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Bioaccumulation and Depuration Pattern of Copper in Different Tissues of *Mystus vittatus*, Related to Various Size Groups

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Abstract The bioaccumulation pattern of copper (Cu) in gill, liver, kidney, and muscle of different sizes (fingerlings and adult age) of healthy *Mystus vittatus* when exposed to their respective sublethal concentrations of Cu-water, containing one-third 96-hr LC_{50} level (6.20 and 15.95 mg L^{-1}) for short-term (120 hr) and one-eighth 96-hr LC_{50} level (2.33 and 5.98 mg L^{-1}) for long-term experimentation, respectively, has been analyzed. The Cu shows a maximum deposition ($p < 0.01$) in the liver (82.12 and 70.65 μg/g) followed by gill (74.35 and 63.69 μg/g) and kidney (61.52 and 54.09 μg/g) both in fingerlings and adult fish, respectively, during 28 days of exposure. The lowest deposition of Cu is found to be 0.83 and 0.93 μg/g in fingerlings and 0.79 and 0.86 μg/g in adult muscle tissue during short-term (120 hr) and long-term (28 days) exposure periods, respectively. Comparing the accumulation of Cu on the two size groups at both exposure levels, it is obvious that the fingerlings showed higher Cu concentration in all tissues than those of adult fish. Another equally important finding is that the depuration of Cu by maintaining the bioaccumulated fish (long-term exposed group) of both size groups in quality dechlorinated ground water reveals that there is a significant ($p < 0.05$) reduction in Cu concentration in different tissues as the day passes. A comparison of the performance of the two size groups in respect of depuration clearly indicates that the fingerlings have taken 24–43 days (gill-kidney), whereas in mature fish it is 21–39 days (gill-kidney) to reach the level of control fish. Among the various tissues in both size groups, gill took the minimum number of days for complete recovery, whereas the muscle tissue did not significantly eliminate Cu even after 30 days of depuration. These data constitute a reference for future studies on the evolution of Cu accumulation and elimination tendency in relation to different size groups of fish in the ecotoxicological testing scheme for hazard assessment.

Keywords Copper · Bioaccumulation · Depuration · *Mystus vittatus* · Size group

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Introduction

Pollution is the negative feedback of the environment that affects living organisms. With increasing industrialization and discharge of effluents, heavy metals are becoming important pollutants in aquatic ecosystems (Joshi *et al.* 2002). Heavy metals may affect organisms directly by accumulating in their bodies or indirectly by transferring to the next trophic level of the food chain (Shah and Altindag 2005). Heavy metals accumulate in the tissues of aquatic animals and may become toxic when accumulation reaches a substantially high level (Kalay and Canli 2000). Knowledge about the uptake, distribution, and persistence in tissues of heavy metals is well documented (Karuppasamy 1999). The degree of developmental detoxification mechanisms are different between young and adult organisms (Rand and Petrocelli 1985). Differences in rates of excretion of toxic chemicals may also be involved in age-dependent toxicity effects. However, this proposition needs to be tested with reference to fish.

Fish exposed to a high concentration of trace metals in water may take up substantial quantities of these metals (Sultana and Rao 1998). Heavy metals can be bioaccumulated by fish, either directly from the surrounding water or by ingestion of food (Patrick and Loutit 1978; Kumar and Mathur 1991). However, when metal-contaminated fishes are transferred to clean water, metal depuration occurs (Rozalio *et al.* 1992; Kuroshima *et al.* 1993; Krishnamoorthy and Subramanian 1995).

