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Research Article

Effects of Heavy Metal Accumulation on the 96-h LC_{50} Values in Tench *Tinca tinca* L., 1758

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Abstract: The effects of already accumulated heavy metals (Hg, Cd, Pb) in the body of tench on the 96-h LC_{50} values of the respective heavy metals were studied. The body concentration of mercury, cadmium and lead was 0.011, 0.32 and 1.59 mg/g respectively, and their 96-h LC_{50} values were 1.0, 6.5 and 300.0, ppm, respectively. The general accumulation order of heavy metals and their respective 96-h LC_{50} values were Hg< Cd< Pb. It was observed that the fish with lowest body concentration of heavy metal showed the lowest 96-h LC_{50} value of the respective heavy metal and vice versa. This close relationship showed that the previous body accumulation of heavy metals has a direct effect on the 96-h LC_{50} values of the respective heavy metals and the susceptibility of fish.

Key Words: Mercury, cadmium, lead, 96-h LC_{50} values, heavy metals, accumulation, *Tinca tinca*.

Kadife Balığı (*Tinca tinca* L., 1758)`nda Biriken Ağır Metallerin 96-saat LC_{50} Değerleri Üzerine Etkileri

Özet: Kadife balığında önceden birikmiş olan ağır metallerin (Hg, Cd, Pb), yine bu ağır metaller için incelenen 96 saatlik LC_{50} değerleri üzerindeki etkileri araştırılmıştır. Balığın vücudundaki Hg, Cd, ve Pb konsantrasyonları sırasıyla 0,011, 0,32 ve 1.59 mg/g ve 96-saatlik LC_{50} değerleri ise 1,0, 6,5, 300 ppm şeklinde bulunmuştur. Bu ağır metallerin genel birikim sırası ve ilgili 96-saatlik LC_{50} degerleri Hg< Cd< Pb olarak bulunmuştur. Vücudunda en az ağır metal birikimi gösteren balıklar için 96-saatlik LC_{50} değerlerinin de düşük olduğu gözlenmiştir. Bu yakın ilişki, balığın vücudunda önceden birikmiş olan ağır metallerin ilgili 96-saatlik LC_{50} değerleri üzerinde direkt bir etkisi olduğunu göstermektedir.

Anahtar Sözcükler: Civa, kadmiyum, kurşun, 96-saat LC_{50} degerleri, ağır metal, birikim, *Tinca tinca*.

Introduction

Heavy metals have long been recognized as serious pollutants of the aquatic environment. They cause serious impairment in metabolic, physiological and structural systems when present in high concentrations in the milieu (1). Heavy metals may affect organisms directly by accumulating in their body or indirectly by transferring to the next trophic level of the food chain. One of the most serious results of their persistence is biological amplification through the food chain (2). In the aquatic environment, heavy metals in dissolved form are easily taken up by aquatic organisms where they are strongly bound with sulfhydril groups of proteins and accumulate in their tissues (3,4). The accumulation of heavy metals in the tissues of organisms can result in chronic illness and cause potential damage to the population (5,6).

The 96-h LC_{50} tests are conducted to measure the susceptibility and survival potential of organisms to particular toxic substances such as heavy metals. Higher LC_{50} values are less toxic because greater concentrations are required to produce 50% mortality in organisms (7). The heavy metals that are toxic to many organisms at very low concentrations and are never beneficial to living beings are mercury, cadmium and lead (8).

Fish absorb dissolved or available metals and can therefore serve as a reliable indication of metal pollution in an aquatic ecosystem (9). Tench (*Tinca tinca*) is considered a good test organism for heavy metal contamination because of its feeding behavior and bottom feeding habits (10). The present study was carried out to observe whether heavy metals already accumulated in the body of tench affect the 96-h LC_{50} values of respective heavy metals.

