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Copper sulfate acute ecotoxicity and environmental risk for tropical fish

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ABSTRACT. The aim of this study was to estimate copper sulfate acute toxicity and to determine death percentage and environmental risk on guppy fish (*Phallocerus caudimaculatus*), zebrafish (*Brachydanio rerio*), mato grosso (*Hyphessobrycon eques*), and pacu (*Piaractus mesopotamicus*). Fish were exposed to 0.01, 0.03, 0.05, 0.07, 0.10, and 0.30 mg L^{-1} (guppy), 0.05, 0.07, 0.10, and 0.30 mg L^{-1} (zebrafish), 0.07, 0.10, 0.20, and 0.30 mg L^{-1} (mato grosso) and 9.5, 10.0, 10.5, 11.0, 11.5, and 12.0 mg L^{-1} (pacu) of copper sulfate, with triplicate control. The estimated 50% average lethal concentrations (LC50; 96 hours) were 0.05 (guppy), 0.13 (zebrafish); 0.16 (mato grosso) and 10.36 mg L^{-1} (pacu). Copper sulfate was extremely toxic for guppy, highly toxic for zebrafish and mato grosso and lightly toxic for pacu and presents environmental risk of high adverse effects on the guppy, zebrafish and mato grosso and moderate adverse effect to the pacu. Therefore, the guppy fish, zebrafish, and mato grosso are important alternatives for copper sulfate toxicity evaluation in waterbodies.

Keywords: bioindicators, algaecide, toxicity.

Ecotoxicidade aguda e risco ambiental do sultado de cobre para peixes tropicais

RESUMO. O objetivo deste estudo foi estimar a toxicidade aguda, determinar a porcentagem de mortalidade e o risco ambiental do sulfato de cobre para o peixe guaru (*Phallocerus caudimaculatus*), zebrafish (*Brachydanio rerio*), mato grosso (*Hyphessobryconeques*) e pacu (*Piaractus mesopotamicus*). Para realização dos ensaios com sulfato de cobre foram utilizados: 0,01; 0,03; 0,05; 0,07; 0,10 e 0,30 mg L^{-1} (guaru), 0,05; 0,07; 0,10 e 0,30 mg L^{-1} (paulistinha), 0,07; 0,10; 0,20 e 0,30 mg L^{-1} (mato grosso) e 9,5; 10,0; 10,5; 11,0; 11,5 e 12,0 mg L^{-1} (pacu) como controle em triplicata. A concentração letal média 50% (CL50; 96h) estimada para o guaru foi de 0,05, para o paulistinha foi de 0,13 para o mato grosso foi de 0,16 e para o pacu foi de 10,36 mg L^{-1}. O sulfato de cobre foi classificado como extremamente tóxico para o guaru, altamente tóxico para o paulistinha e mato grosso e ligeiramente tóxico para o pacu e apresenta risco ambiental de elevado efeito adverso para o guaru, paulistinha e mato grosso e moderado efeito adverso para o pacu. Assim, conclui-se que o guaru, paulistinha e mato grosso são importantes alternativas para avaliação da toxicidade do sulfato de cobre em corpos hídricos.

Palavras-chave: bioindicadores, algicida, toxicidade.

Introduction

Industrial and farm activities generate water pollution by heavy metals. By reaching the aquatic environment, heavy metals participate on physiological and biochemical processes of non-target organisms, causing severe metabolism damage. Among heavy metals, Copper is essential for cell functions and enzyme maintenance (LIU et al., 2006). However, it may be toxic in high concentrations and causes changes in protein synthesis, enzyme denaturation and generation of oxygen reactive metabolites, which may accumulate especially on fish liver and cause changes in hepatic function (DROGE, 2002; PARIS-PALACIOS; BIAGIANTINI-RISBOURG, 2006).

