Bull. Environ. Contam. Toxicol. (2003) 70:106–111

DOI: 10.1007/s00128-002-0162-y

Bulletin of Environmental Contamination and Toxicology

Alterations in the Biochemical Constituents of Muscles of *Cirrhinus mrigala* Following Exposure to and Withdrawal from Metals

S. Virk, A. Sharma

Department of Fisheries, Punjab Agricultural University, Ludhiana (Pb.) 141 004, India

Received: 22 February 2001/Accepted: 13 August 2002

Nickel (used in metal plating, production of alloys and alkaline storage batteries) and chromium (used in tanning, metal plating and as mordant in textiles) released into the aquatic environment during the manufacturing processes, cause contamination of water bodies. Fish are of great nutritional significance and their intoxication by the metals reduces their nutritive value. Nickel and chromium are known to alter the biochemical composition of fish (Vincent et al 1995; Virk & Dhawan 1997; Virk and Kaur 1999; Nanda et al 2000). However, very few attempts have been made to observe if any change occurs in the biochemical constituents of muscles when the fish is transferred from polluted to uncontaminated water (James et al 1996; Chandravathy and Reddy 1996). Hence, the present paper reports on the effect of nickel and chromium on the biochemical composition of muscles of *Cirrhinus mrigala* after exposure to the metals and then after withdrawl from the metals.

MATERIALS AND METHODS

Experimental fish, *Cirrhinus mrigala* measuring 9.00±1.0 cm in length were collected from Fish Farm of Department of Fisheries and acclimated to laboratory conditions for 15 days prior to start of the experiment. Static bioassay experiments were conducted in the laboratory at room temperature (25±1.5°C) to assess the acute toxicity of nickel and chromium to fingerlings of *Cirrhinus mrigala*. Dechlorinated tap water having 5-6 mg/L of dissolved oxygen, 7.6-6.8 pH and 300-310 mg/L alkalinity was used to run the experiments. Stock solutions of nickel and chromium were prepared by dissolving reagent grade nickel chloride ($NiCl_26H_2O$) and chromium chloride ($CrCl_36H_2O$) in distilled water. Various concentrations of the two metals were prepared using dilution technique (APHA 1989). No feeding was done during the bioassay experiments.

In acute toxicity tests, ten fingerlings of *C. mrigala* were released in each plastic bucket (15 L capacity) containing 10 L of the test solution. Experiments were conducted in triplicates. Mortality of the fish was recorded at 24 hr interval through 96 hr exposure. The concentration at which 50% of the test fish died (LC_{50}) was calculated by subjecting the data to Probit Analysis (Finney 1971).The safe concentrations were calculated by using the formula (LC0 ÷ LC 100 x LC 50) given by Basak and Konar (1977).

Correspondence to: S. Virk

Healthy fish (9.00±1.0 cm in length) were collected and divided into five groups of 10 each. The first four groups were exposed to safe and sublethal (1/2 of 96 hr LC 50) concentration of nickel (13.6 and 17.00 mg/L) and chromium (31.5 and 35.00 mg/L) for 45 days and then shifted to metal free water for 18 days. The fifth group served as control. Experiments were conducted in triplicate in glass aquaria (95 x 45 x 45 cm) having water capacity of 150 litres. The fish were starved 24 hr prior to initiation of the experiments to overcome differences if any due to differential feeding. Tap water (water quality parameters given earlier) was used to run the experiments. Fish were fed on rice bran : oil cake (1:3) *ad libitum* on alternate days and water of both the experimental and control aquaria was changed after feeding. Three fish from each of the experimental and control aquaria were sacrificed on 0 and 45 days of exposure and on 18th day after the withdrawl of metals and muscle was collected for the estimation of total proteins (Lowry et al 1951), total carbohydrates (Dubois et al 1956), total lipids (Folch et al 1957), moisture and ash content.

One way ANOVA was used to see the differences among the treatments and the control after the exposure and recovery periods using STATGRAPHICS STATISTICAL PACKAGE.

