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# Pattern of Accumulation and Changes in Some Growth Parameters of Indian Flying Barb, *Esomus danricus* (Hamilton-Buchanan) during Sublethal Cadmium Exposure

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**Abstract:** In the present study, pattern of accumulation of three sublethal concentrations of cadmium (0.006, 0.063 and 0.636 mg $L^{-1}$) in different tissues and their effects on growth and somatic indices were determined in Indian flying barb (*Esomus danricus*) at weekly interval for 28 days. At the end of 28 days of exposure, the total tissue cadmium concentration followed the pattern kidney > liver > gill >bone > flesh > brain for 0.063 and 0.636 mg $L^{-1}$ concentrations while for 0.006 mg $L^{-1}$ exposure, accumulation pattern was kidney > gill > liver > bone > flesh=brain. Cd bio-magnified in all the studied tissues except brain and the rate of uptake of Cd increased with time in the kidney, liver, bone and flesh but decreased in the gill. Cd also decreased body weight, brain and hepatosomatic index and increased kidney somatic index in a dose and exposure dependent manner.

**Key words:** $LC_{50}$ · Cadmium · Accumulation · Biomagnifications · Somatic Index

## INTRODUCTION

Cadmium (Cd) is a nonessential heavy metal which is known to bio-magnify in the food chain and cause adverse effects and death of aquatic organisms [1]. Cd levels in clean natural freshwaters are usually below 0.009 ìM, but in environments impacted by man, concentrations can be several times higher [2]. Fishes had been shown to concentrate Cd in their tissues at concentrations many times greater than ambient levels. However, the metal accumulation capacity is dependent on the assimilation and excretion capacities [3] as well as factors such as temperature, age of fish, interaction with other metals, water chemistry and metabolic activity of the fish concerned [4].

In a metal accumulation study [5], wetlands like floodplain lakes, marshes and swamps of Barak Valley, in Assam state of India, serve as sinks for heavy metals like Cd. Indian flying barb, *Esomus danricus* (Hamilton-Buchanan), a minnow having food and ornamental value, commonly inhabits such water bodies and are susceptible to Cd which target gill, liver, kidney and other tissues. It would, thus, be interesting to study the pattern of accumulation of sublethal doses of this heavy metals in different tissues and note how Cd influence growth rate of *Esomus* for a relatively long term exposure duration.

## MATERIALS AND METHODS

Fishes 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 [6]. 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 Cd was prepared from $CdCl_2$. $H_2O$ (Merck, Germany) and serial dilutions were prepared using chlorine free tap water as per dilution techniques [7]. Static-with-renewal acute toxicity tests were conducted with ten fish in each graded concentration and 96 hours $LC_{50}$ value was found to be 6.36 mg $L^{-1}$ in a prior study [8].

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 L of water. Bowls of group I, II, III and IV contained 0.636, 0.063 and 0.006 mg $L^{-1}$ Cd and control (tap water without Cd) 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 Cd. For example, bowl of group I marked

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'7 d' had 10 fish exposed to 0.636 mg $L^{-1}$ Cd that was sacrificed after 7 days of exposure and so on. Cd treatment was carried out twice daily and on each occasion 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 till constant weight was achieved and dissolved in 10 ml deionised water. Analysis for Cd was carried out in a Perkin-Elmer 3110 atomic absorption spectrophotometer (AAS). 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 deionized water. Digestion of samples was based on a modification of the method of Jayakumar and Paul [9].

For calculating the different organo-somatic indices, wet weights of the whole body as well as that of brain, liver and kidney were measured in an electric balance for control and the three test concentrations exposed fish at the beginning of the experiment and on each exposure interval. The organo-somatic indices were calculated as weight of tissue divided by total weight of fish. Change in somatic index was calculated as somatic index at particular exposure duration minus somatic index at 0 day.