Materials and Methods

Fish (*Tinca tinca* L., 1758) were collected from different sites at Lake Mogan (Figure 1) near the city of Ankara (Turkey) with cast nets and transferred to the experimental laboratory of the Department of Biology, Ankara University, Ankara. The fish were stocked in 150-l capacity aquaria for 2 weeks to acclimatize to laboratory conditions. The water temperature, dissolved oxygen, pH and electric conductivity were measured regularly in the laboratory; however, the other physico-chemical parameters were measured at the DSI, TAKK, Chemical Laboratories, Ankara (Table 1). The fish were not fed during the experiment.

To investigate the effects of heavy metals already accumulated in the body of tench on the 96-h LC_{50} values of the respective heavy metals, the experiments were designed accordingly. For determination of the 96-h LC_{50} (lethal concentration) values, 6 concentrations each of cadmium and lead and 5 concentrations of mercury were checked. A group of 8 fish was used for each concentration of each heavy metal. Separate groups of 8 fish each served as controls for mercury, cadmium and lead. All experiments were run for 96 h and the concentration of each heavy metal that caused 50% mortality in fish was named the LC_{50} value of the respective metal. The mortality was calculated as a percentage once every 24 h. The LC_{50} values for 96 h were 1.0 ppm for mercury, 6.5 ppm for cadmium and 300.0 ppm for lead. The LC_{50} values were found (11,12) and the true concentration of each heavy metal was obtained (13).

Heavy metal salt / Heavy metal = Molecular weight / Atomic weight = 1 g metal + 1 l distilled water = 1000 ppm stock solution.

To determine the concentration of heavy metals in various tissues, the fish were stunned by a blow to the head soon after being transferred to the laboratory where their gills, liver, muscle, testes and ovaries were removed. Each tissue was put in a separate petri dish and dried in an oven (Sterilization Thermostat, FN 400, Nuve company) at 110 °C for 48 h. Some 0.5 g of each dry tissue was weighed (Scaltech SBI 41 Balance) and put in a separate test tube. Then 3 ml of nitric acid (HNO_3) and perchloric acid ($HCOl_4$) in a 2:1 ratio were added to each tissue and digested on a hotplate (Nuve HP 121) at 100 °C for 5 h. After complete digestion, 5 ml of distilled water was added to each sample and the metal concentration in mg/g was studied on an atomic absorption spectrophotometer (Hitachi Z 8200 Polarized Zeeman AAS) (14,15). The concentration of each heavy metal in all tissues was taken as the average body concentration of each metal and compared with respective 96-h LC_{50} values. The relationship between the body concentration of heavy metals and 96-h LC_{50} values was calculated as the percentage difference.

Results

The general accumulation order of the heavy metals was Hg< Cd< Pb. The tissue-wise accumulation of each heavy metal varied: for Hg testes> ovaries> liver> muscles> gills, for Cd testes> gills> muscles> ovaries> liver and for Pb liver> gills> muscles> ovaries> testes. However, a comparison with the 96-h LC_{50} values was made on the basis of the average body concentration of each heavy metal. The average body concentration of mercury, cadmium and lead was 0.011, 0.32 and 1.59 mg/g, respectively.

The 96-h LC_{50} value of each heavy metal was found using concentrations of 5 for Hg (0.5, 0.75, 1.0, 1.1 and 1.25 ppm), 6 for Cd (5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 ppm) and 6 for Pb (200.0, 250.0, 275.0, 300.0, 325.0 and 350.0 ppm) (Figure 2). The 96-h LC_{50} for Hg, Cd and Pb was 1.0, 6.5 and 300.0 ppm, respectively. The mortality rate as a percentage for each concentration of each heavy metal is given in Figure 2.

