Asian Journal of Water, Environment and Pollution, Vol. 6, No. 4, pp. 107-112.

Scientific Note

Effect of Heavy Metals, Mercury and Copper, on Plasma Electrolytes in Freshwater Fish *Cyprinus carpio* var. *communis*

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Received June 22, 2007; revised and accepted November 22, 2008

Abstract: In the present study the effect of heavy metals, mercury and copper, on plasma electrolytes viz., sodium, potassium and chloride have been investigated in economically important fish *Cyprinus carpio* var. *communis*. *C. carpio* were exposed to sub-lethal levels of mercury (0.03 ppm) and copper (0.096 ppm) under strict identical hydro-physiochemical conditions. Significant decline in these three electrolytes were observed when fish were exposed to sub-lethal levels of mercury chloride and copper sulphate during chase period of 7, 14, 21, 28 and 35 days.

The decrease in sodium ions was proportional to exposure time in mercury with maximum observed decrease of 33.0% on 35th day, whereas a maximum decrease of 66.36% was noted on 7th day in copper. Plasma potassium level of *Cyprinus carpio* var. *communis* declined continuously in the mercury treated fish showing a maximum per cent decrease of 54.68 at the end of the 28th day. The plasma chloride level observed a maximum decrease of 46.02% and 80 % at the end of 7th day in the mercury and copper-treated fish respectively. The mercury-treated fish maintained the percent decrease of 22.56, 36.98, 38.08 and 39.02 on the 14, 21, 28 and 35th days. However, in the plasma chloride level in copper-treated fish a gradual recovery during subsequent exposure periods was observed giving a per cent decline of 61.23%, 45.39%, 34.02% and 10.11% at the end of the 14th, 21st, 28th, and 35th days, respectively. Hence, results indicated that pollutants including toxic heavy metals and useful micronutrient when a safe level exceed are reflected by the decrease in the plasma electrolytes of fish. The electrolyte levels in fish can be used for biomonitoring and Ecological Risk Assessment.

Key words: *Cyprinus carpio*, electrolytes, heavy metals, mecury, copper biomonitoring and ecological risk assessment.

Introduction

Cyprinus carpio, 'the swimming pig', is a non-predatory and omnivorous fish which efficiently converts the food ingested into flesh and grows very fast. *C. carpio* is recommended as model for toxicological studies (Anand 2002; Michael et al., 2003; Claire et al., 2004; Xiao et al., 2004; Christopher et al., 2005; Lotte et al., 2007).

In freshwater fish the physiological regulation of the major electrolytes is very sensitive to environmental stressors (McDonald et al., 1989). The ions in plasma of fish are altered in response to pollutants in the environment (Xiao et al., 2004; Christiane et al., 2006; Ufuk et al., 2007). Maintenance of constant internal ion concentration (sodium, potassium, chloride and calcium) is essential for active regulation of water influx and ion

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efflux in aquatic organisms (Mayer et al., 1992; Hoyle, 2007). According to Pic (1978) and Foskett et al. (1983), sodium, chloride and potassium ions have also been shown to play essential roles in osmotic regulation by aquatic species.

Though, there are several reports (Dang, 2000; Anand, 2002; Celik and Oehlenschlager, 2007) available in relation to the effect of different heavy metals on fish, there is no information on the mechanism of plasma electrolytes regulation in economically important freshwater teleost fish *Cyprinus carpio* var. *communis*. In this article we communicated the impact of mercuric chloride, a potential persistent toxic metal, and a micronutrient copper sulphate to evaluate the degree of non-specific stress responses of fish to these toxicants. In addition, changes in plasma electrolytes following the heavy metal treatment were studied for biomonitoring and ecological risk assessment. Substantial changes in plasma ion levels were observed during sub-lethal responses to heavy metals.

Materials and Methods

Healthy specimens of *Cyprinus carpio* were procured from Fisheries Development Limited, Aliyar Fish Farm, Aliyar, Tamil Nadu, India. Fish of the same age and size hatched from the same lot of eggs (broodstock) were collected.

Methods

Acclimatization: Acclimatization was done by stocking fish in a large, rectangular cement tank (4m × 6m ×3m), previously soap washed, disinfected with potassium permanganate and thoroughly rinsed thrice prior to filling with water (APHA et al., 1976, 1985). Fish were acclimatized to laboratory conditions for a fortnight, before being used for experiments. During acclimatization, the stock was maintained at natural photoperiod and ambient temperature and fed *ad libitum* once daily with groundnut oil cake and rice bran, both being powdered in the ratio of 1:2 and given in the form of dough. Water was replaced every 24 h and well aerated in order to reduce any accumulation of excretory products and ensure sufficient oxygen supply to fish. Feeding was withheld for 48 h prior to the commencement of all experiments.