RESULTS AND DISCUSSION

In *Cirrhinus mrigala,* no mortality was recorded at 20 mg/L of nickel & 45 mg/L of chromium whereas hundred per cent mortality was observed at 50 and 100 mg/L of nickel and chromium, respectively. 96 hr LC 60 values were calculated to be 34.0 mg/L for nickel and 70.00 mg/L for chromium wherea safe concentrations were found to be 13.60 mg/L for nickel and 31.50 mg/L for chromium.

The effect of nickel & chromium on the protein, carbohydrate, lipid, moisture and ash content of muscle are given in Tables 1 and 2. The results revealed significant alterations which were concentration dependent. Protein level of muscle was reduced significantly in both the concentration of the metals under study except in fish exposed to safe concentration of chromium. However, nickel caused more decline as compared to chromium. The decline in protein could be attributed to the fact that heavy metals in general interfere with protein synthesis (Syversen 1981). Further, under stress conditions, the dietry protein consumed by fish is not stored in the body tissue (Baskaran & Palanichamy 1990) and hence the treated fish meet out their extra energy requirements from body proteins which are mobilized to produce glucose, the instant energy which is made available for fish by the process of gluconeogenesis (Vasanthi et al 1990). Fall in muscle protein has also been reported in *Mystus gulio* exposed to 10% of 96 hr LC 50 value of lead (Kasthuri and Chandran 1997); in *Cyprinus carpio* exposed to 7.50 and 15.00 mg/L of nickel and 10.00 and 20.00 mg/L of chromium (Virk & Dhawan 1997) and to mixture of 7.5 mg Ni/L & 10.00 mg Cr/L (safe concentration) and 15.00 mg Ni/L & 20 mg Cr/L (sublethal concentration) (Virk and Kaur 1999) and in *Heteropneustes fossilis* exposed to 11 mg/L of nickel (Nanda et al 2000)

Table 1. Changes in protein and carbohydrate (g/100 g) of muscle of *Cirrhinus mrigala* after exposure and withdrawl of nickel and chromium.

Days	C	T_1	T_2	T_3	T_4
			Protein		
0 d	15.89				
Exposure period (45 d)	14.78^a ±0.32	10.17^c ±0.21 (-31.19)	7.99^d ±0.75	14.54^a ±0.20 (-1.62)	11.75^b ±0.32 (-20.50)
Recovery period (18 d)	14.17^a ±0.21	13.45^b ±0.20 (-5.08)	11.99^c ±0.21 (-15.38)	14.46^a ±0.56 (+1.97)	14.78^a ±0.32 (+4.30)
			Carbohydrates		
0 d	1.97				
Exposure period (45 d)	1.82^a 0.08	0.99^c ±0.02 (-45.60)	0.83^d ±0.04 (-54.39)	1.26^b ±0.08 (-30.76)	1.07^c ±0.08 (-41.20)
Recovery period (18 d)	1.70^a ±0.05)	1.37^{cb} ±0.05 (-19.41)	1.25^c ±0.07 (-26.47)	1.37^{cb} ±0.05 (-19.41)	1.42^b ±0.05 (-16.47)

Values are Mean ± SE, C-Control, T-Safe concentration of nickel, T_2- Sublethal concentration of Nickel, T_3- Safe concentration of Chromium, T_4-Sublethal concentration of Chromium, d-day. Figures in parentheses indicate percent variation as compared to control.The values with the same superscript in a row do not differ significantly (P= 0.05).

Carbohydratres in muscle depleted significantly in fish exposed to both the concentrations of the two metals. The depletion may be attributed to enhanced glycogenolysis as reported by Vincent et al (1995) in *Catla catla* exposed to 20, 25, 30 & 35 mg/L of chromium for 30 days. Decline in muscle carbohydrate has also been observed in *Heteropneustes fossilis* exposed to 750 ppb of copper for 21 days (James et al 1995) and in *Mystus gulio* exposed to 10% of 96 hr LC 50 value of lead (Kasthuri & Chandran 1997).