Copper sulfate is one of the chemicals that is frequently used for the control of some fungal, parasite, and bacterial disease of fish in the local environments. It is also used as an algaecide, molluscicide, and herbicide in aquaculture, irrigation, and municipal water treatment systems (Stoskopf 1993). The copper level in natural unpolluted water is as low as 0.5–1 μg L^{-1} (Moore and Ramamoorthy 1984). However, industrial development has contributed to a continuous increase of copper in the aquatic environment. The Environmental Bureau in the United States has adopted the copper limits recommended by the Environmental Protection Agency (USEPA 1984) for the protection of aquatic life as 20 μg Cu L^{-1}. Copper is an essential metal for various physiological activities, but at higher concentration it tends to produce toxic effects (Maiti and Banerjee 2000). The freshwater fish *Mystus vittatus* (Kanagkelluthi) is considered one of the commercial fish both for fisheries and the local inhabitants, for whom it is a potential source of food. Furthermore, it is a hardy fish and an ideal animal model to work with.

Uptake and elimination are two of the most important factors in metal metabolism and hence, metal toxicity, but by far the majority of studies have concerned only uptake. Metal accumulation in the tissues of fish varies according to the rates of uptake, storage, and elimination (Langston 1990; Heath 1987). The elimination routes of metals from fish are generally bile, urine, elimination from the gills, and mucus (Riisgard *et al.* 1985). Both toxicity and bioaccumulation potential of a foreign compound are greatly affected by the rate of elimination from the organism. An unaltered chemical like Cu element can be eliminated rapidly; residues will not accumulate and tissue damage is less likely. Studies carried out with aquatic animals have revealed a different level of elimination of heavy metals. Kalay and Canli (2000) found that *Tilapia zilli* exposed to essential Cu, Zn, and nonessential (Cd, Pb) metal showed different elimination levels of the metals from its tissues.

The aim of this study is to determine the accumulation and elimination of copper from liver, gill, kidney, and muscle tissues of freshwater fish *Mystus vittatus* related to different age group of young fingerlings and adult fish by following short- and long-term sublethal exposure to the copper.

Materials and Methods

Experimental Fish and Acclimation

Healthy fingerlings (1.5 ± 0.5 g body weight and 3.1 ± 0.4 cm in total length) and adult size (8 ± 1 g body weight and 9.1 ± 0.5 cm in total length) of the fish *M. vittatus*, each group with 100 fish, were collected from local freshwater bodies in and around Annamalai University, Annamalainagar. The estimated average background Cu levels of water from the site of fish collection was 3.7 ± 0.831 μg L^{-1}. Fishes of fingerlings and adult age groups were separately maintained during the summer season at a maximum tolerable temperature of 27 ± 1°C in a 1000-L tank with continuous aeration and flowing dechlorinated tap water (pH 7.2–7.4; hardness 185–200 mg L^{-1} as $CaCO_3$; alkalinity 170–175 mg L^{-1} as $CaCO_3$; dissolved O_2 6.8–7.5; ammonia 0.12–0.20 mg L^{-1}, and Cu 2.11 ± 0.43 μg L^{-1}) at least for 15 days prior to the experiments. Fishes were fed boiled chicken eggs and small pieces of earthworm (2% of their body weight) on alternate days and 60% of the water was renewed every day. Feeding was suspended 24 hr before and during the mortality test for the fish, whereas during the accumulation and depuration experiments, the fishes were fed egg and earthworm pieces, once a day for 30 min, before the renewal of test water. After 30 min, the remaining food was removed. Fish were kept under proper environmental condition of 12 hr daylight and 12 hr darkness for both exposed and unexposed fish. Water quality was checked daily for pH, ammonia content, and temperature.

Exposure Chemical

Heavy metal copper (Cu) in the form of copper sulfate ($CuSO_4.5H_2O$-Analar grade, E. Merck) was used in the present study. The 96-hr LC_{50} concentration of copper was 18.62 mg L^{-1} for fingerlings and 47.86 mg L^{-1} for adult fish as calculated by using the probit analysis method (Finney 1978). The fingerlings and adult stage of fish were exposed to their respective one-third and one-eighth of 96 hr LC_{50} concentration of copper. The one third of 96 hr LC_{50} of 6.20 and 15.95 mg L^{-1} for short-term and one eighth of 96 hr LC_{50} of 2.33 and 5.98 mg L^{-1} for long-term levels were used for the experiments in the fingerlings and adult fish, respectively.

