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# Accumulation of Copper in Different Tissues and Changes in Oxygen Consumption Rate in Indian Flying Barb, *Esomus danricus* (Hamilton-Buchanan) Exposed to Sub-lethal Concentrations of Copper

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**Abstract**

In the present work, the accumulation pattern of sub-lethal doses (0.005, 0.0025 and 0.001 µgl$^{-1}$) of copper (Cu) in different tissues of Indian flying barb, *Esomus danricus* and its influence on the rate of oxygen consumption of this fish at the end of 28 days of exposure were studied. It was found that the pattern of accumulation changed with concentration. Cu concentration increased in all the tissues except bone and brain and the rate of uptake of Cu increased with time in the gill, liver and flesh but remained same in kidney. Treatment of 0.005 and 0.0025 µgl$^{-1}$ of Cu produced significant decline in the rates of oxygen consumption of the fish at all exposure duration, while treatment of 0.001 µgl$^{-1}$ produced similar decline only after 14 days of exposure when compared to control. Higher doses of exposure had more severe effects.

***Keywords****:* Teleost, copper, accumulation, oxygen consumption.

## 1. Introduction

Copper (Cu) is an essential heavy metal that possess the ability to enter and concentrate in various tissues many times higher than the ambient levels and alter oxygen consumption rates of fish (Farkas *et al.*, 2002; De Boeck *et al.*, 1995). Free Cu may catalyze the formation of highly reactive hydroxyl radicals, which can result in oxidative damage to cells (Gaetke and Chow, 2003). Major sources of Cu in aquatic environment are sewage, industrial effluents and pesticides (Palacios and Risbourg, 2006). Fishes have shown to concentrate Cu in their tissues but the metal accumulation capacity is dependent on the assimilation and excretion capacities of species concerned (Rao and Patnaik, 1999). In a study, Gupta (1998) had shown that wetlands like floodplain lakes, marshes and swamps of Barak Valley, in Assam state of India, serve as sinks for heavy metals including Cu. Indian flying barb, *Esomus danricus* (Hamilton-Buchanan), a minnow having food and ornamental value, commonly inhabits such water bodies and are susceptible to Cu which target gill, liver, kidney and other tissues. It would, thus, be interesting to study the accumulation pattern of sublethal doses of Cu in different tissues of *E danricus* and its influence on the rate of oxygen consumption of this fish.

## 2. Materials and Methods

### *2.1. Fish and experimental system*

Fish of similar length (46.77 ± 4.30 mm) and weight (0.86 ± 0.16 g) were collected from unpolluted, freshwater ponds near Assam University campus, Barak valley, South Assam, India (Das and Gupta, 2009). They were acclimatized under laboratory conditions seven days prior to experimentation and commercially available fish food was given *ad libitum* twice daily. Temperature, pH, hardness and dissolved oxygen under laboratory condition were 29°C, 6.8, 30 mg l$^{-1}$ and 5.5 mg l$^{-1}$ respectively. Stock solution of Cu was prepared from $CuCl_2.2H_2O$ (Merck, Germany) and serial dilutions were prepared using chlorine free tap water as per dilution techniques (APHA, 2005). Three sub-lethal concentrations (0.001, 0.0025 and 0.005 µg.l$^{-1}$) of Cu were selected based on 96 hours $LC_{50}$ value of Cu for *E. dandricus* (0.01 µgl$^{-1}$) determined in a prior study (Das and Gupta, 2010).

### *2.2. Metal accumulation study*

Two hundred fish were randomly selected into four groups of 50 fish each. The 50 fish in each group were housed in five bowls, each containing ten animals. Each of the bowls contained 2 liters of water. Bowls of group I, II, III and IV contained 0.005, 0.0025 and 0.001 µgl$^{-1}$ and

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control (tap water without Cu) respectively. 5 bowls of each group were marked as 0, 7, 14, 21 and 28d corresponding to the days of exposure to that particular concentration of Cu. For example, bowl of group I marked '7 d' had 10 fish exposed to 0.005 $\mu gl^{-1}$ that were sacrificed after 7 days of exposure and so on. Cu treatment was carried out twice daily, and on each time, fresh water was used to avoid accumulation of waste and to ensure constant metal concentration. During the study period, dead fish (if any) were removed. During exposure periods, the fish were fed twice daily. Feeding was, however, stopped 24 h before each sacrifice. After the specified duration of exposure, fish were sacrificed and the gill, kidney, liver, bone, flesh and brain were excised, dried, weighed and digested in 5ml concentrated $HNO_3$ to dryness in oven and dissolved in 10 ml distilled water. Analysis for Cu was carried out in a Perkin-Elmer 3110 atomic absorption spectrophotometer (AAS). The detection limit of the instrument was 0.01$\mu gl^{-1}$. The readings were checked with those of standard solutions, and contamination errors were minimised by using blanks, acid washed glass wares, analytical grade reagents and double distilled water. Digestion of samples was based on a modification of the method of Jayakumar and Paul (2006). Statistical significance of the differences in rate of Cu incorporation between $7^{th}$ day and subsequent intervals (14, 21 and $28^{th}$ day) of exposure at different Cu concentrations were made by one-way ANOVA and Tukey Test using SYSTAT 13 software for Windows.

