Research article

Determination of Lethal Concentration of Copper Compounds on Nile Tilapia (*Oreochromis niloticus* Linnaeus, 1758) Larvae

NATSIMA TOKHUN*
Environmental Science Program, Faculty of Science and Technology, Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathum Thani, Thailand
Email: natsima@vru.ac.th

KANITTHA PAMONPOL
Environmental Science Program, Faculty of Science and Technology, Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathum Thani, Thailand

CHULEEMAS BOONTHAI IWAI
Integrated Water Resource Management Research and Development Center in Northeast Thailand, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand

ATCHARAPORN SOMPARN
Environment Division, Office of General Education, Udon Thani Rajabhat University, Udon Thani, Thailand
Email: somparn_a@yahoo.com

Received 8 December 2017 Accepted 20 October 2018 (*Corresponding Author)

Abstract Copper is an essential element for cell organisms. However, irregularity copper level could have effect on the growth of organism. In this research, we aim at consideration of copper compounds toxicity against larvae of Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) by statistic assays. Four days old of test fish had an average 7.10±0.03 mm of length and 9.72±0.04 mg of fresh weight. All experiments were carried out for a period of 48 to 96 hours at 27–30°C. The number of dead fish was counted every 24 hours and the mortality rate was determined after 96 hours. The statistical data was evaluated by Finney's probit analysis method. The fish died 50% (LC_{50} 95% confidence limits) after 48, 72, 96 hours of concentration copper chloride ($CuCl_2$) 595.75, 231.11, 124.64 µg/L, respectively, and copper nitrate (Cu $(NO_3)_2$) 1,025.40, 588.79, 456.50 µg/L, respectively. The LC_{50} results clearly show that copper compounds toxicity for fish increased with increasing concentration and exposure time. The percentage of survival decreased with increasing of both copper compound concentrations and it was significantly different from control (p<0.01). This study showed that Nile tilapia larvae were more sensitive to copper chloride than copper nitrate. Further study needs the processes by which these chemicals affect biochemical changes of the fish.

Keywords copper, lethal concentration, LC_{50}, tilapia, toxicity

INTRODUCTION

The copper-gold (Cu-Au) has been consumed for a large quantity in Thailand. The internal main supply could be found in Loei province (Yang et al., 2014). Due to high demand, it was imported in various forms i.e. pure copper, copper matt, copper anode, unwrought and copper products in a large amount for 422,619 metric tons in 2012 (Office of Industrial Economics, 2012). Copper and its compounds has been used in various industries such as electrical products, building construction, chemical and pharmaceutical manufacturing (National Pollutant Inventory, 2014). They are directly discharged from industrial effluents and also from polluted runoff in urban and agricultural areas (FAO, 1992; Yirgu, 2011). Therefore, surface water and groundwater are being polluted from these

effluents and runoff that contains copper and its compounds. Copper has been detected in water range from 1 to 5 mg/L, which is classified as small amounts or not generally considered to be toxic. However, gradually accumulation of copper in body could become large doses enough to cause sickness, and could lead to liver damage in extreme cases. The distribution of copper ions has been determined in the aquatic system to assess the influence of biological processes on the copper estuarine behavior. The copper chloride prominently affects the fish muscles as some amino acids (Histidine, Proline, Glycine, Alanine, Methionine and Valine) are found to be reduced or even completely missing (Alkesh, 2016). Aquatic life is more sensitive 10–100 times to the hazardous effects of copper than mammals (Shah and Vyas, 2015).

Lethal concentration test measure the susceptibility and survival potential of organisms to a particular toxic substances such as heavy metals. Pollutants with higher median lethal concentration (LC_{50}) values are required to induce mortality in organisms (Eaton et al., 2005; Zahedi et al., 2012). Nile tilapia is one of the most common freshwater fish used in toxicological studies (Figueiredo-Fernandes et al., 2006a, b, 2007; Garcia-Santos et al., 2006) because it is easy handling, culture and maintenance in laboratory (Alkobaby and El-Wahed, 2017). Moreover, it promptly responds to environmental alterations so this species is also a well-established model for toxicological research (Almeida et al., 2002; Figueiredo-Fernandes et al., 2006a, 2007). Hence, the aim of this study is to determine the value of copper as chloride and nitrate lethal concentration for Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) larvae in order to determine specific times.