Accumulation Experiment

Accumulation experiments were conducted for 5 days of short-term level and 28 days of long-term level for both the

fingerlings and adult size of *M. vittatus*. The experiments were conducted in a glass aquarium (100 L water capacity) each containing 20 fish in 60 L of test solution. Twenty other fish in each age group were simultaneously used as the control group (control group was held in clean dechlorinated water). The average background Cu level of water used in the experiment at 0 days of exposure was 2.11 ± 0.43. Six replicates were prepared for each treatment period. The 60% water in the control and Cu-containing aquariums was renewed every day in order to minimize decreases in the Cu concentrations. At each interval, after 1, 3, and 5 days under short-term exposure and at 7, 14, 21, and 28 days under long-term exposure, six fish were sampled from each age group for determination of copper in their different organs.

Depuration Experiment

The determination of depuration period for Cu-accumulated fingerlings and adult *M. vittatus* was carried out with 50 animals of each size group, after being exposed to their respective sublethal concentration (one eighth of 96 hr LC_{50}) of Cu for for 28 days. Each size group of fish was separately transferred to dechlorinated control water and were allowed to leach accumulated metal from different tissues of the body; six fish from each group were sacrificed at various intervals for the determination of the Cu content in the respective tissue.

Cu Analysis

The fish were dissected and different organs of liver, gill, kidney, and muscle were taken, washed in double distilled water, and preserved in 10% formalin. Before analysis, fixative was removed using filter paper from each tissue and they were weighed (100 mg) and acid digested with a 10-mL mixture of perchloric acid and concentrated nitric acid in the ratio of 1:2 (vol/vol) (FAO 1975). The digest was diluted with distilled water (to bring it up to 25 mL) so that the Cu concentrations were prepared from stock standard solution of the Cu. The final acid-digested extract was analyzed for Cu concentration using Perkin Elmer Atomic Absorption Spectrophotometer-3100. The Cu concentration in tissue was recorded as $\mu g\ g^{-1}$ wet tissue.

Statistical Analysis

Data analyses were carried out using the SPSS (10.0 version) statistical package. The Dunnett test was used to compare experimental treatment groups against control, although Tukey's one-way analysis of variance was used to compare data that exist among experimental treatments at the 1% level. Analysis of variance was used to determine differences between various data sets. A statistical analysis of the accumulation experiments (at the 5% level) was carried out between various intervals of accumulation and their control value of the Cu while a statistical elimination experiment was carried out between the various intervals of elimination period. The elimination levels of the Cu from the tissues were calculated at the 1% level from the differences between the elimination values of Cu for the various periods of exposure.

Results and Discussion

When fish are exposed to elevated levels of metal in a polluted aquatic ecosystem, they tend to take these metals up from their direct environment. Evaluation of the level of Cu examined in the fingerlings and adults of *M. vittatus* in the present investigation for both short- and long-term tests reveal that the levels have been higher in fingerlings than in the mature animals (Tables 1 and 2), which agrees with the statement of Latif *et al.* (1982) on size-related Cu accumulation in fish. Perhaps the younger fish are less capable to cope with increased energy demands associated with increased metabolism or decreased rate of Cu elimination. One such important factor, which varies within and between populations, is the size of organisms. Although the lethal and sublethal effects of Cu are different for different age groups of aquatic biota (Munkittrich and Dixon 1988), accumulation of Cu by different age groups seems to be relatively stable (Kotze *et al.* 1999). However, the importance of size influences contaminant body burden (Watling *et al.* 1981), but only a few studies have attempted to quantify the Cu level in relation to the mechanism of fish age.

However, examination of Cu levels in the control fish of different size groups indicate the same tendency, with the highest in mature fish (Tables 1 and 2). The inevitable inference could be that these size groups would have accumulated the metal prior to capture from local freshwater bodies, as has been noted by Glynn *et al.* (1992) in minnows in natural habitats differentially in proportion to their duration of stay in habitat, mature for a longer period and fingerlings for a lesser period.

