Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 21(2009) 229–236
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Bioaccumulation and depuration of chromium in the selected organs and whole body tissues of freshwater fish *Cirrhinus mrigala* individually and in binary solutions with nickel

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Received 25 February 2008; revised 06 March 2008; accepted 22 May 2008

Abstract

Contamination of aquatic ecosystems with heavy metals has been receiving increased worldwide attention due to their harmful effects on human health and other organisms in the environment. Most of the studies dealing with toxic effects of metals deal with single metal species, while the aquatic organisms are typically exposed to mixtures of metals. Hence, in order to provide data supporting the usefulness of freshwater fish as indicators of heavy metal pollution, it has been proposed in the present study to investigate the bioaccumulation and depuration of chromium in the selected organs of freshwater fingerlings *Cirrhinus mrigala*, individually and in binary solutions with nickel. The results show that the kidney is a target organ for chromium accumulation, which implies that it is also the "critical" organ for toxic symptoms. The results further show that accumulation of nickel in all the tissues of *C. mrigala* is higher than that of chromium. In addition, the metal accumulations of the binary mixtures of chromium and nickel are substantially higher than those of the individual metals, indicating synergistic interactions between the two metals. Theoretically the simplest explanation for an additive joint action of toxicants in a mixture is that they act in a qualitatively similar way. The observed data suggest that *C. mrigala* could be suitable monitoring organisms to study the bioavailability of water-bound metals in freshwater habitats.

Key words: freshwater fish; *Cirrhinus mrigala*; bioaccumulation; metal mixtures; synergism
DOI: 10.1016/S1001-0742(08)62256-1

Introduction

Contamination of aquatic ecosystems (e.g., lakes, rivers, streams, etc.) with heavy metals has been receiving increased worldwide attention due to their harmful effects on human health and other organisms in the environment. The main sources of heavy metals in aquatic ecosystems are of the anthropogenic type. Metals after entering the water may precipitate or adsorb on the surface of solids, remain soluble or suspended in it or may be taken up by fauna and flora. One of the most important properties of a toxic pollutant is its ability to accumulate in the tissues of organisms. Over a long period, the pollutants present in the environment at very low levels may accumulate within the body of aquatic species by various mechanisms to the extent that they exert toxic effects. Therefore, it is of great importance to know the bioaccumulation potential of a pollutant. Among metals, chromium is considered to be of high priority. Chromium compounds are frequently encountered as environmental pollutants and have been known to produce toxic, mutagenic, and carcinogenic effects in biological systems, although Cr is an essential nutrient (Parlak *et al*., 1999). Plating and electroplating factories, leather tanneries, textile manufacturing facilities, cooling tower blow down, rinse waters, steel producing factories, etc. are most often the anthropogenic sources. Nickel is a ubiquitous trace metal and occurs in soil, water, air, and in the biosphere. It is emitted into the environment from both natural and man-made sources. Once released to the environment, nickel readily forms complexes with many ligands, making it more mobile than most heavy metals. The primary sources of nickel emissions into the ambient air are the combustion of coal and oil for heat or power generation, nickel mining, steel manufacture, and miscellaneous sources, such as cement manufacturing. While nickel is an essential element at low concentrations for many organisms, it is toxic at higher concentrations (Clark and Keasling, 2002). Exposure to nickel may lead to various adverse health effects, such as nickel allergy, contact dermatitis, and organ system-toxicity. Numerous studies have confirmed the carcinogenic potency of nickel compounds in experimental animals (Haitian *et al*., 2005).

The accumulation of heavy metals in water suggests that fish may serve as a useful indicator for contamination metals in aquatic systems, because they respond with great

