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Research Article

Behavioural Abnormalities of *Cyprinion watsoni* on Exposure to Copper and Zinc

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Abstract: *Cyprinion watsoni* were exposed to three treatments of copper (0.03, 0.06, 0.12 mg/L) and three treatments of zinc (0.5, 3.0, 7.0 mg/L) for a period of one week to allow behavioural changes to be observed. None of the treatments caused mortality. Treatment with 0.03 mg Cu/L caused little change in fish behaviour; however, 0.06 mg Cu/L caused increased swimming activity and breathing movements. The highest treatment of copper (0.12 mg Cu/L) caused lethargy and loss of equilibrium in *C. watsoni.* Similarly, the lowest concentration of zinc (0.5 mg/L) caused no visible change in fish behaviour; however, with 3.0 mg Zn/L, fish tended to swim faster and showed an increased breathing rate. The highest concentration of zinc (7.0 mg/L) resulted in increased lethargy and a tendency of loss of equilibrium in fish.

Key Words: *Cyprinion watsoni*, Copper, Zinc, Behavioural abnormalities.

Bakır ve Çinko'ya Maruz Kalan Balıklar (*Cyprinion watsoni*)'da Davranış Anormallikleri

Özet: Bakır (0,03; 0,06; 0.12 mg/L) ve Çinko (0,5; 3,0; 7.0 mg/L)'ya maruz kalan balıklarda (*Cyprinion watsoni*) bir hafta süresince davranış değişimleri incelenmiştir. Uygulanan dozlar mortaliteye sebep olmamıştır. Bakırın uygulanan en düşük dozu (0,03 mg/L) balıkların davranışlarında çok az değişim göstermiş fakat, ikinci dozu (0,06 mg/L) balıkların yüzme aktiviteleri ve nefes alma hareketlerinde artış göstermiştir. Bakırın en yüksek dozu (0,12 mg/L) *C. watsoni*'de halsizliğe ve denge bozukluğuna neden olmuştur. Çinkonun en düşük dozu (0,5 mg/L) balıkların davranışlarında gözle görülebilecek herhangi değişime neden olmamıştır, fakat ikinci dozda (3,0 mg/L) balıklar, hızlı yüzme ve soluma oraanında artış göstermişlerdir. Çinkonun en büyük dozu (7,0 mg/L) balıklarda halsizliğe ve yüzmede dengesizliğe sebep olmuştur.

Anahtar Sözcükler: *Cyprinion watsoni*, Bakır, Çinko, Davranış anormallikleri.

Introduction

Fish constitute a valuable commodity from the standpoint of human consumption. Aquatic pollution undoubtedly has direct effects on fish health and survival. Heavy metals are regarded as serious pollutants of the aquatic environment because of their environmental persistence and tendency to be concentrated in aquatic organisms (1). Most heavy metals released into the environment find their way into the aquatic phase as a result of direct input, atmospheric deposition and erosion due to rainwater. Therefore, aquatic animals may be exposed to elevated levels of heavy metals due to their wide use for anthropogenic purposes (2). Copper and zinc are essential heavy metals (3). Essential metals play an important role in various biological processes including oxidative phosphorylation, gene regulation and free radical homeostasis as essential cofactors (4). However, when their concentration exceeds metabolic requirements, they become harmful (5). Behavioural toxicology is a tool for hazard assessment of water pollution (6,7). Behavioural changes in animals are indicative of internal disturbances of the body functions such as inhibition of enzyme functions (8), impairment in neural transmission (1), and disturbances in metabolic pathways (9,10). The development of response criteria in animals varies from detailed physiological measurements to whole animal response, especially preference/avoidance behaviour (11). The elimination of aquatic animals by small insidious physiological or behavioural changes has been reported to be more serious than a massive fish kill, since it is less likely to be observed and corrected (12). The present study was conducted to investigate the

behavioural abnormalities in cyprinid fish, *Cyprinion watsoni*, on exposure to copper and zinc treatments. The observed changes are discussed.

Materials and Methods

Cyprinion watsoni were captured by cast net from Ramli stream and transported to the experimental fish laboratory of the Department of Biology, Quaid-i-Azam University, Islamabad (Pakistan). They were allowed to acclimatize to the laboratory conditions for at least one week in stocking tanks, already aerated with air pumps for oxygen supply. The physiochemical properties of the water used for the experiments are given in Table 1. Water temperature varied according to the ambient laboratory conditions but averaged 18 °C. A photo-period of 12L: 12D was maintained with fluorescent tubes. The fish were fed daily on commercial fish feed. In order to investigate the behavioural abnormalities in *C. watsoni*, three concentrations of each heavy metal (copper: 0.03, 0.06, 0.12 mg/L; zinc: 0.5, 3.0, 7.0 mg/L) were selected. Before treatment, the fish (average standard length 10.8 ± 2.3 cm and average body weight 11.3 ± 3.5 g) were divided into six groups comprising 10 animals each, placed in individual glass aquaria of 50 litre capacity and used for treatment. An untreated group of 15 fish was maintained in a separate tank as a control group. The desired concentrations of the metals were achieved using copper sulphate ($CuSO_4$. $5H_2O$) and zinc sulphate ($ZnSO_4$. $7H_2O$). The water in the tank was changed daily with dechlorinated water containing the same test concentrations of each metal. The experiments lasted for one week. The control and experimental fish were not fed during this period to avoid any contamination. Any change in behaviour was recorded carefully.

