JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 37, No. 3
September, 2006

Toxicity and Sublethal Effects of Potassium Permanganate in Tambaqui (*Colossoma macropomum*)

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Tambaqui (*Colossoma macropomum*) is a species that has shown promise in different intensive fish farming systems, including nurseries, streams, and cages (Merola and Souza 1988; Andrade et al. 1993; Chellapa et al. 1995; Arbeláez-Rojas et al. 2002).

The parasites most frequently found on tambaquis cultured in cages are monogeneans *Anacanthorus spathulatus* and *Linguadactyloides brinkmanni* (Varella et al. 2003). High infestations of these monogeneans on tambaqui gills can impede gas exchange on the surface of the gill lamellae (Thatcher 1981).

A therapeutic chemical widely used in fish production to control monogenean parasites is potassium permanganate ($KMnO_4$) (Kabata 1985). $KMnO_4$, an oxidizing agent, has been used for many years to prevent gill parasites and bacterial and fungal infections (Kabata 1985; Scott 1993).

The toxicity of potassium permanganate has been determined for several fish species (Marking and Bills 1975; Tucker 1987; Bills et al. 1993), and several factors, including pH, total alkalinity, total hardness, and chemical oxygen demand (COD), have been found to affect the toxicity of this chemical to fish (Tucker 1987; Straus 2004).

Marking and Bills (1975), Tucker (1987) and Straus (2004) suggested that the toxicity of $KMnO_4$ to channel catfish (*Ictalurus punctatus*), rainbow trout (*Oncorhynchus mykiss*) and hybrid striped bass (*Morone chrysops* × *M. saxatilis*) is related to water hardness, pH, and COD. Information about the physiological effects of exposure to sublethal concentrations of $KMnO_4$ is, however, limited. For channel catfish, it has been observed that stress indicators (plasma cortisol, glucose, chloride, and osmolality) were modified when the fish was exposed to 2.19 mg/L concentrations of $KMnO_4$ for 36 h (Griffin et al. 2002). Such information is important to assist in evaluating the risks of using this chemical to treat parasitic diseases in tambaquis.

The objective of this study is to determine the toxicity of potassium permanganate in tambaquis (*C. macropomum*) and to find out some toxic effects of its sublethal concentrations by analyzing physiological parameters.

Materials and Methods

Tambaqui juveniles (standard length of 11.80 ± 0.05 cm and weight of 59.10 ± 1.34 g; average ± SE) were acquired from Santo Antônio farm (Rio Preto da Eva, AM, Brazil). The fish were immediately transported to the experimental site of Embrapa Western Amazon, where they were acclimatized in ponds of 200 m^3. The fish were fed with commercial tambaqui feed (36% of crude protein) until satiation, 6 d a week, during the preexperimental period. The feeding of animals was suspended 24 h before and during the toxicity tests.

Glass aquaria containing 40 L of water under semistatic conditions and constant aeration were used for the tests. The tests were carried out with three replicated group of six animals for each treatment, using a completely random design. After a 24-h acclimatization period, the groups of fish were exposed to 0, 6.5, 7.0, 8.0, 9.0, 10.0, 11.5, and 13.0 mg/L of $KMnO_4$ (concentrations chosen based on preliminary tests).

The physiochemical parameters of water in the aquariums were monitored during the toxicity tests. The pH values (7.16 ± 0.44) were obtained using a pH meter YSI Environmental Model pH100 (Yellow Springs Instrument Co., Yellow Springs, OH, USA). Temperature (26.70 ± 0.11 C) and dissolved oxygen (7.09 ± 0.07 mg/L)

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were taken using a YSI Model 55 portable oxygen meter (Yellow Springs Instrument Co.). Total alkalinity (10.63 ± 1.60 mg/L $CaCO_3$) and total hardness (15.68 ± 1.20 mg/L $CaCO_3$) were determined by titration. Total ammonia (0.98 ± 0.19 mg/L) was determined by endophenol method (APHA 1989) and the biochemical oxygen demand (8.40 ± 2.81 mg/L) by 5d-BOD method (APHA 1989). The COD (8.46 ± 2.82 mg/L) followed the procedures described by APHA (1989) with the proposed modification of Boyd and Tucker (1992).

Experiments for exposure to sublethal concentrations of $KMnO_4$ were carried out for 96 h with three replicated groups of six fish (standard length of 11.68 ± 0.05 cm and weight of 52.27 ± 1.01 g; average ± SE) for each aquarium treated at 0 and 4.30 mg/L $KMnO_4$ (50% of the lethal concentration; LC_{50}). Three water parameters were measured: pH, 7.39 ± 0.07; temperature, 25.67 ±0.13 C; and dissolved oxygen, 7.66 ± 0.11 mg/L.

Fish were anesthetized (100 mg/L benzocaine; Gomes et al. 2001) and blood collected from the caudal vein of each of the six fish in the replications. The hematocrit (Ht), using the method of Goldenfarb et al. (1971), and hemoglobin concentration (Hb) following the cyanmethemoglobin method (Kampen and Zijlstra 1964) were determined, and red blood cells (RBCs) were counted under the light microscope using a Neubauer hemocytometer after blood dilution with formol citrate. Hematimetric equations (Acerete et al. 2004) were used to determine the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC). Glucose levels were measured with a blood glucose monitor (Advantage™; Boehringer Mannheim GmbH, Mannheim, Germany). Plasma chloride levels were determined using a commercially available kit (colorimetric chloride; Doles®, Goiás, Brazil), and a flame photometer (Micronal model B462) was used to determine sodium (Na^+) and potassium (K^+) levels.