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sensitivity to changes in the aquatic environment (Aas *et al*., 2001; Mondon *et al*., 2001). Sreedevi *et al*. (1992) studied the effect of nickel on freshwater fish *Cyprinus carpio* treated to various concentrations. The study indicates that nickel accumulation is more in lethal than in sub-lethal concentrations. Friedrich and Filice (1976) studied the intake and accumulation pattern of nickel on *Mytules edulis* over a period of 4 weeks. The accumulation pattern was found to be varying with time. Solis Heredia *et al*. (2000) studied the accumulation of chromium in the liver, kidney and pancreas in rats and the impact of chromium on metallothionein levels. The results of their study show that treatment with chromium induces metallothionein synthesis in pancreas. A number of studies have been carried out to assess freshwater pollution by the discharge of effluents from industries (Rama Rao *et al*., 1978). It is well-known that the discharge of metals from pollution sources usually consists of mixtures rather than of individual metals. Most of the studies dealing with toxic effects of metals deal with single metal species, while the aquatic organisms are typically exposed to mixtures of metals. Many data show that certain metals affect accumulation of the others in fish. It seems that interactions among various metals are related to their competitive uptake from the environment, and different distribution in fish tissues. Interactions among metals may be different, and therefore, the effects of their various mixtures on fish survival may also differ. The effect of mixtures of two or more chemicals is commonly referred to either as additive, synergistic or antagonistic (Jezierska and Witeska, 2001). Hence, in order to provide data supporting the usefulness of freshwater fish as indicators of heavy metal pollution, it has been proposed in the present study to investigate the bioaccumulation and depuration of chromium in the selected organs of freshwater fingerlings *Cirrhinus mrigala*, individually and in binary solutions with nickel. In the current investigation the freshwater fingerlings *C. mrigala* was used, because it is one of the most common Indian carp and withstands a wide range of experimental conditions. It occurs in the principal rivers of India and is a moderately fast growing freshwater major carp. In addition, it is of great commercial importance and is renowned for its taste.

1 Materials and methods

1.1 Test species

The freshwater fingerlings, *C. mrigala* (8 ± 2 g in weight; 6 ± 1 cm in length), were collected from the freshwater bodies near local fish farm, Puthur, Tamilnadu and acclimatized under laboratory conditions for 7 d. For the entire duration of the experiment the fish were fed with rice bran and earthworm pieces that had no detectable amounts of chromium and nickel.

1.2 Test chemicals

The analytical grade nickel chloride and potassium dichromate purchased from S. D. Fine Chemicals, Bangalore, India, were used without further purification. Nickel test solution was prepared by dissolving 4.047 g of nickel chloride ($NiCl_2 \cdot 6H_2O$) with double distilled water to make 1000 mg/L nickel and then diluted to desired nickel with water. Chromium test solution was prepared by dissolving 2.828 g of potassium dichromate ($K_2Cr_2O_7$) with double distilled water to make 1000 mg/L chromium and then diluted to desired chromium with water. Double- distilled water (nickel and chromium levels are non-detectable) was used as the solvent.

1.3 Lethality studies

The LC_{50} values for nickel ($NiCl_2$), chromium ($K_2Cr_2O_7$) and their mixtures ($NiCl_2$ + $K_2Cr_2O_7$) were determined by using Litchfield and Wilcoxon (1949) method and were found to be 10.83, 18.20, and 8.61 mg/L, respectively. The acclimated fish were stocked in 20 L plastic troughs equipped with continuous air supply. The physico-chemical parameters such as pH, total alkalinity, total hardness as $CaCO_3$, calcium and magnesium were measured according to American Public Health Association (APHA, 2005) and maintained at optimum level (7.1 ± 0.2 mg/L, 256 ± 10.8 mg/L, 200 ± 14.2 mg/L, 68 ± 6.5 mg/L, and 18 ± 1.2 mg/L). The water was changed along with waste feed and fecal materials daily at 4 p.m. by a siphoning system, which caused minimal disturbance to the fish. Metal analysis of water was carried out prior to replacement and was found to be with 95% of the required concentration. Daily the containers were refilled and redosed with metal toxicant.

1.4 Experimental design

In the present study, accumulation of the heavy metal chromium in various tissues of *C. mrigala* was monitored for a period of 4 weeks in the laboratory to ascertain the dynamic nature (i.e., the pattern in time) of the response (Friedrich and Filice, 1976). To study the excretion process of the test fish for another 28 d, we took samples of whole body tissues at intervals of 7 d. To study the effect of heavy metal mixture nickel and chromium, we confined our study to a period of 7 d.

Freshly collected *C. mrigala* fingerlings after acclimatization were divided into three groups of 25 each. The experiments were carried out at 28 ± 1°C in an aerated glass trough. Group 1 fish were placed in a glass trough (45 cm × 32 cm × 25 cm) and kept in dechlorinated tap water. The group 2 and group 3 fish were exposed to one-tenth and one-third of LC_{50} taken as lower (1.82 mg/L) and higher (6.07 mg/L) concentration of chromium. The test fish were exposed to the above-mentioned sub-lethal concentrations separately for a period of 7, 14, 21, and 28 d. At the end of those periods, a group of fish were randomly selected from the experimental tank and kept in dechlorinated tap water (elimination period) for another period of 28 d. At the end of each exposure period, gill, liver, kidney, muscle and whole body tissues were isolated and kept in a freezer (–20°C) prior to analysis. Also, during the elimination period of 28 d, whole body tissues were isolated at a periodic interval of 7 d.

