HISTOPATHOLOGICAL AND HEMATOLOGICAL EVALUATION OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) EXPOSED TO A TOXIC CYANOBACTERIUM (*MICROCYSTIS AERUGINOSA*)

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Abstract. Sub-chronic exposure of Nile tilapia (*Oreochromis niloticus*) to the toxicity of *Microcystis aeruginosa*, a toxic cyanobacterium, was investigated with emphasis on the hepatic histopathology and hematological effects. Histopathology revealed abnormalities of the fish livers exposed to *M. aeruginosa* in their diet showing hemorrhage, congestion, vacuolization, leukocyte infiltration, pyknotic cells and irregular arrangements of hepatocytes. The degree of tissue damage was found to be concentration dependent. An alteration in lymphocyte numbers was also detected in the fish exposed to *M. aeruginosa*.

INTRODUCTION

Microcystis spp are cyanobacteria which grow in marine, brackish and fresh water (Fleming and Stephen, 2001). Besides being a source of nutrition for aquatic life, many of them are capable of producing toxins. *Microcystis aeruginosa* is a toxic species which produces microcystins (Carmichael, 1994), a hepatotoxin and tumor promotor (Fischer *et al*, 2000). Pathologic lesions of the liver have been reported in rats exposed to sublethal doses of microcystin-LR (Guzman and Solter, 2002) and exposure to high levels of this toxin can lead to hepatocyte necrosis and hemorrhage, with severe cases resulting in death (Bhattacharya *et al*, 2002). Since blooming of toxic cyanobacteria has been detected in many ponds and water reservoirs in Thailand (Prommana *et al*, 2001; Peerapornpisal *et al*, 2002), a risk assessment to those aquatic organisms is needed. The aim of this study was to investigate the hepatotoxicity *M. aeruginosa* had on Nile tilapia (*Oreochromis niloticus*). The hematological effects were also investigated.

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MATERIALS AND METHODS

M. aeruginosa

Colonies of cyanobacteria dominated by *M. aeruginosa* forming blooms on the surface of Huay Yuak reservoir, Mueang District, Chiang Mai, Thailand were collected during April-June, 2005. *M. aeruginosa* was identified and isolated for fish diet preparation.

Animals

The Nile tilapia (*O. niloticus*) used in the experiment weighed 10.07±0.03 g. They were obtained from the Faculty of Fisheries Technology and Aquatic Resources, Mae Jo University. During a 7-day-acclimatization period, the fish were fed twice daily with a commercial fish diet. The water temperature range was 25-27ºC and the pH was 7.0-7.2. Chlorine residual was below detection limits.

Experimental design

The fish were randomized into 3 groups (20 each). Two experimental groups were fed a standard fish diet mixed with *M. aeruginosa* at proportions of 25% and 50%, respectively. The control fish received only the standard fish diet. The dialy quantity of food was 3% of the body weight for each group. Three replicates per group were conducted. At the end of the 60-day

experimental period, the fish were anesthetized with MS222 (tricaine methansulphonate) for histological and hematological investigation. Blood was collected from the caudal vein with a heparinized capillary tube. Hematocrits (PCV) and differential white blood cells were determined using standard methods (Dacie and Lewis, 1984). Immediately after each fish was bled, the liver was removed and fixed in Bouin's solution for histopathological examination using a routine histological technique with hematoxyline and eosin for staining (Brancroft and Cook, 1994).

Statistics

Means and standard deviations were calculated. The significances of the differences were analyzed with the Student's *t* -test

RESULTS

Fig 1 shows there were no histological change in the livers of the control fish. In contrast, the fish that received *M. aeruginosa* at 25% and 50% proportions of their daily diet showed severe liver damage and the degree of hepatic tissue damage increased in a concentration-dependent manner. The histopathological changes found in the treated fish livers included irregular arrangements of hepatocytes and dilation of the central

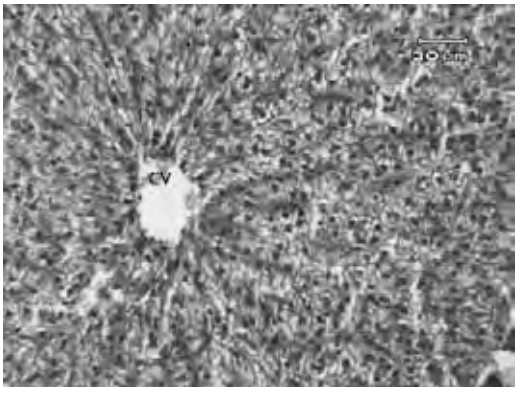

Fig 1- H & E stained section of the liver of a control fish (*O. niloticus*) showing the normal central vein (CV) and regular arrangement of the hepatic sinusoids.