The level of lipid in muscle declined significantly in case of nickel whereas in case of chromium, upsurge was observed. The decline in lipids could be due to utilisation of lipids by fish under stress (Baskaran *et al* 1989) whereas increase could be due to conversion from proteins although no direct evidence is available (Sivaramakrishna et al 1992). Reduction in muscle lipid has been reported in *Mystus guilo* when exposed to 10% of 96 hr LC 50 value of lead (Kasthuri & Chandran 1997) and in *Oreochremis mossambicus* exposed to 0.03, 0.06, 0.09 and

Table 2. Changes in lipid, moisture and ash (g/100 g) of muscle of *Cirrhinus mrigala* after exposure and withdrawl of nickel and chromium.

Days	C	T_1	T_2	T_3	T_4
			Lipids		
0 d	1.80				
Exposure period (45 d)	1.98^b ±0.04	1.64^c ±0.05 (-17.17)	1.50^c ±0.08 (-24.24)	2.27^a ±0.03 (+14.64)	2.42^a ±0.09 (+22.22)
Recovery period (18 d)	2.02^a ±0.04	1.64^b ±0.03 (-18.81)	1.51^c ±0.04 (-25.24)	2.04^a ±0.06 (+0.99)	2.11^a ±0.07 (+4.45)
			Moisture		
0 d	79.06				
Exposure period (45 d)	78.88^a ±1.48	79.81^a ±0.44 (+1.17)	79.95^a ±0.26 (+1.35)	79.13^a ±0.33 (+0.31)	79.49^a ±0.85 (-0.49)
Recovery period (18 d)	80.05^a ±0.56	79.74^a ±0.24 (-0.38)	80.27^a ±0.21 (+0.27)	79.41^a ±1.04 (-0.79)	78.76^a ±1.10 (-1.61)
			Ash		
0 d	1.22				
Exposure period (45 d)	2.54^c ±1.35	7.39^b ±0.56 (+190.94)	9.73^a ±0.71 (+283.07)	2.80^c ±0.14 (+10.23)	6.27^b ±0.76 (+146.85)
Recovery period (18 d)	2.06^b ±0.76	3.80^{ba} ±0.37 (+84.46)	4.98^a ±0.08 (+141.74)	2.73^b ±0.79 (+32.52)	2.93^b ±1.50 (+42.23)

Values are Mean ± SE, C-Control, T-Safe concentration of nickel, T_2- Sublethal concentration of Nickel, T_3- Safe concentration of Chromium, T_4-Sublethal concentration of Chromium, d-day. Figures in parentheses indicate percent variation as compared to control.The values with the same superscript in a row do not differ significantly (P= 0.05).

0.12% of cadmium (Sheela et al 1995). James et al (1995) observed rise in the level of lipids in muscle in *H. fossilis* exposed to 750 ppb of copper for 21 days. Moisture content showed no significant variation whereas the ash content increased during the exposure period.

Test fish when transferred to metal free water after the exposure period showed an improvement in the protein and carbohydrate level of muscle (Table I). For instance the level of protein was 10.16 g/100 g in fish exposed to safe concentration of nickel and it increased to 13.45 g/100 g when the fish was shifted to metal free water. Similarly carbohydrate level increased from 0.99 g/100 g to 1.37 g/100 g under the same conditions. Improvement in the level of proteins may be due to reduced proteolysis as a result of metal elimination and reduced proteolysis could be due to decline in protease activity (Chandravathy and Reddy 1994) whereas recovery in carbohydrate level could be attributed to restoration of regulatory function by the cells and tissues involved in carbohydrate metabolism (Chandravathy & Reddy 1996). Metals have been found eliminated from the intoxicated fish when transferred to normal water (Holcombe et al 1976; Reichert et al 1979). No significant variation was observed in the moisture content whereas ash content decreased during the recovery period.

Recovery in protein & carbohydrate level of muscle suggests elimination of accumulated metals from muscle & improvement in its nutritive value. James et al (1996) observed recovery in the enzyme activities in *H. fossilis* exposed to sublethal levels of mercury but there is paucity of information related to recovery of biochemical constituents of muscle. However, the present study revealed that the recovery period given i.e. 18 days was not sufficient and *C. mrigala* required more time for complete recovery of the biochemical constituents of muscles from metal poisoning.

REFERENCES

APHA (1989) Standard methods for the examination of water & wastewater. 15th Ed. American Public Health Association, New York

Basak PK, Konar S K (1977) Estimation of safe concentrations of insecticides. A new method tested on DDT and BHC. J Inland Fish Soc India 9: 19-29.