For interaction studies, the test animals were divided

into three groups of 25 each. Group 1 was exposed to one-third of LC_{50} (2.87 mg/L) of metal mixtures (nickel and chromium) and groups 2 and 3 were exposed to 2.87 mg/L of nickel and chromium separately for 7 d. Controls were run simultaneously for 7 d. At the end of the exposure periods, various organs were dissected and preserved at −20°C prior to analysis.

1.5 Sample preparation

For the estimation of metals (nickel and chromium), the dried sample tissues were digested with concentrated nitric acid and perchloric acid in the ratio 3:1, respectively, by standard digestion method (Topping, 1973). The concentrations of nickel and chromium were estimated using inductively coupled plasma optical emission spectroscopy (Jobin-Yvon JY24; Instrument SA, France) available at the Centre of Advanced Study in Marine Biology, Annamalai University. All the experiments were conducted in 4 replicates and the average of the values was reported along with standard deviations. The results, expressed in μg metal/g dry tissue (μg/g), were treated statistically using: (1) analysis of variance (ANOVA) for the comparison of several means and (2) student *t*-test for the comparison of two means.

2 Results and discussion

2.1 Bioaccumulation of chromium

Tables 1 and 2 summarize the data of the average chromium concentration in the selected organ tissues and the whole body tissues of *C. mrigala* fingerlings under different exposure periods. The accumulation patterns of chromium are in the order: kidney > liver > gill ≈ muscle, for lower sub-lethal concentration, and kidney > muscle > liver > gill, for higher sub-lethal concentration of chromium. The kidney accumulated the highest level of chromium (97.326 and 162.637 μg/g) for lower as well as higher sub-lethal concentrations. Next to kidney, the liver accumulated the highest level (87.325 ± 3.683 μg/g) for the lower concentration, whereas in the case of the higher concentration, the muscle accumulated the highest level (91.227 μg/g).

Figure 1 represents the accumulation pattern of chromium in the selected organs exposed to the lower and higher sub-lethal concentrations for a period of 28 d. As seen from Fig. 1, the accumulation of chromium increases steadily in all the tissues up to 21 d, for both levels of concentrations and afterwards it remains almost constant in the kidney, whereas in other organs it varies arbitrarily. The maintenance of constant level of accumulation in kidney after a certain period is expected, since kidney is the principal route of excretion for most toxicants. Similar results have been reported by Kendall (1975) for catfish (*Ictalunes punctatus*) exposed to methyl mercuric chloride.

Accumulation and elimination of chromium (μg/g) from the whole body tissues of *C. mrigala* exposed to lower (LSL) and higher (HSL) sub-lethal concentrations are shown in Fig. 2. The increased levels of accumulation during the exposure period suggest relatively rapid absorption of this metal. Similar result has been reported in fresh water isopods exposed to Cu, Pb, and Zn (Van Hattum *et al*., 1993). The depuration experiments were started after 28 d of absorption. During these periods, depuration of chromium was possible due to the subsequent excretion of

Table 1 Accumulation of chromium (μg/g) in the selected organ tissues of *Cirrhinus mrigala* exposed to lower (LSL) and higher sub-lethal (HSL) concentrations ($n = 4$)

Organ	7 d	14 d	21 d	28 d
Control				
Gill	5.231 ± 0.801	5.636 ± 0.224	3.215 ± 0.981	15.436 ± 1.362
Liver	5.487 ± 1.256	7.007 ± 0.826	22.226 ± 1.753	7.687 ± 2.126
Kidney	5.853 ± 0.420	10.442 ± 1.258	26.837 ± 2.636	32.438 ± 1.724
Muscle	6.614 ± 1.263	12.136 ± 0.881	16.241 ± 1.406	20.434 ± 1.325
Lower sub-lethal				
Gill	16.534 ± 1.831	22.932 ± 1.831	28.425 ± 2.664	48.736 ± 3.952
Liver	27.516 ± 3.724	39.217 ± 4.631	57.226 ± 6.425	87.325 ± 3.683
Kidney	21.234 ± 2.156	62.623 ± 5.326	92.617 ± 8.643	97.326 ± 7.963
Muscle	12.226 ± 1.852	30.630 ± 2.773	43.734 ± 3.232	48.635 ± 3.782
Higher sub-lethal				
Gill	19.822 ± 2.207	33.735 ± 1.848	42.682 ± 4.236	36.834 ± 2.332
Liver	51.632 ± 2.336	85.758 ± 4.257	86.474 ± 6.656	78.926 ± 5.437
Kidney	37.718 ± 2.124	82.482 ± 6.352	164.520 ± 9.347	162.637 ± 8.623
Muscle	27.832 ± 1.319	34.670 ± 2.862	73.462 ± 4.833	91.227 ± 5.264

The difference between the control and exposures are statistically significant ($P < 0.05$).

