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Effect of copper and zinc on oxygen consumption of the fresh water fish, *Clarias batrachus* (Linn.)

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Abstract
The environment is impacted by ongoing pollution, caused by both natural factors and human activities such as industrialization and mining. Heavy metals are a major problem because they are toxic and tend to accumulate in living organisms. This study was carried out on juvenile *Clarias batrachus* (L.) to investigate the effects of sub-lethal concentrations of copper and zinc (0.3, 0.4, 0.5 ppm) on the survival rate, oxygen consumption and histopathological changes in the gills of exposed fish. The results showed a decrease in survival rate with increasing concentration of each metal. Copper has the most toxic effect compared with zinc the survival rate has decreased from 50% to 10% for copper and from 70% to 20% for zinc with increasing concentration for 15 days. Oxygen consumption rate decreased with increasing concentration and there was a negative correlation between oxygen consumption and metal concentration. The exposure to each metal caused histopathological changes in the gill and resulted in separation of epithelial secondary gill lamellae, hyperplasia, fusion of secondary lamellae and necrosis.

Keywords: Pollution, heavy metals, oxygen consumption, histopathological changes, *Clarias batrachus*.

1. Introduction
The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades (Vindodhini and Narayanan, 2008) [1]. Increased human activities especially with rapid development of agriculture and industry has resulted in a considerable increase in levels of pollutant such as heavy metals which is the main anthropogenic pollution causing serious and long lasting damage to all living organisms (Sastry and Sukla, 1993; Murugan *et al.,* 2008) [2, 3]. Some toxic metals like copper, which is also essential for cellular metabolism has become extremely toxic for aquatic animals as its concentration increases in water (Carvaho and Fernandes, 2006) [4]. However, zinc which is not an essential element for life, is toxic in low concentrations for all forms of life in the environment (Eisler, 1985) [5], and is an important challenge to toxicologists and ecological transportation (Aardt and Booysen, 2004) [6]. Fish have the ability to accumulate heavy metals in their tissue to higher level than the toxic concentration in their environment by absorption along the gill surface and gut, and their respiratory system differs from all other systems because the gills are the main target of pollutants and damage to gills has immediate impact on the rest of the fish body (Al-Yacoob *et al*., 1994) [7], and human can be at a great risk through contamination of the food chain (Costa and Hartz, 2009) [8]. *C. batrachus* was selected as it is one of the important economic species and easily adapted to laboratory conditions. Many researchers have reported the harmful effects of copper and zinc on aquatic life (Able and Papoutsoglou *et al.,* 1994: Olaifa *et al*., 2004; Muthukumarvel *et al.,* 2007) [9-11]. The aim of this study is to investigate the effect of essential copper (Cu) and toxic zinc (Zn) on the survival rate and oxygen consumption rate after metal exposure for 15 days on juvenile of *C. batrachus*

2. Materials and Methods
Adult and live fish *C. batrachus* were collected from the fish farm Patra and Bhadbhada Bhopal M.P.) Brought to the laboratory, cleaned by using 0.1% $KMnO_4$ to avoid dermal infection. Fishes were acclimatized in glass aquaria for 15 days and were fed with fish food (earthworms) and water in the aquaria was replaced by freshwater at every 24h.
The fish were starved for 24 hr before use and divided into seven groups; three of which were exposed to 0.3, 0.4 and 0.5 ppm of copper and three were exposed to similar concentrations of zinc (six fish in each group), and the last group of fish (six in number) were not exposed to neither copper nor zinc and served as control group. An aqueous stock solution of 1.000 liter

of copper and zinc prepared by dissolving 4.7294 and 7.5466 gm of $CuSO_4$ $5H_2O$ and $ZnSO_4$ $7H_2O$ in a litre of distilled water. Three concentrations of each metal were made these are 0.3, 0.4, 0.5 ppm. The survival rate was recorded for 15 days. The differences are statistically not significant ($P > 0.05$) with the concentration used in experiment ($r = -0.884$ for the copper and - 0.889 for the zinc).

Four fish were taken, each with a weight 5 ± 1.8 gm, and kept singly in one liter conical flask containing dechlorinated tap water and closed firmly. Aeration was made by passing plastic tube through the plastic stopper. Each flask was covered with opaque plastic cover to reduce stress. The acclimation to this enclosed environment was continued for 24hr. The experiment began by stopping aeration and adding one volume of either of the copper and the zinc to one of the flasks in the concentrations of 0.3, 0.4, 0.5 ppm, while the 4th flask was kept as control.

