

Changes of hematological parameters in *Prochilodus lineatus* (Pisces, Prochilodontidae) exposed to sublethal concentration of cypermethrin

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Abstract: Freshwater fish *Prochilodus lineatus* were exposed to sublethal concentrations of cypermethrin (0.3 and 0.6 µg/l) for 2, 5 and 8 days. It was observed that with the increase of exposure time total erythrocyte (RBC), hemoglobin (Hb), hematocrit (Ht) and mean corpuscular hemoglobin concentration (MCHC) values decreased but mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values increased. These reports indicate that hematological parameters, may be useful as a diagnostic test for cypermethrin exposure in aquatic organisms.

Key words: *Prochilodus lineatus*, Cypermethrin, Hematology, Toxicity

Introduction

Alteration of hematological parameters of fish has been associated with their physiological state and may be induced directly by genotoxic compounds. Clinical chemistry analyses are faster and cheaper than analytical chemistry. Since the variation of these parameters have been demonstrated to be sensitive to sublethal concentration of different toxic agents, they can be used for detecting pollutants exposure in the environment (National Research Council, 1989). Pyrethroid insecticides are commonly used to control insect pests and represent about 30% (Amdur *et al.*, 1991) of world insecticide consumption. Their mean life in water is two weeks but they get rapidly absorbed by aquatic organisms with a significant toxicity (Rand and Petrocelli, 1985; Hellawell, 1989; Thomson, 1992; Phillip and Rajasree, 1996). Also, some investigations suggest that they can be absorbed by fish gills even at very low concentration in water (Clark *et al.*, 1985).

Some authors (Reddy and Bashamohideen, 1989; Chauhan *et al.*, 1994; Agarwal and Chaturvedi, 1995; Nath, 1996) have reported a decrease in hematocrit, hemoglobin and red blood cells values of some fish after their exposure to insecticides. Saxena and Seth (2002) showed a significant change in the hematology of the common fresh water fish, *Channa punctatus* on exposure. The information suggests that hematological parameters could be used as potential biomarkers of pyrethroid insecticides.

The aim of present investigation was to study the changes induced by sublethal cypermethrin exposure on some hematological parameters of *P. lineatus*, a widely distributed neotropical fish that represents approximately 60% of ichthyomass of the middle Paraná river (Argentina).

Materials and Methods

Specimens were obtained from pristine environment near Santa Fe city (Argentina). Experimental fish (130-220 mm of length) were acclimatized to laboratory conditions for few days to reduce stressful activity. They were maintained in test tanks (25 litre volume) with aerators at constant temperature of 25°C and 12 hr photoperiod during the experiments.

The experimental sublethal concentrations were 0.3 and 0.6 µg/l of cypermethrin, and the exposure time was of 2, 5 and 8 days. Experiments were performed under semistatic conditions and aquaria water was changed every 24 hr with the appropriate pesticide amount. Groups of ten fish were exposed to each concentration and exposure period (a total of 60 specimens). At the same time, ten specimens, used as controls, were kept in clean water.

At the end of each exposure time blood was collected by dissection of caudal peduncle (Roberts, 1981 ; Reichenbach-Klinke, 1982) with a heparinized micropipette. Hematocrit (Ht) was determined by the microhematocrit technique using capillary tubes by centrifugation at 12,000 rpm for 5 min. Hemoglobin (Hb) was determined spectrophotometrically (540 nm) using cyanomethemoglobin method and expressed as g/100ml. Red blood cell counts (RBC) were made in Neubauer chamber with Hayem dilution solution.

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Ranzani - Paiva (1991).