MATERIALS AND METHODS

The experiment was conducted at Environmental Science Laboratory, Faculty of Science and Technology, Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathum Thani province, Thailand. The acute toxicity of copper compounds for Nile tilapia larvae was determined in terms of 48, 72 and 96 hours LC_{50} and lethal concentrations. The determination of lethal concentration of copper compounds on Nile tilapia larvae was the following.

Test Organism

Two days old of Nile tilapia (*Oreochromis nileticus* Linnaeus, 1758) fish larvae were obtained from the Pathum Thani Inland Aquaculture Fisheries Research and Development Center, Thailand for toxicity testing in an oxygenated plastic bag (Fig. 1). The test was conducted at the Environmental Science Laboratory, Valaya Alongkorn Rajabhat University under the Royal Patronage in January, 2017. The tested fish were acclimatized in distilled water. Each holding tray was aerated by using a portable pump and air stone for a period of 48 hours at a room temperature before experiment use. If survival rate was $\leq$10%, the entire stock was discarded (Boonsomboone, 2004). Test fish fused or toxicity tests had an average 7.10$\pm$0.03 mm of length and 9.72$\pm$0.04 mg of wet weight.

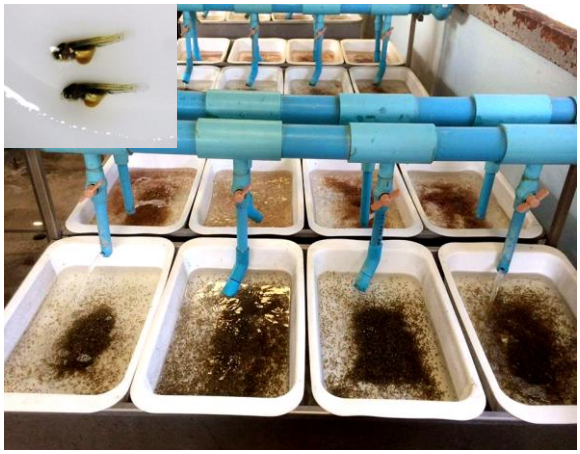

Fig. 1 The test organism, Nile tilapia (*Oreochromis nileticus* Linnaeus, 1758) larvae

Test Media

Analytical grade copper chloride ($CuCl_2 \cdot 2H_2O$) and copper nitrate ($Cu(NO_3)_2 \cdot 3H_2O$) form Sigma-Aldrich were used for preparation of stock solutions that were diluted as desired concentrations. Fish were exposed for 24 to 96 hours, separately, against different concentrations of two copper compounds at 0, 10, 50, 100, 300 and 500 µg/L. The investigation was performed in laboratory conditions at dissolved oxygen (8.06±0.05), temperature (27.77±0.76°C) and pH (6.50±0.50). All physico-chemical parameters were performed by following APHA-AWWA-WEF (1998) on daily basic.

Determination of Lethal Concentration

Four-day static acute toxicity test was performed at five different concentrations (10, 50, 100, 300 and 500 µg/L of copper chloride and nitrate) were used in the test series (10 fish for each). A control group (10 fish) was maintained in deionized water container throughout entire experimental period, the fish were not fed. Fish mortalities were record at 48, 72 and 96 hours after starting of tests, and dead fish were removed immediately from test media. Mortality was assessed in the different copper concentrations along with the control group to determine the 96 hours median lethal concentration (LC_{50}) value by the use of Finney's probit analysis method (Finney, 1971).

