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Patterns of cadmium accumulation in selected tissues of the catfish *Clarias batrachus* (Linn.) exposed to sublethal concentration of cadmium chloride

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ABSTRACT

The pattern of accumulation of cadmium and its affinity to selected tissues - gills, kidneys, liver, skin and muscle - of *Clarias batrachus* exposed to sublethal concentrations (7 ppm) of cadmium chloride was investigated. These tissues showed significant variations in the patterns of accumulation of cadmium. The rate of accumulation varies from tissue to tissue as well as at various exposure periods. The mean rate of accumulation after 60 days of exposure was in the order gills > kidneys > liver > skin > muscle. The difference in the rate of accumulation may be attributed to the proximity of the tissues to the toxicant medium, physiological state of the tissues and presence of ligands in the tissues having an affinity to cadmium.

Key words: bioaccumulation, cadmium chloride toxicity, *Clarias batrachus*, catfish

Introduction

In India, even though industrialization has not reached the level attained in the developed countries, pollution of aquatic habitats seems to be an inevitable problem. More toxic compounds are being increasingly detected in aquatic ecosystems. With the advent of agricultural and industrial revolution, most of the water sources are becoming contaminated (KHARE and SINGH, 2002). Industrial discharges containing toxic and hazardous substances, including heavy metals, (GBEM et al., 2001; WOODLING et al., 2001) contribute tremendously to the pollution of aquatic ecosystems. According to SATYANARAYANAN et al. (1985) the presence of heavy metals on the east coast of India deserves special mention as it almost forms a repository for industrial effluents and city sewages. Among the various heavy metal

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pollutants, cadmium merits special attention due to its potential hazards to aquatic biota (MAYER et al., 1991; BARBER and SHARMA, 1998) as well as to human beings (GROTEN and VAN BLADERON, 1994; VANDERPOOL and REEVES, 2001). This heavy metal is a common aquatic pollutant and is known to be highly toxic to most organisms, even at small concentrations in natural waters (LOVERT et al., 1972).

In general, cadmium is a biologically non-essential, non-biodegradable, persistent type of heavy metal and its compounds are known to have high toxic potentials. Further, continuous, low level cadmium exposure may have a gross biological impact comparable to that of recurring exposures of much greater intensity. In fresh water fish, cadmium uptake is taking place mainly through three routes namely, gills, skin and also from food via the intestinal wall (KARLSSON-NORRGRAN and RUNN, 1985). On the other hand, the metal retention capacity of the fish is dependent on the metal assimilation and excretion capacities of the fish concerned (RAO and PATNAIK, 1999). According to FERARD et al. (1983) aquatic organisms take up heavy metals and concentrate them to amounts considerably higher than those found in the environment. Therefore, it is important to find the pathways of accumulation of heavy metals and their affinity to different tissues, especially in fishes. In this context, the present investigation has been designed to study the pattern of bioaccumulation of cadmium in the gills, liver, kidneys, muscle and skin of the culturable catfish *Clarias batrachus* (Bloch.) exposed to sublethal concentrations of cadmium chloride. *C. batrachus* is highly valued as a table fish throughout the Indian subcontinent and is preferred for culture even in muddy and shallow waters where other culturable fishes may not thrive well.

Materials and methods

Irrespective of sex, healthy specimens of *C. batrachus* having a body weight of 58 ± 2g and length of 18 ± 3 cm were collected locally at Chidambaram, Tamil Nadu, India and acclimated to the laboratory conditions for 30 days in large plastic aquaria containing well water. During acclimation they were fed with minced goat liver every day (d), for 3 hours (h). Water was renewed after every 24 h with routine cleaning of the aquaria, leaving no faecal matter, dead fish (if any) or unconsumed food. Prior to the commencement of the experiment, 96 h median lethal concentration (96 h LC_{50}) of cadmium chloride (E. Merck, India) to *C. batrachus* was estimated following the Trimmed Spearman Kärber method (HAMILTON et al., 1977) and 24 h renewal bio-assay system, and was found to be 70 ppm (95% confidence limit). For the analysis of sublethal toxicity, 24 h renewal bio-assay system was followed. Six groups of 10 fish each were exposed separately in six separate aquaria (marked as 10d, 20d, 40d, 60d, and two extras as ext-1 and ext-2) containing100 litres (L) each of 7 ppm (10% of 96 h LC_{50}) cadmium chloride solution prepared in well water having dissolved oxygen 5. 8 ppm, pH 7.4, water hardness 30.0 mg/L (ANONYM.,