The comparison of the body concentration of each heavy metal with its respective 96-h LC_{50} value showed that mercury with the lowest body concentration (0.011 mg/g) resulted in the lowest 96-h LC_{50} value (1.0 ppm) and lead with the highest body concentration (1.59 mg/g) resulted in the highest 96-h LC_{50} value (300.0 ppm). Cadmium stood in the middle with a body concentration of 0.32 mg/g and a respective 96-h LC_{50} value of 6.5 ppm. The relationship between the body concentration of

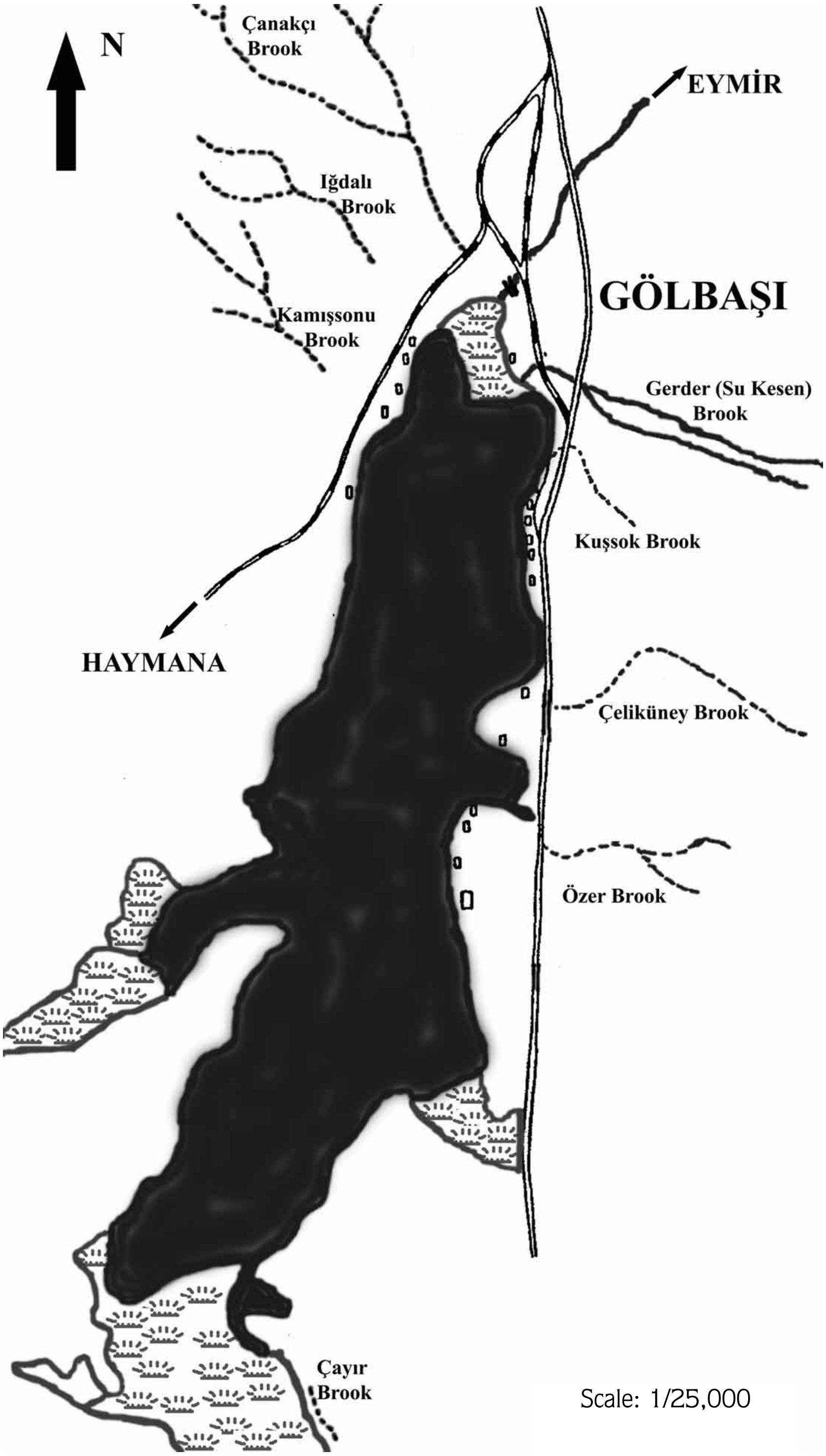

Figure 1. Map of Lake Mogan

Table 1. The physio-chemical parameters of the laboratory water.

