Biosciences, Biotechnology Research Asia Vol. **4**(2), 589-594 (2007)

Bioaccumulation and elimination of chromium in an edible fingerlings of *Cirrhinus mrigala* exposed to sub-lethal concentrations

S. KARTHIKEYAN[1], C. KARTHIK[2] and SELVI SABHANAYAKAM[3]

[1]Department of Physics, St. Joseph's College of Engineering, Chennai - 600 119 (India)
[2]Department of Biotechnology, St. Joseph's College of Engineering, Chennai - 600 119 (India)
[3]Department of Zoology, Annamalai University, Annamalainagar - 608 002 (India)

(Received: August 14, 2007; Accepted: September 21, 2007)

ABSTRACT

The bioaccumulation of chromium in different organs of freshwater fingerlings *Cirrhinus mrigala* exposed to two different sub-lethal concentrations for a period of 28 days has been studied using Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES). Further, uptake and elimination process of chromium has also been studied. The degree of accumulation of chromium was found to be different in different organs of the fish. Accumulation pattern was time dependent and elimination process was not in order. The results indicate that they have potential as bioindicators of pollution for chromium. These observations were consistent with earlier findings.

Keywords: Bioaccumulation, elimination, chromium, fish.

INTRODUCTION

The increasing industrialization and development of activities to cope with the population explosion have brought inevitable water crisis (Rao and Rameshwari, 1998). The discharge of industrial effluents into natural water bodies alters the chemical composition of the natural aquatic environment leading to severe water pollution. The heavy metals are considered to be known toxicants, which inflict acute disorder in aquatic organisms. Heavy metals include a great variety of chemical elements that typically occur in low or trace amounts in the environment, all which have the potential to provoke toxic effects in organisms. Leland (1975) has reviewed the literature about the sources and effects of metal pollutant in aquatic environment.

Chromium is a highly reactive transition metal occurring widely in environmental matrices including rocks, soil, sediments, air, water and biological materials. This metal is essential to plant and animal nutrition at trace level but becomes toxic at high concentrations (Abbasi *et.al.*, 1991). Over exposure of chromium will cause irritation, corrosion of skin and respiratory tract in humans and adverse effects on plants and aquatic life (Srivastava *et.al.*, 2001). Chromium occurs in aqueous system as both trivalent and hexavalent forms, the latter being of particular concern because of high oxidizing potential, high solubility and its ability to cross biological membranes (Levy and Venitt, 1986). The main sources of chromium to the aquatic environment are wastes from metal finishing industries, dumping of solid wastes and municipal wastes (Eisler, 1986).

Fishes have occupied top of the aquatic food chains and may accumulate metals and pass them to human beings through food, causing chronic or acute diseases (Forstner and Wittman, 1981 and Fowler *et.al.*, 1993). The bioaccumulation of heavy metals depend on availability and persistence of the contaminants, water, food and physiochemical

properties of the toxicants. Heavy metals being non-biodegradable primarily necessitates the knowledge in their uptake, distribution and persistence in tissues of various organisms. In view of this, it has been proposed in the present study to evaluate the bioaccumulation and elimination of chromium in the selected tissues of an edible fish, *Cirrhinus mrigala* for 28 days and subsequent elimination of chromium for another 28 days.

MATERIAL AND METHODS

The chemicals used were analytical grade. A stock chromium (VI) solution (1000mg/l) was prepared in distilled water using potassium dichromate. All the working solutions were prepared by diluting the stock solution with distilled water. *Cirrhinus mrigala* fingerings of length (6±1) cm and weight (8±1) gm were collected from the freshwater bodies near local fish farm, Puthur, Tamilnadu and acclimatized under laboratory conditions (29±1)°C for seven days. Boiled eggs, rice bran and earthworm pieces were fed on every alternate days.

The LC_{50} for chromium (as $K_2Cr_2O_7$) for 96h was found out by using Probit method (Finney, 1971). The 1/10th and 1/3rd of LC_{50} were taken as lower (1.82ppm) and higher (6.06ppm) concentrations, respectively. The test fishes were exposed to above two sub-lethal concentrations separately for a period of 7, 14, 21 and 28 days. At the end of the periods, a group of fishes were randomly selected from experimental tank and were kept in clear water for another period of 28 days. At the end of every exposure periods, gill, liver, kidney and muscle and whole body tissues were isolated and kept in hot air oven at 85°C for 24 hours. Also, during the elimination period of 28 days, whole body tissues were isolated at a periodic interval of 7 days and dried using the method mentioned above. The dried samples were digested with concentric nitric and perchloric acid in 3:1 ratio. The chromium concentration in each sample was estimated using Inductively Coupled Plasma Atomic Emission Spectrometer (ISA JOBIN YVON 24 model) available at CAS in Marine Biology, Annamalai University. The data were analysed by using standard student t-test (Siegel, 1956).

