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BIOACCUMULATION OF SOME HEAVY METALS IN THE FISH, *CLARIAS BATRACHUS* (LINN.)

S. S. Gandhewar[1*], S. B. Zade[2]

1. Jankidevi Bajaj College of Science, Wardha, Maharashtra, India.
2. P.G. Dept. of Zoology, RTMNU, Nagpur, Maharashtra, India.

ABSTRACT: The excessive occurrence of heavy metals in natural loads has now-a-days become a matter of concern for the health of aquatic ecosystem. Heavy metals like Copper, Lead, Mercury, Cadmium, Nickel and Chromium are mainly responsible for water pollution. Metal pollution is a global problem growing at an alarming rate and aquatic organisms are continuously exposed to elevated levels of metals seriously threatening the whole ecosystem. The current experimental study of bioaccumulation of Copper, Lead and Cadmium in fish *Clarias batrachus* (Linn.) was conducted on 10^{th}, 20^{th} and 30^{th} days of exposure. The physico-chemical characterization of water was done as per standard methods (APHA, 2012). The tissues samples were analyzed using Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICP-AES) and the results were expressed as µg/gm. Enhanced bioaccumulation of all the heavy metals was observed with increased time duration. Experimental tissues of kidney and liver were found to accumulate highest amounts of heavy metals in comparison to the control tissues of respective organs. Accelerated release of heavy metals into the aquatic environment leads to serious water pollution problems, persistence and bioaccumulation. Therefore, it is important to know the various sources of discharge of heavy metals into aquatic ecosystems throughout the world. The present study was undertaken to assess bioaccumulation of some heavy metals in the fish, *Clarias batrachus* (Linn.) and to address its correlative impacts.

KEYWORDS: Aquatic ecosystem, Bioaccumulation, *Clarias batrachus,* Heavy metals.

Corresponding Author: Dr. S. S. Gandhewar* Ph.D.

Jankidevi Bajaj College of Science, Wardha, Maharashtra, India.

Email Address: sanjeevgandhewar@gmail.com

1.INTRODUCTION

Disposal of waste material or waste water leads to the contamination of water bodies which persistently affects the flora and fauna. Heavy metals such as arsenic, cadmium, chromium, cobalt, copper, gold, iron, lead, manganese, mercury, nickel, platinum, silver, zinc etc., are a total of 23 heavy metals out of the 35 metals which are of concern to human beings due to their occupational or residential exposure [1]. These heavy metals are generally present in diet and environment. These heavy metals in diminutive quantities are often required for maintaining good health, but in higher amounts they may turn out to be toxic or dangerous. Heavy metal toxicity can lower energy levels and damage the functioning of kidney, brain, liver, lungs, blood composition as well as other vital organs. Long-term exposure can lead to gradually progressing physical, muscular, and neurological degenerative processes that imitate diseases such as multiple sclerosis, Parkinson's disease, Alzheimer's disease and muscular dystrophy. Repeated long-term exposure of some metals and their compounds may even cause cancer [2]. The most frequently found heavy metals in waste water include cadmium, arsenic, chromium, lead, copper, zinc and nickel. All of these can cause potential risks for human health and the environment [3]. Lead poisoning mainly affects the gastrointestinal tract and central nervous system [4]. Constant exposure of lead can result in birth defects, mental retardation, psychosis, allergies, autism, dyslexia, hyperactivity, weight loss, paralysis, brain damage, kidney damage, muscular weakness and may also cause death [5]. Heavy metals like lead, mercury and cadmium accumulate in organisms over the time and can cause sickness [6]. Some metals such as lead, cadmium and mercury are deleterious to aquatic life and usually occur in lower concentrations, whereas some of the heavy metals are biologically important [7]. Heavy metals and other pollutants are released in complex form into the aquatic environment and not as a single entity. The disquiet for environmental protection in connection to the aquatic environment emerges from the fact that, various chemicals are being discharged into surface water bodies which later disturb their fragile ecology and ultimately ends with the loss of biodiversity. A majority of non-biodegradable xenobiotics released along with industrial effluents enter into the animal and plant systems and continuously exert their toxic effect due to slow release of toxicants [8,9]. “Mina mata Bay” mercury pollution disaster during (1960 and 1970), draw a special attention to the dreadful consequences of methyl mercury on health. The Japanese Chisso Corporation during (1932 and 1968), discharged approximately 27 tonnes of methyl mercury with its wastewater into the bay and contaminated it. Subsequently, severe damage of the central nervous system was observed among the members of human population with higher consumption of fish and shellfish from the bay. Similarly, in Japan during 1912, Itai-itai disease was caused due to cadmium poisoning; which directly affected the bones and caused kidney failure. Heavy metal (Cr, Cd, Cu, Pb, Fe, Zn) concentration of six Mediterranean fishes in the muscle, gill and liver revealed higher concentration of most of the metals in liver [10].