Oxygen consumption in each flask was determined by using DO meter (OSI 325-A-SET) at intervals of 30, 60, 90, 120 and 180 minutes. Oxygen consumption rates were calculated as $mgO_2/g/h$ (Sumich, 1996)[12]. Gill specimens from the control and experimental animals were taken from juveniles exposed to 0.5 ppm of each metal (copper and zinc) for 6 days (LT_{50} for the highest concentration), fixed in Bouin's solution and dehydrated in graded series of alcohol, cleared in xylene and embedded in paraffin wax. Sectioning was carried out using rotating microtome to 5-7µ, and stained with hematoxylin-eosin method (Humason, 1979) [13].

3. Results

The results showed a decrease in the LT_{50} from 14 days at 0.3 ppm to 6 days at 0.5 ppm for the copper. Similarly a decrease in the survival rate was observed with increasing concentration from 50% at 0.3 ppm down to 10% in 0.5 ppm after 15 days. Zinc, likewise, showed similar results, LT_{50} decreased from >15 days at 0.4 ppm to 8 days at 0.5 ppm and the survival rate has decreased from 70% at 0.3 ppm to 20% at 0.5 ppm (Table 1). There was a decrease in the oxygen consumption rate of *C. batrachus* with increasing concentration of each of exposure, ranging from 0.348 at 0.3 ppm to 0.226 $mgO_2/g/h$ at 0.5 ppm for the copper and from 0.392 at 0.3 ppm to 0.345 $mgO_2/g/h$ at 0.5 ppm for zinc, compared with the control 0.492 $mgO_2/g/h$ (Figure 1). Fish gill arches carry two rows of filaments known as primary lamellae or gill filaments, on the upper and lower surface of each primary lamellae there is a row of secondary lamellae covered by a thin layer of epithelial cells (Plate 1).

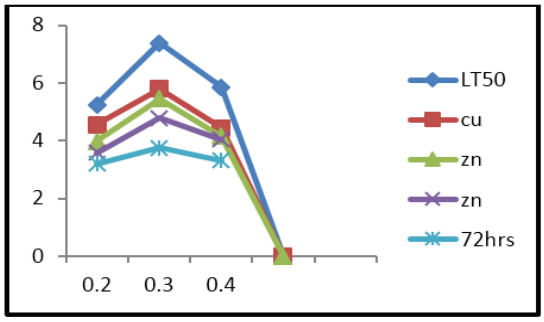
The gills of *C. batrachus* were exposed to 0.5 ppm of each metal (copper and zinc) for 6 days. The results indicated the separation of the epithelium of the secondary lamellae, hyperplasia, fusion of secondary lamellae and necrosis (Plate 2-6).

Tables

Table 1: Survival rate % and half lethal time (LT_{50}) of exposed *C. batrachus* to three levels of copper and zinc for 15 days

S. No.	Concentration of copper (ppm)					Concentration of zinc (ppm)
	0.3	0.4	0.5	0.3	0.4	0.5
LT50 (days)	14	11	6	>15	13	8
Survival rate (%)	50%	35%	10%	70%	40%	20%

Graphs



Graph. 1 Oxygen consumption rate in *C. batrachus* after 24h of exposure to different levels of copper and zinc.

Figures

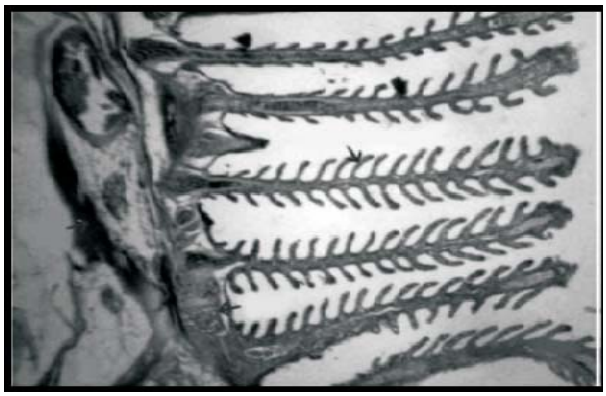


Plate 1: Longitudinal section in the gill of normal *C. batrachus* shows the primary lamellae (A), and secondary lamellae (B) (200 X).

