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Abstract: *Tilapia zilli* were exposed to the same concentration (1 mg/L) of essential (Cu, Zn) and non-essential (Cd, Pb) metals for 10 days so that these metals would accumulate in the liver, gill, brain and muscle tissues. Subsequently, the animals were transferred to uncontaminated water for a period of elimination, during which samples were taken at days 1, 7, 15 and 30.

Cadmium and lead accumulated in all the tissues and the tissue concentrations increased many times compared to the levels of these metals in the control fish (<0.05 μg/g d.w.). The brain also accumulated lead to a high level. Copper also accumulated significantly (P<0.01) in all the tissues studied except for muscle tissue. The accumulation of zinc was not significant (P>0.05) in any tissue. The accumulation of the metals was found to be considerably different when the tissues and the metals were compared with each other.

After a 30-day elimination period, the levels of cadmium, lead and copper in the gills decreased 21.5 (P<0.001), 3.02 (P<0.05) and 7.37 (P<0.05) times, respectively. Cadmium and copper were not eliminated from the liver. On the contrary, the levels increased during the elimination period. Lead was the only metal that was eliminated to a significant extent from the liver. Elimination of the metals also showed considerable differences in terms of both the tissues and the metals. The elimination levels of cadmium and copper from the gills were higher than the elimination level of lead, while the opposite was true for the liver.

Key Words: RCu, Zn, Cd, Pb, Accumulation, Elimination, *Tilapia zilli*

Bir Tatlısu Türü Olan *Tilapia zilli*'nin Dokularında Biriken Gerekli (Cu, Zn) ve Gerekli Olmayan (Pb, Cd) Metallerin Atılımı

Özet: *Tilapia zilli* aynı derişimdeki (1 mg/L) gerekli (Cu, Zn) ve gerekli olmayan (Cd, Pb) metallerin etkisine 10 gün bırakılarak karaciğer, solungaç, beyin ve kas dokularındaki metal birikimine bakılmıştır. Bunu takiben deney hayvanları metal içermeyen suya alınarak 1, 7, 15 ve 30. günlerde çalışılan dokulardaki metal atılım düzeyi saptanmıştır.

Kadmiyum ve kurşun bütün dokularda birikmiş ve doku konsantrasyonları kontrol balıklarına göre birkaç kat artmıştır (<0.05 μg/g k.a.). Beyin dokusu yüksek düzeyde kurşun biriktirmişitr. Bakır kas dokusu dışındaki tüm dokularda önemli düzeyde birikmiştir (P<0.01). Çinko, diğer metallere göre tüm dokularda düşük düzeyde birikmiştir. Genel olarak metallerin birikim düzeyi, metale ve incelenen dokuya göre değişim göstermiştir.

Atılım peryodunun 30. gününde solungaç dokusu Cd, Pb ve Cu metalleri düzeyi sırasıyla 21.5 (P<0.001), 3.02 (P<0.05), ve 7.37 (P<0.05) kat düşüş göstermiştir. Atılım süresince kadmiyum ve bakırın karaciğer dokusundaki düzeyi, düşüş göstereceğine artış göstermiştir. Karaciğer dokusunun kurşunu en yüksek düzeyde attığı saptanmıştır. Solungaç dokusu kurşuna göre bakır ve kadmiyumu daha iyi elemine ederken, karaciğer dokusu için tam tersi bir durum olduğu belirlenmiştir. Atılım düzeyi, dokuya ve metale göre değişim göstermiştir.

Anahtar Sözcükler: Cu, Zn, Cd, Pb, Birikim, Atılım, *Tilapia zilli*

Introduction

Cadmium and lead have no known role in biological systems, whereas copper and zinc are essential components of enzymes or metalloproteins in fish metabolism. Heavy metals are natural trace components of the aquatic environment, but background levels in the environment have increased, especially in areas where industrial, agricultural and mining activities are

widespread (1-4). Most heavy metals released into the environment find their way into the aquatic phase as a result of direct input, atmospheric deposition and erosion due to rainwater. Therefore, aquatic animals may be exposed to elevated levels of heavy metals due to their wide use for anthropogenic purposes.