The present results have clearly proved the increasing accumulation of Cu in tissues over the long-term exposure rather than in the short-term exposure of examined fish of both size groups, which can be regarded as an indication of cumulative contamination (Muller and Serder 2002). Age-related changes then represent a crucial variability component in the present studies. The significantly varying

Table 1 Short-term (120 hr) accumulation of copper in different tissues (μg g^{-1} wet wt) of copper -exposed fingerlings (6.20 mg L^{-1} Cu) and adult (15.98 mg L^{-1} Cu) fish, *Mystus vittatus*

Tissues	Exposure group	Fingerlings				Adult			
		Exposure periods (hr)			Significant level (between exposure groups)	Exposure periods (hr)			Significant level (between exposure groups)
		24	72	120	*F*-value	24	72	120	*F*-value
Liver	Control	2.54 ± 0.040[a]	2.48 ± 0.060[a]	2.60 ± 0.098[a]	4581.509**	2.81 ± 0.063[a]	2.77 ± 0.058[a]	2/85 ± 0.079[a]	1334.188**
	Treated	16.13 ± 0.166[b]	58.70 ± 1.265[d]	76.26 ± 2.123[f]		12.72 ± 0.148[b]	31.53 ± 0.252[c]	63.68 ± 2.242[d]	
Gill	Control	1.65 ± 0.032[a]	1.72 ± 0.089[a]	1.58 ± 0.103[a]	769.154**	1.79 ± 0.440[a]	1.83 ± 0.380[a]	1.75 ± 0.510[a]	386.095**
	Treated	13.84 ± 0.125 [b]	39.62 ± 0.622[c]	62.44 ± 1.029[d]		11.54 ± 0.142 [b]	28.72 ± 0.421 [c]	53.77 ± 1.436 [f]	
Kidney	Control	2.35 ± 0.024[a]	2.38 ± 0.038[a]	2.32 ± 0.051[a]	168.885**	2.46 ± 0.054[a]	2.52 ± 0.061[a]	2.40 ± 0.079[a]	1765.922**
	Treated	9.82 ± 0.318 [b]	32.67 ± 0.390 [c]	43.81 ± 0.941[d]		9.18 ± 0.713[b]	26.92 ± 0.827[c]	38.66 ± 1.035[d]	
Muscle	Control	0.16 ± 0.003[a]	0.13 ± 0.001[a]	0.19 ± 0.004[a]	2.154[NS]	0.18 ± 0.004[a]	0.20 ± 0.002[a]	0.16 ± 0.011[a]	2.240[NS]
	Treated	0.48 ± 0.004 [b]	0.53 ± 0.013 [b]	0.83 ± 0.007[d]		0.41 ± 0.003[b]	0.52 ± 0.004[c]	0.79 ± 0.003[d]	

Values are expressed as mean of six individual ± SD

**F-value $\leq$ 0.01 $\Rightarrow$ significant at 1% level

Different letter designations denotes significant at 5% level

NS not significant

accumulation level of tissues in relation to the fish age/or weight has been reported for numerous fish species for copper (Kotze *et al.* 1999; Latif *et al.* 1982). Accumulation of Cu in the young fingerling does not permit a statistically significant separation of Cu accumulation in the adult, indicating that there is an increased Cu level in both age groups of this species. Accumulation is strongly dependent on the period of exposure, which is in close agreement with the report of Ruparelia *et al.* (1992) and Karuppasamy (2004) on heavy-metal-exposed fish.

In the present investigation, the maximum levels of copper accumulation have been observed in liver compared to other organs in both fingerlings and adult fish during short- and long-term exposure. These results are equal to the effects of copper on bioaccumulation of liver in fish *Cyprinus carpio* after short-term and long-term (30 days) exposure (Peyghan *et al.* 2003). According to Sultana and Rao (1998), the liver is the principal site involved in the storage of metals. Kotze *et al.* (1999) have also reported a higher accumulation of copper in liver tissue than any other organ in *Oreochromis mossambicus* as well as in *Clarias gariepinnus.* The higher accumulation of Cu in the liver of both size groups of test fish exposed to copper indicate that the storage of Cu might be due to sequestering of this metal by the metallothioneins (Dallinger 1995) and the liver may also play a role in detoxification of Cu (Hogstrand and Haux 1991).