Parameter	Values
Temperature	20.67 ± 0.49 °C
Dissolved oxygen	7.68 ± 0.13 mg/l
pH	7.49 ± 0.9
Photoperiodicity	12 L: 12 D
Electric conductivity	0.29 ± 0.02 mS/cm
Bicarbonates	97.6 mg/l
Total alkalinity ($CaCO_3$)	80.0 mg/l
Chlorine	10.3 mg/l
Sulfates	26.1 mg/l
Calcium	29.0 mg/l
Magnesium	1.2 mg/l
Total hardness ($CaCO_3$)	77.5 mg/l
Turbidity	2
Mercury	<0.005 mg/l
Cadmium	<0.005 mg/l
Lead	<0.005 mg/l

each heavy metal and its respective 96-h LC_{50} values in terms of percentage difference was 95.07% for Cd, 98.90% for Hg and 99.47% for Pb, i.e. Pb> Hg> Cd (Table 2).

In general, the relationship between the body concentration of each heavy metal and its respective 96-h LC_{50} value was close and sequenced; however, the relationship in terms of the percentage difference of each heavy metal differed. It was lower in Cd (95.07%) and higher in Pb (99.47%).

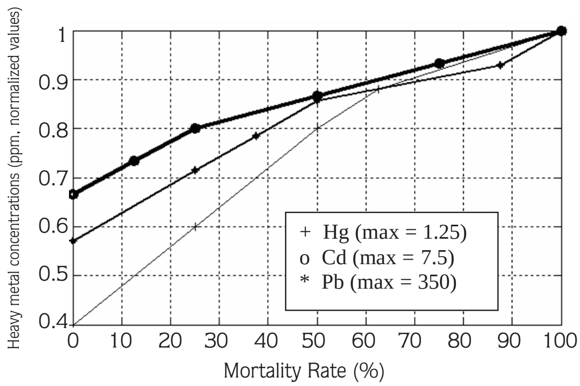

Figure 2. Lethal concentrations of heavy metals, mortality rate and 96-h LC_{50} values of *Tinca tinca*.

Discussion

It is evident from the results that the previous body accumulation of heavy metals has a direct effect on the LC_{50} values of the respective metals in tench. The fish with a lower body concentration of a heavy metal had a lower 96-h LC_{50} value of the respective metal and vice versa.

The concentration of heavy metals in fish is related to several factors, such as the food habits and foraging behavior of the organism (16), trophic status, source of a particular metal, distance of the organism from the contamination source and the presence of other ions in the milieu (17), bio-magnification and/or bio-diminishing of a particular metal (6), food availability (18), metallothioneins and other metal detoxifying proteins in

Table 2. Percentage differences between the body concentrations of heavy metals and their respective 96-h LC_{50} values.

Fish tissues	Heavy metal concentration (mg/g)		
	Hg	Cd	Pb
Gills	0.006 ± 0.003	0.38 ± 0.09	1.86 ± 0.56
Muscle	0.007 ± 0.002	0.33 ± 0.08	1.47 ± 0.06
Liver	0.008 ± 0.003	0.23 ± 0.07	2.55 ± 0.57
Testes	0.017 ± 0.0004	0.42 ± 0.03	0.78 ± 0.04
Ovaries	0.015 ± 0.001	0.24 ± 0.009	1.33 ± 0.07
Average Body Concentration (mg/g)	011 ± 0.002	0.32 ± 0.06	1.59 ± 0.26
96-h LC_{50} values (ppm)	1.0	6.5	300.0
% difference	98.90	95.07	99.47

the body of the animal (19), temperature, transport of metal across the membrane and the metabolic rate of the animal (20), physical and chemical properties of the water (21) and the seasonal changes in the taxonomic composition of different trophic levels affecting the concentration and accumulation of heavy metal in the body of fish (16).