Calculation of BCF and excretion rate constants

Bioconcentration factor and excretion rate constant (Taizo *et al.*, 1989, 1995; Karl Fent and Peter 1995) were calculated by the following equation

$$BCF = \frac{\text{Concentration of chromium in each part of fish}}{\text{Concentration of chromium in water}}$$

and $$C = C_0 e^{-kt}$$

where the symbols have their usual meanings.

RESULTS AND DISCUSSION

Tables 1 and 2 summarize the data on chromium concentration in various organs and whole body tissues respectively under various exposure periods. Figs. 1-4 represent accumulation pattern of chromium in various organ tissues of *Cirrhinus mrigala* fingerlings. In order to get information on bioaccumulation as well as the dynamics and fate of chromium in the exposed organisms, the bioconcentration factor (BCF), uptake rate (k_1) and elimination rate (k_2) constants were also calculated (Taizo *et. al.*, 1989, 1995) and reported in table 3.

It has been observed from Fig. 1 that gill tissue has a substantial amount of chromium. Also, the accumulation varies with the exposure period and environmental concentrations. At low concentration, the accumulation of chromium was in accordance with exposure time. But at higher concentration (6.06 ppm), there was a gradual increase of chromium upto 21 days and afterwards

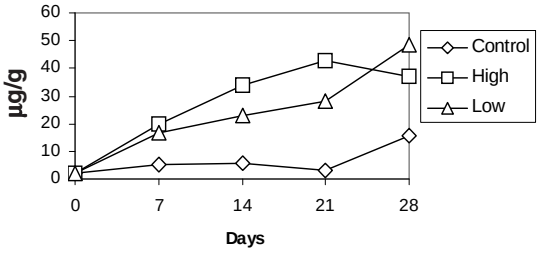

Fig. 1: Accumulation of Cr in the gill tissues of *Cirrhinus mrigala*

Table 1: Accumulation of chromium (µµg/g) in the organs of *Cirrhinus mrigala* exposed to lower and higher sublethal concentrations. Standard deviation : P: Level of significance

Organs	Control				Lower Sublethal Conc.				Higher Sublethal Conc.			
Days	7	14	21	28	7	14	21	28	7	14	21	28
Gill ±	5.231	5.636	3.215	15.436	16.534	22.932	28.425	48.736	19.822	33.735	42.682	36.834
	±0.801	±0.224	±0.981	±1.362	±1.246	±1.831	±2.664	±3.952	±2.207	±1.848	±4.236	±2.332
Liver ±	5.487	7.007	22.226	7.687	27.516	39.217	57.226	87.325	51.632	85.758	86.474	78.926
	±1.256	±0.826	±1.753	±2.126	±3.724	±4.631	±6.425	±3.683	±2.336	±4.257	±6.656	±5.437
Kidney	5.853	10.442	26.837	32.438	21.234	62.623	92.617	97.326	37.718	82.482	164.520	162.634
±	±0.420	±1.258	±2.636	±1.724	±2.156	±5.326	±8.643	±7.963	±2.124	±6.352	±9.347	±8.623
Muscle	6.614	12.136	16.241	20.434	12.226	30.630	43.734	48.635	27.832	34.670	73.462	91.227
±	±1.263	±0.881	±1.406	±1.325	±1.852	±2.773	±3.232	±3.782	±1.319	±2.862	±4.833	±5.264

The differences between controls and exposures are statistically significant (P<0.005) (n=4)

Table 2: Accumulation and elimination of chromium (µg/g) from the whole body tissues of *Cirrhinus mrigala*: Standard deviation: P : Level of significance

Time (in days)		Control	Accumulation LSL	Accumulation HSL	Elimination LSL	Elimination HSL
7		12.427	29.662	58.614	94.660	187.053
	±	± 0.961	± 2.937	± 4.626	± 6.262	± 10.726
14		18.886	84.124	113.763	56.426	106.634
	±	± 1.723	± 6.453	± 8.262	± 3.762	± 8.724
21		27.102	121.625	181.228	69.835	111.624
	±	± 4.635	± 7.607	± 8.437	± 3.836	± 9.215
28		22.320	132.429	217.260	38.421	79.836
	±	± 2.216	± 9.638	± 10.433	± 3.263	± 6.432