Sub-lethal Studies

Three glass aquaria of 125 L capacity which were cleaned, previously disinfected with potassium permanganate, sun dried and filled with clean water were taken for sub-lethal toxicity studies. Analytical grade mercuric chloride 1/10th of (24 h) LC 50 concentration of mercury chloride (0.03 ppm) and copper sulphate (0.069 ppm) were obtained from Fisher Inorganic & Aromatics Limited, Chennai, India and used (Sprague, 1971; Barse et al., 2006). A control (100 fish) without heavy metal was maintained simultaneously.

Experiments were conducted for 35 days. At weekly intervals (7, 14, 21, 28 and 35 days), 20 fish were randomly selected from control and experimental aquaria and sacrificed without being anesthetized for analysis. Blood was drawn from cardiac region by cardiac puncture using plastic disposable syringe fitted with 26 gauge needle which was already moisted with heparin (Beparine^{â}, heparin sodium, 1P 2000 IU ml^{-1}, derived from beef intestinal mucosa containing 0.15% W/V cholesterol IP preservative), an anticoagulant manufactured by Biological E Limited, Hyderabad, India. Blood collected from treatment and control was expelled into the separate heparinized plastic vials and placed immediately on ice. Pooled blood sample was used for determination of all the parameters. Blood was centrifuged for 15 min. at 10,000 rpm and plasma was withdrawn and transferred into clean vials for further analysis viz. sodium, potassium and chloride.

Statistical Analysis

The statistical analysis was made individually on each sample and the mean value of five individual observations was taken for each parameter. The standard error for the sample mean was calculated and they are given in tables.

Results

Changes in Plasma Ion Concentration

The changes observed in plasma ions (sodium, potassium and chloride) concentrations following mercury and copper treatment are presented in Tables 1, 2 and 3. Significant decline in these three candidate ions (sodium, potassium and chloride) was observed in the fish owing to the exposure to sublethal levels of mercury chloride and copper sulphate.

Overall the plasma sodium level in mercury-treated fish showed gradual decrease of 10.65%, 11.19%, 17.19%, 32.66% and 33.00% from control on 7th, 14th, 21st, 28th, and 35th days respectively. The copper-treated fish sodium level gradually recovered showing a value of 35%, 14% and 10% at the end of 14, 21 and 28 days, respectively. After 28th day, plasma sodium level once again decreased showing a per cent decrease of 29.80 at the end of 35th day of treatment (Table 1 and Figure 1).

Table 1: Changes in plasma sodium ion concentration following sub-lethal doses of mercury and copper

Exposure (days)	*Control* / *Metal ion*	*Sodium ion concentration (μg/ml)*		*Percent change in the concentration of plasma sodium ion*	
		Mercury	Copper	Mercury	Copper
7	97.600 ± 3.355	87.200 ±2.373	31.232 ± 2.443	-10.655	-66.368
14	88.100 ±1.60	78.237±1.930	57.265 ± 1.334	-11.195	-30.835
21	89.732 ±1.229	74.300±272	77.17 ± 1.990	-17.190	-14.000
28	95.200 ± 2.090	64.100±1.732	85.585 ± 1.536	-32.660	-9.619
35	90.600 ±1.457	60.700±1.040	60.793 ± 1.857	-33.002	-29.807

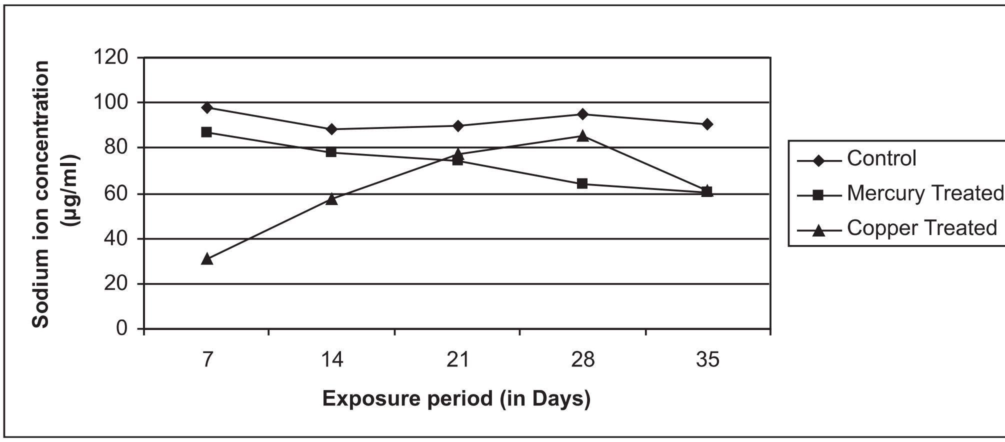

Figure 1: Changes in plasma sodium concentration.