Similarly, the 96-h LC_{50} values of fish vary from species to species and from metal to metal. Gill and Pant (22), Kirubagaran and Joy (23), Veena et al. (12) and Iliopoulou-Georgudaki and Kotsanis (24) reported 96-h LC_{50} values of 0.181, 0.51, 0.13 and 0.51 ppm Hg, for *Barbus conchonus, Clarius batrachus, Etrophus maculatus* and *Salmo gairdneri*, respectively. Spehar (25) found 96-h LC_{50} values of 2.5 and 28.0 ppm Cd for *Jordanella floridae* and *Mugil cephalus*, respectively. Johansson-Sjobeck and Larsson (26) and Gill and Pant (27) found 96-h LC_{50} values of 20.0 and 12.65 ppm Cd for *Puntius conchonius* and *Pleuronectes flesus* respectively. Das and Banerjee (11) reported 175.0 and 300.0 ppm Cd for *Heteropneustes fossilis* and *Lebio rohita*, respectively. However, Smet and Blust (28) observed 100% mortality in *Cyprinus carpio* after 21-29 days of exposure to 20 mm of Cd. Holcombe et al. (5) found 4.1 and 3.36 ppm total and dissolved Pb, respectively, for the 96-h LC_{50} values of *Salvalinus fontinalis*, and Srivastava and Mishra (29) recorded 19 ppm Pb for the 96-h LC_{50} of *Colisa fasciatus*. However, Hodson et al. (30) found 2.4 ppm Pb for the 21-day LC_{50} of *Salmo gairdneri*.

The susceptibility of fish to a particular heavy metal is a very important factor for LC_{50} values. The fish that is highly susceptible to the toxicity of one metal may be less or non-susceptible to the toxicity of another metal at the same concentration of that metal in the milieu. Similarly, the metal which is highly toxic to one organism at low concentration may be less or non-toxic to other organism at the same or even higher concentration. Das and Banerjee (11) reported 300.0 ppm Cd for the 96-h LC_{50} of *Heteropneustes fossilis*, whereas in the present study the 96-h LC_{50} for *Tinca tinca* was 6.5 ppm Cd. However, the same concentration of LC_{50} (300.0 ppm) was recorded for Pb. It shows that *H. fossilis* and *T. tinca* have same degree of susceptibility to different heavy metals, or 2 different heavy metals have different levels of toxic effects on 2 different species.

Because of the lack of available data on the effects of body concentrations of heavy metals on the respective LC_{50} values, the results of the present study have not been compared with those of other studies and discussed accordingly. However, some justifications have been provided following various studies. The degree of susceptibility of *T. tinca* to lower concentrations of Hg and Cd and the higher concentration of Pb may be attributed to the altered physiological response of fish to the specific metal and the level of solubility of metals. The fish exposed to metal can compensate for the stressors. If it cannot successfully compensate for stressor effects, an altered physiological stage may be reached in which the organism continues to function and, in extreme cases, the acclimation response may be exhausted with a subsequent effect on fitness (31). In the present study, it is possible that the fish compensated for the higher level of lead, but this process may have been exhausted very early on exposure to mercury and cadmium. It is also possible that lead as compared to mercury and cadmium is less soluble in water and because of non-solubility a higher concentration may be required to cause effects resulting in higher LC_{50} values. Dense insoluble clusters of lead were seen at the bottom of the experimental aquaria in the present study.

Although the LC_{50} values vary from species to species and the accumulation of heavy metals in the body of fish depends upon several factors, it is evident from the present study that previous body concentrations of heavy metals affect the LC_{50} values of fish. It may be due to the increased resistance of fish to the heavy metal through acclimatization. During acclimatization, some proteins, such as metallothioneins (19), are released in the body of the organism and detoxify the metal ions. This may cause higher concentrations of heavy metals being required to cause effects, resulting in higher LC_{50} values. In conclusion, for the determination of LC_{50} values, the previous accumulations of heavy metals in the body of animal must not be ignored and for accurate LC_{50} values the experimental fish must be devoid of previous heavy metal body load. Furthermore, the present study shows the adaptation capability of tench to heavy metal load. The previous body accumulations of heavy metals may enhance the resistance of the animal to added heavy metal load in the environment.

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