The use of copper sulfate ($CuSO_4$) as agricultural, algaecide, macrophyte herbicide and as therapy agent in bacterial and ectoparasite diseases on fish has increased the concentration of this heavy metal in water bodies. Industrial effluents and accidental or deliberate discharge aggravates this situation (OLIVEIRA-FILHO et al., 2004).

The ecotoxicological assessment is essential to identify potential negative impacts on waterbodies and to regulate the use and potential environmental risk classification (USEPA, 2002). Sentinels or bioindicator organisms, which represent the many environmental levels, are used to environmental screening (ARUN et al., 2005). The characterization of bioindicator organisms requires a series of information, such as sensitivity, reproductive cycle, distribution, test optimization price and operation ease.

Copper ecotoxicological assessment is described for some fish species, such as commom curimbatá (*Prochilodus scrofa*) (MAZON; FERNANDES, 1999), tilapia (*Oreochromis mossambicus*) (JAMES; SAMPATH, 2003), blue tilapia (*Oreochromis aureus*) (STRAUS, 2003), guppy (*Poecilia reticulata*) (BOOCK; MACHADO-NETO, 2005), zebrafish (*Brachydanio rerio*) (PARIS-PALACIOS; BIAGIANTINI-RISBOURG, 2006), tiger bass (*Terapon jarbua*) (VIJAYAVEL et al., 2006) and lesser bleeding heart tetra (*Hyphessobrycon socolofi*) (DUARTE et al., 2009). However, further studies are required for the characterization and identification of the ecotoxicological risk of copper sulfate on tropical fish.

Among the species that can be used for ecotoxicological tests, mato grosso (*Hyphessobrycon eques*), zebrafish (*Brachydanio rerio*), guppy (*Phallocerus caudimaculatus*) and pacu (*Piaractus mesopotamicus*) present all the bioindicators organisms' features, in addition of living in lentic environment more susceptible to the xenobiotics adverse effects. Thus, the aim of this study was to evaluate the copper sulfate sensitivity (LC50) and to determine the mortality percentage and the environmental risk for guppy (*P. caudimaculatus*), mato grosso (*H. eques*), zebrafish (*B. rerio*) and pacu (*P. mesopotamicus*).

Material and methods

Fish from a breeding in Jaboticabal region were kept in a 30 days quarantine for disease observation. Thereafter, they were acclimatized in 250 L boxes in a bioassay room, at 25.0 ± 2.0ºC, with a 12 hours photoperiod for 10 days, at continuous water flow and air induction with air pump and fed on commercial feed once a day.

Initially, potassium dichromate sensitivity assays were made for evaluation of fish health according to Cruz et al. (2008), and the response pattern of sensitivity was similar to that described by this author.

The concentrations of copper sulfate used were 0.05, 0.07, 0.10, and 0.30 mg L^{-1} for *B. rerio*; 0.01, 0.03, 0.05, 0.07, 0,10, and 0.30 mg L^{-1} for *P. caudimaculatus*; 0.07, 0.10, 0.20, and 0.30 mg L^{-1} for *H. eques*; 9.5; 10.0; 10.5, 11.0, 11.5, and 12.0 mg L^{-1} for *P. mesopotamicus*. A control group was established for acute toxicity tests, all in triplicate, with three fish per rehearsal on 1.0 g L^{-1} max density (ABNT, 2004).

Initial water quality variables were: pH of 6.5 to 7.5; over 5.0 mg L^{-1} dissolved oxygen; 170.0 to 180.0 μS cm^{-1} electrical conductivity; 10.0 to 60.0 mg $CaCO_3$ L^{-1} hardness and 200.0-210.0 mg $CaCO_3^{-1}$ alkalinity (ABNT, 2004). Fish were exposed to copper sulfate for 96 hours, without feeding, with daily basis mortality evaluation and removal of dead fish. There was no water exchange or debris removal during the exposure.

The acute toxicity, expressed as LC50 (50% of lethal concentration), was estimated through the mortality evaluation, using the software 'Trimmed Spermam Karber' (HAMILTON et al., 1977).

