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Residue of cadmium and mercury in liver and muscle of a freshwater catfish *Heteropneustes fossilis* (Bloch)

Dhananjay K. Srivastava*, Seema Pandey, Saumya, Arun K. Srivastava and Ram Singh[1]

Fish Physiology and Toxicology Lab., S. M. M. Town P. G. College Ballia, 277001 India
[1]S.C.P.G. College, Ballia

Abstract :*Toxic metals viz., cadmium and mercury in liver and muscle tissues of a freshwater catfish, H. fossilis were measured by graphic mode and atomic absorption spectrophotometer (AAS). Fish were exposed to fractions of 96h LC 50 value. Acute (1/5th of 96h LC_{50}), subacute (1/10th of 96h LC_{50}) and sublethal (1/15th of 96h LC 50) concentrations for acute (96h), short (10-20 days) and long (30-60 days) terms. The accumulation of both the metals in liver and muscle was significant in respect of dose and duration. The cadmium residue was found to be higher in liver than muscle but mercury was more in muscle than liver. It was also observed that, the relationship between the body concentration of each heavy metal and its respective 96h LC_{50} value was close and sequenced.*

Key word: Residue, Metal toxicity, Liver / Muscle, Atomic absorption spectroscopy, *H. fossilis*

Introduction

Metals viz., cadmium and mercury can alter many physiological and biochemical parameters, either in blood or in tissue, including structural deformations in aquatic animals especially in fish (Barlas, 1999; Al- Yousuf *et al.,* 2000; Canli *et al.,* 2001; Cengiz and Unlu, 2002) being non-biodegradable. They can be concentrated along the food chain, producing their toxic effects at points after, away from the source of pollution (Fernandez *et al.,* 2000; Berzins and Bundy, 2002; Kishe and Machiawa, 2003).

The cadmium and mercury play no known role in metabolism, as no enzyme has been identified which specially, requires cadmium and mercury as a co-factor. Thus both metals are however, extremely hazardous to life of aquatic as well as terrestrial animals through food chain.

Laboratory studies suggest that metal concentration in a given fish species depends on many factors, such as exposed dose and duration (Hansen *et al.,* 2002) as well as metal speciation and the concentration of competing ions in exposure media (Playle *et al.,* 1993). In laboratory metals exposure are at high concentrations and for shorter durations than would be the case in nature.

The objectives of this paper was to estimate the concentration of non-nutritional metals like cadmium and mercury in liver and muscle of a freshwater catfish, *Heteropneustes fossilis* after exposure to different dose and time intervals. This information would be an useful tool for the affection management and control of natural area with respect to the input of toxic metals and their bioavailability.

Materials and Methods

The catfish, (Average wt. 32.50 ± 2.25g; length 12.25 ± 1.50cm) were collected locally and acclimatized in tap water (pH 7.8, chloride 7.5m mg/L, hardness 146.65 mg/L as $CaCO_3$ and BOD 17.50 mg/L) for 10 days under natural photoperiod and ambient temperature in glass

*E-Mail: dhan.sri3@gmail.com

aquaria. They were fed daily *ad libitum* on wheat flour and pellets of ground dried shrimp. The stock solution of cadmium chloride and mercuric chloride was prepared in water, both the metals are of technical grade (95 to 98% purity).

Acute toxicity bioassays were performed for the determination of median lethal concentrations for cadmium chloride and mercuric chloride for 24, 48, 72 and 96h exposure periods under static test condition (APHA, 2005). Ten fish were exposed to metals concentrations. The LC_{50} values as well as 95% confidence limits were calculated according Litchfield and Wilcoxon, (1949). The fish were not fed during the bioassays experiments. The presumably harmless (safe) concentrations of both the metals were estimated by the formula of Hart *et al.,* (1945) which was found to be 0.036 mgl^{-1} for cadmium chloride and 0.039 mgl^{-1} for mercuric chloride respectively.

The fish were exposed to acute ($1/5^{th}$ of 96h LC_{50}), subacute ($1/10^{th}$ of 96h LC_{50}) and sublethal ($1/15^{th}$ of 96h LC_{50}), concentrations of cadmium chloride and mercuric chloride for acute (96h), short (10-20 days) and long (30-60 days) terms.