Couture and Rajotte (2003) argue that Cu metabolism is found in fish under homeostatic regulatory control because Cu is an essential element. Furthermore, they have suggested that the liver Cu concentrations are usually regulated below 50 μg/g dry weight. However once this threshold is exceeded, the Cu homeostatic control mechanism becomes overloaded and liver Cu concentrations can increase. Data of the present study support this idea. In the only cases, after the postexposure periods of short- and long-term experiments, the liver Cu concentration exceeding the 50 μg/g threshold occurs in both fingerlings and adult fish. The level of excess liver Cu concentration, in relation to the usual regulated level, was approximately 64.20% in fingerlings and 41.30% in adult of *M. vittatus* at 28 days of exposure. These results demonstrate the loss of regulatory control of liver Cu in these fish. Moreover, the weak partial correlation between exposure concentration and concentration measured in tissues also suggests that internalized Cu is tightly regulated at the initial periods of exposure (Watanabe *et al.* 1997).

The essential metal can be taken up by fish through both waterborne and dietary routes, with branchial uptake probably being more important (Kock and Bucher 1997). In regard to the level of Cu accumulation in gill tissue, it also seems to be a favorite site for both size groups of *M. vittatus*, next to the liver tissue. The present results are in

Table 2 Long-term (28 days) accumulation of copper in different tissues (μg g^{-1} wet wt) of copper exposed fingerlings (2.33 mg L^{-1} Cu) and adult (5.98 mg L^{-1} Cu) age of fish, *Mystus vittatus*

Tissues	Exposure group	Fingerlings					Adult				
		Exposure periods (days)				Significant level (between exposure groups)	Exposure periods (days)				Significant level (between exposure groups)
		7	14	21	28	*F*-value	7	14	21	28	*F*-value
Liver	Control	2.69 ± 0.065[a]	2.73 ± 0.050[a]	2.66 ± 0.048[a]	2.68 ± 0.033[a]	2746.152**	2.95 ± 0.069[a]	2.92 ± 0.081[a]	2.99 ± 0.043[a]	2.94 ± 0.055[a]	951.730**
	Treated	12.36 ± 0.211[b]	38.84 ± 0.529[c]	61.18 ± 1.050[d]	82.12 ± 2.322[e]		10.63 ± 0.335[b]	41.58 ± 1.635[c]	51.83 ± 1.732[d]	70.65 ± 2.033[e]	
Gill	Control	1.81 ± 0.046[a]	1.78 ± 0.052[a]	1.86 ± 0.022[a]	1.79 ± 0.019[a]	1275.683**	1.85 ± 0.053[a]	1.82 ± 0.062[a]	1.87 ± 0.077[a]	1.86 ± 0.051[a]	1211.887**
	Treated	11.35 ± 0.191[b]	34.29 ± 0.757[c]	57.43 ± 1.035[d]	74.35 ± 2.195[e]		9.84 ± 0.638[b]	20.30 ± 0.864[c]	39.52 ± 0.944[d]	63.69 ± 1.078[e]	
Kidney	Control	2.45 ± 0.053[a]	2.41 ± 0.049[a]	2.50 ± 0.036[a]	2.44 ± 0.061[a]	7851.823**	2.69 ± 0.064[a]	2.70 ± 0.028[a]	2.65 ± 0.019[a]	2.72 ± 0.066[a]	6367.056**
	Treated	8.73 ± 0.121[b]	26.76 ± 0.344[c]	46.22 ± 0.943[d]	61.52 ± 2.081[e]		8.77 ± 0.134[b]	12.16 ± 0.334[b]	33.17 ± 0.635[c]	54.09 ± 1.043[d]	
Muscle	Control	0.18 ± 0.003[a]	0.16 ± 0.001[a]	0.21 ± 0.003[a]	0.17 ± 0.002[a]	116.127**	0.18 ± 0.001[a]	0.19 ± 0.001[a]	0.18 ± 0.003[a]	0.17 ± 0.002[a]	262.382**
	Treated	0.47 ± 0.004 [b]	0.71 ± 0.002[c]	0.89 ± 0.007 [d]	0.93 ± 0.002 [d]		0.32 ± 0.004[b]	0.048 ± 0.007[b]	0.66 ± 0.005[c]	0.86 ± 0.004[d]	