Statistical Analysis

The entire experiment was analyzed using a completely randomized design (CRD) method. Mortality data for 48, 72 and 96 hours was calculated for LC_{50} values (95% confidence limits) by Finney's probit analysis (Finney 1971) using the Statistical Analysis System (SPSS) version 11.5 program. Standard error of mean value (SEM) of replication sampling (n=12) was taken for each analysis. A significantly different result was established by a one-way ANOVA, and mean comparisons of different treatments were carried out by least significant difference (LSD) on Duncan multiple test of Statistix 8 Software (Analytical Software, Tallahassee, FL). The acceptance level of significance was a probability value less than 0.05 ($p<0.05$).

RESULTS AND DISCUSSION

Median lethal concentration (LC_{50}, 95% confidence limits) for copper chloride and copper nitrate on the larvae of Nile tilapia (*Oreochromis nileticus* Linnaeus, 1758) calculated by Finney's probit analysis method and SPSS Statistical Software at 48, 72 and 96 hours of exposure was shown in Table 1 and Fig. 2.

Table 1 LC_{50} value with 95% confidence limit of copper chloride and copper nitrate on fish

Exposure times (hour)	Lethal concentration (µg Cu/L, lower-upper values)	
	Copper chloride ($CuCl_2$)	Copper nitrate ($Cu(NO_3)_2$)
48	595.754 (391.083 – 1,118.644)	1,025.395 (668.913 – 2,207.740)
72	231.110 (171.199 – 335.477)	588.785 (418.447 – 988.509)
96	124.637 (94.508 – 167.266)	456.504 (322.974 – 755.933)

Note: LC_{50} is concentration of copper compounds that caused dead 50% of the exposed fish at a specific time of observation (e.g. 48 hours LC_{50}), (expressed as µg/L of copper concentrations)

Results showed that the 48 hours LC_{50} (lower–upper values) for copper chloride was 595.754 (391.083–1,118.644) µg/L which sharply declined to 231.110 (171.199–335.477) and 124.637 (94.508–167.266) µg/L at 72 and 96 hours, respectively. LC_{50} value for copper chloride was lower than copper nitrate at exposure times. At 48, 72 and 96 hours LC_{50} values for copper nitrate on fish

were 1,025.395 (668.913–2,207.740), 588.785 (418.447–988.509) and 456.504 (322.974–755.933) µg/L, respectively. This study showed that copper chloride was more toxic than copper nitrate on Nile tilapia larvae.