2. MATERIALS AND METHODS

To conduct the toxicological experiments, three different heavy metal salts *viz.* lead acetate, cadmium chloride and copper sulphate of analytical reagent grade were used.

Use of dilution water for Conducting Experiments

Chlorine-free tap water stored for over ten days in a large overhead tank was used to carry out the experiments. The physico-chemical characteristics of the chlorine-free tap water were analysed as per the standard methods[11] (Table-1).

Table 1: Details of the physico-chemical characteristics of water

Sr. No.	Physico-chemical characteristics of dilution water	Range
1	pH	7.2-7.4
2	Temperature (ºC)	24-28
3	Dissolved Oxygen (mg/lit.)	6.9-7.1
4.	Alkalinity (as $CaCO_3$) (mg/lit.)	160-170
5	Total Hardness (as $CaCO_3$) (mg/lit.)	160-180

Healthy fishes weighing about 40 to 50 gm were obtained from local natural sources and transported to the laboratory for examination of pathological symptoms. All the selected healthy fishes were treated with 1% $KMnO_4$ solution to keep away any type of external infection, subsequently maintained in chlorine-free tap water. The fishes were fed once daily especially in the morning with milled goat liver and tubifex worms. The fishes selected were separately maintained in two different aquaria; one as control and other as experimental. The concentration of heavy metals (lead, copper and cadmium) were examined in different organs like gills, kidney, liver and muscle in both; the experimental as well as control fish. The analysis for accumulation of heavy metals in *C. batrachus* was done at the end of the experimental period.

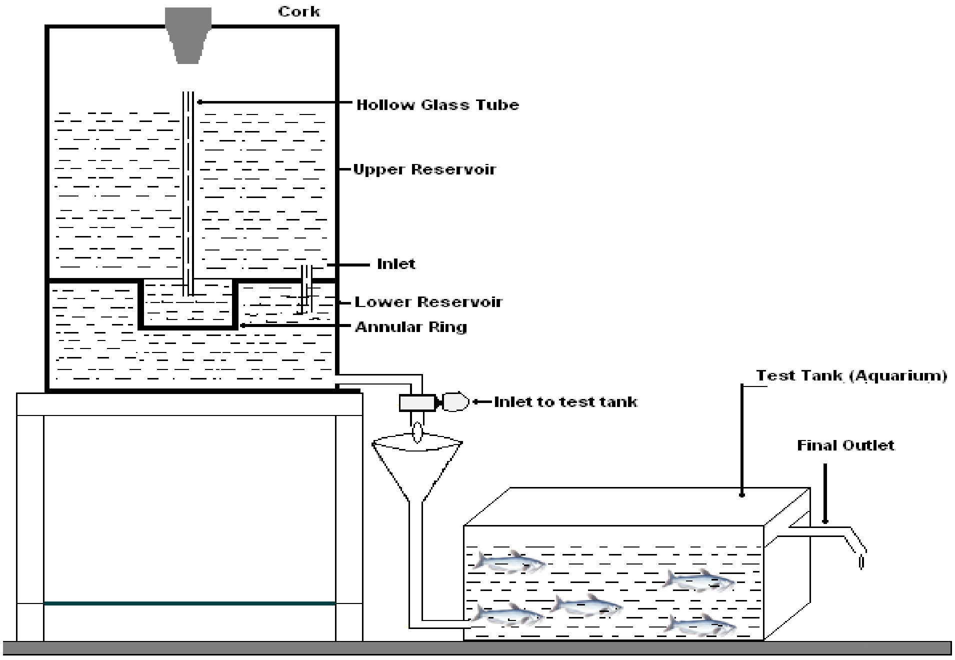

Figure-1: Schematic representation of dosing apparatus used for conducting long duration (chronic) toxicity experiments