Table 2 Accumulation and elimination of chromium (μg/g) from the whole body tissues of *C. mrigala* exposed to concentrations ($n = 4$)

Time (d)	Control	Accumulation		Elimination	
		LSL	HSL	LSL	HSL
7	12.427 ± 0.961	29.662 ± 2.937	58.614 ± 4.626	94.660 ± 6.626	187.053 ± 10.726
14	18.886 ± 1.723	84.124 ± 6.453	113.763 ± 8.262	56.426 ± 3.762	106.634 ± 8.724
21	27.102 ± 4.635	121.625 ± 7.607	181.228 ± 8.437	69.835 ± 3.836	111.624 ± 9.215
28	22.320 ± 2.216	132.429 ± 9.638	217.260 ± 10.433	38.421 ± 3.263	79.836 ± 6.432

The difference between the control and exposed are statistically significant ($P < 0.05$).

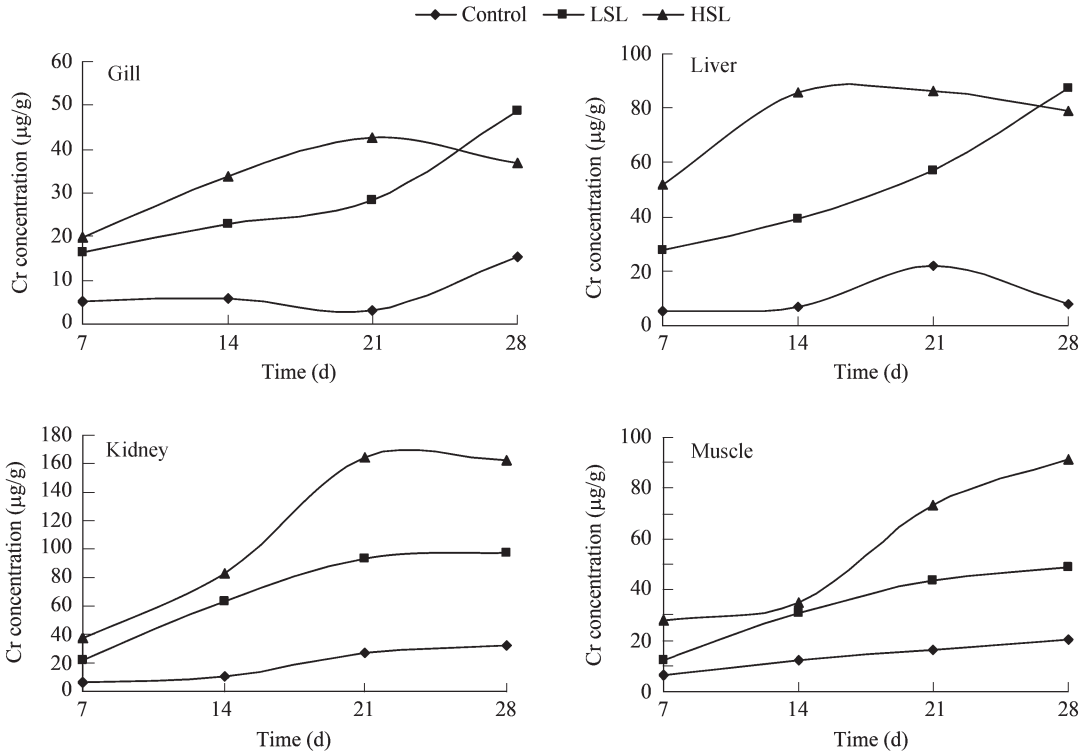

Fig. 1 Accumulation of chromium in the selected organ tissues of *C. mrigala* exposed to LSL and HSL concentrations.

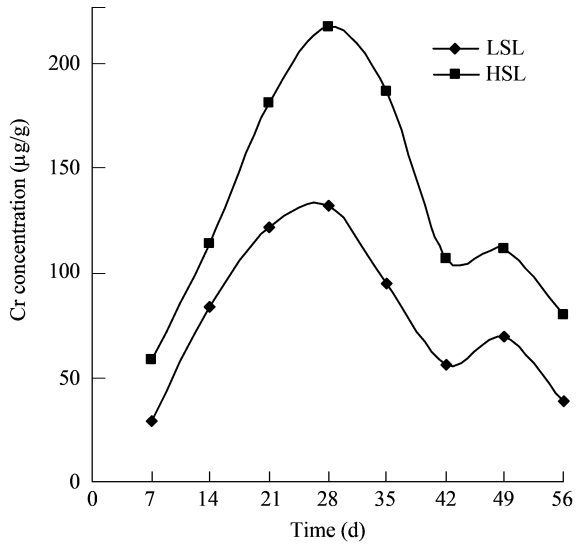

Fig. 2 Accumulation and elimination of chromium from the whole body tissues of *C. mrigala* exposed to LSL and HSL concentrations.