For growth studies, ten fish each were individually reared in 0.636, 0.063 and 0.006 mg $L^{-1}$ of Cd and dechlorinated tap water that served as control. Test and control fish were weighed in an electrical balance at the beginning of the experiment and on 7, 14, 21 and 28 days. Statistical significance of the differences in weight changes between control and exposed fish at different Cd concentrations was determined; and comparisons among the brain-somatic index (BSI), hepato-somatic index (HSI) and kidney-somatic index (KSI) values in control and the three test concentrations at the beginning of the experiment and on 7, 14, 21 and 28 days were also made by one-way ANOVA and Tukey Test using SYSTAT 13 software for Windows.

## RESULTS

The level of the metal accumulated by each tissue after 28 days increased as the exposure concentration increased. At all the exposure levels the metal was not detected in the liver after 7 days of exposure. While, for all the three exposure concentrations, bone and flesh showed no metal accumulation up to 14 days and brain did not show any accumulation up to 28 days of exposure (Table 1). At the end of 28 days of exposure, the total

Table 1: Accumulation (mg $kg^{-1}$ dry weight) and concentration of Cd in selected tissues of *Esomus danricus* exposed to sublethal concentrations over time

| Exposure duration | Tissue Cd level (mg $kg^{-1}$ dry weight) | Control | 0.006 mg $L^{-1}$ Cd | 0.063 mg $L^{-1}$ Cd | 0.636 mg $L^{-1}$ Cd |
|---|---|---|---|---|---|
| 7-d | Gill (Concentration) | ND | 0.029±0.004 (X 4.8) | 0.619±0.019 (X 10.3) | 9.69±0.641 (X 16.1) |
| | Kidney (Concentration) | ND | ND | ND | 2.28±0.05(X 3.8) |
| | Liver | ND | ND | ND | ND |
| | Bone | ND | ND | ND | ND |
| | Flesh | ND | ND | ND | ND |
| | Brain | ND | ND | ND | ND |
| 14-d | Gill(Concentration) | ND | 0.019±0.002(X 3.1) | 0.436±0.042(X 7.26) | 5.43±0.072(X 9) |
| | Kidney (Concentration) | ND | ND | 0.15±0.013(X 2.5) | 4.9±0.277(X 8.1) |
| | Liver (Concentration) | ND | ND | ND | 0.719±0.044 |
| (X1.19) | Bone | ND | ND | ND | ND |
| | Flesh | ND | ND | ND | ND |
| | Brain | ND | ND | ND | ND |
| 21-d | Gill (Concentration) | ND | 0.0167±0.0009(X 2.78) | 0.302±0.002(X 5) | 4.53±0.088(X 7.5) |
| | Kidney (Concentration) | ND | 0.02±0.002(X 3.3) | 0.449±0.022(X 7.5) | 8.19±0.445(X 13.65) |
| | Liver (Concentration) | ND | ND | 0.09±0.021(X 1.5) | 1.8±0.261(X 3) |
| | Bone(Concentration) | ND | ND | 0.015±0.001(X 0.25) | 0.388±0.037(X 0.65) |
| | Flesh (Concentration) | ND | ND | ND | 0.05±0.016(X0.08) |
| | Brain | ND | ND | ND | ND |
| 28-d | Gill (Concentration) | ND | 0.009±0.001 (X 1.5) | 0.171±0.014 (X2.85) | 2.09±0.073 (X 3.49) |
| | Kidney (Concentration) | ND | 0.027±0.003 (X 4.5) | 0.726±0.05 (X 12) | 11.5±0.485 (X 19) |
| | Liver (Concentration) | ND | 0.006±0.002 (X 1) | 0.199±0.028 (X 3.3) | 2.78±0.229 (X 4.6) |
| | Bone (Concentration) | ND | 0.0006±0.0002 (X 0.1) | 0.03±0.014 (X 0.5) | 0.68±0.175 (X 1) |
| | Flesh (Concentration) | ND | ND | 0.0006±0.0002(X0.01) | 0.06±0.017(X0.1) |
| | Brain | ND | ND | ND | ND |

ND-Not detectable; Concentration=cadmium level in tissue/corresponding exposure concentration.