1992) and a water temperature of 27 ± 2 ^{0}C. Parallel groups of 10 fish each were kept in separate aquaria containing 100 L of well water (without the addition of cadmium chloride) as controls. Feeding was allowed in the experimental as well as control groups every day for a period of 3 h before renewal of the medium throughout the period of the experiment. Random checking of goat liver for the presence of cadmium on 10 different days during the experiment did not reveal any detectable amount. After the expiry of 10th, 20th, 40th and 60th days of exposure, 3 fish each from the respectively marked experimental, as well as control aquaria, were sacrificed. For estimating the cadmium content, first a pair of gills, kidneys, liver, pieces of skin and muscle were excised from the experimental fish as well as control fish separately and the tissues were placed in separate Petri dishes to dry at 80 °C until reaching a constant weight. Five hundred mg each of the dried tissues were placed in separate digestion flasks and nitric-perchloric acid (4:1) mixture was added. The digestion flasks were gradually brought to and kept at 130 °C on a hotplate until all materials were dissolved and the digests were diluted with deionized water. All the dissection instruments and glassware were acid washed and rinsed with deionized water. Metal concentrations in samples were measured using a Perkin Elmer A Analyst 800 atomic absorption spectrophotometer and is given in ppm. Obtained data were subjected to standard statistical processing based on random sampling of three different samples of experimental, as well as control groups, of each tissue at each sampling period. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was performed (BRUNING and KINTZ, 1968) to determine whether the bioaccumulation of cadmium in the various tissues studied was influenced significantly by the exposure periods. Since there were no significant variations in the values of the respective control tissues collected at the various exposure periods, the average value of each of the control tissues was taken into account.

Results

Macroscopic (behavioural) observation. On exposure to the sublethal concentration of cadmium chloride, the toxic stress on the fish was manifested in the form of restlessness and jerky and erratic swimming movements. The exposed fish also showed increased ventilatory movements of operculam and increased gulping activity. Instantaneous secretion of excessive mucus all over the body surface of the exposed fish was also noticed. Copious amounts of mucus were later released into the media at various stages of exposure in the form of streaks, along with rejected flakes of epithelial cells and other cell debris. Even though the exposed fish rejected the food provided, especially in the earlier stages (up to 5th day) of exposure, they started consuming the food (with hesitation) from the 6th day onwards and gradually resumed feeding activity to near normal situation by the 13th day. Throughout the duration of the experiment no significant behavioural or macroscopic

changes were observed in the control groups. No death occurred either in the control or in the experimental groups during the whole period of the experiment.

Cadmium accumulation study. A summary of the analysis of variance showing the level of significance of accumulation of cadmium in the various tissues studied is documented in Table 1 along with the alterations in the pattern of bioaccumulation (Table 2) as revealed by Duncan's multiple range test.

Table 1. Summary of analysis of variance (ANOVA) showing the level of significance in the pattern of bioaccumulation of cadmium in gills, liver, kidneys, muscle and skin of *Clarias batrachus* in various exposure groups (10, 20, 40 and 60 days) of 7 ppm cadmium chloride exposure.

Parameter (Tissues)	Source	Sum square (ss)	df	Mean square (ms)	F	P
Gills	Total	2.61	14	-	-	-
	Between groups	2.59	04	0.65	325	< 0.001
	Within groups	0.02	10	0.002	-	-
Liver	Total	1.54	14	-	-	-
	Between groups	1.51	04	0.3775	125.83	< 0.001
	Within groups	0.03	10	0.003	-	-
Kidneys	Total	1.7	14	-	-	-
	Between groups	1.69	04	0.4225	422.5	< 0.001
	Within groups	0.01	10	0.001	-	-
Muscle	Total	0.02	14	-	-	-
	Between groups	0.01	04	0.0015	15	< 0.001
	Within groups	0.01	10	0.001	-	-
Skin	Total	0.48	14	-	-	-
	Between groups	0.47	04	0.1175	117.5	< 0.001
	Within groups	0.01	10	0.001	-	-

Gills. Of all the tissues investigated, the rate of accumulation of cadmium was maximum in gills of exposed fish, and no detectable amount of cadmium was observed in the gills of the control fish (Table 2). The mean rate of accumulation of cadmium in gills after 60 days of exposure was 0.98 ± 0.03 ppm (Fig. 1). After an initial surge, metal concentration decreased gradually until the 40th day. However, the fag end of the experiment registered an increasing trend in the accumulation pattern (Table 2).