metal-rich residual bodies. However, with respect to the rate of accumulation and absorption, there is no consistency. Also, the level of chromium was not completely reversible and after a period of 14 d of the elimination phase, it was still found to be irregular. A considerable amount of chromium was found even at the end of the depuration period and did not reach the normal (control) level; this might be due to the maintenance of a constant level of chromium for a basic physiological requirement (Goyer, 1986). It has been reported (Evtushenko *et al.*, 1986; Watras *et al.*, 1985) that the level of accumulated metals in tissues remained invariably a plateau even when the organisms were exposed to them continuously for a sufficiently long period. It has also been reported that a considerable level of concentration of nickel and chromium was observed in control animal and it could be due to the presence of nickel (0.21 μg/g) and chromium (0.14 μg/g) in the water used in the present study which was drawn from the general source.

2.2 Depuration of chromium

In order to get information on bioaccumulation as well as dynamics and fate of chromium in the exposed organism, the bioconcentration factor (BCF), uptake rate (k_1) and elimination rate (k_2) constants (Darryl *et al.*, 1986) were also calculated and reported in Table 3. In general, it was observed in the present study that elimination rate constant (k_2) under all treatments was smaller for both levels of concentration (0.003–0.005). Further, it could be seen from Table 3, that the highest level of chromium was observed in kidney (BCF = 53.46 or 26.83) for both levels of concentration. Further the uptake and elimination rate constants were found to be: k_1 = 0.225 h^{-1} and k_2 = 0.004 h^{-1} for kidney; k_1 = 0.197 h^{-1} and k_2 = 0.004 h^{-1} for liver; and k_1 = 0.077 h^{-1} and k_2 = 0.003 h^{-1} for muscle. A considerable level of chromium also accumulated in the gill tissues at both levels (BCF = 26.75 or 6.07). The uptake and excretion rate constants for gill tissues were: k_1 = 0.088 h^{-1} and k_2 = 0.003 h^{-1} for lower; k_1 = 0.018 h^{-1} and k_2 = 0.003 h^{-1} for higher concentration respectively. The BCF values recorded for the whole body tissues were 72.74 and 35.84 for lower and higher concentration respectively.

Table 3 Bioconcentration factor (BCF), uptake rate (k_1) and elimination rate (k_2) constants in the selected organ tissues exposed to chromium for 28 d (n = 4)

Organ	Lower sub-lethal concentration		
	BCF	k_1 (h^{-1})	k_2 (h^{-1})
Gill	26.75±2.10	0.088±0.004	0.003±0.001
Liver	47.96±2.60	0.197±0.012	0.004±0.003
Kidney	53.46±3.20	0.225±0.022	0.004±0.001
Muscle	26.70±1.80	0.077±0.003	0.003±0.002
Whole body	72.74±3.20	0.255±0.013	0.004±0.001
	Higher sub-lethal concentration		
	BCF	k_1 (h^{-1})	k_2 (h^{-1})
Gill	6.07±0.90	0.018±0.003	0.003±0.004
Liver	13.01±1.30	0.051±0.001	0.004±0.002
Kidney	26.83±1.80	0.132±0.012	0.005±0.001
Muscle	15.05±1.20	0.059±0.002	0.004±0.001
Whole body	35.84±3.40	0.154±0.032	0.004±0.001

k_1 was calculated by $k_2 \times$ BCF.

2.3 Bioaccumulation of nickel and chromium mixtures

Tables 4 and 5 show respectively the comparative toxicity of nickel, chromium and their mixture (1:1) and the BCF values in selected organs of the test fish. It could be seen from the results that the bioaccumulation of both nickel and chromium was in the order: kidney > liver > gill > muscle. Further, the metal accumulation of binary mixtures of chromium and nickel were substantially higher than those of the individual metals, indicating synergistic interactions between the two metals. The highest level of accumulation (BCF = 49.71 for nickel and 18.03 for chromium) was found in the kidney. The liver occupied the second position (BCF = 40.98 for nickel and 15.42 for chromium) next to kidney. The gill also accumulated a considerable level of nickel and chromium, BCF = 30.82 and 8.31, respectively. However, the muscle recorded the least value (BCF = 14.03 or 7.88) for both metals. These results are in line with those of Ishimatsu *et al*. (1995), who found the kidney to be an organ with the highest Ni deposition in rats, next in the lung and liver.