The LC_{50} value of $KMnO_4$ was determined by the Trimmed Spearman–Karber method (Hamilton et al. 1977), using the mean mortality of the fish. Results were expressed as mean ± SEM. Significant differences among means from physiological parameters of control versus animals exposed to sublethal concentrations of $KMnO_4$ were established by Student's t test ($P < 0.05$) (Zar 1999).

Results and Discussion

Mortality rates in tambaquis were directly related to higher concentrations of $KMnO_4$ in the water (Fig. 1). The higher mortality rates occurred within 24 h with few changes after 24 h, which conform to the findings of Marking and Bills (1975). The tambaquis exposed to concentrations above 6.5 mg $KMnO_4$/L became agitated in the first hours of exposure to the chemical, with an intense mucus excretion and alterations in the opercular movements. After 24 h of exposure, the tambaquis showed signs of lethargy and loss of equilibrium. The same changes in behavior were observed by Straus (2004) for hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) exposed to $KMnO_4$ for 24 h.

LC_{50}-96 h of $KMnO_4$ for tambaquis was calculated to be 8.60 mg/L (Fig. 1). This value indicated that the tambaqui is more tolerant of $KMnO_4$ than channel catfish (*I. punctatus*), striped bass (*M. saxatilis*), common carp (*Cyprinus carpio*), and American eel (*Anguilla rostrata*) (LC_{50}-96 h of $KMnO_4$ were 4.5, 4.0, 3.05, and 7.52 mg/L, respectively) (Hughes 1971; Hilton and Eversole 1980; Tucker 1987; Das and Kaviraj 1994).

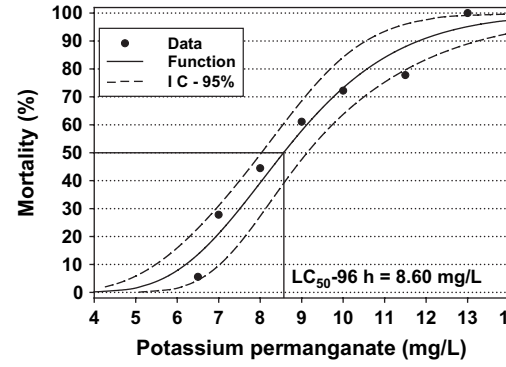

FIGURE 1. *The effect of potassium permanganate concentration on the mortality of Colossoma macropomum in 96 h.*

TABLE 1. *Physiological parameters of tambaqui,* Colossoma macropomum*, after exposure to sublethal concentration of $KMnO_4$ for 96 h.*

Parameters	Potassium permanganate concentration	
	0 mg/L	4.30 mg/L
Ht (%)	31.89 ± 1.15	31.92 ± 1.71
Hb (g/dL)	8.65 ± 0.23	8.76 ± 0.31
RBC (10^6/mm^3)	1.74 ± 0.12	1.75 ± 0.08
MCV (μm^3)	161.49 ± 17.93	150.13 ± 12.96
MCH (pg)	49.73 ± 3.58	52.61 ± 2.84
MCHC (%)	27.50 ± 0.86	27.98 ± 0.72
Glucose (mg/dL)	50.90 ± 3.90	63.50 ± 3.44*
Chloride (mmol/L)	117.24 ± 4.81	136.11 ± 4.00*
Sodium (meq/L)	126.92 ± 2.56	136.10 ± 2.54*
Potassium (meq/L)	5.48 ± 0.32	4.89 ± 0.36

Ht = hematocrit; hb = hemoglobin concentration; RBC = red blood cell; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration. Values are means (±SEM) of six fish of each aquarium. Means followed by asterisks are significantly different at $P < 0.05$ by Student's t test.

For tambaquis, significant alterations were not observed in the Ht, Hb, RBC, MCV, MCH, and MCHC values after 96 h of exposure to the sublethal concentrations (4.30 mg/L) of $KMnO_4$ (Table 1). However, a significant increase in glucose, chloride, and plasmatic sodium levels was observed in tambaquis exposed to 4.30 mg/L of $KMnO_4$ (Table 1); a decrease in the plasmatic potassium values, although not significant, was also observed for fish exposure to $KMnO_4$ (Table 1). Griffin et al. (2002) found changes in the glucose and plasmatic chloride levels in channel catfish exposed for 36 h to 2.19 mg/L of $KMnO_4$, which suggests a disturbance in the ionic balance of both species.

Potassium-permanganate-treated tambaquis showed moderate increases in the secondary stress indicators: glucose, Na^+, and Cl^- and a decrease in K^+. Hyperglycemia in fish under stress conditions results from the rapid mobilization of energy reserves, which makes it possible for the animal to survive through the period of disturbance (Morgan and Iwama 1997). According to Mazeaud and Mazeaud (1981), disturbances in concentrations of Na^+ and Cl^-, in stress situations, result from increased adrenaline level in the blood.

It is important to determine the toxicity of $KMnO_4$ in tambaquis and establish the tolerance limits on using this chemical because 2–5 mg/L long baths are frequently used to treat parasitic diseases in fish (Kabata 1985; Scott 1993). The results of the present study show that a sublethal concentration of $KMnO_4$ (4.30 mg/L) activates some secondary indications of stress, provoking alterations in the homeostasis of the tambaquis. Exposure of up to 4.30 mg/L of $KMnO_4$ may have much more deleterious effects on the fish.

Acknowledgments

This study was supported by the "TANRE/FINEP/FUCAPI" and "Tanque-rede BASA" Projects. The authors are thankful to José Pereira and Márcia Pessoa, from Embrapa Western Amazon, for their assistance in the experiments and water quality analyses. We also thank M. V. Garcia for design of graphics. A. L. F. d. S., L. D. d. A., C. R. S., and F. R. B. are recipients of student fellowship from the National Council for Scientific and Technological Development (CNPq) and Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM).

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