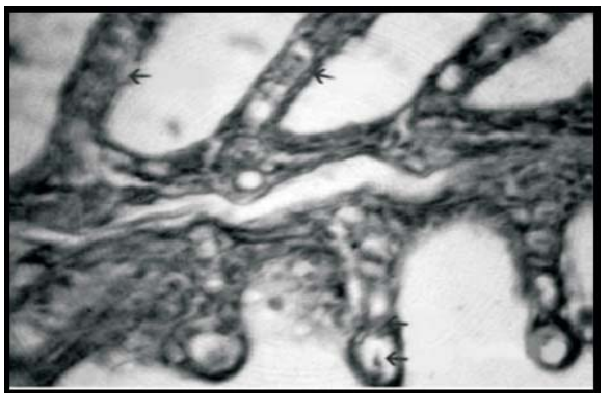


Plate 2: Longitudinal section in the gills of *C. batrachus* exposed to 0.5 ppm of copper for 6 days shows fusion of secondary lamellae (A) and hyperplasia (B) (400 X).

Plate 3: Longitudinal section in the gills of *C. batrachus* exposed to 0.5 ppm of copper for 6 days shows separation of epithelial gill lamellae (A), hyperplasia (B), fusion of secondary lamellae (C) and necrosis (D) (400 X**)**

Plate 4: Longitudinal section in the gills of *C. batrachus* exposed to 0.5 ppm of copper for 6 days, demonstrates separation of epithelial gill lamellae (A) and necrosis (B) (400 X).

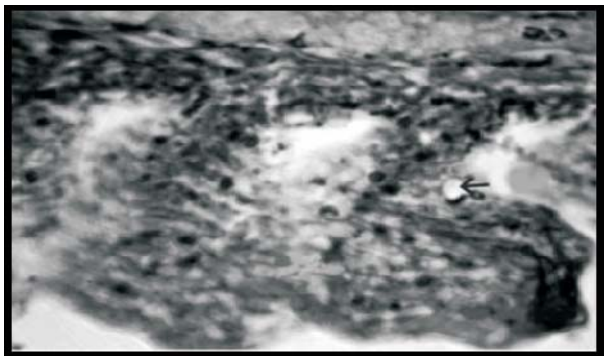


Plate 5: Longitudinal section in the gills of *C. batrachus* exposed to 0.5 ppm of zinc for 6 days illustrates separation of epithelial gill lamellae (A) and hyperplasia (B) (400 X).

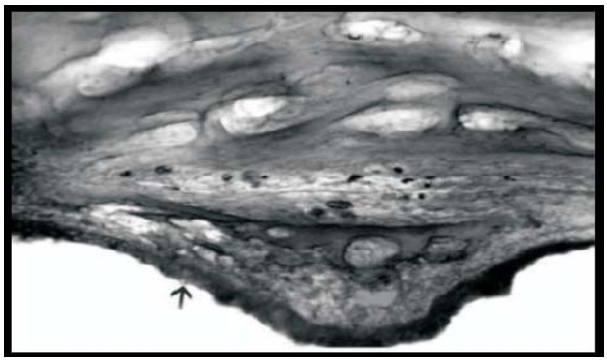


Plate 6: Longitudinal section in the gills of *C. batrachus* exposed to 0.5 ppm of zinc for 6 days, shows fusion of secondary gill lamellae and necrosis (B) (200 X).

4. Discussion

In the present study the survival rate was decreased from 50% to 10% for the copper and from 70% to 20% for the zinc after exposing *C. batrachus* to sub-lethal concentrations of each of both metals for 15 days. Similar results were reported by other studies, Abdullah and Ahmed (1998) [14] on *Cyprinus carpio*, Hassan (2005) [15] on *C. carassius* and Vutukuru (2005) [16] when exposed *Labeo rohita* to different concentrations of chromium.

The increase of death with increasing concentration and increasing of the duration of exposure could be because of the accumulation of metals in different tissues of body especially in the gills which are important sites for the entry of metals, therefore causing lesions and gill damage and failure of metabolic activities (Bols *et al*., 2001; James *et al*., 2003) [17, 18]. So it is possible that the cumulative action of copper and zinc at various metabolic sites is responsible for the death of the fish (Basa and Rani, 2003) [19].

The main reason of death in fish exposed to heavy metals is the hypoxia because the metals act on the gill function and structure causing damage of the gill epithelia, disturbances in osmo-regulation process, decrease of oxygen consumption and then death (Albaster and Lioyd, 1982; Peuranen *et al*., 1994; Hassan, 2005) [20, 21, 22]. The gills are very susceptible to water-born metals and often show various metal induced lesions. This leads not only to osmotic imbalance but may also caused an impairment of the respiratory system function of the fish which differs according to the type of metal and site of action (Jezierska and Sarnowski, 2002; Dobreva *et al*., 2008 [23, 24].