In addition, the highest accumulation was also observed in the kidney for metal mixtures. This might be due to strong irrigation and in relation to the function of excretion. Tulasi *et al*. (1987) have also reported high accumulation of the metal in the kidney of *Barytelpus guerini* than in any other organs. Since the kidney is the principal organ involved in the storage of metal, highest level of the accumulation is observed in all the experimental treatments.

Next to the kidney, a significant concentration of metal was observed in the liver, the prime site of metal binding and release in fishes, for all the treatments. Metal concentration in the liver reflects its multifunctional role in detoxification and storage processes. This may be due to the increased synthesis of metallothionein and its storage as a constituent of liver cytoplasm resulting in increased accumulation of metal in the liver. Similar results have been reported in the study of liver tissues of white catfish *Ictalurus punctatus* exposed to cadmium (Rowe and Massarao, 1974).

Further, appreciable levels of nickel and chromium were observed in the gill in all the treatments. This may be due to the passive exchange of metals that occurs between animals and their environment through the epithelium of the bronchial gill (Ay *et al*., 1999). In addition, gill tissues play an important role in ion regulation, gas exchange, acid balance, excretion and elimination of nitrogenous wastes, which signify the key role it plays as the interface with the aquatic environment. The observed accumulation of metals may probably be due largely to the absorption of heavy metal to the gill surface, the absorption being dependent on the availability of proteins to which the metals bond.

The low trace metal values found in the muscle may be the result of the richness of contractile proteins, which have a high affinity for calcium (Schiaffino and Reggiani, 1996), therefore, should have a low affinity for heavy metals according to the general rules of organometallic chemistry. Although it is generally recognized that freshwater fish muscle is not considered a metal accumulating tissue, a least quantum of metal has got accumulated in all treatments under varying levels of pH and hardness (Karthikeyan *et al*., 2007). These factors have to be considered in bio-monitoring programs because they are consumed by the general public as food.

To determine the toxicity of metal mixtures, the additive toxicity index developed by Marking and Dawson (1975)

Table 4 Accumulation of nickel and chromium during Ni, Cr, and Ni + Cr treatments in the selected organs of *C. mrigala* (n = 4)

Organ	Nickel (μg/g)			Chromium (μg/g)		
	Control	Ni treatment	Ni + Cr treatment	Control	Cr treatment	Ni + Cr treatment
Gill	11.625±0.930	72.426±4.550	92.681±3.536	5.231±0.801	19.532±2.570	21.382±2.651
Liver	7.836±0.425	96.314±3.526	120.602±5.216	5.487±1.256	36.240±2.855	40.276±3.472
Kidney	10.714±0.628	116.824±6.431	130.275±7.860	5.853±0.420	42.374±3.682	45.276±2.225
Muscle	3.652±0.226	32.962±2.458	36.283±1.626	6.614±1.263	18.536±1.938	20.713±1.860

The difference between controls and exposures is statistically significant ($P < 0.05$).

Table 5 BCF in the selected organs of *C. mrigala* exposed to nickel, chromium and their mixtures for 7 d

Organ	BCF for nickel		BCF for chromium	
	Ni treatment	Ni + Cr treatment	Cr treatment	Ni + Cr treatment
Gill	30.82 ± 2.85	39.44 ± 3.27	8.31 ± 1.05	9.10 ± 1.42
Liver	40.98 ± 3.46	51.32 ± 4.05	15.42 ± 1.96	17.14 ± 2.42
Kidney	49.71 ± 3.86	55.44 ± 4.86	18.03 ± 2.25	19.27 ± 2.88
Muscle	14.03 ± 1.95	15.44 ± 2.08	7.88 ± 1.06	1.81 ± 1.30

is used. The toxicity unit, or sum of the biological effects of metal mixtures, is calculated by the following equation.

$$\frac{A_m}{A_i} + \frac{B_m}{B_i} = S \quad (1)$$

where, A and B represent metals; i and m represent toxicities (the 96 h LC_{50} values) of the individual metal and metal mixtures respectively; S represents sum of biological effects. The following relation is used to arrive at an appropriate index:

$\frac{1}{S} - 1$ when $S \leqslant 1$ (greater than additive toxicity)

$S \pm 1$ when $S \geqslant 1$ (less than additive toxicity)

When an index is negative, it indicates less-than additive toxicity, zero indicates additive toxicity and a positive value, more-than-additive. If the 95% confidence limits overlap zero, it is considered simple additivity. The additive index calculated for the 96 h LC_{50} (Cr + Ni) mixture was +1.04, indicating more than additive interaction. Theoretically, the simplest explanation for an additive joint action of toxicants in a mixture is that they act in a qualitatively similar way. It further suggests that Ni and Cr may follow different uptake pathways into organisms.