Values are expressed as mean of six individual ± SD

**F-value $\leq 0.01 \Rightarrow$ significant at 1% level

Different letter designations denotes significant at 5% level

NS not significant

equal trend to the report of Cu accumulation in the gills of rainbow trout after short- and long-term exposure to Cu (Kuroshima 1992). According to Kotze *et al.* (1999), the gills play a distinct role in Cu uptake from the environment. Similar findings have been observed by Rajamanickam (1992) in the same age adult fish with Cu treatments. Also, an examination of the Cu content in various organs of *Scylla serrata* indicates that gills have a very high value of Cu (John and Fernandez 1998). The above authors have suggested that this may possibly be because of the active uptake of Cu from gills. It is thought that Cu enters the epithelial cell via the lanthanum-sensitive Na channels (Taylor *et al.* 2000). The resultant absorption and binding of copper ions to the branchial surface might increase the concentration of copper in gills (Stagg and Shuttleworth 1982). Furthermore, the present result supports the view of Benoit (1975), who has suggested that the Cu accumulation essentially occurs in the osmotic and ionic-regulating organs such as gills.

The appreciable levels of copper found in gills of both size groups have increased as the exposure duration increases in both short- and long-term experiments. The observed time-dependent increases of the level of Cu is probably due in large part to the long time of direct contact, to absorption of Cu to the gill surfaces, and also to absorption being dependent on the availability of protein

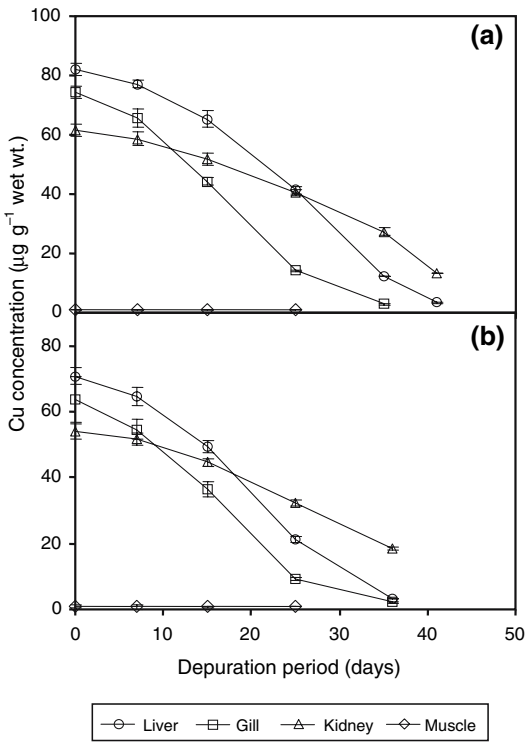

Fig. 1 Depuration of Cu in different tissues of **(a)** fingerlings and **(b)** adult of Mystus *vittatus* previously exposed to Cu during 28 days. The values are given as mean ± SD, $n = 6$

(Mazon and Fernandes 1999). Furthermore, such increased accumulation of Cu in gill tissue is in close agreement with the statement of Karuppasamy (2004). He has suggested that the rapid accumulation of heavy metal toxicant in gill is probably due to the large volume of water that passes though the gill to supply O_2 under stress.