Table 2: Rate of incorporation of Cd into selected tissues of *Esomus danricus* over time

| Exposure duration | Tissue Cd level (mg $kg^{-1}$ dry weight/day) | 0.006 mg $L^{-1}$ Cd | 0.063 mg $L^{-1}$ Cd | 0.636 mg $L^{-1}$ Cd |
|---|---|---|---|---|
| 7-d | Gill | 0.004±0.0006 | 0.088±0.002 | 1.38±0.091 |
| | Kidney | ND | ND | 0.325±0.007 |
| | Liver | ND | ND | ND |
| | Bone | ND | ND | ND |
| | Flesh | ND | ND | ND |
| | Brain | ND | ND | ND |
| 14-d | Gill | 0.0013±0.0001 | 0.031±0.003 | 0.388±0.005 |
| | Kidney | ND | 0.01±0.0009 | 0.353±0.019 |
| | Liver | ND | ND | 0.051±0.003 |
| | Bone | ND | ND | ND |
| | Flesh | ND | ND | ND |
| | Brain | ND | ND | ND |
| 21-d | Gill | 0.0007±0.00004 | 0.014±0.00009 | 0.215±0.004 |
| | Kidney | 0.0009±0.00009 | 0.021 ±0.001 | 0.390±0.021 |
| | Liver | ND | 0.004±0.001 | 0.085±0.012 |
| | Bone | ND | 0.0007±0.00004 | 0.018±0.001 |
| | Flesh | ND | ND | 0.002±0.0007 |
| | Brain | ND | ND | ND |
| 28-d | Gill | 0.0003±0.00004 | 0.006±0.0005 | 0.074±0.002 |
| | Kidney | 0.0009±0.0001 | 0.026±0.002 | 0.41±0.017 |
| | Liver | 0.0002±0.00007 | 0.077±0.001 | 0.099±0.008 |
| | Bone | 0.00002±0.000007 | 0.001±0.0005 | 0.024±0.006 |
| | Flesh | ND | 0.00002±0.000007 | 0.002±0.0006 |
| | Brain | ND | ND | ND |

Table 3: Weekly changes in weight (g) of control and test *Esomus danricus* during a 28-day exposure to Cd

| | Weight change (g ± SD) from 0 day | | | |
|---|---|---|---|---|
| Cd Conc. (mg $L^{-1}$) | 7 day | 14 day | 21 day | 28 day |
| Control | 0.014±0.003 | 0.019±0.004 | 0.024±0.004[a] | 0.03±0.006[a] |
| 0.006 | -0.001±0.0009* | -0.007±0.002* | -0.01±0.002* | -0.013±0.002*[b] |
| 0.063 | -0.015±0.002* | -0.027±0.003* | -0.031±0.005*[c] | -0.047 ±0.003*[c] |
| 0.636 | -0.029±0.006* | -0.033±0.007* | -0.049±0.011*[d] | -0.08±0.017*[d] |

'-' Indicates decrease; *Significant different from Control at each interval ($p<0.05$); values with different alphabets in superscript at the same column indicate significant differences from corresponding value at day 7 ($p<0.05$).

Table 4: Weekly changes in somatic indices of control and test *Esomus danricus* during 28 days of exposure to Cd