Liver. As with the case of gills, cadmium could not be traced in the liver of the control fish. In the case of the experimental groups, even though the quantity of accumulated cadmium was less in the case of liver when compared to gills, the pattern of accumulation showed a more or less continuous increasing trend until the 40th day of exposure (Table 2; Fig. 1). However, after 60 days the quantity of cadmium in the tissue decreased. The mean rate of accumulation was 0.77 ± 0.01 ppm (Fig. 1).

Table 2. Alterations in the bioaccumulation of cadmium (in ppm) in gills, liver, kidneys, muscle and skin of *Clarias batrachus* at various stages of 7 ppm cadmium chloride exposure

Parameter (Bioaccumulation of Cd in various tissues)	Control	Length of experimental exposure (days)			
		10	20	40	60
Gills	0.00 ± 0.00	1.15 ± 0.04 a**	0.92 ± 0.05 a** b**	0.75 ± 0.01 a** b**	1.09 ± 0.01 a** b**
Liver	0.00 ± 0.00	0.65 ± 0.02 a**	0.78 ± 0.02 a** b*	0.92 ± 0.01 a** b*	0.72 ± 0.00 a** b**
Kidneys	0.00 ± 0.00	0.77 ± 0.01 a**	0.83 ± 0.01 a** b^{NS}	0.63 ± 0.01 a** b**	0.99 ± 0.01 a** b**
Muscle	0.00 ± 0.00	0.02 ± 0.00 a**	0.04 ± 0.01 a** b**	0.04 ± 0.00 a** b^{NS}	0.05 ± 0.00 a** b^{NS}
Skin	0.00 ± 0.00	0.33 ± 0.01 a**	0.53 ± 0.01 a** b**	0.24 ± 0.00 a** b**	0.13 ± 0.01 a** b*

Note: $\bar{X}$ ± SEM where number of fish taken into account in each exposure group (n) = 3; a = between the respective experimental group and control group; b = between the respective experimental group and preceding experimental group; NS = Not significant; * = P<0.05; ** = P<0.01.

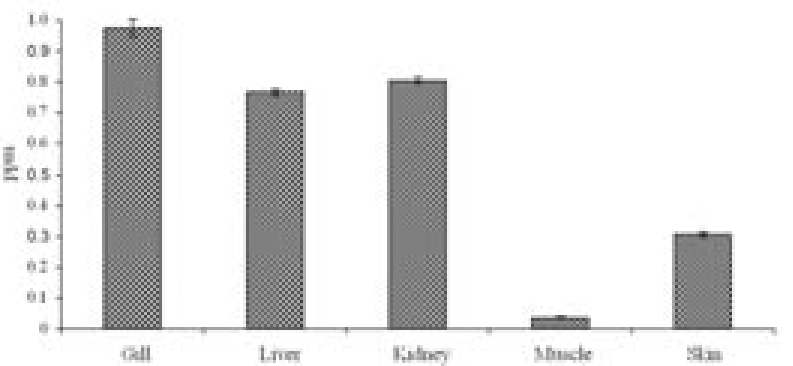

Fig. 1. Mean rate of bioaccumulation of cadmium (in ppm) in gills, liver, kidneys, muscle and skin of *Clarias batrachus* during 7 ppm cadmium chloride exposure. $\bar{X}$ ± SEM

Kidneys. The rate of accumulation of cadmium in kidneys increased along with exposure time, with an exception only after 40 days (Table 2). The mean rate of accumulation of cadmium in kidneys during the sublethal exposure was 0.81 ± 0.01 ppm, which is next to that of gills. Cadmium was not detected in the control fish.

Muscle. Cadmium was not detectable in the muscle of the control fish and only traces of the metal were present in the case of the experimental tissue (Table 2). Even after 60 days of continuous exposure, only 0.05 ± 0.00 ppm cadmium was present in the muscle tissue (Table 2) of the exposed fish. The mean rate of accumulation of cadmium in muscle was 0.04 ± 0.00 ppm (Fig. 1).