To determine the concentrations of residue of the nutritional metals cadmium chloride and mercuric chloride in tissues of liver and muscle, the fish were stunned by a blow to the head soon after being transferred to laboratory of Central Inland Fisheries Research Institute (CIFRI) Barrackpur, Kolkata, (W. B.) in a ice box. Liver and muscle were removed. Each tissues was put in a separate petri dish and dried in an oven at 110° C for 48 h. Some 5g of each dry tissue was weighed and put in a separate test tube. Then 3 ml of conc. nitric acid and perchloric acid in a 2:1 ratio were added to each test tube and digested on a hot plate at 160°C for 5 h. After complete digestion, 5ml of distilled water was added to each sample and the metals concentration were presented in (ppm). The mercury was measured on an atomic absorption spectrophotometer (AAS) and cadmium with a graphic mode instead of flame mode.

Results and Discussion

The concentrations of cadmium and mercury in the liver and muscle of control catfish was 0.014±0.001 and 0.002±0.001 ppm as well as 0.012±0.001 and 0.003±0.001 ppm respectively. The general accumulation order of the heavy metals was Cd >Hg. In the present study the fish exposed to cadmium chloride and mercuric chloride at acute, subacute and sublethal concentrations for acute (96h), short (10-20 days) and long (30-60 days) terms accumulate significant amount of cadmium and mercury in liver and muscle. The amount of cadmium in liver was more in camparison to muscle at each concentrations and time intervals (Table 1). Contrary to mercury which is more in the muscle than liver (Table 2). The concentration of cadmium residue was maximum at subacute concentration for long (30-60 days) term in respect of acute and sublethal concentrations for acute (96h) and short (10-20 days) terms. The fish showed more mercury residue in muscle instead of liver at subacute concentration for short (10-20 days) term. The accumulation of residue was more at low concentrations for long term exposure in comparison to high concentrations for short terms.

Table 1. Relationship between the body concentrations of heavy metals at acute concentration and their respective 96h LC_{50} values.

Heavy metal concentration (ppm)		
Tissue	$HgCl_2$	$CdCl_2$
Liver	0.010±0.002	0.35±0.03
Muscle	0.015±0.002	0.20±0.02
Average body concentration (ppm)	0.0125± 0.002	0.275± 0.025
LC_{50} value (mg/L)	0.85	0.75

Table 2. Residue of cadmium (ppm) in liver and muscle of a catfish, *H. fossilis* on exposure to acute, subacute and sublethal concentration for acute (96h) and both short (10-20days) and long (30-60days) terms.

Tissue	Control	Experimental								
		Acute	Subacute concentration				Sublethal concentration			
			Short term		Long term		Short term		Long term	
		96h	10days	20days	30days	60days	10days	20days	30days	60days
Liver	0.014	0.35±	0.38±	0.42±	0.75±	0.86±	0.32	0.34±	0.37±	0.42±
	0.001	0.03*	0.04*	0.05**	0.02**	0.04*	0.02*	0.04*	0.04*	0.05*
Muscle	0.012	0.20±	0.22±	0.26±	0.30±	0.32±	0.20	0.24±	0.26±	0.28±
	0.001	0.028	0.03*	0.04**	0.05*	0.04**	0.02*	0.04**	0.05*	0.04*

Note: Value are mean ±SE (n=6), P<0.05, P<0.01, P<0.001

The 96h LC_{50} value for mercury and cadmium were 0.85 mgl^{-1} and 0.75 mg/L respectively. Comparison with the 96h LC_{50} values was made on the basis of the average body concentration of each heavy metal. The average body concentration of mercury and cadmium were 0.0125 ± 0.002 ppm and 0.275 ± 0.025 ppm respectively.

The comparison of the body concentration of each heavy metal with its respective 96h LC_{50} value showed that mercury with lowest body concentration (0.0125 ppm) resulted in the highest 96h LC_{50} value (0.85 mg/L), cadmium with the highest body concentration (0.275 ppm) resulted in the lowest 96h LC_{50} value (0.78 mg/L). In general, the relationship between the body concentration of each heavy metal and its respective 96h LC_{50} value was close and sequenced (Table 3).