Heavy metals accumulate in the tissues of aquatic animals and may become toxic when accumulation reaches a substantially high level. Accumulation levels vary considerably among metals and species (5). Toxic effects occur when excretory, metabolic, storage and detoxification mechanisms are no longer able to counter uptake. This capacity, however, also varies between different species and different metals (4,5). Studies carried out with different fish species have revealed that both essential and non-essential metals can produce toxic effects in fish by disturbing physiological activities (6-9), biochemical processes (10-14), reproduction and growth (15,16), and mortality (17-19).

The tropical freshwater fish *T. zilli* is an important culture fish because it is easily reproduced and does not have feeding problems. 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 concentrated on uptake. Therefore, it would be useful to compare the elimination of essential and non-essential metals. The aim of this study was to determine the elimination of two essential (Cu and Zn) and two non-essential (Cd and Pb) metals from the liver, gill, muscle and brain tissues of *T. zilli*, following sublethal exposure to these metals.

Materials and Methods

Specimens of the tropical freshwater fish *T. zilli* were obtained from uncontaminated (metal concentrations were undetectable with the current method) fish culture pools filled with ground water at Çukurova University, and transferred to the laboratory where the experiments were conducted. The animals were put in aquariums (30x40x120 cm) for one month in order to acclimatize them to the new conditions. The experimental room was air conditioned (25±1.1°C) and illuminated with two fluorescent lamps (daylight 65/80 W) for 12 hours. The tap water used for the experiment had a pH value of 8.0±0.3 and a total hardness of 231±2.2 mg $CaCO_3$/L. The aquariums were aerated with air stones attached to an air compressor for oxygen saturation (7.5±0.7 mg O_2/L). The animals were fed once a day with an artificial fish food which did not contain cadmium or lead in measurable amounts (<0.05 μg/g d.w.). The mean fork length (cm) and standard deviation in the fish used in this study were 15.3±1.2 cm. There was no statistical difference between the study group and controls regarding the size of the fish. This may be important because smaller animals are generally more active than large ones, so metal uptake and elimination could also be higher in smaller animals (5,8,20).

The experiments were conducted in glass aquariums (145 L water capacity) each containing 20 fish in 100 L of contaminated test solution or tap water (for the control and elimination experiments). The fish were exposed to the metals separately and for each metal exposure and control three replicate tanks were used. The fish were first exposed to a nominal concentration of 1 mg/L of Cd ($CdCl_2$. H_2O), Pb ($Pb(NO_3)_2$), Cu ($CuSO_4$. $5H_2O$) or Zn ($ZnSO_4$. $7H_2O$) for 10 days. The water in the control and metal-containing aquariums was changed every two days in order to minimize decreases in the metal concentrations. At the end of the 10-day exposure period, 4 sample fish were taken from each replicate tank (a total of 12 fish for each metal exposure and control) and killed by a blow to the head. The fork length of each fish was measured to the nearest mm and then the fish were dissected with clean equipment in order to obtain muscle, gill, liver and brain tissues. Tissue samples from two fish of the same exposure groups were pooled (to make 6 subsamples) and dried in an oven set to 110°C until they reached constant weights. The remaining fish were transferred to clean aquariums which were filled with tap water for the elimination experiments. The tap water was also changed every two days during the elimination period. After 1, 7, 15 and 30 days, 12 fish were taken from each treatment group and processed as explained above. Thus, 300 fish were used in total.

The dry tissue samples were weighed to the nearest mg and put into digestion tubes. To each tube, 3 ml nitric acid and 1.5 ml perchloric acid (Merck) were added and the tubes were placed on a hotplate at 120°C (gradually increased) for 3 hours. The digest was diluted with distilled water so that the metal concentrations were in the range of the standards which were prepared from a stock standard solution of the metals (B.D.H.). The metal concentrations were measured using a Perkin Elmer AS

3100 flame atomic absorption spectrophotometer. The metal concentration in tissue was recorded as µg metal/g dry weight. In order to check the validity of the measurements, reference material (TORT 1 lobster hepatopancreas, National Research Council, Canada) was also included in the measurements so that the results could be compared with the reference data. The results showed that the present measurements were within a 10% range of the reference data.