Skin. Even though skin constitutes the single tissue having maximum surface area and direct contact with toxicant medium, the rate of accumulation was less when compared to other tissues, such as gills, kidneys and liver (Table 2; Fig. 1). No cadmium was traceable in the skin of the control fish. In the case of the experimental fish, the rate of accumulation decreased during the later periods (Table 2), leading to an overall decrease in the mean rate (0.31 ± 0.01) of bioaccumulation of cadmium in the skin tissue (Fig. 1).

Discussion

In the present investigation, the mean rates of cadmium accumulation in the various tissues studied was in the order gills > kidneys > liver > skin > muscle and it may be inferred that the pattern of accumulation of cadmium differs from tissue to tissue. Critical analysis revealed that the rate of accumulation in the various tissues in comparison to their respective controls is influenced by the duration of exposure (Table 1). However, there occurred significant variations in the concentrations of accumulated cadmium in the various tissues studied at different exposure periods (Table 2) when compared with the respective preceding exposure periods. This difference in accumulation may be attributed to the proximity of the tissue to the toxicant medium, the physiological state of the tissue, presence of ligands having an affinity to cadmium and/or to the role of the tissue in the detoxification process.

From the point of view of proximity to toxicant of the various tissues analysed, gills and skin are in direct contact with the toxic medium, whereas liver, kidneys and muscle are exposed through media effect. Even though the gills and skin come into direct contact with the ambient toxicant, the pattern of bioaccumulation showed considerable differences between them (Table 2, Fig. 1). While gill was the organ accumulating the maximum cadmium, skin accumulated a far lower amount. Even though reports indicate a correlation between bioaccumulation of metals and exposure concentration, along with exposure time, (GILES, 1988), such a correlation did not exactly seem to fit data from the present study, as both skin and gill are faced with the same concentration of the toxicant and the same exposure period. A probable reason for the observed difference in the metal

accumulating capacity of gills and skin may be the physiological state of the tissue and/or structural and functional organization of these organs. In the case of fish and crustaceans, as well as molluscs, gills are one of the target organs to suffer instantaneously from ambient toxicants. One of the basic reasons for the gills to act as the primary site for cadmium accumulation, as observed in the present study, is its external position and its proximity to the ambient toxicants. In addition, the highly branched structural organization of the gill and the resultant highly increased surface area, along with the large volume of water passing through the gill surface and the highly vascular physiological state and the relatively small biomass when compared to their surface area (MAYER et al., 1991) make the gill a prime site for cadmium accumulation.

As far as the presence of various ligands in the tissues is concerned, being an oxyphilic and sulphophilic element, cadmium undergoes multiple bonding in the body (MOORE and RAMAMOORTHY, 1984), forming stable complexes with a variety of organic compounds. In the present study the increased mucogenesis under the influence of toxicant, as also reported by RAJAN and BANARJEE (1991), might result in the formation of a mucous trap over the gills for the Cd^{2+} ions due to the preferential attraction of cadmium to the -SH groups present in the mucus. The cation binding capacity of the fish mucus is also reported by INGERSOLL et al. (1990). However, according to PAUL and BANERJEE (1997), due to the constant and increased ventilatory movements of the operculam under the influence of the xenobiotics, the protective mucous plug inside the opercular chamber is quite often discharged into the medium. Such discharges of mucous plug might make the gills a more vulnerable site for accumulation of cadmium. All these structural and functional peculiarities of the gills, along with the high vascularization, might be responsible for the highest rate of accumulation of cadmium.

On the contrary, the skin, which also comes under the direct contact stress of the toxicant, shows a far lesser rate of bioaccumulation. According to RAJAN and BANERJEE (1991) and PAUL and BANERJEE (1996), the mucogenic activity of the body skin epithelium in fish is very high when compared to gills (HEMALATHA and BANERJEE, 1997). This increased mucogenesis may play a crucial role in preventing the cadmium ions from entering the body, as the coagulated mucus all over the body might be acting as a protective ion trap. Further, unlike gill, discharge of the body mucus into medium is not an active process as there are no ventilatory movements in the skin epithelium, and the rejected epithelial cells, along with the proteniaceous contents of the other degenerating cells, form a protective scab over the skin. Such a protective covering may act as an efficient trap for the Cd^{2+} ions and, at a later stage, when the cellular debris along with the mucous mass is released into the medium, the entire accumulated cadmium ions might be rejected into the medium itself and thereby greatly retard their entry into the body skin. Moreover, the regeneration of the exhausted and sloughed mucous cells is quite quick in the case of body skin when compared to the opercular epithelium (PAUL and BANERJEE, 1996 and 1997) and gills

(HEMALATHA and BANERJEE, 1997), leaving less time for the accumulation of cadmium on the body skin epithelium. The intermittent increases observed in the concentration of cadmium in skin and gill (Table 2) at various stages of exposure may be attributed, at least partially, to the temporary breakdown of the mucogenic barrier due to the exhaustion of the mucous cells after their hyper-activity.