The copper residue in kidney is also significantly ($p < 0.01$) increased in both age groups of *M. vittatus*, exposed to their respective concentrations of copper after short- and long-term investigations (Tables 1 and 2). The researcher's results clearly indicate the kidney as a target organ for Cu in *M. vittatus.* According to Thomas *et al.* (1985), kidney has shown a good potential for accumulation of metals. Accumulation of Cu in the kidney is proportionally increased with duration of exposure in both size groups, which is more consistent with the results of Topashka-Ancheva *et al.* (1998) and Mazon and Fernandes (1999), who have suggested that these increased Cu contents in the kidney are possibly from selective reabsorption of essential electrolytes, glucose, as well as essential metals such as Cu from urine at the proximal tubules.

According to results in the present study, the copper content in muscle tissue is significantly ($p < 0.05$) higher than in the control groups after short- and long-term exposure. Although these levels in test fishes are significantly higher than those in the control fish, they are within the approved limits for human consumption. The maximum level limit of 20 µg/g of Cu concentration in muscle tissue on edible fish has been reported by Cossa *et al.* (1979).

In general, Cu concentrations in muscle tissue of copper-exposed fish are considerably lower ($p < 0.05$) than those in other tissues, which is more consistent with other studies (Bradley and Morris 1986; Rajotte and Couture 2002). Muscle Cu concentrations were significantly lower relative to the higher concentrations in liver tissue, which is in agreement with the results of Wong *et al.* (1999) on silver sea bream under Cu toxicant. It is therefore possible that lower muscle Cu may be related to increased deposition of Cu in the liver of *M. vittatus.* That is, increased Cu ligands in liver (Bingham *et al.* 1984) could result in decreased muscle Cu concentration. Such increased Cu level in muscle tissue of both fingerlings and adult fish are directly proportional to the exposure periods, reaching maximum increase of 0.83 and 0.79 µg g^{-1}, respectively, at 120 hr of short-term exposure and 0.93 and 0.86 µg g^{-1}, respectively, at the 28th day of long-term exposure. This trend of results coincides with the studies of Cu accumulation in liver and muscle of common carp *C. carpio* over short- and long-term exposure (Peyghan *et al.* 2003) and *Tilapia zilli* (Kalay and Canli 2000).

Depuration studies carried out with *M. vittatus* using Cu by maintaining the bioaccumulated (for 28 days) test fish of both fingerlings and adult size in quality dechlorinated

ground water reveal that there is significant ($p < 0.01$) reduction in metal concentration in the different tissues as days progressed (Figure 1a and 1b). It appears that in general, more particularly in the fingerlings, the rate of depuration is a relatively slow process to begin with, which speeds up during the later phase of the experiment. It is closely in accord with the report of Devi (1990), who worked on *O. mossambicus* with Cu, that the phenomenon of depuration of Cu functions as a slow process in the initial phase of the study and becomes active during the later phase of the experiment. Cu is one of the tightly bound metals that forms a ligand to metallothionein and complete Cu release, which depends upon the turnover rate that can take months or even years (Roesijadi and Robinson 1994).

Previously, Kalay and Canli (2000), who have worked on *T. zilli* with Cu, have reported that Cu is rapidly eliminated from the tissues, showing a shorter period of biological half-life of Cu. However, in the present study, the original level has been achieved within 21–43 days among both size groups. A comparison of the performance of the two size groups of *M. vittatus* with respect to depuration clearly indicates that the fingerlings have taken 24–43 days to reach back to the original level, whereas the mature fish have taken 21–39 days. Similarly, Calmari *et al.* (1982) have also reported a marked drop in metal concentration from the tissue after the exposure was terminated. Within an individual fish, the kinetics of metal accumulation and release are expected to be very complex because physical and chemical parameters, water temperature, salinity, diet, fish species, and many other parameters may affect the rate of metal release in aquatic animal (Lemus and Chung 1999). For most metals, the rate at which they are released appears to be directly related to the rate of accumulation.