Nutritional metals, viz., cadmium and mercury are considered a major source of pollution in natural water. They have inherent toxicity to the living aquatic forms. Being non- biodegradable, they reach the aquatic environment and remain accumulated in various forms of exposed animals, especially in liver and muscle.

The concentration of heavy metal in fish is related to several factors, such as the food habits and foraging behavior of the organism (Chen and Folt, 2000), trophic status, source of a particular metal, distance of the organism from the contamination source and the presence of other ions in the milieu (Giesy and Wiener, 1977), bio- magnification and/ or bio-diminishing of a particular metal (Barlas, 1999), food availability (Dethloff, 2001), metallothioneins and other metal detoxifying proteins in the body of the animal (Deb and Fukushima, 1999), temperature, transport of metal across the membrane and the metabolic rate of the animal (Macleod and Pessah, 1973), physical and chemical properties of the water (Wepner *et al.,* 1992) and the seasonal changes in the taxonomic composition of

Table 3. Residue of mercury (ppm) in liver and muscle of a catfish, *H. fossilis* on exposure to acute, subacute and sublethal concentration for acute (96h) and both short (10-20days) and long (30-60days) terms.

Tissue	Control	Experimental								
		Acute	Subacute concentration				Sublethal concentration			
			Short term		Long term		Short term		Long term	
		96h	10days	20days	30days	60days	10days	20days	30days	60days
Liver	0.002 0.001	0.010± 0.002	0.025± 0.004	0.028± 0.003	0.030± 0.001	0.032 0.002	0.015± 0.002	0.018± 0.003	0.022± 0.004	0.025± 0.002
Muscle	0.003 0.001	0.015± 0.002	0.026± 0.002	0.030± 0.001	0.034± 0.002	0.038± 0.003	0.012± 0.002	0.020± 0.001	0.023± 0.003	0.028± 0.002

Note. Value are mean ±SE (n=6), *P<0.05, **P<0.01, ***P<0.001

different trophic levels affecting the concentrations and accumulation of heavy metals in the body of fish (Chen and Folt, 2000).

The cadmium is a non-essential, non beneficial metal recognized to be of high toxic potential. The highest cadmium accumulation was measured in kidney followed by liver (Giguere *et al*., 2004). In the present study the fish exposed to cadmium chloride at acute, subacute and sublethal concentrations for acute (96h), short (10-20 days) and long (30-60 days) terms accumulate significant amount of cadmium in liver and muscle. The amount of cadmium in liver is more in comparision to muscle at each concentrations and time intervals. Similar observation was also made by several workers (Mason, 1987; Barak and Mason, 1990; Al-yousuf *et al*., 2000 and Chale, 2002, Cicik and Engin, 2005).

The presence of unusually high level of nutritional metals such as cadmium has many deleterious effects. Contamination above permissible limits is responsible for the reduction of fish skeleton, gill deformities, liver tumors, ulcers and finrot (Misra *et al.,* 2000). Cadmium is very toxic to fish and other aquatic organisms. However, temperature, pH and water hardness are factors that influence its toxicity and uptake by fish (Hontela *et al*., 1996). The concentration of cadmium residue was maximum at subacute concentration for long (30-60 days) term in respect of acute and sublethal concentrations for acute (96h) and short (10-20 days) terms.

The most relevant form of mercury is methylmercury (MeHg). The clinical symptoms of methylmercury poisoning are mainly neurological impairments. The neurologic signs of MeHg poisoning are parenthesis (numbness and tingling in the extremities), ataxia, neurasthenia, loss of vision/hearing, tremor, coma and finally to death (Abou –Dania, 1992; Atchison and Hare, 1994; Sorensen, 1991; Sveinsdottir, 2005; Mason and Robert, 2006).

The level of mercury residue in exposed fish liver muscle was high at acute, subacute and sublethal concentrations for acute (96h) and both short ((10-20 days) and long (30-60 days) terms. The fish showed more mercury residue in muscle instead of liver at subacute concentration for short (10-20 days) term.

