the mortality rate with respect to Cu concentration.

Experiments on bioaccumulation of Cu were made in six bowl shaped earthen vats. The vats were stocked with sufficient quantity of crustacean zooplankton and oligochaet worms collected from unpolluted sources and were conditioned for 15 days for the planktons and worms to grow. Three vats were then treated with a sub-lethal concentration of Cu (0.1 mg/L) and three vats were kept as controls. The sub-lethal concentration selected was based on 50% of the LC_{50} value of nominal concentration of Cu for the crustacean *D. forbesi* and the teleost fish *C. carpio*. After 96 h of exposures, samples of water were carefully collected from at least 10 cm above bottom soil of each vat by a 1 L high density polyethylene (HDPE) bucket and passed through a plankton net made up of bolting silk no.25 (mesh size 64 μm) to collect the crustacean zooplankton. Sediment samples were collected from each vat by a hand dredger and were spread in a HDPE tray to collect worms. Sediment samples were then dried to constant weight in oven at 98 ± 2°C. Collected samples were preserved by the methods described by Guhathakurta and Kaviraj (2004). Then each vat was stocked with ten fingerlings of the fish *C. carpio* and was kept in the vat for another 96 h in order to determine accumulation of Cu from ambient concentration of Cu and food organisms. Fish samples were collected after 96 h of its exposure, i.e., 192 h of the whole experiment. All samples collected from the experimental vats were immediately digested or preserved for determination of Cu.

Water samples were digested in strong nitric acid as described above. The sediment soils were digested in nitric acid and hydrochloric acid (Guhathakurta and Kaviraj 2000). Fish samples were divided into two groups. From one part of the sample, whole fish was digested while fish from another part of the sample were dissected to collect gill, liver, gut, kidney and muscle tissues. The whole fish and each tissue samples were digested in nitric acid, sulphuric

acid and perchloric acid (Guhathakurta and Kaviraj 2004). Cu in the digested samples was determined in flame AAS using blank and standards as described above. Precision and accuracy of the analytical methods were checked by analyses of a standard reference material of Cu in aqueous solution (NIST, SRM No 3114) and soil samples spiked by Cu (Nafde et al. 1998). Adopted analytical procedures yielded 96 ± 2 % recovery of Cu from the SRM and spiked samples tested. Detection limit of Cu in the instrument was found at 0.01 mg/L.

Results and Discussion

Deposition of Cu in water in respect of time has been presented in Fig. 1. Dissolved Cu in water gradually decreased with the period of exposure. The concentration of Cu after 96 h of exposure was 0.04, 0.43 and 0.66 mg/L, respectively for the treatments 1.5, 2.5 and 3.5 mg/L thereby showing 2.7 %, 17.2 % and 18.9 % of Cu in water after 96 h in respective treatments.

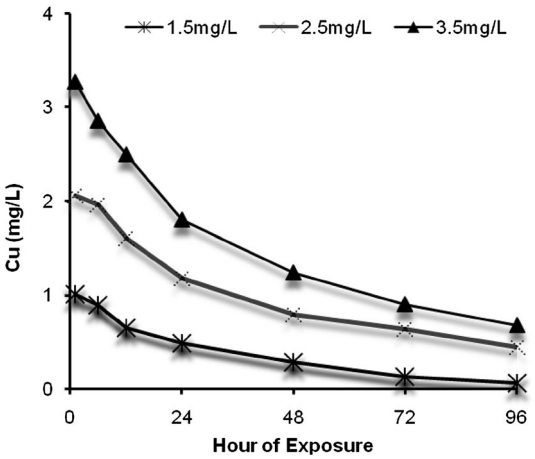


Fig. 1 Change in concentration of Cu with respect to time (actual observation)

