



ORIGINAL RESEARCH PAPER

Zoology

EFFECTS OF CADMIUM STRESS ON THE CAT FISH (CLARIAS BATRACHUS) OF RIVER KOSI AND SOME PONDS OF SAHARSA DISTRICT: A CRITICAL ANALYSIS.

KEY WORDS: *Clarias batrachus*, cadmium, bioaccumulation, concentrations.

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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 studied. 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.

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. Industrial discharges containing toxic and hazardous substances, including heavy metals, contribute tremendously to the pollution of aquatic ecosystems. Among the various heavy metal pollutants, cadmium merits special attention due to its potential hazards to aquatic biota as well as to human beings. 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.

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. On the other hand, the metal retention capacity of the fish is dependent on the metal assimilation and excretion capacities of the fish concerned. 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* 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

Healthy specimens of *C. batrachus* having a body weight of 58 ± 2 g 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 LC50) of cadmium chloride (E. Merck, India) to *C. batrachus* was estimated following the Trimmed Spearman Kärber method 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) containing 100 litres (L) each of 7 ppm (10% of 96 h LC50) cadmium chloride solution prepared in well water having dissolved oxygen 5.8 ppm, pH 7.4, water hardness 30.0 mg/L and a water temperature of 27 ± 2 °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 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 operculum 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.

Analysis of Cadmium accumulation.

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.

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	-	-

Liver

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 ^{NS}	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: $\bar{x} \pm SEM$ 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.

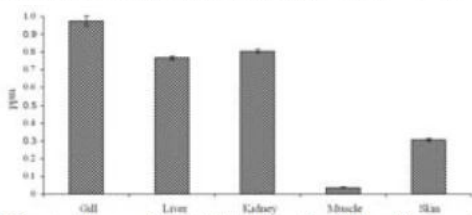


Fig. 1. Mean rate of bioaccumulation of cadmium (in ppm) in gills, liver, kidneys, muscle and skin of *Clarias batrachus* during 7 ppm cadmium chloride exposure. $\bar{x} \pm SEM$

Kidneys

Accumulation increased along with exposure time, with an exception only after 40 days (Table 2). Cadmium was not detected in the control fish.

Muscle

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

Skin

The rate of accumulation was less. No cadmium was traceable in the skin of the control fish. In the experimental fish, the rate of accumulation decreased during the later periods

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 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, forming stable complexes with a variety of organic compounds. In the present study the increased mucogenesis under the influence of toxicant, might result in the formation of a mucous trap over the gills for the Cd²⁺ ions due to the preferential attraction of cadmium to the -SH groups present in the mucus. 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, shows a far lesser rate of bioaccumulation. 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 proteinaceous 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 Cd²⁺ 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 and gills 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.

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. 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. 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 and therefore it is not surprising that the metal ions are complexed in these organs.

Moreover, liver and kidneys, being the major organs of metabolic activities including detoxification, 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. 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. 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.

References

1. ANONYMOUS (1992): (American Public Health Association), AWWA (American Water Works Association), WPCF (Water Pollution Control Federation) (1992): Standard methods for the examination of water and waste water. 19th Ed., Washington, D. C.
2. BARBER, D., M. S. SHARMA (1998): Experimentally induced bioaccumulation and elimination of cadmium in fresh-water fishes. *Poll. Res.* 17, 99-104.
3. BHATTACHARYA, T., A. K. RAY, S. BHATTACHARYA (1985): Response of *Channa punctatus* under short term and long term exposure to industrial pollutants. Induction of histopathology in the kidney. *Z. Mikrosk. Anat. Forsch (Leipzig)*. 899, 327-334.
4. BRUNING, J. L., B. L. KINTZ (1968): Computational Hand Book of Statistics. Foresman and Company, Dallas, USA. pp. 1-308.
5. DORIAN, C., V. H. GATTONE (1992): Renal cadmium deposition and injury as a result of accumulation of cadmium metallothionein (CdMT) by proximal convoluted tubules - A light microscope autoradiographic study with CdMT. *Toxicol. Appl. Pharmacol.* 114, 173-181.
6. FERARD, J. F., J. M. JOUANY, R. TRUHAUT, P. VASSEUR (1983): Accumulation of cadmium in a freshwater food chain experimental model. *Ecotoxicol. Environ. Safe.* 7, 43-52.
7. GBEM, T. T., J. K. BALOGUN, F. A. LAWAL, P. A. ANNUNE (2001): Trace metal accumulation in *Clarias gariepinus* Teugules exposed to sublethal levels of tannery effluent. *Sci. Total. Environ.* 271, 1-9.
8. GILES, M. A. (1988): Accumulation of cadmium by rainbow trout, *Salmo gairdneri*, during extended exposure. *Can. J. Fish. Aquat. Sci.* 45, 1045-1053.
9. GROTEN, J. P., P. J. VAN BLADEREN (1994): Cadmium bioavailability and health risk in food. *Trends Food Sci. Technol.* 5, 50-55. HAMILTON, M. A., R. C. RUSSO, R. V. THURSTON (1977): Trimmed Spearman Kärber method for estimating medium lethal concentrations in toxicity bioassays. *Environ. Sci. Tech.* 11, 714-719.
10. HEMALATHA, S., T. K. BANERJEE (1997): Histopathological analysis of sublethal toxicity of zinc chloride to the respiratory organs of the air-breathing catfish *Heteropneustes fossilis* (Bloch.) *Biol. Res.* 30, 11-21.
11. INGERSOLL, C. G., D. A. SACHES, J. S. MEYER, D. D. GUTLEY, J. E. TIETIGE (1990): Epidermal response to pH, aluminium and cadmium exposure in brook trout (*Salvelinus fontinalis*) fry. *Can. J. Fish. Aquat. Sci.* 47, 1616-1622.
12. KARLSSON-NORRGREN, L., P. RUNN (1985): Cadmium dynamics in fish: Pulse studies with ¹⁰⁹Cd in female Zebrafish, *Brachydanio rerio*. *J. Fish. Biol.* 27, 571-581.
13. KENT, C. (1998): Basic Toxicology, John Wiley Sons., Inc, New York, pp. 1-402.
14. KHARE, S., S. SINGH (2002): Histopathological lesions induced by copper sulphate and lead nitrate in the gills of fresh water fish *Nandus nandus*. *J. Ecotoxicol. Environ. Monit.* 12, 105-111.