The concentration of mercury in the present study was similar to that observed by other workers reported in several other fishes (Mwachiro and Durve, 1997; Kannan *et.al*., 1998 Misra *et al.,* 2000, Ruelas and Paezosuna, 2005). Barak and Mason (1990), also measured the concentrations of mercury, cadmium and lead in the fish and liver of freshwater fishes. Mercury levels in the flesh of fishes was about twices the levels in the liver, but cadmium and lead levels were several time higher in liver than flesh. Higher mercury concentrations were reported in tench from Britain (MAFF, 1973). The highest cadmium concentration for perch of an ages were measured in the kidney followed by the liver gastrointestinal tract, gills and cascass. However, in indigenous yellow perch the liver and kidney may be important sites for chronic toxicity since these organ concentrated cadmium to the highest levels (Giguere *et al.,* 2004).

Similarly, the 96h LC_{50} values of fish vary from species to species and from metal to metal. Gill and Pant, 1985; Kirubagaran and Joy, 1988; Veena *et al*., 1997 and Illopoulou *et al*., 2001 reported 96h LC_{50} values of 0.181, 0.51,0.13 and 0.51 ppm Hg, for *Barbus conchonus, Clarius batrachus, Etrophus maculates* and *Salmo gairdneri*, respectively. Spehar (1976), found 96h LC_{50} values of 2.5 and 28.0 ppm Cd for *Jordanellla floridae* and *mugil cephalus*, respectively. Susceptibility of fish to a particular heavy metal is very importat factor for LC_{50} values. The fish that is highly susceptible to the toxicity of one metal may be less or non-susceptible to the toxicity of another metal at the same concentration of that metal in the milieu. Similarly, the metal which is highly toxic to one organism at low concentration may be less or non- toxic to other organism at the same or even- higher concentration. Das and Banerjee (1980) reported 300.0 ppm cadmium chloride for the 96h LC50 of *Heteropneustes fossilis*, whereas Shah and Altindac (2006), reported the 96h LC_{50} for *Tinca tinca* was 6.5 ppm cadmium chloride.

Because of the lack of available data on the effects of body concentrations of heavy metals on the respective LC_{50} values, the results of the present study have not been compared with those of other studies and discussed accordingly. But the degree of susceptibility of *H. fossilis* to lower concentration of mercury and higher concentration of cadmium may be attributed to the altered physiological response of fish to the specific metal and the level of solubility of metals. The fish exposed to metal can compensate for the stressors. If it can not successfully compensate for stressors effects an altered physiological stage may be reached in which organism continues to function and, in extreme cases, the acclimation response may be exhausted with a subsequent effect on fitness (Mayer *et al*., 1992.). Cadmium and mercury, a nutritional toxicant accumulated in the liver and muscle of exposed fish, *H. fossilis* which were investigated by graphic mode and AAS. The cadmium accumulation was greater in liver than in muscle, contrary to mercury which was greater in muscle than liver. Furthermore, the present study shows the adaptation capability of *H. fossilis* to heavy metal load.

References

Abou-Dania, M. (1992) Metal, In : *Beurotoxicology.Boca. Raton*., 370-373.

Al- Yousuf M.H., El- Shahawi, M.S. and Ghais, Al- S.M. (2000) Trace metal in liver, skin and muscle of *Lethrinus lengan* fish species in relation to body length and sex. *Sci. Total Environ*.*,* **256**, 87-94.

APHA, (2005) American Water Works Association and Water Pollution Control Federation (21st Edi.).Washington.

Atchison, W. and Hare, M. (1994) Mechanisms of methylmercury induced neurotoxicology. *FASEB, J.*, **8**, 622-629.

Barak, N.A.E. and Mason, C.F. (1990) Mercury, cadmium and lead concentrations in five species of freshwater fish from eastern England. *Sci. Total Environ.*, **92**, 257-263.

Barlas, N.E. (1999) A pilot study of heavy metal concentration of various environment and fishes in upper sakarya basin, *Turkey Environ. Toxicol.*, **14**, 367-373.

Berzins, D. W. and Bundy, K. J. (2002) Bioaccumulation of lead in *Xenopus laevis* tadpoles from water and sediment.*Environ. Int.*, **28**, 69-77.