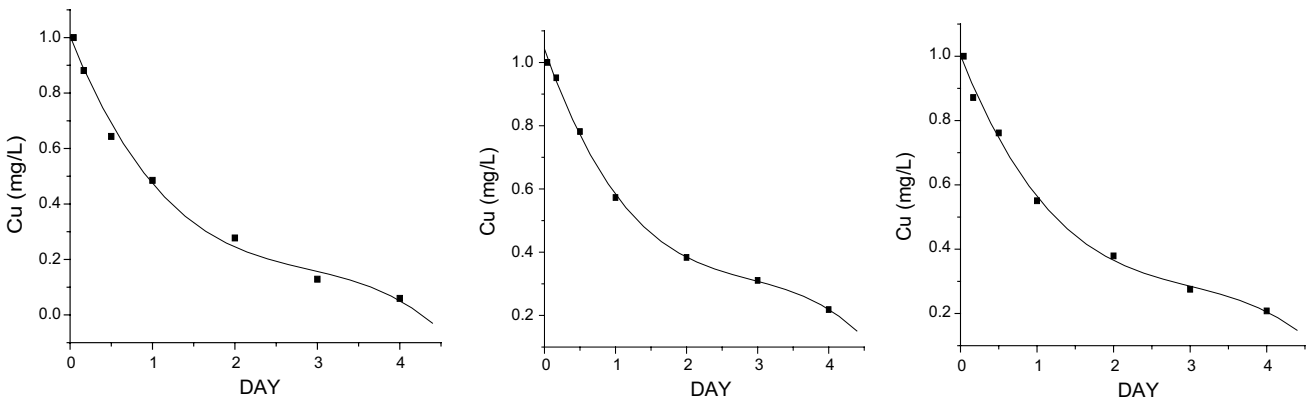
By normalizing the data of deposition of Cu with respect to time and then fitting the data in cubic regression equation the following relationships were obtained between concentration of Cu and time of observation (Fig. 2a–c):

$$\mathrm{Cu} = 1.0019 - 0.7311\mathrm{T} + 0.2291\mathrm{T}^2 - 0.02643\mathrm{T}^3 \quad \left(\mathrm{R}^2 = 0.994 \text{ and } \mathrm{SD} = 0.0214\right) \tag{1}$$

$$\mathrm{Cu} = 1.0396 - 0.6309\mathrm{T} + 0.1967\mathrm{T}^2 - 0.02256\mathrm{T}^3 \quad \left(\mathrm{R}^2 = 0.999 \text{ and } \mathrm{SD} = 0.0135\right) \tag{2}$$

$$\mathrm{Cu} = 1.0005 - 0.5953\mathrm{T} + 0.1783\mathrm{T}^2 - 0.01978\mathrm{T}^3 \quad \left(\mathrm{R}^2 = 0.996 \text{ and } \mathrm{SD} = 0.0291\right) \tag{3}$$

Now by differentiating Eq. (1) with respect to time (T), the rate of change for concentration for treatment of 1.5 mg/L of Cu was computed as $Y_1 = \frac{dCu}{dT} = -0.7311 + 2\times0.2291T - 3\times0.02643T^2$. This equation was again differentiated with respect of time and by solving $\frac{dY_1}{dT} = 0$, we obtained time as T = 2.88939 days or 69.34 h. Because, $\left.\frac{d^2Y_1}{dT^2}\right|_{T=2.88939} = -6\times0.02643 < 0$, i.e., Y_1 (treatment of 1.5 mg/L Cu) had maximum reduction at 69.34 h. By applying similar approach on Eq. (2), it was observed that Y_2 (treatment of 2.5 mg/L Cu) had maximum reduction at T = 2.90632 or 69.75 h. Finally, from Eq. (3) it



(a) 1.5 mg/L Cu **(b)** 2.5 mg/L Cu **(c)** 3.5 mg/L Cu

Fig. 2 Cubic regression of Cu concentration against time

Table 1 LC_{50} values of copper (mg/L) with 95% confidence limit in parentheses for three aquatic organisms representing three different taxa and niche