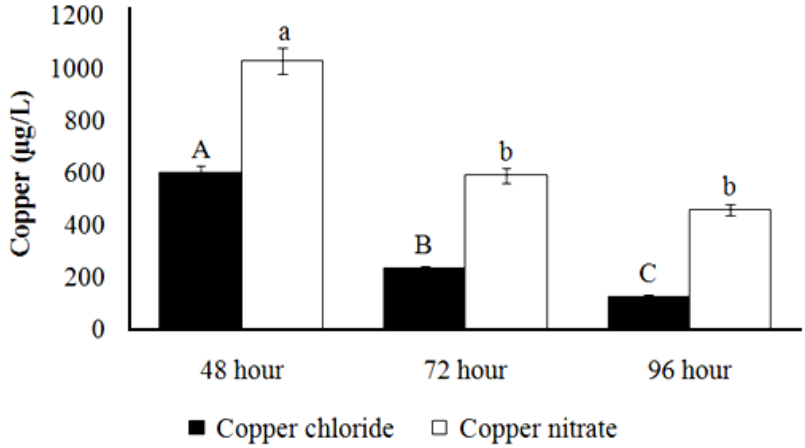

Fig. 2 Shown LC_{50} value of both copper compounds on fish

The 96 hours LC_{50} value for copper chloride in Nile tilapia larvae was found 124.637 µg/L in the present work, and here we report copper chloride to be highly toxic to fish. The Material Safety Data Sheet (MSDS) reports copper chloride acute toxicity to carp fish (*Cyprinus carpio*) in laboratory tests, in the average range LC_{50} value of 120–230 µg/L (Sigma-Aldrich Corporation, 2014). Our result is in good agreement with this report. These values are considerably lower than 96 hour LC_{50} (900 µg/L) of Bluegill (*Lepomis macrochirus*), indicating higher vulnerability to copper chloride in the former two species (Sigma-Aldrich Corporation, 2014; NTU chemistry Deportment, 2017). Conversely, Warrin et al. (2009) found that copper chloride in sediment media had higher toxic on *Chironomus tentans* than copper sulfate and copper nitrate. The results were obtained by Taweel et al. (2013) reported that mean of 48, 72 and 96 hours LC_{50} value for copper sulfate in fingerlings of Nile tilapia (*Oreochromis nileticus*) were 1,863 (1,031–5,194), 1,368 (741–2,802) and 1,093 (581–1,613) µg/L, respectively. According to Iwai et al. (2016) estimates of 48, 72 and 96 hour LC_{50} value for copper sulfate to Nile tilapia (*Oreochromis nileticus*) larvae were 1,869 (1,157–5,235), 1,740 (–) and 1,383 (859–2,718) µg/L, respectively, these results are higher than present study (Table 1). On the other hand, LC_{50} for copper oxide on Barnacle (*Balanus improvises*) was 350, 140 and 20 µg/L for 48, 72 and 96 hours, respectively (U.S.EPA, 2009a). For copper sulfate, the larva was the most sensitive stage of development of cobia (*Rachycentron canadum*): eggs, larvae (one-day-old fish), juveniles (20 days old) and young fish (40 days old) in toxicity tests, with an 96 hours LC_{50} of 91 (56–134), 60 (29–96), 87 (49–133) and 240 (119–378) µg/L, respectively as the early stages were more sensitive than the later stages, (Le et al., 2005). In addition, copper was most toxic to fingerling tilapia fish and the toxicity ranking of four heavy metals was copper > lead > cadmium > zinc (Taweel et al., 2013). Also, copper was more toxic than cadmium for common carp (*Cyprius carpio* L.) embryos and larvae (Jezierska et al., 2009).

The observed percentage of Nile tilapia mortality for copper in static tests continuous for different hours and different concentrations were shown in Table 2. There was positive relationship between the mortality and concentration levels; when concentration increased, the mortality rate increased as well. Similar, the percentage of survival decreased with increasing of both copper compound concentrations and exposure times. The result showed significant decreased percentage of fish survival in copper chloride and copper nitrate when compared with control (p<0.01). The fish tests significant responded to both copper concentrations (50 µg/L) in percent survival (72.0–85.6%) at specific times in Table 2. The copper concentrations that caused 50% of the exposed fish at 96 hour were found 100 µg/L of copper chloride and 300 µg/L of copper nitrate. Similar to Howell et al. (1984) reported that copper (94 µg/L) reduced the filtration rate of mussels (*Mytilus edulis*) by half, probably through reducing the ciliary beation via the bronchial nerves. Chen et al.

(2012) reported that even short-term pulsed exposure to low levels of copper (1.6–2.0 µg/L) reduced growth rate of tilapia (*Oreochromis mossambicus)* larvae. Shah and Vyas (2015) reported that at 2 mg/L of copper chloride concentration caused damage to cell membrane and vaculation of *Labeo rohita* on 15 day, nucleus was affected on 30 days and on 45 days the cell membrane was wrinkled and damaged.