It could be seen from Table 4 that nickel levels as high as 50 times the nickel concentration in water (2.35 μg/g) were found in kidney exposed to nickel, whereas it was only 18 times that of the chromium concentration in water (2.35 μg/g) in kidney exposed to chromium. It could also be seen that there is a slightly higher nickel content in fish from the Ni + Cr treated groups compared to those exposed to nickel alone, leading to the conclusion that the combined exposure induced higher nickel accumulation in fish by 10%–28%. Similarly, an increase of 7%–12% chromium was observed in the presence of nickel. It could also be seen from the Figs. 3 and 4 that the combined effect of the metal mixtures was greater than that of individual exposure which exhibited the synergistic tendency of the metal. The possible explanation for the increased toxicities of mixtures might include increase in the rate of uptake, formation of toxic metabolites, reduction in excretion, alteration of distribution, and inhibition of detoxification.

3 Discussion

The present study indicates that the bioaccumulation of chromium in the liver tissue was time and dose dependent. At lower concentrations, there was a gradual absorption of chromium and at higher concentrations, the accumulation was found to level to a plateau after 14 d. This is evident by reduced uptake rate at higher

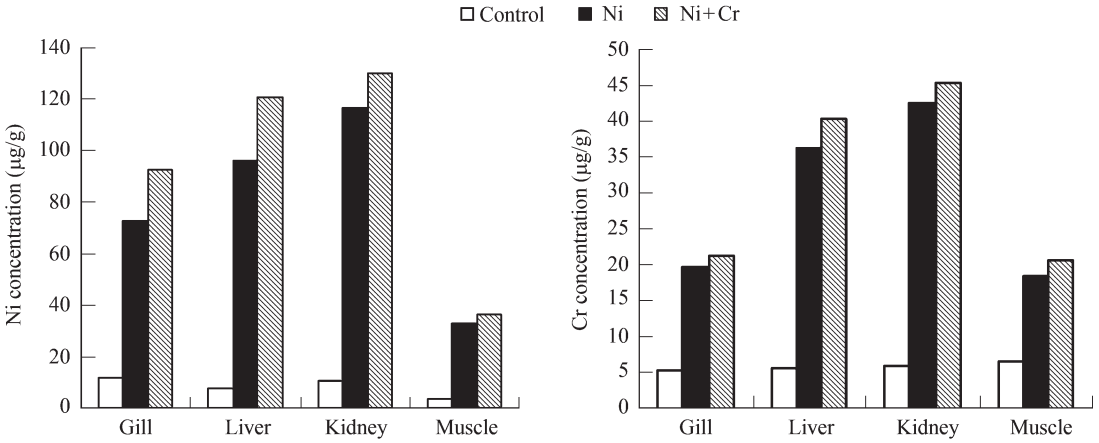

Fig. 3 Accumulation of nickel and chromium during Ni, Cr, and Ni + Cr treatments in the selected organs of *C. mrigala*.

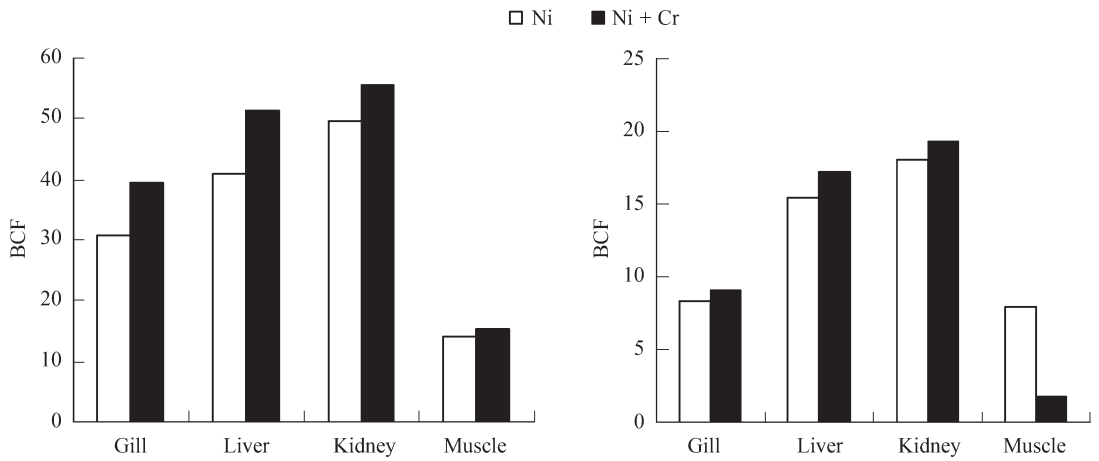

Fig. 4 BCF for nickel and chromium in the selected organs of *C. mrigala* exposed to nickel, chromium and their mixtures for 7 d.