Hour of exposure	*Diaptomus forbesi* (Crustacea)	*Branchiura sowerbyi* (Oligochaeta)	*Cyprinus carpio* (Teleostomi)
24			
N	0.75 (0.58–0.94)	0.52 (0.48–0.57)	0.37 (0.27–0.48)
A	0.63 (0.49–0.76)	0.46 (0.42–0.49)	0.33 (0.24–0.42)
48			
N	0.52 (0.32–0.74)	0.46 (0.42–0.52)	0.29 (0.19–0.39)
A	0.46 (0.28–0.64)	0.40 (0.36–0.45)	0.26 (0.17–0.34)
72			
N	0.37 (0.18–0.51)	0.42 (0.38–0.46)	0.24 (0.16–0.32)
A	0.32 (0.16–0.45)	0.37 (0.33–0.40)	0.21 (0.14–0.28)
96			
N	0.22 (0.14–0.33)	0.39 (0.35–0.44)	0.20 (0.15–0.29)
A	0.19 (0.12–0.29)	0.35 (0.31–0.38)	0.18 (0.13–0.25)

Values presented are based on both nominal (N) and actual concentration of Cu after 1 h of treatment (A)

was observed that Y_3 (treatment of 3.5 mg/L Cu) had maximum reduction at T = 3.00421 days or 72.11 h.

LC_{50} values of Cu with 95% confidence limit for the three species of aquatic organisms have been presented in Table 1. The values were determined based on nominal concentration as well as on actual concentration of Cu determined after 1 h of exposure. There was no mortality of any organism in control. The 96 h LC_{50} values of Cu for the three test organisms ranged from 0.20 to 0.39 mg/L of nominal concentration and 0.18–0.35 mg/L of actual 1 h concentration of Cu in water, thereby showing only 10% difference in LC_{50} values between nominal and actual concentration. All the test organisms were highly sensitive to Cu, worm being relatively more tolerant than other two organisms.

The 96 h LC_{50} value of Cu for the crustacean *Mysis* sp (1.44 mg/L) and the teleost fish *Mugil cephalus* (4.4 mg/L) recorded by Zyadah and Abdel-Baky (2000) are slightly higher than the LC_{50} values of Cu for the crustacean *D. forbesi* and the fish *C. carpio* recorded in the present study, while 48 h EC_{50} of Cu to the crustacean *Daphnia similis* (0.013–0.046 mg/L) and fish *Danio rerio* (0.038–0.095 mg/L) (Filho et al. 2004) are much lower than those recorded in the present study. While sensitivity of the aquatic organisms to Cu varies from species to species, it is influenced strongly by the physicochemical parameters including pH, water hardness and dissolved organic matter (Kim et al. 2001; Kiyani et al. 2013). Using species sensitivity distribution curve by log-logistic model Xin et al. (2015) observed that invertebrate taxa were more sensitive to Cu than vertebrates. But results of the present study revealed that there was no significant difference in 96 h LC_{50} values between the fingerlings of teleost fish *C. carpio* and the crustacean *D. forbesi*. The fish species was even more susceptible to Cu than the oligochaet worm *B. sowerbyi*. However, relative susceptibility of the three organisms to Cu changed with exposure period. Toxicity of metals to aquatic organisms depends on bioavailability of the metal and mode of its entry into the body. Playle et al. (1992) observed that adult fathead minnows (*Pimephales promelas*) had the ability to modify its local chemical microenvironment at their gill surface and regulate accumulation and toxicity of Cu. It is not known if small fry of *C. carpio* (mean weight 0.52g ± 0.03) used in the present study had such regulation. Ability to regulate accumulation of Cu from solution is not uniform among crustaceans. Though certain species of cladocerans (Choueri et al. 2009) can efficiently regulate Cu in their body ability of *D. forbesi* or copepods, in general, to regulate Cu is not known.