15. KLAVERCAMP, J. E., W. A. MC DONALD, D. A. DUNCAN, R. WAGENANN (1984): Metallothionein and acclimation to heavy metals in fish, a review. In: Contaminant effects on fisheries (Cairns, V. W., P. V. Hodson, J. O. Nriagu, Eds.). Wiley, New York, pp. 99-113.
16. LOVERT, R. J., W. H. GUTENMANN, I. S. PAKKALA, W. D. YOUNGS, D. J. LISK, G. E. BURDICK, E. J. HARRIS (1972): A survey of total cadmium content of 406 fish from 49 New York state fresh waters. *J. Fish Res. Board-Can.* 29, 1283-1290.
17. MAYER, W., M. KRETSCHMER, A. HOFFMANN, G. HARISH (1991): Biochemical and histochemical observations on effects of low level heavy metal load (Lead, Cadmium) in different organ systems of the freshwater crayfish, *Astacus astacus* L. (Crustacea: Decapoda). *Ecotoxicol. Environ. Safe.* 21, 137-156.
18. MOORE, J. W., S. RAMAMOORTHY (1984): Heavy Metals in Natural Waters, Applied Monitoring and Impact Assessment. Springer Verlag, Tokyo. pp. 1-268.
19. NARAYANAN, K. R., P. S. LYLE, S. AJMAL KHAN (1997): Pattern of accumulation of heavy metals (Mercury, Cadmium and Zinc) in the mud crab *Scylla serrata*. *J. Ecotoxicol. Environ. Monit.* 7, 191-195.
20. PAUL, V. I., T. K. BANERJEE (1996): Analysis of ammonium sulphate toxicity in the catfish *Heteropneustes fossilis* using mucocyte indexing. *Pol. Arch. Hydrobiol.* 43, 111-125.
21. PAUL, V. I., T. K. BANERJEE (1997): Histopathological changes induced by ambient ammonia (ammonium sulphate) on the opercular linings of the live fish *Heteropneustes fossilis* (Bloch). *Dis. Aquat. Org.* 28, 15-161.
22. PROTASOWICKI, M., A. CHODYNEICKI (1992): Bioaccumulation of cadmium in some organs of carp, *Cyprinus carpio* L., in case of per os administration. *Arch. Pol. Fish.* 1, 61-66.
23. RAJAN, M. T., T. K. BANERJEE (1991): Histopathological changes induced by acute toxicity of mercuric chloride on the epidermis of freshwater catfish *Heteropneustes fossilis* (Bloch). *Ecotoxicol. Environ. Safe.* 22, 139-152.
24. RAO, L. M., R. PATNAIK (1999): Heavy metal accumulation in the catfish *Mystus vittatus* (Bloch.) from Mehadragedda stream of Visakhapatnam, India. *Poll. Res.* 19, 325-329.
25. REMA, L. P., B. PHILIP (1997): Accumulation of an essential metal (Zinc) and a non-essential metal (Mercury) in different tissues of *Oreochromis mossambicus* (Peters). *Ind. J. Exp. Biol.* 35, 67-69.
26. SATYANARAYANAN, D., I. M. RAO, B. R. PRASADA REDDY (1985): Chemical oceanography of harbour and coastal environment of Visakhapatnam (Bay of Bengal). Part I: Trace metals in water and particulate matter. *J. Mar. Sci.* 14, 139-146.
27. SMITH, L. S., G. R. BELL (1976): A practical guide to the anatomy and physiology of Pacific Salmon. *Fish Mar. Serv. Misc. Spec. Publ.* 27, 14.
28. VANDERPOOL, A., G. REEVES (2001): Cadmium absorption in women fed processed edible sunflower kernels labeled with a stable isotope of cadmium, ¹¹³Cd1. *J. Environ. Res. Sec. A.* 87, 69-80.
29. WOODLING, J. D., S. F. BRINKMAN, B. J. HORN (2001): Non uniform accumulation of cadmium and copper in kidney's of wild brown trout *Salmo trutta* populations. *Arch. Environ. Contam. Toxicol.* 40, 381-385.