Table 2 Percent survival of exposed Nile tilapia larvae at specific times

Concentrations (µg /L)	% Survival for Nile tilapia larvae (n=120)					
	Copper chloride ($CuCl_2$)			Copper nitrate ($Cu(NO_3)_2$)		
	48 h	72 h	96 h	48 h	72 h	96 h
0	94.7 ± 4.2a	90.2 ± 3.5a	87.4 ± 4.2a	100.0 ± 0.0a	100.0 ± 0.0a	98.9 ± 3.3a
10	90.1 ± 5.2ab	84.7 ± 3.8a	79.0 ± 6.3b	98.9 ± 3.3ab	95.6 ± 5.3ab	92.2 ± 6.7ab
50	85.6 ± 5.1b	77.3 ± 7.2b	72.0 ± 5.0c	92.2 ± 6.7bc	85.6 ± 7.3b	81.1 ± 9.2bc
100	79.0 ± 6.1c	68.6 ± 8.5c	56.6 ± 9.9d	92.2 ± 8.3bc	88.9 ± 7.8b	82.2 ± 10.9c
300	61.2 ± 7.0d	47.3 ± 6.1d	39.4 ± 8.7e	88.9 ± 7.8c	73.3 ± 13.2c	66.7 ± 14.1d
500	47.1 ± 9.8e	31.8 ± 9.7e	21.8 ± 3.9f	57.8 ± 13.0d	43.3 ± 19.4d	37.8 ± 17.2e
LSD	**	**	**	**	**	**
CV (%)	8.56	11.11	11.40	8.71	13.24	14.67

*Note: ** showed significantly different from control ($p \leq 0.01$)*

CONCLUSION

Based on the acute toxicity tests performed in this study, Nile tilapia larvae was shown to be more sensitive to copper chloride than copper nitrate which reducing LC_{50} value and survival rate of fish tests in copper chloride concentrations. The LC_{50} determination of copper compounds on fish larvae was copper toxicity increased with increasing concentration and exposure time. The copper concentrations that caused 50% of the exposed fish at 96 hours were found 100 µg/L of copper chloride and 300 µg/L of copper nitrate.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to Environmental Science Program, Faculty of Science and Technology, and Research and Development Institute, Valaya Alongkorn Rajabhat University under the Royal Patronage, Environment Division, Office of General Education, Udon Thani Rajabhat University, and Integrated Water Resource Management Research and Development Center in Northeast Thailand, Faculty of Agriculture, Khon Kaen University, Thailand for financial support.

REFERENCES

Alkesh, I.S. 2016. Impact of heavy metal copper chloride on fish *Labeo rohita*. Int. J. Adv. Res., 4 (10), 461-466.

Alkobaby, A.I. and Abd El-Wahed, R.K. 2017. The acute toxicity of copper to Nile tilapia (*Oreochromis niloticus*) fingerlings and its effects on gill and liver histology. J. Aquac. Res. Development, 8 (1), 1-6.

Almeida, J.A., Diniz, Y.S., Marques, S.F., Faine, L.A., Ribas, B.O., Burneiko, R.C. and Novelli, E.L.B. 2002. The use of the oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to in vivo cadmium contamination. Environ. Int., 27, 673-679.

APHA-AWWA-WEF. 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington DC, USA.

Boonsomboon, S. 2004. A selection of appropria test conditions for whole effluent toxicity testing with juvenile Nile tilapia (*Orecochromis niloticus*). M.Sc. Thesis, Graduated School, Mahidol University, Thailand.

Chen, W.Y., Lin, C.J., Ju, Y.R., Tsai, J.W. and Liao, C.M. 2012. Coupled dynamics of energy budget and population growth of tilapia in response to pulsed waterborne copper. Ecotoxicology, 21 (8), 2264-2275.

FAO. 1992. Committee for inland fisheries of Africa. Report of the third Session of the Working Party on Pollution and Fisheries, Accra, Ghana, 25-29 November 1991. FAO Fisheries Report, 471, Rome, FAO.

Figueiredo-Fernandes, A., Fontaínhas-Fernandes, A., Monteiro, R.A.F., Reis-Henriques, M.A. and Rocha, E. 2006a. Effects of the fungicide mancozeb in the liver structure of Nile tilapia, *Oreochromis niloticus*-Assessment and quantification of induced cytological changes using qualitative histopathology and the stereological point-sampled intercept method. Bull. Environ. Contam.Toxicol., 76 (2), 249-255.