Results of the present study indicated that *D. forbesi* could regulate Cu up to 48h more efficiently than the *C. carpio* fry and the *B. sowerbyi*. Accordingly, *D. forbesi* was more tolerant than either *C. carpio* or *B. sowerbyi* up to 48 h. As actual mechanism of toxicity of Cu to the three test organisms used in the present study is not generally known we applied a different technique to compare mortality pattern of the three aquatic organisms with respect to time. The lethal values of Cu (based on 1 h actual concentration) were normalized and plotted against time (Fig. 3). It was observed that lethal values of Cu sharply decreased with the increase of time for the crustacean *D. forbesi*, while the lethal values decreased moderately for the fish *C. carpio* and slowly for the worm *B. sowerbyi*. Accordingly, *D. forbesi* became more sensitive to Cu at 96 h than either *B. sowerbyi* or *C. carpio*. Although such difference in sensitivity between *D. forbesi* and *B. sowerbyi* was discernible from 96 h LC_{50} values the difference between *D. forbesi* and *C. carpio* was not reflected in LC_{50} values.

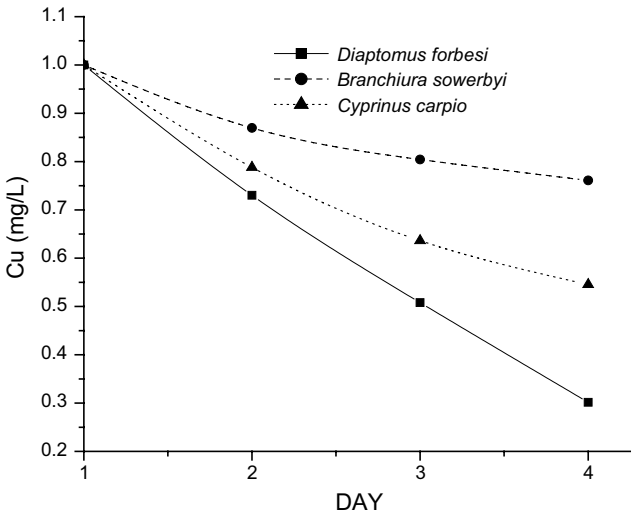


Fig. 3 Change in lethal values of Cu with respect to time

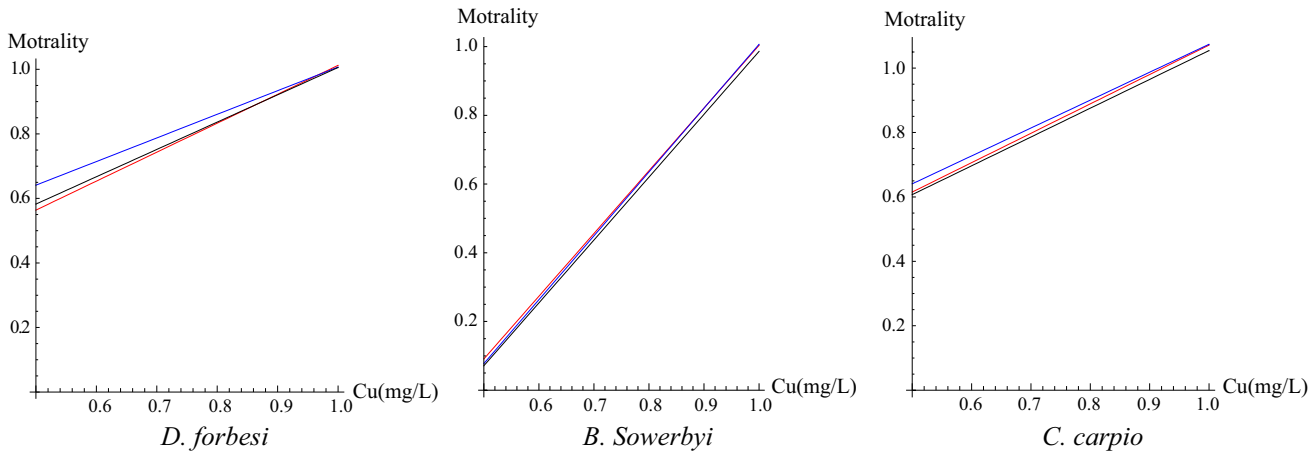


Fig. 4 Regression of mortality of the three organism at 48 h (*red*), 72 h (*blue*) and 96 h (*black*)

Table 2 Partitioning of Cu in different components of the experimental system after 96 h of treatment with 0.1 mg/L of Cu