| Exposure duration | | Somatic Indices | Control | 0.006 mg $L^{-1}$ Cd | 0.063 mg $L^{-1}$ Cd | 0.636 mg $L^{-1}$ Cd |
|---|---|---|---|---|---|---|
| 7-d | Changes in somatic indices (g±SD) from 0 day | BSI | 0.0026±0.006 | -0.009±0.001 | -0.033±0.009 | -0.097±0.035* |
| | | HSI | 0.003±0.003 | -0.029±0.003 | -0.11±0.029* | -0.796±0.043* |
| | | KSI | 0.0001±0.0002 | 0.0002±0.00005 | 0.0067±0.002* | 0.012±0.002* |
| 14-d | | BSI | 0.0072±0.009[a] | -0.022±0.005[a] | -0.053±0.013*[a] | -0.171±0.045*[b] |
| | | HSI | 0.005±0.003[a] | -0.103±0.011*[a] | -0.239±0.056*[b] | -1.329±0.046*[b] |
| | | KSI | 0.0009±0.001[a] | 0.0015±0.0002*[b] | 0.01±0.002*[a] | 0.017±0.0008*[b] |
| 21-d | | BSI | 0.01±0.008[a] | -0.049±0.029*[a] | -0.117±0.033*[b] | -0.248±0.045*[b] |
| | | HSI | 0.011±0.005[a] | -0.172±0.017*[b] | -0.448±0.114*[b] | -1.53±0.049*[b] |
| | | KSI | 0.001±0.001[a] | 0.003±0.0003*[b] | 0.014±0.003*[a] | 0.026±0.002*[b] |
| 28-d | | BSI | 0.016±0.01[a] | -0.105±0.012*[b] | -0.187±0.048*[b] | -0.279±0.056*[b] |
| | | HSI | 0.023±0.015[a] | -0.252±0.079*[b] | -0.549±0.117*[b] | -1.59± 0.052*[b] |
| | | KSI | 0.001±0.002[a] | 0.005±0.0005*[b] | 0.019±0.004*[b] | 0.034±0.002*[b] |

'-' Indicates decrease; *significantly different from Control at $P<0.05$; 'a' indicate not significant and 'b' indicate significant differences at $P<0.05$ amongst values.

tissue Cd concentration followed the pattern kidney>liver>gill>bone>flesh>brain for 0.06 and 0.636 mg $L^{-1}$ concentrations while for 0.006 mg $L^{-1}$ exposure, accumulation pattern was kidney > gill> liver >bone> flesh=brain. The study reveals that the uptake of Cd is tissue specific. The data for the rate of uptake of Cd from the ambient water by the gill and the influx into the other organs due to inter-organ redistribution are presented in Table 2. After 7 days, the rate of uptake Cd by the gill and kidney was concentration dependent and a similar trend was observed in all the tissues after 28 days. With exposure dose and duration, the rate of uptake of Cd decreased in gill and increased in kidney, liver, bone and flesh, while brain did not show any incorporation. Besides, Cd concentration showed maximum of 19 fold increases in kidney after 28 days of exposure to 0.636 mg $L^{-1}$ (Table 1).

In this study, waterborne Cd exposure resulted in a reduction of flying barb growth rate and had an inverse relationship between growth rate and Cd concentration. Body weight of control fish after 28 days of exposure increased significantly ($p<0.05$) by 3.8 percent where as body weight of 0.636, 0.063 and 0.006 mg $L^{-1}$ of Cd exposed fish decreased gradually by 7.98, 5.28 and 1.57 percent respectively after same duration. However, for the lowest dose (0.006) and two higher doses (0.636 and 0.063), no significant decline in weight was discernible up to 21 and 14 days of exposure respectively (Table 3). BSI, HSI and KSI values at the beginning of the experiment (0 day) and in the four successive weekly measurements were not significantly different among one another in control. 0.006, 0.063 and 0.636 mg $L^{-1}$ Cd at the end of 28 days of exposure decreased BSI by 9.62, 15.42 and 25.36 per cent and HSI by 13.42, 30.69 and 85 percent but increased KSI by 2.97, 8.06 and 19.33; respectively (Table 4). Statistical analysis revealed significant difference in BSI from 7 day onwards in 0.6 mg $L^{-1}$ exposure. While, 0.063 mg $L^{-1}$ exposed fish did not show significant difference in BSI up to 7 day and 0.006 showed significant decline in BSI only after 14 days of exposure as compared to control ($p<0.05$). Significant difference in HSI and KSI was discernible from 7 day onwards in the 0.636 and 0.063 mg $L^{-1}$ exposure ($p<0.05$). While, 0.006 mg $L^{-1}$ Cd exposed fish did not show significant difference in HSI and KSI at 7 day, as compared to control (Table 4). The observed differences probably reflect the nature of response of different organs to different toxicants. It is also possible that the variations in response are also governed, at least partly, by the concentration of the toxicant or duration of exposure.