Figueiredo-Fernandes, A., Fontaínhas-Fernandes, A., Peixoto, F., Rocha, E. and Reis-Henriques, M.A. 2006b. Effect of paraquat on oxidative stress enzymes in tilapia *Oreochromis niloticus* at two levels of temperature. Pest. Biochem. Physiol., 85 (2), 97-103.

Figueiredo-Fernandes, A., Ferreira-Cardoso, J.V., Garcia-Santos, S., Monteiro, S.M., Carrola, J. and Motos, P. 2007. Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. Pesq. Vet. Bras, 27 (3), 102-109.

Gadagbui, B.K.M., Addy, M. and Goksøyr, A. 1996. Species characteristics of hepatic biotransformation enzymes in two tropical freshwater teleosts, tilapia (*Oreochromis niloticus*) and mudfish (*Clarias anguillaris*). Comp. Biochem. Physiol., 114 (3), 201-211.

Howell, R., Grant, A.M. and Maccoy, N.E. 1984. Effect of treatment with reserpine on the change in filtration rate of *Mytilus edulis* subjected to dissolved copper. Mar. Pollut. Bull., 15 (12), 436-439.

Iwai, C.B, Soeung, L. and Houng, T. 2016. Acute toxicity testing of copper on four species of tropical freshwater biota (*Moina macrocopa, Chironomus javanus, Ctenopharyngodon idella* and *Oreochromis niloticus*). Khon Kaen Agr. J., 44 (Suppl. 1), 1006-1013.

Jezierska, B., Sarnowski, P., Witeska M. and Ługowska K. 2009. Disturbances of early development of fish caused by heavy metals (a review). Electron. J. Ichthyol., 5 (2), 76-96.

Le, Q.D., Nguyen, M.C., Nguyen, T.H. and Nguyen D.C. 2005. Acute toxicity test to determine the effects of copper, zinc and cyanide on cobia (*Rachycentron canadum*) resources in North Vietnam. Australasian J. Ecotoxicol., 11, 163-166.

National Pollutant Inventory 2014. Copper and compounds. Department of the Environment and Energy, Australia Government, http://www.npi.gov.au/resource/copper-and-compounds (retrived on 27 October, 2017).

Office of Industrial Economics. 2012. Copper industrial situation 2012. http://www.oie.go.th/sites/default/files/attachments/industry_ overview/copper-industrialsituation-2555.pdf

Reddy, P.P., Jagadeshwarlu, R. and Devi, G.S. 2015. Determination of lethal concentration (LC_{50}) of copper to *Sarotherodon mossambica*. Int. J. Fish. Aqua. Stud., 4 (1), 172-175.

Shah, A.I. and Vyas, B.M. 2015. Relative study of copper toxicity on aquatic life and human health. J. Environ. Res. Develop., 9 (34A), 884-888.

Taweel, A., Shuhaimi-Othman, M. and Ahmad, A.K. 2013. *In vivo* Acute toxicity tests of some heavy metals to Tilapia fish (*Oreochromis niloticus*). J. Biol. Sci., 13 (5), 365-371.

Yang, S., Wang, G., Du, W. and Huang, L. 2014. Remote sensing and GIS-based prediction and assessment of copper-gold resources in Thailand. The 35th International Symposium on Remote Sensing of Environment (ISRSE35), 22-26 April, 2013, Beijing, China, IOP Publishing: IOP Conference Series, Earth and Environmental Science, 17 (1), 1-4.

Yirgu, Z. 2011. Accumulation of certain heavy metals in Nile tilapia (*oreochromis niloticus*), Fish species relative to heavy metal concentrations in the water of Lake Hawassa. M.Sc. Thesis, Graduate School, Addis Ababa University.

Zahedi, S., Mehrpoosh, M., Vaezzade, H., Dagesaracki, M.S. and Hosseini, S.M. 2012. LC_{50} determination and copper and cadmium accumulation in the gills of Kutum (*Rutilus frisii kutum*) fingerlings. Terr. Aquat. Environ. Toxicol., 6 (2), 71-76.