Similarly, the plasma potassium showed the percent decrease of 57.94, 53.66, 54.68 and 51.87 on the 14, 21, 28 and 35th days. The plasma potassium in copper-treated fish showed a maximum percent decrease of 50.1 on 7th day and a minimum percent decrease of 5.00 at the end of 28th day. After 28th day, plasma potassium level registered a decrease of 15% at the end of 35th day of treatment (Table 2 and Figure 2).

The plasma chloride level registered a maximum decrease of 46.02% and 80% at the end of 7th day in the mercury and copper-treated fish respectively. The mercury-treated fish maintained the percent decrease of 22.56, 36.98, 38.08 and 39.02 on the 14, 21, 28 and 35th days. However, in the plasma chloride level in copper-treated fish, a gradual recovery during subsequent exposure periods was observed giving a per cent decline of 61.23%, 45.39%, 34.02% and 10.11% at the end of the 14th, 21st, 28th, and 35th days, respectively.

The control fish when analyzed for varying exposure periods, depicted that all the exposure periods were equal to each other, thus showing a homogeneity in value of plasma sodium, potassium and chloride levels (Table 3 and Figure 3).

Discussion

In the present study the observed depression and low depression levels of ions from plasma (viz., Na^+, K^+ and Cl^-) during mercury and copper treatment represents a direct response of fish to recognize potentially harmful substances in the first phase similar to those reported earlier. (Wedemeyer, 1969; Benjamin et al., 2006, Ramon et al., 2006). Here in *C. carpio* plasma electrolytes significantly alter during sub-lethal mercury and copper toxicity and are in agreement with those reported earlier in *Salmo gairdneri* (Van der Putte et al. 1983; Witters, 1986; Goss and Wood, 1988; Reid and McDonald, 1988, Aline et al., 2005, Borgmann et al., 2005) which is due to impaired uptake and diffusion losses of ions *via* gills and kidney as described by Stagg and Shuttleworth (1982), Lisa et al. (2005) and Jonathan et al. (2006).

The initial dip on 7th day in the plasma electrolytes (alarm phase) seems to be a direct response of the fish as a result of exposure to potentially harmful substances in water. The elevation in the decreased level of electrolytes on 14, 21 and 28th days apparently represents a resistance phase in case during which period the animals try to

Table 2: Changes in plasma potassium ion concentration following sub-lethal doses of mercury and copper

Exposure (days)	*Control* / *Metal ion*	*Potassium ion concentration (μg/ml)*		*Percent change in the concentration of plasma Potassium ion*	
		Mercury	*Copper*	*Mercury*	*Copper*
7	10.437±0.254	5.000±0.505	5.215 ± 0.444	-52.23	-50.10
14	9.600±0.152	4.037±0.411	6.1248 ± 0.423	-57.94	-36.20
21	8.780±0.259	4.068±0.252	7.892 ± 0.325	-53.66	-10.12
28	10.980±0.271	4.976±0.363	10.431 ±0.322	-54.68	-5.00
35	10.245±0.154	4.930±0.349	8.709 ±0. 389	-51.87	-15.00

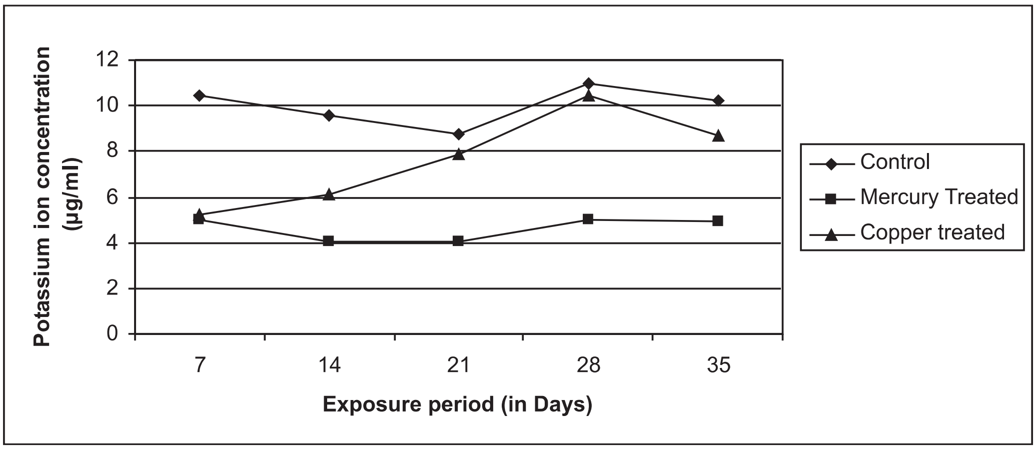

Figure 2: Changes in plasma potassium ion concentration.