Component	Unit	Control	Treatment
Water	mg/L	BDL	0.017 ± 0.002
Sediment	mg/kg	1.79 ± 0.37	10.57 ± 0.98
Plankton	µg/g	<0.01	1.46 ± 0.51
Worm	µg/g	<0.01	4.38 ± 1.12

BDL below detection limit

Table 3 Bioaccumulation of Cu (µg/g) in whole body and in different tissues of fish *C. carpio*. Values are mean of three replicates ± SD

Tissue	Control (96 h)	Treated (192 h)
Whole body	0.29 ± 0.24	2.44 ± 0.20
Muscle	<0.01	0.11 ± 0.11
Kidney	0.51 ± 0.29	1.92 ± 1.22
Gill	1.14 ± 0.26	4.01 ± 0.8
Gut	4.96 ± 0.76	16.99 ± 2.08
Liver	17.28 ± 1.14	53.9 ± 6.82

As maximum reduction in the concentration of Cu in water was found between 69.34 ~ 72.11 h, the mortality data were normalized and linear regression curves were drawn for 48, 72 and 96 h (Fig. 4). It was revealed that mortality rate at 72 h was higher compared to 48 and 96 h exposure period for *D. forbesi* and *C. carpio*, but the difference in mortality of *B. sowerbyi* among the three exposure periods was not substantial.

In the bioaccumulation experiment, Cu was found to partition differentially between abiotic and biotic components of the experimental medium (Table 2). After 96 h of exposure, concentration of Cu in water was only 0.017 ± 0.002, which was lower than the permissible level of Cu in surface water prescribed by Bureau of Indian Standards (Anonymous 2012). But Cu deposited over bottom sediment and the concentration became as high as 10.57 ± 0.98 $mg.kg^{-1}$. However, increase in concentration of Cu in sediment does not necessarily increase toxicity of Cu to oligochaet worms because sediment bound Cu is not always bioavailable to these organisms (Ankley et al. 1994). Critical body residue of Cu in which 50% of reduction in survival or autotomy or reproduction occurred in the oligochaet *Tubifex tubifex* was 2–11 times higher in sediment than water only medium (Mendez-Fernandez et al. 2013). Based on LC_{50} values of some sediment organisms Roman et al. (2007) calculated "No Observed Effect Concentration" (NOEC) of Cu in the sediment that produced little effects to sediment organisms and it ranged between 3 and 47 mg/kg of the sediment. Based on this NOEC value, sediment concentration of Cu in the present study appeared to render little risk to worms, which accumulated 4.38 ± 1.12 µg/g Cu during 96 h exposure. Accumulation of Cu in planktonic organisms and whole body of fish were also only 1.46 ± 0.51 and 2.44 ± 0.20 µg/g respectively, indicating that food chain concentration of Cu was not prominent.

However, Cu accumulated in high concentration in gut and liver (Table 3) indicating that Cu accumulation in *C. carpio* was principally through food. Possibility of depuration of Cu from these tissues was minimum, because fish samples were immediately preserved after collection from the experimental vats. This was one of the possible reasons of high concentration of Cu in these tissues. High concentration of Cu in liver indicates potential of detoxification of Cu in this tissue (Nazi et al. 2014). Copper often accumulates and becomes toxic to aquatic organisms at concentrations slightly over the level in which it is required in the body.

It is concluded from the present study that irrespective of concentration at which Cu is evaluated in laboratory test water, the time required for its maximum reduction in concentration range between 69 and 72 h. Relative sensitivity

of the aquatic organisms *C. carpio, D. forbesi* and *B. sowerbyi* to Cu change with exposure period and 96 h LC_{50} value alone is not adequate to compare their sensitivity to Cu. Cubic regression of the data of Cu deposition in water and normalization of the mortality data followed by linear regression can better explain the pattern of Cu retention in water and its toxicity to aquatic organisms. The bioaccumulation experiment indicates that the fish *C. carpio* probably accumulates Cu mostly from food, but food chain concentration of the metal is not evident from this experiment.

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