Data analyses were carried out using the SPSS statistical package. The Mann-Whitney U test was used for data with two groups, while the Kruskall-Wallis one-way Analysis of Variance was used to compare data with more than two groups. A statistical analysis of the accumulation experiment was carried out on the 10-day accumulation values and the control values of the metals at the same time while a statistical analysis of the elimination experiment was carried out using the 10-day accumulation values and values from each elimination period. The elimination levels of the metals from the tissues were calculated from the differences between the 10-day accumulation values and 30-day elimination values.

The mean metal concentrations and standard errors are given in the figures. The tissue concentrations of the metals were also compared statistically and the results are shown in the figures. These were down between the 10-day control values and 10-day accumulation values, (0) indicated as A, or between the 10-day accumulation value and 30-day elimination values, indicated as E. Comparisons between the accumulation values and values from the other elimination periods were also made and these are explained in the results.

Results

The results of the experiment are shown in Figures 1-4. Accumulation (between 10 days control and 10-day accumulation treatment experiments: A) and elimination (between the values after 10 days' accumulation and the values after the 30-day elimination period: E) are shown in the figures.

The cadmium concentrations in the tissues of the control fish were below the detection limit of the instrument (<0.05 ppm) throughout the experiments. NS=not significant (P>0.05). The ratios between the wet weights and dry weights of the tissues were calculated and these were 3.52, 4.10 and 3.09 for liver, gills and muscle respectively (Figure 1).

The tissues of *T. zilli* (Figures 1a and b) accumulated significant amounts of cadmium and the tissue metal concentrations increased (P<0.001) many times when compared to the control values (<0.05 µg/g d.w.) for cadmium. The accumulation of cadmium after 10 days' exposure to the metal produced tissue cadmium concentrations in the order: gill > liver > muscle > brain. Significant eliminations of cadmium occurred in the gills from the first day (P<0.001) of the elimination period to the 30th day (P<0.001), while the liver, muscle and brain did not show any elimination of cadmium (P>0.05). There was an increase (P<0.05) in the cadmium concentration of the brain during the 1st day of

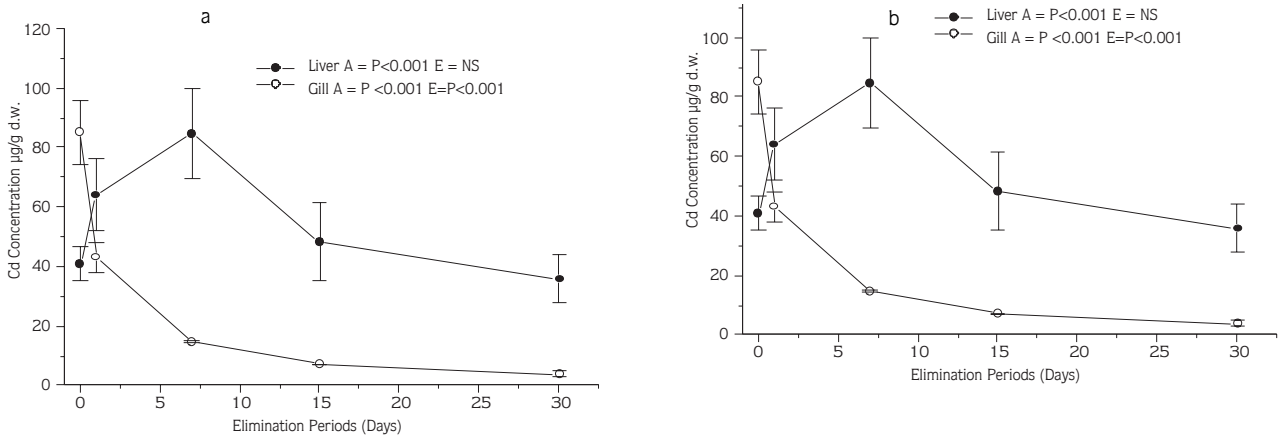

Figure 1. 10-day accumulation (0) and 30-day elimination (1-30) values of cadmium in the liver and gill (a) or in the brain and muscle (b) of *Tilapia zilli*.

elimination. When compared to the 10-day accumulation data, cadmium levels in the gill were found to have decreased 21.5 times (P<0.001) after 30 days of elimination.