Canli, M. and Kalay, M., Ay, O. (2001) Metal (Cd, Pb, Cu, Cr Ni) concentration in tissue of a fish *Sardina pilchardus* and a *Peaenus japonicus* from stations on the Medeterranean Sea. *Bull. Environ. Contam. Toxicol.,* **67**, 75-82.

Cengiz E.I. and Unlu, E. (2002) Histopathological changes in the gill of mosquito fish *Gambusia affinis* exposed to endosulfan. *Bull. Environ. Contam. Toxicol.*, **68**, 290-296.

Chale, R. M. (2002) Trace metal concentration in water, sediment and fish tissue from lake Tanganyika. *Sci. Total Environ.*, **299**, 115-121.

Chen, C.Y. and Folt, C.L. (2000) Bioaccumulation and diminution of arsenic and lead in a freshwater food web. *Environ.Sci. Technol.*, **34**, 3878-3884.

Cicik, B. and Engin, K. (2005) The effects of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle of *Cyprinus carpio. J. Turk. J. Vet. Anim. Sci.*, **29**, 113-117.

Das, K.K. and Bagerjee, S.K. (1980) Cadmium toxicity in fishes. *Hydrobiol.*, **75**, 117-121.

Deb, S.C.and Fukushima, T. (1999) Metals in aquatic ecosystems: Mechanism of uptake, accumulation and release*. Int. Environ. Stud.,* **56**, 385-33.

Dethloff, G.M., Bailey, H.C. and Maier, K. J. (2001) Effects of dissolved copper on select haematological, biochemical and immunological parameters of wild rainbow trout, *Oncorhynchus mykiss*. *Arch. Environ. Contam. Toxicol.*, **40**, 371-380

Fernandez, M., Cuestas, O., Jimenez, M.A., Gareia, L.M., Fernandez, M.L., marina, M.L. and Gonzalaz, M.J. (2000) Organochloride and heavy metal residue in the water/ sediment system of the southest regional park in Madrid, Spain *Chemospher.*, **41**, 801-812.

Giesy, J.P. and Wiener, J.G. (1977) Frequency distributions of trace metal concentrations in five freshwater fishes *Trans.Am. Soc.,* **106**, 393-403.

Giguere, A., Peter, G.C. Campbell, Landis Hare, Gardon McDonald, D. and Joseph, B. R. (2004) Influence of lake chemistry and fish age on cadmium, copper and zinc concentrations in various organs of indigenous yellow perch (*Perca flavescens*). *Can. J. fish Aquat. Sci.*, **61**, 1702-1716.

Gill, T.S. and Pant, J.C. (1985) Mercury induced blood anomalies in the fresh water teleost, *Barbus conchonius*. *Water Air Soil Pollut.*, **24**, 165-171.

Hansen, J. A., Lipton, J. and Welsh, P.G. (2002) Relative sensitivity of bull trout (*Salvelinus confluentus*) and rainbow trout (*Oncorhynchus mykiss*) to acute copper toxicity. *Environ. Toxicol. Chem.,* **21**, 633-639.

Hart, W.B., Doudoroff, P. and Greenbank, J. (1945) The evaluation of the toxicity of industrial water, chemicals and other substances to freshwater fish. *Atlantic Refining Co. Phil.*, **31**.

Hontela, A., Danial, C. and Recard, A.C. (1996). Effect of acute, subacute exposure to cadmium on the interrenal and thyroid function on rainbow trout, *Onchorhynchus mykiss*. *Aquat. Toxicol.*, **35**, 171-182.

Illopoulou–Georgudaki, J. and Kotsanis, N. (2001) Toxic effects of cadmium and mercury in rainbow trout (*Oncorhynchus mykiss*): A short term bioassay. *Bull. Environ. Contam. Toxicol.*, **66**, 77-85.

Kannan, K., Smith Jr., R.G., Lee, R. F., Windom, H.L, Heitmullar, P.T., Macaulay, J.M. and Summers, J.K. (1998) Distribution of total mercury and methylmercury in water, sediment and fish from south *Florida Estuaries*., **34**, 109-118.