The ecotoxicity was classified accordingly to the method described by Zucker and Jonhson (1985), and the environmental risk was calculated by quotient (Q) (URBAN; COOK, 1986). The quotient value (Q) was calculated by the estimated environmental concentration value (2.0 mg L^{-1}) (BOYD; MASSAUT, 1999) divided by LC50 values obtained on the acute toxicity tests. Environmental risks were ranked as no risk with no adverse effects ($Q \leq 0.1$); moderate adverse effects risk ($0.1 \leq Q \leq 10$) and high adverse effect risk ($Q > 10$).

Results and discussion

Phallocerus caudimaculatus presented greater copper sulfate sensitivity, whereas *P. mesopotamicus* presented the lower sensitivity. *H. eques* and *B. rerio* exhibited intermediate sensitivity after 50% lethal concentration estimative. The sensitivity rank was as follows: *P. caudimaculatus* > *B. rerio* > *H. eques* > *P. mesopotamicus* (Table 1).

Table 1. Average acute toxicity values (LC50; 96 hours), upper and lower limit and standard deviation of copper sulfate exposed fish.

Copper sulfate Mg L^{-1}	Guppy *P. caudimaculatus*	Mato grosso *H. eques*	Zebrafish *B. rerio*	Pacu *P. mesopotamicus*
Upper limit	0.08 ± 0.02	0.21 ± 0.03	0.24 ± 0.13	10.62 ± 0.02
LC50; 96 hours	0.05 ± 0.01	0.16 ± 0.03	0.13 ± 0.04	10.36 ± 0.03
Lower limit	0.03 ± 0.01	0.13 ± 0.02	0.10 ± 0.03	10.12 ± 0.05

Phallocerus caudimaculatus presented 0.05 mg L^{-1} LC50 (0.03 mg L^{-1} metallic copper sulfate), which is more toxic than 0.1 mg L^{-1} copper oxychloride (0.06 mg L^{-1} metallic copper) on *P. reticulata* described by Boock and Machado-Neto (2005). LC50 values difference may be caused by the use of different salts (copper sulfate and chloride) resulting in different anions concentrations that can affect the biomarkers in a direct or indirect interaction with the cation influencing its bioavailability, or due to interspecific differences in sensitivity between fish (KWOK et al., 2008).

There was no *P. caudimaculatus* mortality on control and at 0.01 mg L^{-1}; the mortality rate was between 13.33 and 40.0% at 0.03 mg L^{-1}; 33.33 to 44.45% at 0.05 mg L^{-1}; 40.0 to 46.67% at 0.07 mg L^{-1}; 66.67 to 80.0% at 0.10 mg L^{-1} and 100.0% mortality at 0.30 mg L^{-1} (Figure 1a).