The lead concentrations in the tissues of the control fish were below the detection limit of the instrument (<0.05 ppm) throughout the experiments (Figure 2). The tissues of *Tilapia zilli* (Figure 2a and b) also accumulated lead and the tissue metal concentrations increased (P<0.001) many times when compared to the control values (<0.05 μg/g d.w.) of lead. The accumulation of lead after 10 days' exposure to the metal produced tissue lead concentrations in the order: gill > liver > brain > muscle. The elimination of lead occurred in the gills (P<0.05) and liver (P<0.05), beginning from the 7th day of the elimination period, whereas the muscle and brain did not show any significant elimination of lead (P>0.05). When compared to the levels in the 10-day accumulation data, the lead levels in the gills and liver were found to have decreased 3.02 times (P<0.05) and 2.42 times (P<0.05) respectively after 30 days of elimination.

The mean concentrations and standard errors (in parentheses) of copper in the liver, gills, muscle and brain of the control fish during the experiments ranged between 12.2 (2.55) and 15.16 (3.76), 4.17 (2.82) and, 1.51 (0.25) and 2.74 (0.25), and 4.74 (2.84) and 7.58 (1.81) respectively. There were no statistical differences in the copper concentrations in the control tissues between sampling points (Figure 3).

With the exception of muscle tissue, copper was (P<0.01) accumulated by all the tissues studied (Figure 3a and b). The order of copper concentrations in the tissues after 10 days exposure to the metal was liver > gills > brain as the copper concentrations increased 19.9, 4.24 and 3.40 times respectively. Significant eliminations of copper occurred only in the gills (P<0.001) as the copper concentrations in the gills decreased 7.37 times (P<0.05) after 30 days of elimination. Copper elimination from the gills was significant from day 1 (P<0.05) and also at the other sampling times (P<0.001).

Mean concentrations and standard errors (in parentheses) of zinc in the liver, gill, muscle and brain tissues of the control fish during the experiments ranged between 59.80 (3.40) and 73.10 (10.68), 78.97 (11.00) and 100.1 (8.17), 70.01 (5.45) and 85.56 (5.30), and 66.53 (8.63) and 90.42 (16.39) μg/g respectively. There were no statistical differences in the copper concentrations of the control tissues between sampling times (Figure 4).

Zinc accumulation in the tissues was not significant (P>0.05) after 10 days' exposure to the metal (Figures 4a and b). No overall changes in tissue zinc levels were measured over the 30-day cleansing period, although the zinc concentrations in the brain at days 1 and 7 were significantly lower (P<0.05) than the initial value.

Discussion

Metal accumulation in the tissues of fish varies according to the rates of uptake, storage and elimination (4,5). This means that metals which have high uptake and low elimination rates in the tissues of fish are expected to