The difference between controls and exposures are statistically significant (P < 0.005) (n=4)

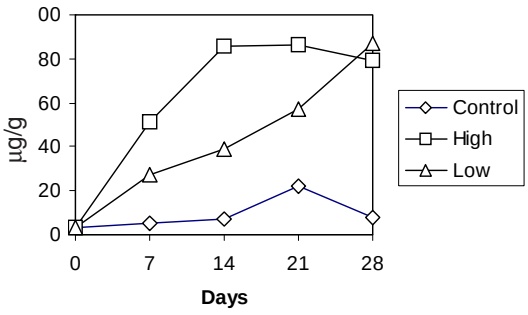

Fig. 2: Accumulation of Cr in the liver tissues of *Cirrhinus mrigala*

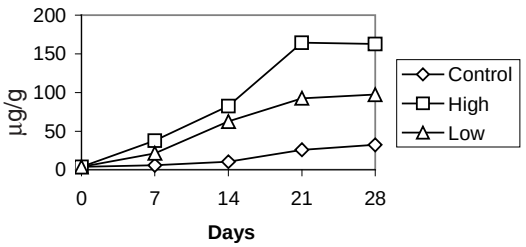

Fig. 3: Accumulation of Cr in the kidney tissues of *Cirrhinus mrigala*

Table 3: Bioconcentration factor (BCF), uptake (k_1) and elimination(k_2) rate constants exposed to chromium for 28 days

Organs	Lower Sublethal Concentration			Higher Sublethal Concentration		
	BCF	$K_1(h^{-1})$	$K_2(h^{-1})$	BCF	$K_1(h^{-1})$	$K_2(h^{-1})$
Gill	26.75	0.088	0.003	6.07	0.018	0.003
±	± 2.10	± 0.004	± 0.001	± 0.90	± 0.003	± 0.004
Liver	47.96	0.197	0.004	13.01	0.051	0.004
±	± 2.60	± 0.012	± 0.003	± 1.30	± 0.001	± 0.002
Kidney	53.46	0.225	0.004	26.83	0.132	0.005
±	± 3.20	± 0.022	± 0.011	± 1.80	± 0.012	± 0.001
Muscle	26.70	0.077	0.003	15.05	0.059	0.004
±	± 1.80	± 0.003	± 0.002	± 1.20	± 0.002	± 0.001
Whole	72.74	0.255	0.004	35.84	0.154	0.004
Body ±	± 3.20	± 0.013	± 0.001	± 3.40	± 0.032	± 0.001

K_1 was calculated by $K_2 \times$ BCF (n=4)

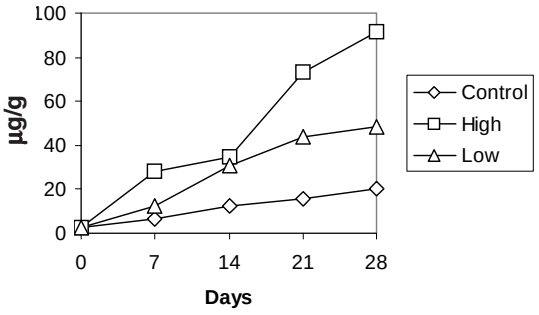

Fig. 4: Accumulation of Cr in the muscle tissues of *Cirrhinus mrigala*

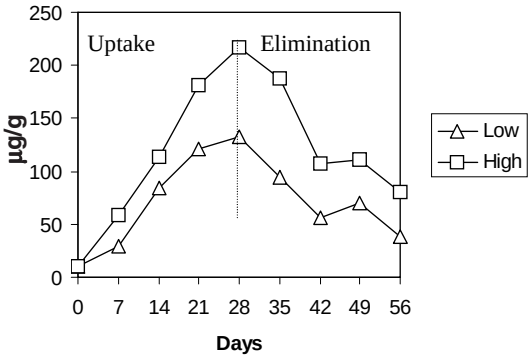

Fig. 5: Accumulation of Cr in the whole body tissues (µg/g dry weight) of *Cirrhinus mrigala*