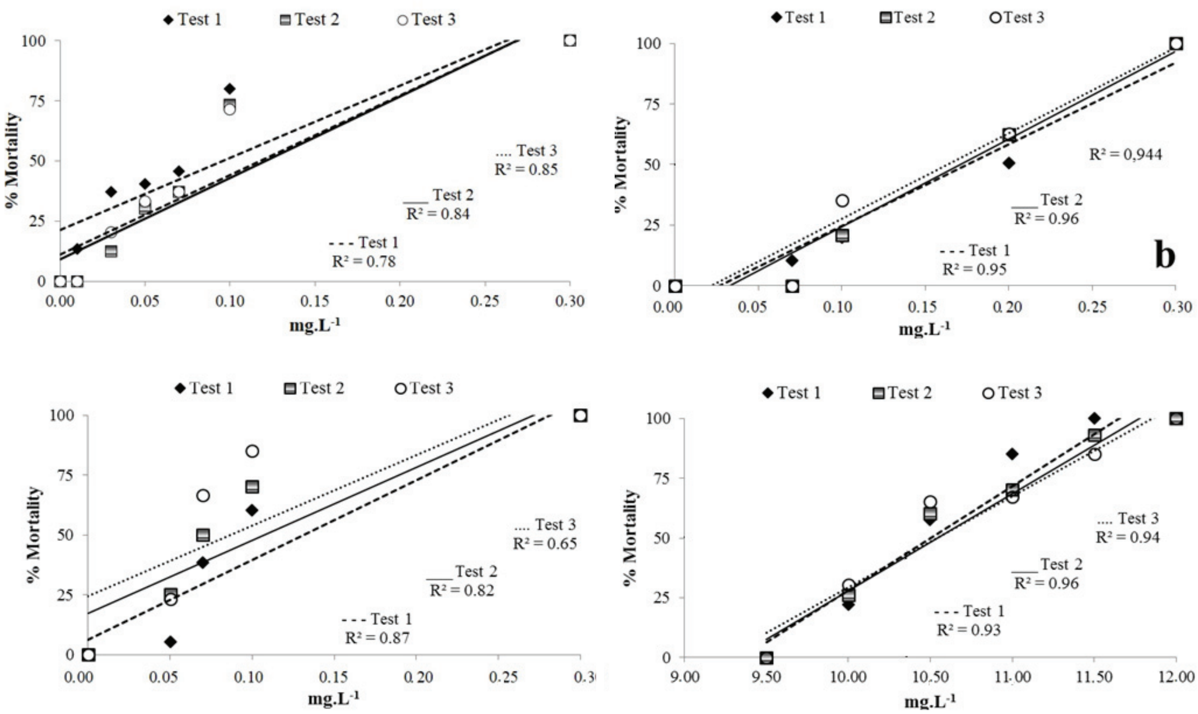

Figure 1. Percent mortality of fish *P. canaliculata* (a); *H. eques* (b); *B. rerio* (c) and *P. mesopotamicus* (d) exposed to copper sulfate (mg L^{-1}).

Hyphessobrycon eques copper metal (copper sulfate) LC50 results (0.16 mg L^{-1}) was about 150 times lower than in an iron metal effluent treatment plant (22.89 mg L^{-1}) (SOTERO-SANTOS et al., 2007). Copper, however, along with iron, may have its toxicity raised. This compound is a reaction catalyst which oxides unsaturated lipids, with direct effect on cell membrane structure and function (NUNES et al., 2010). *Hyphessobrycon socolofi* fish exposed to copper presented greater sensitivity (0.03 μg L^{-1}) (DUARTE et al., 2009) than that obtained in this research (0.16 mg L^{-1}). The sensitivity differences may be due to the 5.09 mg $CaCO_3$ L^{-1} water hardness usage compared to 10.0 to 60.0 mg.$CaCO_3$ L^{-1} on this test, since metal solubilization in aquatic environments is associated to abiotic factors such as pH and hardness, which influence toxicity (LINNIK; ZUBENKO, 2000). Elevated calcium levels reduce copper toxic effects by specific site attachments on the gill surface and membrane permeability control, helping to maintain homeostasis (HUNN, 1985).

After 96 hours of copper sulfate exposure, *H. eques* mortality percentage varied from 0 to 10.12% at 0.07 mg L^{-1}, 20.4 to 33.33% at 0.1 mg L^{-1}; 50.6 to 66.67% at 0.2 mg L^{-1} and 100.0% mortality at 0.3 mg L^{-1} (Figure 1b).

Lethal concentration (LC50;96 hours) for *Brachydanio rerio* was lower (0.13 mg L^{-1}) than the obtained for the same test organism exposed to copper oxychloride (0.18 mg L^{-1}) (OLIVEIRA-FILHO et al., 1997). The lower test organism acute toxicity value (LC50) obtained showed greater copper sulfate sensitivity, compared to copper oxychloride. The mortality percentage of fish exposed to copper sulfate in this study is presented at Figure 1.