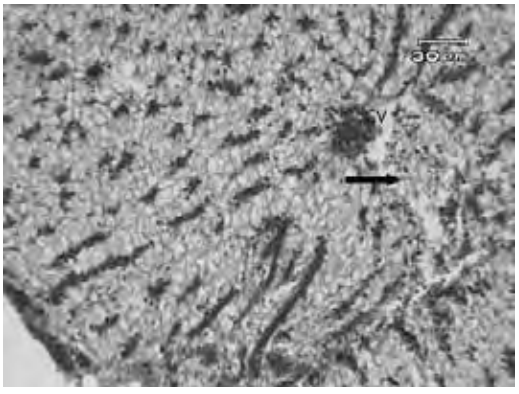

Fig 2- H & E stained section of the liver of *O. niloticus* exposed to *M. aeruginosa* showing dilation of the central vein (CV) and blood congestion (arrow). Notice the irregular arrangement of the hepatic sinusoids.

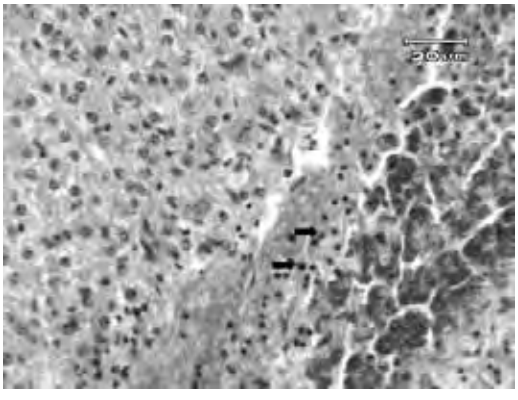

Fig 3- Leukocyte infiltration detected in hepatic tissue of *O. niloticus* exposed to *M. aeruginosa* (arrow) (H & E stain).

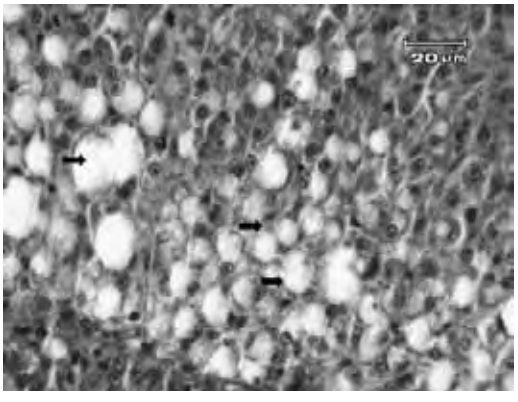

Fig 4- Numerous vacuolated cells (arrows) distributed in the hepatic tissue of *O. niloticus* exposed to *M. aeruginosa* (H & E stain).

Table 1
Defferential white blood cell counts in *O. niloticus* exposed to 25% and 50% *M. aeruginosa* for 60/days compared to controls.

Groups	White blood cells (Cells/ml^3)				
	Monocytes	Lymphocytes	Neutrophils	Eosinophils	Basophils
Control	12,968.9 ± 7,074.0[a]	76,929.4 ± 8,946.7[a]	26,232.6 ± 1,128.8[a]	884.2 ± 1,148.3[a]	294.7 ± 589.5[a]
25% *M. aeruginosa*	26,340.7 ± 14,207.8[a]	92,568.6 ± 20,937.7[a]	30,354.5 ± 1,229.0[a]	501.2 ± 777.3[a]	501.2 ± 772.3[a]
50% *M. aeruginosa*	26,935.2 ± 11,163.2[a]	48,312.4 ± 12,485.1[a]	48,882.2 ± 3,270.7[a]	3,277.9 ± 3,277.1[a]	855.1 ± 1,282.6[a]

[a] = significant differences at $p \leq 0.05$

vein accompanied by blood congestion (Fig 2). Leukocyte infiltration and vacuolization were also detected in the hepatic tissues of the treated fish (Figs 3 and 4). The results of the hematological examinations are presented in Table 1. A significant decrease in lymphocyte counts was found in the fish receiving *M. aeruginosa*, compared to controls. The PCV results in the subjects did not differ from those in the control fish (data not shown).

DISCUSSION

Along with worldwide water eutrophication, cyanotoxins are of environmental concern and fish mortality is associated with *M. aeruginosa* blooms. The histopathological finding in *O. niloticus* treated with *M. aeruginosa* indicate that cyanobacteria produce potent hepatotoxins which alter the architecture of hepatocytes and may impair their function. The congested blood vessels indicated clear evidence of hemorrhages. Although the PCV values in the treated fish were not different from those of controls, the decrease in lymphocyte number and leukocyte infiltration in hepatic tissue also supports the evidence of tissue inflammation. Histopathological and hematological alterations in *O. niloticus* exposed to *M. aeruginosa* may lead to the loss of fish homeostasis and place them under stress. The elevation of gluthatione (GSH) and malodialdehyde (MDA), important biomarkers of oxidative stress, was detected in silver carp (*Hypophthalmichthys molitrix* Val.) grown in *M. aeruginosa* water blooms (Bláha *et al*, 2004). The vacuolization of hepatocytes may also indicate an imbalance in the rate of synthesis of substance(s) in the cells and release into the systemic circulation. Inhibition of protein phosphatase in the liver of rainbow trout exposed to microcystin-LR has also been reported previously (Fischer *et al*, 2000). Since fish serve as a protein source for humans, the accumulation of hepatotoxins from *M. aeruginosa* in their organs may lead to serious problems for fish consumers.

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