there has been a fall in concentration of chromium. At higher concentration, the uptake rate (k_1) is less than lower sub-lethal concentration values by 5%. This reduced uptake is due to inhibition of accumulation caused by gill damage as evidenced by histopathological studies (Palaniappan *et. al*., 2002). Since gills are the most common entry point of water-soluble toxicant in fishes and have direct contact with pollutant medium, there has been considerable concentration of chromium at both treatments. The bioaccumulation of chromium in liver tissue was also found to be time and dose dependent (Fig. 2). At lower concentration, there was a gradual uptake of chromium and at higher concentration, the accumulation was found to be almost plateau after 14 days. This may be evidenced by reduced uptake rate at higher concentration (k_1=0.051±±0.001h^{-1}) compared to lower concentration (k_1=0.197±±0.012h^{-1}). The high value of bioconcentration factor in liver (47.96±±2.6) reflects the affinity between the metal and tissues. Also liver is the prime site of metal binding and releases in fishes (Barber and Sharma, 1998).

The highest accumulation of chromium was recorded in kidney (BCF=53.46±±3.2 & 26.83±±1.8) at both the level of concentrations. At higher concentration, accumulation remains constant after 21 days as shown in Fig.3. The decrease or the maintenance in the level of accumulation after a

certain period is the expected one since, kidney is the principal route of excretion through which most toxicants are excreted. Similar results have been reported by Kendall (1975) for *catfish* exposed to methyl mercuric chloride. In muscle, accumulation pattern varies with degree of toxicants. The low value of accumulation (BCF=15.05±±1.2) at higher sub-lethal concentration may be due to mucus secretion (Heath, 1991). Further, the uptake rate was low for higher treatment (k_1=0.059±±0.002h^{-1}) when compared to lower treatment (k_1=0.077±±0.003h^{-1}). This reduced uptake and lower bioconcentration factor (Table3) of chromium was not surprising since these tissues were not considered to be specific physiological sites for the storage of chromium. Similar results have been reported in abdominal muscles and digestive tissues of *crayfish* exposed to copper (Shaheen Zia and Alikhan 1989). The accumulation pattern during the period of study was in the order:

kidney > liver > gill ≈ muscle for lower concentration and kidney > muscle > liver > gill for higher sub-lethal concentration of chromium. A significant concentration of chromium observed in control animals could be due to presence of chromium (0.14±±0.07ppm) in the tap water used in the present study.

The whole body burden of chromium (µµg/g dry animal weight) recorded during the uptake and elimination phases are shown in Fig.5. The increased levels of accumulation during the exposure period suggest relatively rapid uptake rate for this metal. Similar results have been reported for fresh water *isopodes* exposed to Cu, Pb and Zn (Van Hattum *et. al*., 1993). The depuration experiments were started after 28 days of uptake. During these periods, depuration of chromium is possible due to subsequent excretion of metal rich residual bodies (George and Viarengo, 1985). However, with respect to the degree of accumulation and uptake rate, no consistency exists between different data. Also, the elimination of chromium was not completely reversible and after the period of 14 days of elimination phase, it was found to be irregular. A considerable amount of chromium was found at the end of depuration period and did not reach the normal (control) level and this may be due to maintenance of constant level of chromium for its basic physiological requirements (Goyer, 1986 and Eisler 1986). It has been reported (Ward 1982; Evtushenko *et. al*., 1986 and watras *et. al*., 1985) that tissue levels of accumulated metals remains invariably plateau when the organisms were exposed continuously for a sufficiently long period.

CONCLUSION

The results of the study clearly demonstrated the ability of *Cirrhinus mrigala* to accumulate the metal (Cr) from the medium. This ability of fish to regulate the metal against changes in external toxicants indicate that they have potential as bioindicators of pollution for chromiun. Hence it may be concluded that to assess the regulatory process existing among the various organs in the animals, it is necessary to determine the relationship between rates of metal uptake at different concentration gradients.

REFERENCES

1. Abbasi S.A., Baji V., Madhavan V.K. and Soni R. Impact of chromium (VI) on *catfish wallago attu*. *I. J. Env. Hlth*. **33**: 336-340 (1991).
2. Barbar D and Sharma M.S. Experimentally induced bioaccumulation and elimination of cadmium in fresh water fishes. *Poll. Res.* **17**: 99-104 (1998).
3. Eisler R. Chromium hazards to fish, wildlife and invertebrates. A synoptic review: Biological report 85(1-6), 60pp. United States fish and wildlife research centre, Laurei, M.D (1986).
4. Evtushenko Z.S., Belcheva M.N. and Lukyanoval O.N. Cadmium accumulation in organs of the scallop *Mizuhopecten yessonensis - II.* Subcellular distribution of metals and metal binding proteins. *Comp. Biochem. Physiol.* **83**: 377-383 (1986).