3. RESULTS AND DISCUSSION

Bioaccumulation of Heavy Metals

The concentration of heavy metals (copper, lead, cadmium) was analysed in different organs (gills, kidney, liver and muscle) of both experimental as well as control fish. The accumulation of heavy metals in *C. batrachus* was analysed at the end of specific experimental period 10, 20 and 30 days respectively. At the end of 10^{th}, 20^{th} and 30^{th} day of exposure, fish from experimental as well as control aquaria were sacrificed in order to estimate the content of copper, lead and cadmium in the gill, kidney, liver and muscle. The organs were excised from the experimental as well as control fish and dissolved in 5 ml concentrated nitric acid (E.Merck). The samples were kept overnight and the volume was made up to 25 ml by adding 20 ml double distilled water. The samples of heavy metals in the tissues were analyzed using Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICP-AES) and the results were expressed in μg/gm. The bioaccumulation of heavy metals, copper sulphate, lead acetate and cadmium chloride in various tissues of catfish, *Clarias batrachus* after 10, 20 and 30 days of exposure are presented in Tables 2, 3 and 4 respectively.

Table 2: Bioaccumulation of Copper (μg/g wet wt.) in the tissues of fish, *Clarias batrachus* exposed to sublethal concentration of copper sulphate

Tissue	Set	Exposure Period in days		
		10	20	30
Liver	Control	0.21±0.10	0.21±0.41	0.21±0.35
	Expt.	0.97±0.35*	1.40±0.24	1.96±0.57**

Kidney	Control	0.24±0.11	0.24±0.26	0.24±0.33*
	Expt.	0.98±0.36*	1.38±0.31	1.58±0.37**
Gill	Control	0.31±0.093*	0.31±0.23	0.31±0.23*
	Expt.	0.60±0.11*	0.75±0.14	0.98±0.14*
Muscle	Control	0.11±0.03	0.11±0.03*	0.11±0.11*
	Expt.	0.38±0.50^{NS}	0.40±0.16*	0.48±0.14*

All values are expressed as mean, µg/g wet wt. ±SE

* P<0.05; ** P<0.01; NS: Non-significant

Table 3: Bioaccumulation of Lead (µg/g wet wt.) in the tissues of fish, *Clarias batrachus* exposed to sublethal concentration of lead acetate

Tissue	Set	Exposure period in days		
		10	20	30
Liver	Control	0.21±0.81*	0.21±0.38*	0.21±0.48**
	Expt.	0.89±0.007	1.30±0.26*	1.80±0.34**
Kidney	Control	0.25±0.25*	0.25±0.34*	0.25±0.29*
	Expt.	0.97±0.31*	1.37±0.37*	1.57±0.44*
Gill	Control	0.31±0.17*	0.31±0.20	0.31±0.32*
	Expt.	0.81±0.10*	0.98±0.27	1.27±0.30*
Muscle	Control	0.11±0.30*	0.11±0.30*	0.11±0.27*
	Expt.	0.37±0.60	0.39±0.80	0.42±0.36*

All values are expressed as mean, µg/g wet wt. ±SE * P<0.05; ** P<0.01; NS: Non-significant

Table 4: Bioaccumulation of Cadmium (µg/g wet wt.) in the tissues of fish, *Clarias batrachus* exposed to sublethal concentration of cadmium chloride