The present study also reported on a decrease in the oxygen consumption during exposure to either metal. Copper seems to have more toxic effect than zinc, as it has caused the highest mortality and reduction in the oxygen consumption. Goss and Wood (1988) [25] suggested that heavy metals act on gill function resulting in a decrease in oxygen consumption rate because of ion regulatory and acid-base disturbance. The same result was reported for the common carp by DeBoeck *et al*., (1995) [26] when they exposed fish to sub-lethal concentration of copper, and Jezierska and Sarnowski (2002) [27] when they exposed *Cyprinus carpio* larvae to mercury, copper and zinc, and reported that short-term copper exposure resulted in a strong decline of oxygen consumption by the larvae of *C. carpio* compared with zinc. In addition, a decrease in oxygen consumption rate was reported by Dobreva *et al*. (2008) [28] after exposing crussian carp *C. gibellio* to growing increase in the concentration of zinc for 96 hr, and Vutukuru (2005) [29] when exposed major Indian carp *Labeo rohita* to chromium for 96 hr and suggested that there is an alteration in cellular components as a cause of depression in the respiratory activity in fish exposed to metallic stress. Reduction of oxygen consumption rate in fish exposed to heavy metals indicate the onset of hypoxia under metallic stress (James, 1990) [30], because metals accumulate in gill epithelium and induce lesions like necrosis, thickening and separation of respiratory epithelium (Peuranen *et al*., 1994; Hassan, 2005) [31, 32], also it may resulted in an increase of diffusion distance between the water and blood which makes oxygen absorption difficult (Dalzell and MacFurtan, 1994; Aardt and Booysen, 2004) [33, 34]. In addition, metals may impair the respiratory surface function by reducing the respiratory surface area through the atrophy and fusion of secondary lamellae, as well as the internal action of metal which enhances the action of respiratory inhibiting factors. (Muthukumarvel *et al*., 2007; Shereena and Logswamy, 2008) [35, 36].

The present histopathological study showed that the exposure to each copper and zinc has caused a damage in the gill of juvenile *C. batrachus* because fish gills are always in contact with water and they have a very thin epithelial layer of a few microns separating the interior of the fish from the external environment, that makes the gills are important target for pollutants and strongly affected by environmental contaminants (Gross *et al.,* 1987) [37]. Researchers divide the fish gill lesions into two groups, the direct effect of irritants and the defence responses of fish. The direct effects of metals were necrosis and rupture of the gill epithelium, because the exposure to heavy metals leads to the formation of insoluble protein compound which are toxic to the gill (Albaster and Lioyd, 1982) [38].

The results showed separation of epithelial gill lamellae, hyperplasia and lamellae fusion. These changes in the gill epithelial layer may be explained as a kind of protection against pollutants, because separation of the epithelial gill lamellae increases the distance through which the metals has to travelled to reach the blood stream, where hyperplasia results in fusion of secondary lamellae which could be protective for a larger vulnerable gills surface area (Leino *et al*., 1987; Pandey *et al*., 1997; Wangsongsak, 2003) [39, 40, 41]. A similar result was obtained by Muhvich (1995) [42] when he exposed gold fish *Carassius auratus* to sub-lethal concentration of copper sulphate for 96 hr, the results showed hyperplasia in the top of the filament, and Pandey *et al*. (1997) [43] when they exposed estuarine mullet *Liza parsia* to sub-lethal concentration of lead nitrate 0.5 ppm for 15 days, the results indicated a separation of the epithelial lining cells from the basement membrane of the secondary lamellae, hyperplasia and complete fusion of secondary lamellae. Wangsongsak (2003) and Hassan (2005) [44, 45] reported that the exposure of *Puntius gonionotus* and *Carassius carassius* to sub-lethal concentrations of zinc resulted in the separation of the epithelial secondary lamellae, fusion of secondary lamellae and necrosis.

5. Conclusion

In conclusion the exposure of the freshwater *C. batrachus* to sub-lethal concentrations of copper and zinc tends to increase mortality with increasing exposure time and concentration, decrease the level of oxygen consumption rate, histopathological changes in gill epithelia. In addition the strongest effect of copper on juvenile *C. batrachus* resulted from copper uptake by the gills which induced epithelial lesions and gill disorders more than zinc.

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