*2.3 Oxygen Consumption*

Three test chambers (each of 3 litre capacity) were marked A, B and C containing 0.005, 0.0025 and 0.001 $\mu gl^{-1}$ of Cu respectively. Each test chamber contained ten fish. At the beginning (0 day), each fish of test chamber A was transferred to respiratory chamber, which was also numbered in accordance with the test chamber and the experiment was run for a period of 1h. After the experiment, the fish was weighed and replaced in its respective test chamber. The same process was repeated for other fishes of the test chamber A (ten replicates) and for 7d, 14d, 21d and 28day. Controls were also run simultaneously in chlorine-free tap water to obtain information on the oxygen consumption of the fish in normal state. Similarly, the process was repeated for fish in test chambers B and C. Respiratory measurements were made by the closed chamber method (Fitch, 1975) and the dissolved oxygen was estimated adopting Winkler's method. Rate of oxygen consumption was measured in ml/hr/100g tissue. Statistical significance of the differences in oxygen consumption between control and exposed fish at different Cu concentrations were made by one-way ANOVA and Tukey Test using SYSTAT 13 software for Windows.

## 3. Results

The initial level (on 0 day) of Cu for all tissues was below detection limit. However, the level of Cu accumulated by each tissue after 28 days of exposure was directly proportional to the exposure concentration of Cu. Cu, irrespective of exposure level, was never detected in the flesh within7 days of exposure and in bone and brain within 28 days of exposure (Table 1).

**Table 1**. Accumulation ($\mu g\ g^{-1}$ dry weight) of Cu in selected tissues of *Esomus danricus* exposed to sub-lethal concentrations over time

| Exposure concentration of Cu ($\mu g\ l^{-1}$) | Tissue | Concentration (µg.g-1) after days of exposure (d) | | | |
|---|---|---|---|---|---|
| | | 7d | 14d | 21d | 28d |
| Control | Gill | BDL | BDL | BDL | BDL |
| | Kidney | BDL | BDL | BDL | BDL |
| | Liver | BDL | BDL | BDL | BDL |
| | Flesh | BDL | BDL | BDL | BDL |
| | Bone | BDL | BDL | BDL | BDL |
| | Brain | BDL | BDL | BDL | BDL |
| 0.001 | Gill | BDL | BDL | 0.0003± 0.0001 | 0.0008± 0.0001 |
| | Kidney | BDL | BDL | BDL | BDL |
| | Liver | BDL | 0.0007± 0.0002 | 0.0015± 0.0001 | 0.0026± 0.002 |
| | Flesh | BDL | BDL | BDL | BDL |
| | Bone | BDL | BDL | BDL | BDL |
| | Brain | BDL | BDL | BDL | BDL |
| 0.0025 | Gill | 0.0004± 0.0006 | 0.0019± 0.001 | 0.004± 0.002 | 0.007± 0.002 |
| | Kidney | BDL | BDL | BDL | 0.0013± 0.001 |
| | Liver | 0.0008± 0.0001 | 0.0027± 0.0003 | 0.006± 0.002 | 0.015± 0.002 |
| | Flesh | BDL | BDL | BDL | 0.001± 0.0004 |
| | Bone | BDL | BDL | BDL | BDL |
| | Brain | BDL | BDL | BDL | BDL |
| 0.005 | Gill | 0.0035± 0.0008 | 0.008± 0.001 | 0.015± 0.003 | 0.021± 0.004 |
| | Kidney | 0.0015± 0.0006 | 0.0029± 0.001 | 0.004± 0.002 | 0.008± 0.002 |
| | Liver | 0.005± 0.002 | 0.016± 0.002 | 0.03± 0.014 | 0.05± 0.016 |
| | Flesh | BDL | BDL | 0.0005± 0.0002 | 0.004± 0.001 |
| | Bone | BDL | BDL | BDL | BDL |
| | Brain | BDL | BDL | BDL | BDL |

BDL – Below detection limit

At the end of 28 days of exposure, the total tissue Cu concentration followed the pattern liver>gill>kidney>flesh>bone=brain for exposure to 0.005 and 0.0025 $\mu gl^{-1}$ Cu and liver>gill; kidney for 0.001 $\mu gl^{-1}$ Cu, bone, flesh and brain showing no detectable values. The study reveals that the uptake of Cu is tissue specific. Evaluating the rate of accumulation (tissue concentration / days of exposure) it was revealed that for exposure to 0.005 $\mu gl^{-1}$ Cu, the accumulation of Cu in gill and liver was similar up to 14 days but increased thereafter up to 28 days ($p<0.05$). Kidney, on the other hand, showed similar rate of accumulation for all exposure durations for the same concentration ($p<0.05$). For 0.0025 $\mu gl^{-1}$, the rate of accumulation of Cu in gill up to 14 days was similar but increased thereafter, up to 28 days. Kidney and liver, on the other hand, showed similar rate of accumulation for all exposure durations for the same concentration ($p<0.05$). For exposure to 0.001 $\mu gl^{-1}$Cu, the rate of accumulation of Cu in gill, kidney and liver showed similar pattern for all exposure durations ($p<0.05$). However, the rate of accumulation increased significantly with increase in concentration. Besides, bone and brain did not show Cu accumulation at any dose and flesh did not show any accumulation at the lowest dose up to 28 days of exposure.