concentration (k_1 = 0.051h^{-1}) compared with that of the lower concentrations (k_1 = 0.197 h^{-1}). The higher value of the bioconcentration factor observed in the liver (47.96) reflects the affinity of the metal to the tissues for both the levels of concentration. Since the liver is a major producer of metal binding proteins, the induction of low molecular weight metal binding proteins, such as metallothionein can be closely related to heavy metal exposures and this metal taken up from the environment is possibly detoxified by their binding on to proteins; this results in the higher concentration of metal mixtures in the liver.

It could be also seen that the gill tissue has in it a substantial amount of chromium during chromium treatment. Also, accumulation varies with exposure period and environmental concentrations. At low concentration, the accumulation was in accordance with exposure time. But at higher concentration (6.06 μg/L), there is a gradual increase of chromium up to 21 d and then the concentration tends to decrease. At higher concentrations, the uptake rate (k_1) is less than that in the lower sub-lethal concentration values by 5%. This reduced uptake may be due to inhibition of accumulation caused by the gill damage.

In the case of bioaccumulation of chromium in the muscle, the accumulation pattern varies with the degree of concentration of the toxicants. The low value of accumulation (BCF = 15.05) at higher sub-lethal concentration may be due to mucus secretion (Graney *et al*., 1984). Further, the uptake rate is low when treated with higher concentration (k_1 = 0.059 h^{-1}) when compared to lower one (k_1 = 0.077 h^{-1}). This reduced uptake and lower bioconcentration factor of chromium may be due to basic physiological function. Similar results have been reported in the abdominal muscles and digestive tissues of crayfish exposed to copper (Alikhan and Zia, 1989).

In general, a linear relationship between chromium uptake and the level of exposure has been observed in the whole body of the animal, implying little metabolic control over absorption at the two levels tested. However, chromium absorption was different in different organs, suggesting different functional capability to regulate metal absorption. Also, during the elimination process, no significant excretion of metal has observed and this may be due to the concentration of metals in the body, despite changes in metal availability in the environment.

The results of the present study show that the toxicity of nickel (LC_{50} = 10.83 mg/L) to *C. mrigala* is less than the toxicity of chromium (LC_{50} = 18.20 mg/L). Also, the highest no observed effect concentration (NOEC) observed for Ni was 4 μg/g, while for Cr it was 5 μg/g. Further, it can be seen that accumulation of nickel in all the tissues of *C. mrigala* is higher than that of chromium. It suggests that the cell membranes are more permeable to nickel than to chromium which no doubt accounts for greater accumulation of the former element to these organisms. The toxicity of nickel may be due to nickel being in contact with the skin (body surface), penetrating the epidermis and combining with body protein (Nielson, 1977). But in the case of chromium Cr(VI) after entering the cell it is readily reduced to Cr(III). This intracellular reduction of Cr(VI) to Cr(III) helps maintain a low level of chromium (Cohen *et al*., 1993) and this may possibly explain the reduced bioavailability of chromium when compared to that of nickel. Also, when the fish are exposed to nickel and chromium simultaneously, increased absorption of nickel and chromium is observed in all the tissues. These observations are in agreement with the increased uptake of nickel and chromium reported by Van Hoof and Nauwelaers (1984) in the freshwater fish *Rutilus*. Increased bioavailability of nickel and chromium in the case of their combined exposure signifies the greater toxicity of the metal mixture compared to that of the individual metal exhibiting synergism among these metals.

4 Conclusions

The results of the present study show that the kidney is a target organ for chromium accumulation, which implies that it is also the "critical" organ for toxic symptoms. The results further show that accumulation of nickel in all the tissues of *C. mrigala* is higher than that of chromium. In addition, the metal accumulations of the binary mixtures of chromium and nickel are substantially higher than those of the individual metals, indicating synergistic interactions between the two metals. Theoretically the simplest explanation for an additive joint action of toxicants in a mixture is that they act in a qualitatively similar way. In conclusion, the observed data indicate that *C. mrigala* could be suitable monitoring organisms to study the bioavailability of water-bound metals in freshwater habitats.

Acknowledgments

We thank the anonymous referees, who significantly contributed to improving the contents and style of the manuscript.

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