In the present investigation, the depuration of Cu clearly indicates that all the selected tissues have taken a greater number of days to return to a normal level than the days required for such level of Cu accumulation. The elimination routes of metals from fish are generally bile, urine, gills, and mucus (Varanasi and Markey 1978; Viarengo *et al.* 1985; Riisgard *et al.* 1985). It seems that although there are more elimination routes than uptake routes (Heath 1987), Cu accumulations are greater than Cu elimination, suggesting that once Cu has accumulated in tissue it is difficult to eliminate it from the body, which is evidenced in the work of Kalay and Canli (2000).

It is known that size and life stage of fish have a profound impact on the sensitivity to metal toxicity. Thus, in the present study, the different size groups of *M. vittatus* that are exposed to Cu have shown the fingerlings to have more susceptibility than the adult fish in terms of depuration period. These observations are in line with the findings of Glynn and Olssoni (1991). Mance (1987) opines that juvenile fish are generally more susceptible to metals than adults are.

Among the various parts (gill, liver, kidney, and muscle) examined for depuration, gill has taken the minimum number of days (24 and 21) for complete recovery from the initial levels, followed by liver (41 and 36) and kidney (43 and 39), respectively, for fingerlings and adult fish. This may indicate that metals have a shorter biological half-life in the gills than in the liver (Kalay and Canli 2000), possibly because they are removed from the gills either back to water (adsorbed metals) or transferred to other tissues, particularly liver, for detoxification. Devi (1990), who has studied depuration of Cu in *O. mossambicus*, reports that the initial level of Cu in gill tissue could be reached after a period of 39 days. According to Anderson and Spear (1980), the accumulated Cu in the gills of pumpkin seed sunfish displays a monophasic elimination. It is known that aquatic organisms have ligands of various binding strengths that may facilitate metals transport across the membranes (Simkiss *et al.* 1982).

In the present findings, liver and kidney also showed a significant ($p < 0.01$) level of Cu elimination next to the gill tissue in both age groups of test fish. Thus, the liver seems to be more efficient next to gill in eliminating the Cu compared to kidney and muscle. Similar observations have been made by Devi (1990) who discovered, while working with *O. mossambicus* exposed to Cu, that among the different parts of the test animals, liver exhibits a higher rate of loss of the Cu than any other parts. Cu detoxification must in the short-term occur via the cytosolic protein normally present in the liver of fish, with induced metallothionein playing an increasingly important role when the input of toxic metal ions becomes chronic (Roesijadi and Robinson 1994). The above-mentioned process may also be the reason for the fastest depuration of Cu in liver than in kidney in the present study.

The muscle of both fingerlings and adult fish does not significantly eliminate copper even after a 30-day depuration period. Similar observations are made by Kalay and Canli (2000) who report that muscle Cu concentration is not eliminated during the 30-day depuration period.

Conclusion

The most salient feature of this study is that Cu has a similar path of accumulation and elimination in tissue of young fingerlings and adult size of *M. vittatus.* Bioaccumulation response is powerful because it integrates a wide range of toxicological factors. It is quite amazing to note that Cu is stored preferentially in liver, gill, and kidney, which are not the edible parts, whereas in the edible part of

muscle, the Cu concentration is lower than international standards for human consumption even after a month of exposure to their lower sublethal concentration in both age groups of fish. The difference in Cu accumulation is usually not significant between both size groups of fish. Generally, body metal regulation is said to occur when body Cu concentration stays at constant levels, but rises in Cu concentration in both size groups after 28 days of exposure, indicating the absence of body Cu regulation system in the organism concerned. As the results of depuration study showed that the gills of the fishes are the first organs for quick elimination of Cu than others, suggesting that Cu in the gill might be released from the gill surface to water medium and adsorbed into the circulation system, which moves to other parts of the body. Moreover, the result of this study suggests that once the Cu accumulated in tissues of *M. vittatus*, it is more difficult to be eliminated from the body in young fish compared to in adult fish. Furthermore, it is hoped that these data on the Cu accumulation and elimination tendency in various tissues of test fish in relation to different size group could be used as a guideline in the ecotoxicology testing scheme for hazard assessment and other purposes.

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