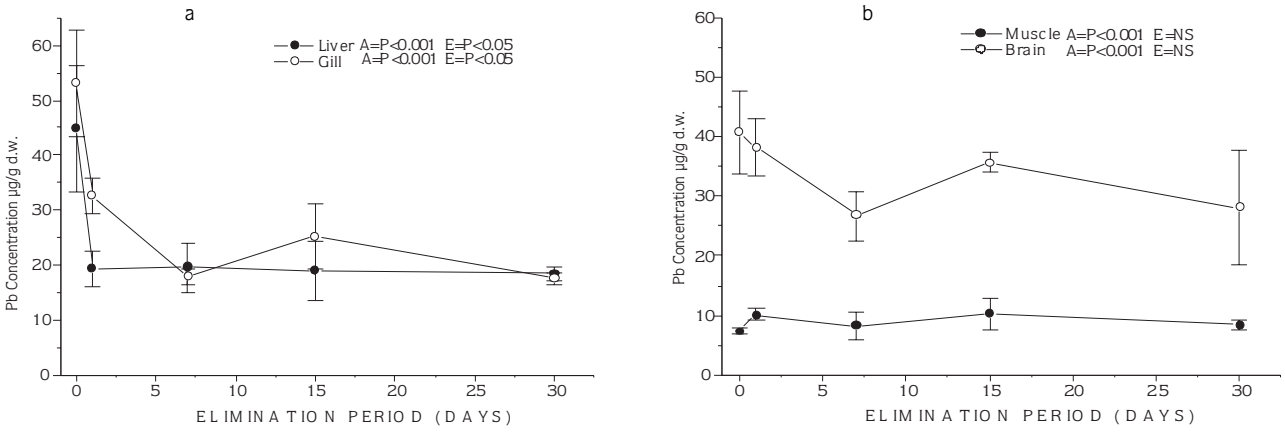

Figure 2. 10-day accumulation (0) and 30-day elimination (1-30) values of lead in the liver and gill (a) or in the brain and muscle (b) of *Tilapia zilli*.

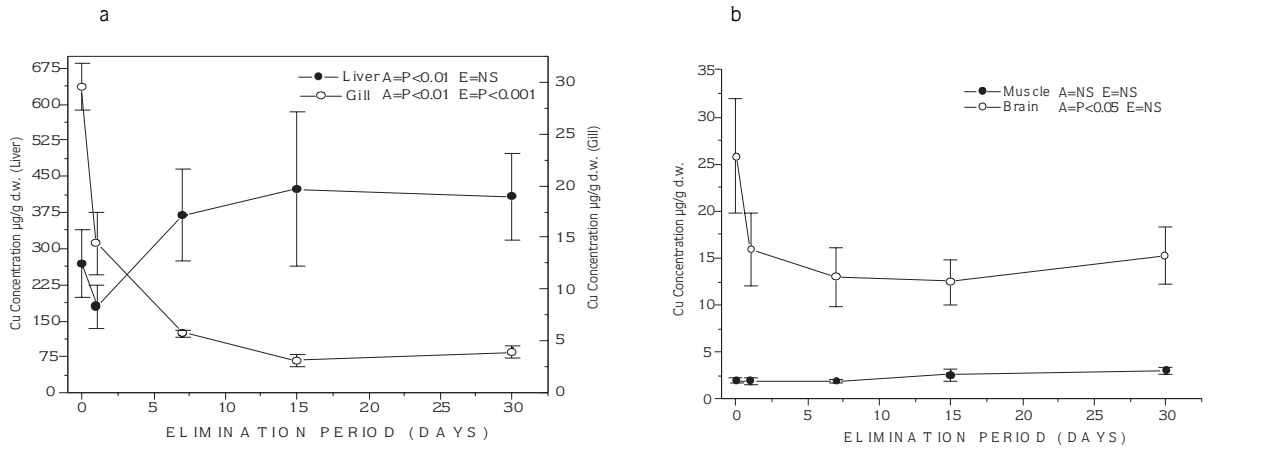

Figure 3. 10-day accumulation (0) and 30-day elimination (1-30) values of copper in the liver and gill (a) or in the brain and muscle (b) of *Tilapia zilli*