The present study revealed that the flying barb responded to Cu by reducing the rate of oxygen consumption. It was observed that the oxygen

consumption rate of flying barb decreased with the increase in concentration of Cu, exposure duration also played a crucial role and oxygen consumption rate was found to decline with the increase in exposure period at 0.005 and 0.0025 µg $l^{-1}$ of Cu, whereas, 0. 001 µ$gl^{-1}$ Cu showed no significant changes in oxygen consumption upto14 days of exposure, but declined significantly thereafter up to 28 days of exposure (Table 2).

**Table 2.** Effect of Copper on rate of oxygen consumption in *Esomus danricus*

| Cu conc. (µg $l^{-1}$) | Oxygen Consumption (ml/hr/100g tissue) | | | |
|---|---|---|---|---|
| | 7 d | 14 d | 21 d | 28 d |
| Control | 38.94± 0.46 | 39.19± 0.29 | 39.03± 0.24 | 39.06± 0.15 |
| 0.001 | 38.39± 0.61** | 38.01± 0.64** | 30.31± 1.25* | 24.11± 1.76* |
| 0.0025 | 29.24± 1.15* | 24.87± 1.64* | 21.70± 1.43* | 19.20± 1.70* |
| 0.005 | 27.27± 1.0* | 21.67± 1.25* | 19.70± 1.93* | 14.84± 1.94* |

*Significant;

** Not significant at $p<0.05$

## 4. Discussion

Gill is the primary route for Cu uptake in fish due to its direct exposure to toxicants in water (Jayakumar and Paul, 2006; Kamunde *et al.*, 2002). In flying barb, Cu accumulated progressively in gills in concentration dependent manner, reaching4.4 fold increase after 28 days of exposure to 0.005 µ$gl^{-1}$ of Cu and only 0.8 fold magnification observed at 0.001 µ$gl^{-1}$ of Cu. Though both liver and kidney are typically important for metal accumulation and storage in fish (Gigue`re *et al.*, 2004), Cu metabolism is controlled chiefly by the liver. The liver not only tends to accumulate Cu from medium, but also plays an important role in Cu homeostasis (Grosell *et al.*, 1997). In flying barb, Cu concentration was 10 fold in liver after 28 days of exposure to 0.005 µ$gl^{-1}$ (Table 1). In rainbow trout exposed to radioactive Cu, the liver was shown to be the major target organ while the kidney was less important for Cu accumulation (Clearwater *et al*., 2000). Similarly, Cu concentrations were seven times higher in the liver than the kidney after 70 day of exposure in yellow perch *Perca flavescens* (Kraemer *et al.*, 2005). Thus, Cu was predominantly removed from the body and accumulated in the liver over time. Apart from the bone and brain, which had no detectable level of Cu throughout the study, the flesh accumulated the lowest level of Cu, even after 28 days of exposure (Table 1). This may be connected with the fact that the flesh and bone are not concerned with detoxification and therefore the transportation of Cu from other tissues to flesh and bone may not arise. Lack of a detectable level of Cu in the brain can be due to blood–brain barrier that prevents the entry of Cu into the brain (Crowe and Morgan, 1997).

The measurement of oxygen consumption of fish in presence of pollutant is the best index of its activity (Delhaye and Cornett, 1975). In the present study, flying barb responded to Cu by reducing the rate of oxygen consumption. Adverse effects of Cu on respiratory capabilities, as seen in the present study, were also studied in *Tilapia sparrmanii* (Van Aardt and Hough, 2006) and in *Esomus danricus* (Vutukuru *et al*., 2005). Cu had a depressing effect on oxygen consumption in *Cyprinus carpio* (De Boeck *et al*., 1995) and in *Labeo capensis* and *Micropteris salmoides* (Van Aardt and Hough, 2007). Cu reduced oxygen consumption by common carp and rainbow trout larvae in a concentration-dependent way (Jezierska and Sarnowski, 2002). In this study, all the sublethal doses of Cu induced excessive mucous secretion in gills. This phenomenon of mucous secretion can also impair gas exchange across the secondary lamellae epithelium (Handy and Eddy, 1989).

In conclusion, the present study indicates that Cu is accumulated at alarming level in gill and liver in Indian flying barb when exposed to level of Cu above 0.001 µ$gl^{-1}$. The fish responds to such high accumulation by lowering oxygen uptake.

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