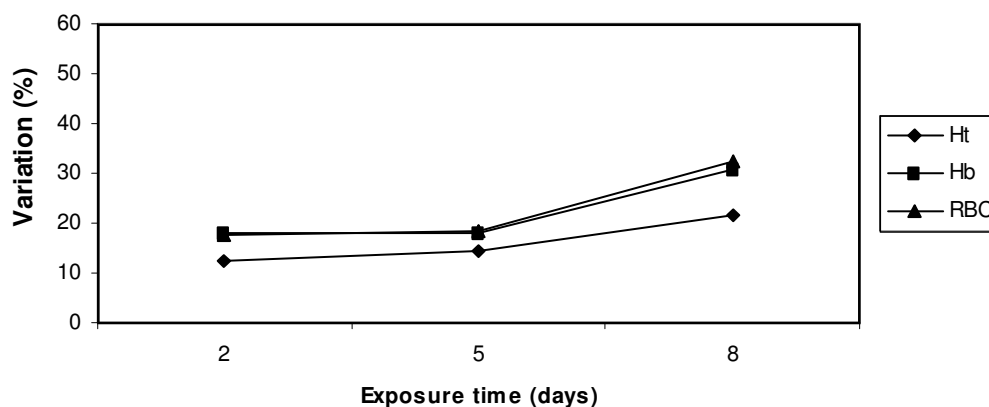
One way analysis of variance (ANOVA) and a Tukey HSD comparison post tests were applied in statistical evaluation of the results (Zar, 1996).

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Table - 1: Mean hematological parameters of *Prochilodus lineatus* exposed to sublethal concentration (0.3 and 0.6 µg/l) of cypermethrin

Parameter	Control	Concentration cypermethrin	Exposure (days)		
			2	5	8
RBC (10 ³)mm ³	1,403±167	0.3µg/l	1,1585±156	1,142±125	950±119
Ht (%)	35±3	0.6µg/l	739±249	892±105	722±100
Hb (g/100ml)	7±1	0.3µg/l	30±2	30±6	27±3
MCV (µm ³)	248±25	0.6µg/l	21±7	23±2	20±2
MCH (µµg)	51±3	0.3µg/l	6±1	6±1	5±0.5
MCHC (%)	21±3	0.6µg/l	4±2	5±0.3	4±0.5
		0.3µg/l	264±28	60±48	288±36
		0.6 µg/l	257±95	264±19	274±7
		0.3µg/l	52±10	52±5	53±7
		0.6µg/l	53±20	52±2	53±2
		0.3µg/l	20±3	20±4	18±1
		0.6µg/l	18±7	20±1	19±1

**Fig. 1:** Hematological parameters variation in *Prochilodus lineatus* exposed to 0.30 µg/l of cypermethrin

Results and Discussion

The normal values of hematological parameters in the control group can be seen in Table 1. Quantitative changes of hematological parameters in fish blood exposed to both sublethal concentration of cypermethrin (0.3 and 0.6 µg/l) after 2, 5 and 8 days are also given in Table 1. Significant decrease in Ht, Hb, RBC and MCHC levels were observed, whereas the MCV and MCH levels showed an increment, with increase in the concentration of cypermethrin.

Erythrocyte counts of fish exposed to 0.3µg/l, indicated a 32.3% decrease as compared to control values at the end of the experiment. Reduction in hemoglobin (30.7%) was accompanied by lowest hematocrit value (21.6%) (Fig. 1). At 0.6 µg/l concentration the reduction of RBC counts (48.5%), hemoglobin (47.2%) and hematocrit (43.0%) was observed after 8 days of exposure. In blood parameters, a significant difference ($p < 0.05$) between control and each concentration respectively, was found. Fish blood reflects pathophysiological status and its parameters are important in diagnosis of the structural and functional status of fish exposed to toxicants (Sampath *et al.*,

1998). Hematological values measured in the present study in control fish are in agreement with those reported by Parma de Croux (1994) as normal values. Hematological findings after exposure to cypermethrin revealed toxic manifestations with evident effects after 8 days.

Pyrethroids belong to a lipophilic insecticides class which are easily degraded in natural environments, but they prove to be toxic for fish due to the poor ability of the fish to metabolise these compounds (Demoute, 1989). In fish, the toxic compounds gain access via the gills directly into blood stream, persist there and cause alterations in hematology. A decrease in important blood parameters has been reported during exposure to various pesticides in fishes (Reddy and Bashamohideen, 1989; Chauhan, *et al.*, 1994; Agarwal and Chaturvedi, 1995; Nath, 1996; Saxena and Seth, 2002). Changes in hematological parameters might have been brought about by cypermethrin as an anemic condition due to decreased synthesis of Hb and RBC number in bone marrow cells. Hematology of *P. lineatus* has not been much documented, so this paper would provide an important contribution to the knowledge of the specimen constitution and

variation. Finally, hematological alterations may be used for diagnosis in the field to assess pollution related pathophysiological alterations in fish.

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