Tissue	Set	Exposure period in days		
		10	20	30
Liver	Control	0.22±0.16*	0.22±0.41*	0.22±0.39*
	Expt.	0.81±0.20*	1.28±0.44*	1.34±0.43*
Kidney	Control	0.25±0.27^{NS}	0.25±0.35*	0.25±0.51*
	Expt.	0.88±0.32^{NS}	1.33±0.42*	1.48±0.27*
Gill	Control	0.31±0.29^{NS}	0.31±0.47*	0.31±0.60*
	Expt.	0.96±0.74^{NS}	1.41±0.36*	1.90±0.54*
Muscle	Control	0.12±0.55^{NS}	0.12±0.045^{NS}	0.12±0.09*
	Expt.	0.34±0.44^{NS}	0.36±0.22^{NS}	0.44±0.16*

All values are expressed as mean, µg/g wet wt. ±SE * P<0.05; ** P<0.01; *** P<0.001; NS: Non-significant

The metal accumulation in tissues of aquatic animals depend on the heavy metal concentration and exposure period duration, and also depend on the factors such as salinity, temperature, alkalinity, hardness, interacting agents and metabolic activity of tissue and also the rate of uptake, storage and elimination [12, 13, 14]. In the present investigation, the tissues of *C. batrachus* accumulated heavy metals from the ambient medium i.e. water, although the levels of metal accumulation differed considerably from metal to metal. The gills and liver exhibited highest metal accumulation, while the muscle exhibited least metal accumulation. Bioaccumulation of heavy metals for the exposure period of 30 days, the copper concentration in the tissues was in the following order, liver > kidney > gill > muscle in the fish under study. In fish, the liver is the selective organ for storage of copper. Copper exhibited its highest levels in the liver and the lowest values in the muscles. The high accumulation of copper in the liver could be attributed to the specific metabolic processes and enzyme catalyzed reactions involving copper, taking place in the liver. Copper is an essential element and tends to accumulate to a greater extent than other elements, such as zinc and iron [12, 13, 14]. The induction of low molecular weight metal -binding proteins, such as metallothionein is closely related to heavy metal exposure and metals acquired from the environment can be detoxified by binding to these proteins [15]. Therefore, tissues like liver which is a major producer of metal-binding proteins showed higher concentrations of most of the heavy metals [16, 17, 18]. Copper persisted more in liver even after 30 days of exposure (1.25 μg/g) in the present investigation. Thus, the present results are congruent with the reports presented by earlier workers [16, 17]. The greatest accumulation of copper occurring in the liver, reinforces the view that the liver of fish plays a protective role against chronic heavy metal exposure by producing metallothioneins[19, 15], acting as a storage site and as a vital organ in the regulation of copper [20]. The findings of De Boeck *et al*. (1997) who studied the effects of sublethal copper exposure in common carp support the present results [21]. The concentrations of heavy metals are more in detoxifying organs like liver and kidney. Muscle being non- detoxifying organ, accumulated the least concentrations of copper revealed in this study. The accumulation patterns of metals in fish are found to be metal dependent. Copper demonstrated more significant uptake through gill. Gupta (1999) suggested that deposited copper or cadmium in the gills of experimental fishes is further translocated to kidney through blood [22]. The translocated copper ions in kidney might have excreted out due to normal renal function, therefore, low copper accumulation values might have been obtained in the kidney in comparison to liver in 30 days exposure. According to Stokes (1979), fish muscle normally contains low concentration of copper and, even at high levels of copper exposure, muscle does not often reflect increases of copper [23]. In the present investigation, low concentration of heavy metals copper, lead and cadmium in muscle tissue were noticed. Similar observations were also recorded in Egyptian fishes [23, 24]. The bioaccumulation of the three heavy metals in the fish tissues was found to be highest in liver and lowest in muscle and were in the order of liver > kidney > gill > muscle.