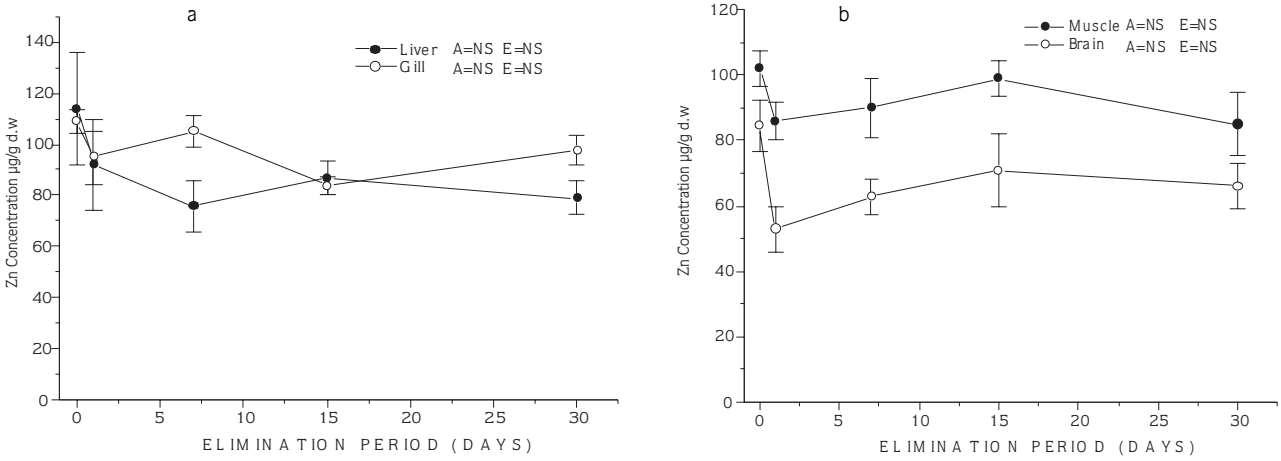

Figure 4. 10-day accumulation (0) and 30-day elimination (1-30) values of zinc in the liver and gill (a) or in the brain and muscle (b) of *Tilapia zilli*.

be accumulated to higher levels. Metal uptake is dependent upon the exposure concentration and period as well as other factors such as salinity, temperature, interaction with other metals, the complexing agent (such as EDTA) and metabolic activity (5,20-24). The accumulation of non-essential metals may occur at very low environmental concentration because fishes are not able to regulate their levels (1,4,5,8). Background levels of cadmium and lead in the tissues of *Tilapia zilli* were also found to be undetectable with the current method, possibly due to the fact that the fish were obtained from uncontaminated culture pools. The tissues of *Tilapia zilli* accumulated cadmium and lead from the medium, and the tissue concentrations rose sharply, although metal accumulation differed considerably among the tissues, the liver and gills showing the greatest accumulation. Similar patterns of cadmium and lead accumulation have also been reported in other studies carried out on fish (24-28). Lead is also known to have a special affinity for the brain as lead accumulation is also high in this tissue (27,28). Similarly, lead also accumulates in or adsorbs on the scales of fish at considerably high levels and the adsorption ratio of lead may be more than some other heavy metals like cadmium (29). Of the essential metals, only copper was accumulated by the tissues, primarily the liver of *Tilapia zilli*, whereas zinc accumulation was not significant.

The accumulation of essential metals is normally smaller than the accumulation of non-essential metals and can be significant only beyond a threshold metal concentration in the water (5). This may be due to a low affinity for essential metals as a result of higher background levels (e.g. zinc) and also shorter half-lives in the tissues as a result of better regulation (4,5,8,26,30). The different accumulation levels of metals in different tissues may be primarily due to different metabolic activities. Tissues like the liver, spleen, kidney and gills are highly active in fish metabolism and therefore may accumulate metals to higher levels than other tissues like the muscle, as has been shown in this study and studies carried out with other fishes (8,19,24,26,28,30). The gill tissue is also exposed to environmental metals to a greater extent than the other tissues and this might cause more accumulation and adsorption of the metals in or on the gill surface.

It has been shown that the induction of low molecular weight metal-binding proteins such as metallothionein is closely related to heavy metal exposure, indicating that metals taken up from the environment can be detoxified by binding on these proteins, though each metal has a different binding capacity (30-35). Therefore, tissues like the liver, which is a major producer of metal-binding proteins, show high concentrations of most heavy metals (19,24,26,28,31,36). The accumulation of lead in the gills is also great, but in the liver, lead accumulation levels are relatively low when compared to other non-essential metals (27,28). There is a lack of evidence on the induction of lead-binding proteins related to metallothioneins in fish tissues (25,33), and this may be the reason for relatively little accumulation of lead in the liver.