After 96 hours of exposition to copper sulfate, *B. rerio* percentage of mortality at 0.05 mg L^{-1} was 5.4 to 25.1%; 43.65 to 66.67% at 0.07 mg L^{-1}; 60.0 to 90.0% at 0.1 mg L^{-1}; and 100.0% at 0.3 mg L^{-1} (Figure 1c).

Average 50% lethal concentration obtained for *P. caudimaculatus*, *H. eques* and *B. rerio* were similar to those obtained for curimbatá (*Prochilodus scrofa*) (0.098 and 0.015 mg L^{-1}) and for piauçu (*Leoporinus macrocephalus*) (0.09 mg L^{-1}) (CARVALHO; FERNANDES, 2006; NUNES et al., 2010; TAKASUSUKI et al., 2004). Mitochondrial respiratory enzymes and proteins responsible for respiratory functions lose effectiveness on energy conversion and stop oxidative phosphorylation when attached to metals, causing cell damage (DROGE, 2002).

The LC50 for *P. mesopotamicus* in this study was lower (10.36 mg L^{-1}) than for *Colossoma macropomum*, *Oreochromis mossambicus* and for tiger bass (*Terapon jarbua*) (0.74, 4.27, and 2.5 mg L^{-1}, respectively), (JAMES; SAMPATH, 2003; VIJAYAVEL et al., 2006) and higher than that for blue tilapia

(*Oreochromis aureus*) (43.06 mg L^{-1}) (STRAUS, 2003). LC50 differences may have been due to the difference on the sensitivity of test organisms and to the use of hard water (concentrations above 150.0 mg $CaCO_3$) on the tests (RODRIGUES; ALMEIDA, 2011).

There was no *P. mesopotamicus* mortality at 9.50 mg L^{-1}; 22 to 33% mortality at 10.0 mg L^{-1}; 70.0 to 88.1% at 11.0 mg L^{-1}; 89.9 to 100.0% at 11.5 mg L^{-1}; and 100.0% at 12.0 mg L^{-1} (Figure 1d).

Copper sulfate was extremely toxic for *P. caudimaculatus* due to LC50 value below 0.1 mg L^{-1}; highly toxic for *H. eques* and *B. rerio* (LC50 between 0.1 to 1.0 mg L^{-1}) and lightly toxic for *P. mesopotamicus* due to LC50 between 10.0 to 100.0 mg L^{-1} (ZUCKER; JONHSON, 1985).

Copper sulfate is widely used as fungicide/insecticide, on algae and macrophyte infestation control and as fish farming therapy agent with 0.5 to 2.0 mg L^{-1} pulverizations (BOYD; MASSAUT, 1999; TAO et al., 2002). Due to its many uses, copper sulfate may present hazard for non-target organisms, causing direct changes on periphyton communities and fish habitats (ARUN et al., 2005; CRUZ et al., 2008), such as for *P. caudimaculatus*, which inhabits creeks and lentic streams and is the most sensitive species to copper sulfate on this study, conversely to *P. mesopotamicus*, which was the most resistant (Table 1). Despite its resistance, it should be noted that the last one is an economically important species, besides being reophylic (ROMAGOSA; NARAHARA, 2002) and being affected by a xenobiotic toxicity, should not generate offspring.

As for the environmental risk classification, copper sulfate belongs to a high adverse effect risk class for *P. caudimaculatus*, *H. eques* and *B. rerio*, considering the estimated environmental coefficient values (Q) that are below 0.1 mg L^{-1}. However, it was classified as moderate adverse effect risk for *P. mesopotamicus*, due to 0.19 mg L^{-1} coefficient Q (URBAN; COOK, 1986).

Conclusion

Guppy (*P. caudimaculatus*), zebrafish (*B. rerio*) and mato grosso (*H. eques*) have wide applicability in copper characterization studies and ecotoxicological assessment due to their high sensitivity to copper sulfate.

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