Lead accumulation in the tissues of *Clarias batrachus* was in the order: liver > kidney > gill > muscle. Similar type of bioaccumulation trend was reported for cadmium in 30 days exposure. It has been found that, metal accumulation is mainly affected by some of the water quality parameters that affect toxicity. So it is potentially one of the most valuable tools for identifying and quantifying the impact of metals in aquatic environment [25, 26, 27, 28]. Clements and Rees (1997) stated that, bioaccumulation of metals in the field is complex and may be influenced by several factors, like abiotic variables and metal availability [29]. Cadmium has proved to be highly toxic to aquatic fauna even at fairly low concentrations [30, 31, 32, 33]. It displaces zinc in many vital enzymatic reactions, causing disruption or cessation of activity. The transformation of one form of metal to another, which occurs at tissue level, generally modifies the level of toxicity of metal. The result of this process is called detoxification. Cadmium tends to accumulate more in liver and kidney [34] through its strong binding with cysteine residues of metallothionein [35].

4. CONCLUSION

The deleterious concentration of heavy metals has been found to accumulate in the vital organs of fish. Liver was found to accumulate comparatively higher amount of heavy metals. The effects of higher concentrations of heavy metals have been proved highly hazardous to health experimentally as well as clinically. This study revealed the accumulation of heavy metals in fish *Clarias batrachus* which may act as an indicative measure to address the production, bioaccumulation and health hazardous issues of heavy metals.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

REFERENCES

1. Mosby CV, Glanze WD, Anderson KN. Mosby Medical Encyclopedia, The Signet: Revised Edition. St. Louis. 1996.
2. Jarup L. Hazards of heavy metal contamination. British medical bulletin. 2003; 68:167-82.
3. Lambert M, Leven BA, Green RM. New methods of cleaning up heavy metal in soils and water. Environmental science and technology briefs for citizens. Kansas State University, Manhattan, KS. 2000.
4. Markowitz M. Lead Poisoning. Pediatr Rev. 2000; 21:327–335.
5. Martin S, Griswold W. Human health effects of heavy metals. Environmental Science and Technology briefs for citizens. 2009;15:1-6.
6. Hawkes SJ. What is a" heavy metal"?. Journal of Chemical Education. 1997; 74:1374.
7. Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN. Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary toxicology. 2014; 7:60-72.

8. Adham KG, Hamed SS, Ibrahim HM, Saleh RA. Impaired functions in Nile tilapia, Oreochromis niloticus (Linnaeus, 1757), from polluted waters. Acta hydrochimica et hydrobiologica. 2001; 29:278-88.
9. Olojo EA, Olurin KB, Mbaka G, Oluwemimo AD. Histopathology of the gill and liver tissues of the African catfish Clarias gariepinus exposed to lead. 2005.
10. Canli M, Atli G. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environmental pollution. 2003; 121:129-36.
11. APHA. Standard Methods for the examination of water and waste water. 2012.
12. Heath AG. Water pollution. Fish Physiology, CRC Press, Boca Raton, FL, USA. 1987;245.
13. Langston WJ. Toxic effects of metals and the incidence of marine ecosystems. Heavy metals in the marine environment. CRC Press, New York. 1990; 256.
14. Roesijadi G. and Robinson WE. Metal regulation in aquatic animals, mechanisms of uptake, accumulation and release. In: Aquatic Toxicology; molecular, Biochemical and cellular perspectives. Ceds; Melins DC, Ostrander GK, Lewis Publisher, London. 1994; 539.
15. Canli M, Stagg RM, Rodger G. The induction of metallothionein in tissues of the Norway lobster Nephrops norvegicus following exposure to cadmium, copper and zinc: the relationships between metallothionein and the metals. Environmental pollution. 1997; 96:343-50.
16. Thomas DG, Cryer A, Solbe JF, Kay J. A comparison of the accumulation and protein binding of environmental cadmium in the gills, kidney and liver of rainbow trout (Salmo gairdneri Richardson). Comparative biochemistry and physiology. C, Comparative pharmacology and toxicology. 1983;76:241-6.
17. Amiard JC, Amiard-Triquet C, Berthet B, Metayer C. Comparative study of the patterns of bioaccumulation of essential (Cu, Zn) and non-essential (Cd, Pb) trace metals in various estuarine and coastal organisms. Journal of Experimental Marine Biology and Ecology. 1987;106:73-89.
18. Allen P. Distribution of mercury in the soft tissues of the blue tilapia Oreochromis aureus (Steindachner) after acute exposure to mercury (II) chloride. Bulletin of environmental contamination and toxicology. 1994; 53:675-83.
19. McCarter JA, Roch M. Hepatic metallothionein and resistance to copper in juvenile coho salmon. Comparative Biochemistry and Physiology Part C: Comparative Pharmacology. 1983; 74:133-7.
20. Buckley JT, Roch M, McCarter JA, Rendell CA and Athieson AT. Chronic exposure of coho salmon to sublethal concentrations of copper-I. Effect on growth, accumulation and distribution of copper, and on copper tolerance. Comp. Biochem. Physiol, Part C. 1982; 72:15-19.
21. De Boeck G, Vlaeminck A, Blust R. Effects of sublethal copper exposure on copper accumulation, food consumption, growth, energy stores, and nucleic acid content in common carp. Archives of environmental contamination and toxicology. 1997; 33:415-22.