Kirubagaran, R. and Joy, K.P. (1988) Inhibition of testicular 3b- Hydroxy-D5 steroid dehydrogenase (3b-HSD) activity in catfish, *Clarius batrachus* (L.) by mercurials. *Ind. J. Exp. Biol.*, **26**, 907-908.

Kishe, M.A. and Machiawa, J. F. (2003) Distribution of heavy metals in sediment of Mwanza gulf of lakes Victoria, Tanzania, *Environ. Int.,* **28**, 619-625.

Litchfield, J. T. and Wilcoxon, F. (1949) A simplified method of evaluating dose effect experiments. *J. Pharmacol. Exp.Ther.*, **96**, 99-113.

Macleod, J.C. and Pessah, E. (1973) Temperature effects on mercury accumulation, toxicity and metabolic rate in rainbow trout, *Salmo gairdneri*. *J. Fish. Res. Board Can.,* **30**, 485-492.

MAFF. (1973) Survey of mercury in food: A supplementary report. Third report of the working party on the monitoring of food stuffs for heavy metals. *Ministry* *of Agriculture, Fisheries and Food. HMSO, London.*

Mason, C.F. (1987) A survey of mercury, lead and cadmium in muscle of British freshwater fish. *Chemosphere*., **16**, 901-906.

Mason, C.F. and Robert, P. (2006) Methylmercury concentration in fish from tidal waters of the Chesapeake *Bay. Environ. Contam. Toxicol*., **2**, 97-101.

Mayer, F.L., Versteeg, D.J., McKee M.J. and Folmar, L.C. (1992) Physiological and non – specific biomarkers. In :Biomarkers: Biochemical, physiological and histological markers of anthropogenic stress. Huggett, R.J. Kimerle, R.A. Mehrle, P.M. Jr., Bergman, H.L. (eds), Lewis Publishers, Boca Raton, PL. 5-85.

Misra, S. M., Borana, K., Pannis, A. and Bajpai, A.K. (2000) Assessment of heavy metals concentration in grass carp (*Ctenopharyngodon idella*). *Poll. Res*., **21**, 69-71.

Mwachiro, E.C. and Durve, V.S. (1997) Heavy metal status of the reservoir bari near Udaipur (Rajasthan) and the accumulation of the metals in the fish organs. *Poll. Res.,* **16**, 67-74.

Playle, R.C., Dixon, D.G. and Burnison, K. (1993) Copper and cadmium binding to fish gills: modification by dissolved organic carbon and synthetic ligands. *Cant. Fish Aquat. Sci.,* **50**, 2667-2677.

Ruelas- Inzunaza, J. and Paezosuna, F. (2005) Mercury in fish and shark tissue from two coastal lagoons in the gulf of California, Mexico. *Bull. Environ. Contam. Toxicol.,* **24**, 294-300.

Shah, S.L. and Altindag, A. (2005). Effects of heavy metal accumulation on the 96h LC50 values in Tench *Tinca tinca* L., 1758. *Turk. J. Vet. Anim. Sci*., **29**, 139-144.

Sorenson, E.M.E. (1991) Metal poisoning in fish , C.R.C. *Press. Inc. Boca*., 370-373.

Spehar, R.L. (1976) Cadmium and zinc toxicity in flagfish, *Jordanella floridae*. *J. Fish. Res. Board Can*., **33**, 1939-1945.

Sveinsdottir, A. Yr. (2005) Factor controlling mercury concentrations in largemouth Bass (*Micropterus salmoides*) and other fish form of Maryland reservoir. *Bull. Environ. Contam. Toxicol*., **49**, 4.

Veena, K.B. Radhakrishnan, C.K. and Chacko, J. (1997) Heavy metal induced biochemical effects in an estuarine teleost. *Indian J. Mar. Sci.,* **26**, 74-78.

Wepner, V. Vanvuren, J.H.J. and Dupreez, H.H., (1992) Effect of manganese and iron at a neutral and acidic pH on the haematology of the banded Tilapia, *Tilapia sparrmanii*. *Bull. Environ. Contam. Toxicol*., **49**, 613-619.