## DISCUSSION

Cd accumulated maximally in gill after 7 days and as highest tissue load was reached in the gill, it was redistributed to the kidney and subsequently to the liver (after 28 days). This pattern of redistribution is evident when a comparison of the rate of incorporation of Cd into these six tissue types was made (Table 2). This finding corroborates the works on *Clarias gariepinus* [10] but in tilapia [11] and rainbow trout [12], Cd accumulation in gill increased with exposure period. Thus, flying barb's kidney, followed by liver eventually accumulates a higher proportion of the body burden of Cd subsequent to an initial build-up in the gill filaments. Gill appears to have a capacity for retaining a higher initial level of Cd than the kidney and liver in flying barb. Gill is the primary site for Cd uptake due to its proximity to toxicants [9]. Later, kidney and liver accumulates Cd as these organs are involved in detoxification process [13]. It is assumed that Cd-metallothioneins (MTs) released from the liver cell is then gradually redistributed to the kidney, which is the main target organ for chronic cadmium toxicity [14]. The kidney is thus the final destination of all the Cd from various tissues as it has also been shown that Cd-MT is filtered through the glomerulus and is reabsorbed by the proximal tubular cells, possibly by endocytosis [15]. Within these cells the complex is taken up by lysosomes and degraded by proteases to release Cd, which may result in renal accumulation of the metal. This might also explain the reason for increase in weight of kidney at all the exposure durations and concentration in flying barb. Similar results were observed in carp [16] and eel [17]. However, in perch [18], carp [19], stickleback [20] and olive flounder [21], Cd mainly accumulated in liver. Apart from the brain, which had no detectable level of Cd throughout the study, the flesh accumulated the lowest level of cadmium, even after 28 days of exposure (Tables 1, 2). This may be connected with the fact that the flesh is not concerned with detoxification and therefore the transportation of Cd from other tissues to flesh may not arise. Bone, on the other hand, accumulated higher Cd, perhaps, manifesting in bone deformity as acute effect of this metal. Lack of a detectable level of cadmium in the brain can be due to blood-brain barrier that prevents the entry of cadmium into the brain [22]. However, brain showed decline in weight due to Cd exposure (Table 4). This can be because of the fact that energy available during long term Cd stress was devoted mostly to sustaining normal metabolism, indirectly effecting growth.

Effect of pollutants on fish has been considered by many workers using a variety of different approaches including changes in growth rate [23] and somatic indices [24]. Loss of weight due to pollutant exposure may be due to starvation [25] but in the present study, the exposed fish maintained their feeding regime; hence the loss of weight cannot be correlated with starvation but may be due to intoxication [26]. Besides, 0.006, 0.063 and 0.636 mg $L^{-1}$ Cd showed no significant decline in weight up to 21, 14 and 14 days of exposure respectively (Table 3). This is probably due to the fact that fish utilized the reserve sources of energy available and maintained the body weight before the toxicity of Cd became prominent. Such retarded growth due to 0.5-1.5 mg $L^{-1}$ Cd was also seen in guppies after 30 days exposure [27] and in rainbow trout to 10 and 25 ìg $L^{-1}$ Cd [28]. It was suggested that reduced growth was mainly due to an indirect effect of Cd on macromolecular syntheses which are secondary effects induced by physiological stress [27].

In conclusion, the present study indicates that the accumulation of Cd and its effect on growth in Indian flying barb *E. danricus* is dependent on concentration, time and tissue type.

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