Baskaran P, Palanichamy S, Arunachalam S (1989) Effects of textile dye effluent on feeding energetics, body composition and oxygen consumption of the fresh water fish *Oreochromis mossambicus*. J Ecobiol 9: 203-14.

Baskaran P, Palanichamy S (1990) Impact of agricultural (Ammonium chloride) fertilizer on physiology & biochemistry of fresh water teleost fish, *Oreochromis mossambicus*. J Ecobiol 2: 97-106.

Chandravathy VM, Reddy SLN (1994) *In vivo* recovery of protein metabolism in gill and brain of a freshwater fish, *Anabas scandens* after exposure to lead nitrate. J Environ Biol 15: 75-82.

Chandravathy VM, Reddy SLN (1996) Lead nitrate exposure changes in carbohydrate metabolism of freshwater fish. J Environ Biol 7: 75-79.

Dubois M, Gilles KA, Hamitton JK, Roberts PA, Smith F (1956) Calorimetric method for the determination of sugars and related substances. Anal Chem 28: 350-56.

Finney DJ (1971) Probit Analysis. Cambridge Univ. Press, London, England.

Folch J, Lees M, Sloane BGH (1957) A simple method jfor the isolation and purification of total lipids from animal tissues. J Biol Chem 226: 497-509.

Holcombe GW, Benoit DA, Leonard EN, Mckim JM (1976) Long term effects of lead exposure on three generations of brook trout, *Salvelinus fontinalis*. J Fish Res Bd Canada 33: 1731-41.

James R, Sampath K, Siva KV, Babu S, Shanmuganandam P (1995) Toxic effects of copper and mercury on food intake, growth and proximate chemical composition in *Heteropneustes fossilis*. J Environ Biol 16: 1-6.

James R, Sampath K, Deakiamma, Pattu VJ (1996) Recovery of dehydrogenase enzymes activities in *Heteropneustes fossilis* (Bloch) exposed to sublethal levels of mercury. Indian J Fish 43: 293-297.

Kasthuri J, Chandran MR (1997) Sublethal effect of lead on feeding energetics, growth performance, biochemical composition and accumulation of the estuarine catfish, *Mystus gulio* (Ham.) J Environ Biol 18: 95-101.

Lowry DH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent. J Biol Chem 193: 265-75.

Nanda P, Panda BN, Bahera MK (2000) Nickel induced alterations in protein level of some tissues of *Heteropneustes fossilis*. J. Environ Biol 21:117-119.

Reichert WL, David AF, Malins CD (1979) Uptake and metabolism of lead and cadmium in coho salmon, *Onchorhynchus kisutch*. Comp Biochem Physiol Pharmacol 63: 229-34.

Sheela M, Mallika G, Muniandy S (1995) Impact of cadmium on food utilisation, growth and body composition in the fish, *Oreochromis mossambicus*. Environ Ecol 13: 410-14.

Sivarama Krishna B, Suresh A, Venkataraman P, Radhakrishnaiah K (1992) Copper induced changes in lipid metabolism in the tissues of fresh water teleost, *Labeo rohita*. Biochem Intern 26: 333-335.

Syversen TLM (1987) Effects of methyl mercury on protein synthesis *in vitro*. Acta Pharmacol Toxicology 49: 422-26.

Vasanthi R, Baskaran P, Palanichamy S (1990) Influence of carbofuran on growth and protein conversion efficiency in some fresh water fishes. J Ecobiol 2: 85-88.

Vincent S, Ambrose T, Cyrill L, Selvanaygam M (1995) Biochemical responses of the Indian Major carp, *Catla catala* (Ham.) to chromium toxicity. Indian J Environ Hlth 37: 192-96.

Virk S, Dhawan A (1997) Effect of nickel and chromium on flesh quality of *Cyprinus carpio* (Linn.). Indian J Ecol 24: 1-9.

Virk S, Kaur K (1999) Impact of mixture of nickel & chromium on the protein content of flesh & liver of *Cyprinus carpio* during spawning and post-spawning phases. Bull Environ Contam Toxicol 63: 499-502.