22. Gupta AK. Short-term toxicity of copper to Channa punctatus(Bloch). Journal of Environmental Biology. 1999; 20:227-9.
23. Stokes PM. Copper accumulation in freshwater biota. In: Nriagu J.O.(ed) copper in the environment. John Wiley and Sons. Inc., New York, USA. 1979; 357-381.
24. Gomaa MN, Abou-Arab AA, Badawy A, Khayria N. Distribution pattern of some heavy metals in Egyptian fish organs. Food chemistry. 1995; 53:385-9.
25. Borgmann U, Norwood WP. Kinetics of excess (above background) copper and zinc in Hyalella azteca and their relationship to chronic toxicity. Canadian Journal of Fisheries and Aquatic Sciences. 1994;52:864-74.
26. Birungi Z, Masola B, Zaranyika MF, Naigaga I, Marshall B. Active biomonitoring of trace heavy metals using fish (Oreochromis niloticus) as bioindicator species. The case of Nakivubo wetland along Lake Victoria. Physics and Chemistry of the Earth, Parts A/B/C. 2007; 32:1350-8.
27. Shinn C, Dauba F, Grenouillet G, Guenard G, Lek S. Temporal variation of heavy metal contamination in fish of the river lot in southern France. Ecotoxicology and Environmental Safety. 2009; 72:1957-65.
28. Alert AL, Fairchild JF, Schmitt CJ, Besser JM, Brumbaugh WG and Olson SJ. Effects of mining-derived metals in riffle-dwelling benthic fishes in Southeast Missouri, USA. Ecotoxicol. Environ. Safety. 2009; 72: 1642-1651.
29. Clements WH, Rees DE. Effects of heavy metals on prey abundance, feeding habits, and metal uptake of brown trout in the Arkansas River, Colorado. Transactions of the American Fisheries Society. 1997;126:774-85.
30. Marshall JS. Cadmium toxicity to laboratory and field populations of Daphnia galeata mendotae. Bulletin of environmental contamination and toxicology. 1979 Jan 1;21(1):453-7.
31. Alabaster JS and Lloyd R. Cadmium, in: water quality criteria for freshwater fish. 1980; 221-251.
32. Fu H, Steinebach OM, Van den Hamer CJ, Balm PH, Lock RA. Involvement of cortisol and metallothionein-like proteins in the physiological responses of tilapia (Oreochromis mossambicus) to sublethal cadmium stress. Aquatic toxicology. 1990;16:257-69.
33. Chong K, Wang WX. Comparative studies on the biokinetics of Cd, Cr, and Zn in the green mussel Perna viridis and the Manila clam Ruditapes philippinarum. Environmental Pollution. 2001;115:107-21.
34. Suzuki Y. Cadmium metabolism and toxicity in rats after long-term subcutaneous administration. J. Toxicol. Environ. Health. 1980; 6: 469-482.
35. Friberg L, Kjellstrom T, Nordberg G and Piscator M. Cadmium, in: Handbook on the toxicology of metals (eds.) Friberg et al., Elsevier/ North-Holland Biomedical Press. 1979; 355-381.