5. Finney D.J. *Probit Analysis*, pp.333, 3rd Ed., Cambridge University press, Cambridge.
6. Forstner V. and Wittman G. T. W. (1981) *Metal pollution in the aquatic environment*, Berlin Heidelberg, Springer Verlag, Germany 272-286 (1971).
7. Fowler S.W., Readmen J.W., Cregion B., Villeneurve J.P. and Mckay K. Petroleum hydrocarbons and trace metals in near shore Gulf sediments and biota before and after the 1991 war. An assesment of temporal and spatial trends. *Mar. Poll. Bult*. **27**: 171-182 (1993).
8. George S.G and Viarengo A. An integration of current knowledge of the uptake, metabolism and intracellular control of heavy metal in mussels. in: Vernberg FJ, Thurberg FP, Calabrese A and Vernberg WB (Eds) *Marine Pollution and Physiology Recent advances*,. University of South Carolina press, Columbia. 125-144 (1985).
9. Goyer R.A. Toxic effects of metals in C.D. Klaassen, M.O. Ambur and J. Doull (eds). *The basic science of poisons*., Macmillan, New York. 582-635 (1986).
10. Heath A.G., *Water pollution and fish physiology*, pp.359 Lewis publishers, Boca Raton, Florida, USA. (1991) .
11. Karl Fent and Peter W. Looser., Bioaccumulation and bioavailability of tributyltin chloride : Influence of pH and humic acid. *Wat. Res*. **29**: 1631-1637 (1995).
12. Kendall M.W. Acute effects on methyl mercuric toxicity ion channel catfish. (*Ictalnes punctatus*) *Bull. Environ. Contam. Toxicol*. **13**: 570-575 (1975).
13. Leland H.V.,Heavy metals and other trace elements. *J. wat. Poll. control. Fed*. **47**: 1635-1656 (1975).
14. Levy L.S and Venitt S.C.,arcinogenicity and Mutagenicity of chromium compounds: The association between bronchial metaplasia and neoplasia, *Carcinogenesis*, **7**: 831-835 (1986).
15. Palaniappan PL.RM., Karthikeyan S. and Selvi Sabhanayakam. Effects of sublethal concentration of chromium on gill tissues of an edible fish *Cirrhinus mrigala*, *J. current Sciences*, **2**(2): 271-274 (2002).
16. Rao L.M. and Rameshwari K., Incidence of heavy metal pollution in Mehadrigedda stream of visakhapatinam, *Poll. Res*. **17**: 153-155 (1998).
17. Shaheen Zia and Alikhan M.A. Copper uptake and regulations in a copper tolerant *Decapod Cambarus bartoni (Fabricius Decapoda crustacea) Bull. Environ. contam. Toxicol.* **42**: 103-110 (1989).
18. Siegel S. *Non-parametric statistics for behavioural sciences*. McGraw Hill, London (1956).
19. Srivastava R.K., Ayachi A.K. and Mono Mishra. Removal of Chromium (VI) by utilization of Bedi leaves. *Poll. Res.* **20**: 639-643 (2001).
20. Taizo Tsuda, Strigeru Aoki, Mihoko Kojima and Hiroyceki Harada Bioconcentration and excretion of Benthiocarb and Simetryne by carp. *Wat. Res.* **23**: 529-531(1989).
21. Taizo Tsuda, Shigeru Aoki, Tomohiro Inove and Mihoko Kojima Accumulation and excretion of Diazinon, Fenthion and Fenitrothion by kill fish: comparison of individual and mixed pesticides. *Wat. Res*. **29**: 445- 448 (1995).
22. Van Hattum B., Korthals G., Van Straalen W.M., Govers H.A.J. and Joosse E.N.G. Accumulation patterns of trace metal in freshwater isopodes in sediment, Bioassays-Influence of substrate characteristics, Temperature and pH. *wat. Res*. **27**: 669-684 (1993).
23. Ward T.J., Laboratory study of the accumulation and distribution of cadmium in the Sydney rock *oyster saccostrea commercialli (I & R). Aust. J. Mar. Freshwater Res.* **33**: 33-34 (1982).
24. Watras C.J., Mac Farlane J. and Morel F.M.M. Nickel accumulation by "*Sonedesmus*" and "*Daphnia*": Food chain transport and geochemical implications. *Can. J. Fish Aquatic. Sci.* **42**: 724-730 (1985).