Table 1. Physio-chemical properties of the water used in the laboratory.

Parameter	Unit/Value
Temperature	18 °C
pH	7.85
Electric Conductivity	250 µm/cm
Total Dissolved Solids	187.50 mg/L
Hardness	150 mg/L
Calcium	60.12 mg/L
Magnesium	21.93 mg/L
Bicarbonates	274.6 mg/L
Chlorides	34.7 mg/L
Sulphates	23.0 mg/L

Results and Discussion

The lowest treatment of copper (0.03 mg/L) caused little change in fish behaviour, which may be the avoidance behaviour of animals to pollutants in the receiving water. A significant response to 0.06 mg Cu/L was noted: the swimming activity and breathing rate of the fish increased. In the highest treatment (0.12 mg Cu/L) fish became lethargic and lost equilibrium. Zinc, at the lowest treatment (0.5 mg/L), did not cause any visible change in fish behaviour. However, in 3.0 mg Zn/L, the fish tended to swim faster and showed an increased breathing rate. In the highest treatment of zinc (7.0 mg Zn/L), the fish showed a lethargic response and lost equilibrium (Table 2). It is evident from the observed behavioural changes in *Cyprinion watsoni* that both heavy metals have the same pattern of effects. The lowest treatments of both copper and zinc did not cause any significant change in fish behaviour. The second treatments resulted in increased swimming activity and breathing rate and the highest treatments caused lethargic conditions and loss of equilibrium in exposed animals.

Table 2. Behavioural parameters determined in *Cyprinion watsoni*, on exposure to copper and zinc treatments. (+) indicates an increase.

Parameter	Copper (mg/L)			Zinc (mg/L)		
	0.03	0.06	0.12	0.5	3.0	7.0
Locomotion	+	+			+	
Breathing rate		+			+	
Lethargy			+			+
Loss of equilibrium			+			+

Behavioural abnormalities in various fish species on exposure to heavy metals have been reported by several researchers. Ghatak and Konar (13) observed frequent surfacing with irregular opercular movement and loss of equilibrium in *Tilapia mossambica* when exposed to cadmium. Similarly, hyperactivity, erratic swimming, and loss of equilibrium in Brook trout, *Salvalinus fontinalis*, in response to lead treatment have been reported by Holcombe *et al.* (14). The loss of equilibrium, frequent surfacing and sinking, burst of erratic swimming and

gradual onset of inactivity in Rainbow trout, *Salmo gairdneri*, on mercury exposure, have also been determined (15). Golden shiner, *Notemigonus crysoleucus*, when exposed to 5 ppm copper piped at the surface, became restless, failed to school, became sluggish and finally lost equilibrium (16). The locomotor activity of Bluegill sunfish, *Lepomis macrochirus*, treated with 0.04, 0.08 and 0.4 ppm copper, decreased to 67, 61 and 44% respectively (10). Lethargic response and frequent surfacing along with gulping of air in exposure to 0.25 ppm copper were observed in *Heteropneustes fossilis* (17). *Etroplus maculatus* on exposure to copper, mercury and selenium showed irregular erratic swimming, frequent surfacing, gulping of air, revolving, convulsions, and accelerated ventilation with rapid arhythmic opercular and mouth movements (1).

Behavioural abnormalities have been attributed to nervous impairment due to blockage of nervous transmission between the nervous system and various effector sites (18), the enzyme dysfunctions that may cause paralysis of respiratory muscles and/or depression of respiratory centre (8), and disturbances in energy pathways which result in depletion of energy (10).

In the case of the present study, the small change in fish behaviour in 0.03 mg/L copper may be the avoidance behaviour of the animal to metal. Fish exposed to concentrations of metal do not reach the stage of exhaustion, rather they accomodate and adapt to the stressor (19). Increase in swimming activity with increased breathing rate, lethargic condition and loss of equilibrium in *Cyprinion watsoni* exposed to copper (0.06 and 0.12 mg/L) and zinc (3.0 and 7.0 mg/L) are attributed probably to the disturbances in the metabolic reactions resulting in the depletion of energy. It is possible that animals which have higher metabolic activity could require higher levels of oxygen and thus would have a higher respiration or breathing rate (20). Lethargy and loss of equilibrium may be due to depletion of energy in the body of the animal. A drop in the metabolic production of cellular energy in the form of high-energy bond in Bluegill sunfish, *Lepomis macrochirus*, on exposure to copper has been reported (10). Decreased and increased glucose levels on cadmium exposure have been reported in *Hetropneustes fossilis* and *Labeo rohita* respectively (9). The varying levels of blood glucose are indicative of abnormal carbohydrate metabolism and are possibly the result of impaired hormonal control (21). The release of corticosteroid hormones in Sockeye salmon, *Oncorhyncus nerka*, when treated with copper has been reported (19). In the present study, the decrease and increase in glucose levels in *Cyprinion watsoni* treated with copper and zinc (published elsewhere, 22) reveal the impairment of the carbohydrate metabolism, which resulted in the depletion of energy, causing lethargy and loss of equilibrium in fish.

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