Like accumulation, several factors also influence the elimination of metals from the tissues, such as duration, temperature, interaction with other heavy metals and metabolic activity of animals, as well as the tissue concerned (5,8,36-38). The elimination routes of metals from fish are generally bile, urine, elimination from the gills, and mucus (29,39-42). It seems that although there are more elimination routes than uptake routes (5), metal accumulations are greater than metal eliminations, suggesting that once metals have accumulated in tissues, it is difficult to eliminate them from the body, especially the non-essential metals. In the present study, the gills showed the greatest elimination of metals whereas the liver eliminated only lead to a significant degree, and the brain and muscle did not eliminate the metals significantly after 30 days. On the contrary, there were increases in the levels of liver cadmium and copper during elimination. This may be due to the movement of metals from the gills and from the other tissues to the liver for detoxification (5,31,33). In a previous study, it was shown that in cod, (*Gadus morhua*) mercury concentrated from food first moved to the liver and later gradually to the muscle, as the muscle concentration of mercury rose even though the fish were no longer exposed to mercury (38). The results of the present study suggest that metals in the gills are largely from metals adsorbed onto the gill surface and in the circulation system, because most of the metal accumulated was eliminated within one week. This may indicate that metals have a short biological half-life in the gills and a long biological half-life in the liver, possibly due to the fact that they are removed from the gills either back to water (adsorbed metals) or transferred to other tissues, particularly the liver, for detoxification. A well-known detoxification mechanism for heavy metals is that of metal-binding proteins, such as metallothionein, which can be induced by heavy metal exposure (32,33,35).

Studies carried out with aquatic animals have revealed different levels of elimination of heavy metals. Viarengo et al. (40) found that *Mytilus galloprovincialis* exposed to Cu and Cd showed different elimination levels of the metals from its tissues. Cu was rapidly eliminated from the gill and digestive gland cells, showing a biological half-life of 10 days, whereas Cd was released from the tissues much more slowly. The authors reported that metal-binding proteins also followed similar patterns. The levels of elimination of mercury were also found to be very low in the bivalves *Mytilus edulis* and *Macoma balthica*, indicating that the biological half-life in a chronically contaminated area was higher (293 d) than that in a temporarily contaminated area (53 d) (41). Cadmium in the blue tilapia *Oreochromis aureus* was retained 34 days after the cadmium exposure was stopped. The highest concentration was found in the kidney, followed by the liver, and the lowest concentration was in the muscle (36). These results also support the results of the present study.

Conclusion

The results of this study shows that essential and non-essential metals had different accumulation and

elimination patterns in the tissues of *Tilapia zilli*, although the path that copper followed was similar to that of the non-essential metals rather than the path of zinc. When compared to the control values, the levels of accumulation of the non-essential metals were generally higher than those of the essential metals. The tissues accumulated the metals, especially cadmium, copper and lead from the medium and the liver and gills showed the greatest accumulation. However, apart from lead elimination from the liver, the gills were the only tissue that showed significant elimination of cadmium, copper and lead. As the results show, the elimination levels of metals from the gills were also the highest, suggesting that metals in the gills might be a combination of metals adsorbed onto the gill surface and metals in the circulation system. As a result of this, metals accumulated in the gills removed from the first day of the cleansing period to the 30th day, especially metals adsorbed on the gills probably returned to the water, and metals in the circulation system moved to other parts of the body, particularly to the liver for detoxification. Although the level of elimination of metals from the gills seems very high, the overall elimination of metals from the whole body may not actually be so considerable because of the sizes of the muscle and liver. Therefore, the results of this study suggest that once non-essential metals and copper accumulate in the tissues of fish, it is difficult to eliminate them from the body. However, zinc behaved different than the other metals as its tissue levels did not change during either the accumulation elimination experiment, suggesting that the tissues of *Tilapia zilli* can regulate their zinc levels much better than the levels of non-essential metals and also copper.

Acknowledgments

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