Even though liver and kidneys do not come into direct contact with the medium, the cadmium accumulation pattern in them followed more or less the same pattern as that of gills (Table 2). Kidneys are next to gill (Fig. 1) quantity-wise in the accumulation of cadmium. SMITH and BELL (1976) have observed higher rates of heavy metal accumulation, especially in the posterior kidney, a tissue primarily involved in the excretory function. Many other workers have also reported the increased cadmium accumulation capacities of kidneys, liver and gills of aquatic organisms (PROTASOWICKI and CHODYNIECKI, 1992; NARAYANAN et al., 1997). One of the main reasons attributed to the increased presence of heavy metals in these organs is their capacity to accumulate cadmium brought by blood from other parts of the body and induce the production of the metal binding protein, metallothionein, which is believed to play a crucial role against the toxic effects of heavy metals by binding them (BHATTACHARYA et al., 1985). According to KLAVERCAMP et al. (1984) the gill and the liver, along with kidney, are the main sites of metallothionein production and metal retention. This may be yet another main reason for the enhanced presence of cadmium in the gills, kidneys and liver. In addition, all these tissues are rich in the cadmium binding-SH groups (REMA and PHILIP, 1997) and therefore it is not surprising that the metal ions are complexed in these organs.

According to KENT (1998) the liver and kidneys are involved in the detoxification and removal of toxic substances circulating in the blood stream. Moreover, liver and kidneys, being the major organs of metabolic activities including detoxification (KLAVERCAMP et al., 1984), cadmium might also be transported into these organs from other tissues, including gills and muscle, for the purpose of subsequent elimination. Such transportation might lead to higher rates of accumulation in these two organs. The possibility of such detoxification/elimination-related mobilization of accumulated cadmium may be one reason for the intermittent reductions in the quantity of accumulated cadmium in gills, as well as skin (Table 2) at various stages of exposure. Further, according to DORIAN and GATTONE (1992) unbound metals, such as cadmium and mercury, can be reabsorbed by active transport mechanism in the cells of the proximal convoluted tubule, and once they are in the cells they bind to metallothionein, resulting in their accumulation. All these observations justify the possibility of transporting the trace amounts of cadmium from the various tissues to kidneys.

Of all the tissues investigated in the present study, the muscle accumulated the lowest level of cadmium, even after 60 days of the experiment (Table 2; Fig. 1). This finding

confirmed the existing reports (PROTASOWICKI and CHODYNIECKI, 1992; BARBER and SHARMA, 1998). In the light of all these observations, and also from the results of the present study, it may be inferred that the residue of cadmium present in the fish muscle may not be exactly correlated to its concentration in the ambient water medium. Various reasons may be attributed to the lower rate of bioaccumulation of cadmium in muscle. First of all, the muscle does not come into direct contact with the toxicant medium as it is totally covered externally by the skin, which in many ways helps the organism to ward off the penetration of the toxicant, as already discussed. Another probable reason may be the fact that even though muscle is the most valued edible tissue, it is not an active site for detoxification and therefore transport of cadmium from other tissues to muscle (as in the case of liver and kidney) does not seems to arise.

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SAŽETAK

Istražen je model nakupljanja kadmija i njegov afinitet prema tkivu škrga, bubrega, kože i mišićja u somova *Clarias batrachus* izloženima subletalnim koncentracijama (7 ppm) kadmijeva klorida. Pretražena tkiva pokazivala su značajne razlike u načinu nakupljanja kadmija. Udio nakupljenog kadmija ovisi o vrsti tkiva i trajanju izloženosti. Prosječni stupanj nakupljenosti nakon 60 dana od izlaganja kretao se prema sljedećem redoslijedu škrge>bubrezi>jetra>koža>mišići. Razlika u udjelu nakupljenosti može se pripisati blizini tkiva toksičnom mediju, fiziološkom stanju tkiva i prisutnosti liganada u tkivu koje ima afinitet prema kadmiju.

Ključne riječi: biokoncentracija, toksičnost kadmijeva klorida, *Clarias batrachus*, som