Table 3: Changes in plasma chloride ion concentration following sub-lethal doses of mercury and copper

Exposure (days)	*Control* / *Metal Ion*	*Chloride ion concentration (μg/ml)*		*Percent change in the concentration of plasma Chloride ion*	
		Mercury	Copper	Mercury	Copper
7	124.600±2.596	67.250±1.188	24.920 ± 1.123	-46.027	-78.25
14	119.200±1.857	74.256±1.994	46.214 ± 1.655	-22.56	-52.23
21	120.376±1.669	73.444±1.238	65.738 ± 1.231	36.98	
28	115.425±2.190	71.470±2.531	75.950 ±1.562	-38.08	
35	125.500±2.250	76.520±2.395	123.250 ± 1.256	-39.02	-7.38

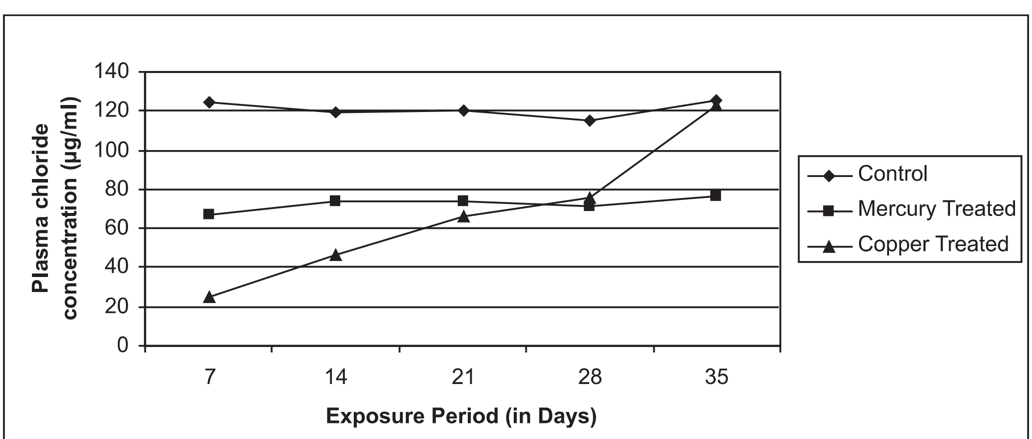

Figure 3: Changes in plasma chloride concentration.

counteract the physiological changes. By the 35th day fish were exhausted; hence during this exhaustion phase the subsequent fall in plasma electrolytes levels were observed resembling the results depicted by Anand (2002).

Decline of Na^+ levels is due to the effect of metals directly or indirectly interfering with Na^+ transport (Malley et al., 1988), an imbalance between influx (inhibition) and efflux (McDonald et al., 1989), reduction in epithelial permeability to Na^+ and renal damage (Lauren and McDonald, 1987).

In sub-lethal treatments, blood potassium level decrease is due to liver damage as reported by Hilmy et al. (1982) in mercury-exposed Cyprinodont *Aphanius dispar*, cadmium-exposed flounder *Platichthys flesus* L. (Larsson et al., 1981) and chromium-exposed rainbow trout *Salmo gairdneri* (Van der Putte et al., 1982).

Regulation of plasma chloride levels and hypochloremia in teleostan fish subjected to stressful conditions is direct response of disruption of gills and failure of kidney to obstruct excess loss of Cl^- from body (Selye, 1950; Bently, 1971; Pic, 1978). In the present study, there is greater efflux of chloride during initial stages of sub-lethal treatment. However, this recovery in the continuous presence of copper indicates that the gills and chloride cells also underwent same physiological and biochemical changes, which may be analogous to those reported for rainbow trout *Salmo gairdneri* exposed to copper (Lauren and McDonald, 1987), *Hyalella azteca* exposed to copper (Borgmann et al., 2005), tilapia *Oreochromis mossambicus* exposed to cadmium (Fu et al., 1990) and *Colossama macropomum* exposed to copper and cadmium (Aline et al., 2005).

From the foregoing discussion it is clear that many of the trace metal contaminants of polluted waters have impact on concentrations of ions in plasma of fish. By measuring changes in plasma ion levels, sub-lethal responses to pollutants can be effectively monitored; such measurements are easily made and the methods are inexpensive, fairly sensitive and provide good indices of pathological changes in ion regulating tissues.

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