Bull. Environ. Contam. Toxicol. (2006) 77:551–558

DOI: 10.1007/s00128-006-1099-3

Bulletin of Environmental Contamination and Toxicology

Toxic Effects of Lead on the Liver and Gills of *Oncorhynchus mykiss* WALBAUM 1792

M. Suiçmez,[1] M. Kayım,[2] D. Köseoğlu,[3] E. Hasdemir[4]

[1] Department of Biology, Faculty of Art and Science, University of Gaziosmanpaşa, Tokat, 60240, Turkey
[2] Fisheries Department, College of Almus, University of Gaziosmanpaşa, Tokat, 60240, Turkey
[3] Department of Pathology, Faculty of Medicine, University of Gaziosmanpaşa, Tokat, 60240, Turkey
[4] Department of Chemistry, Faculty of Art and Science, University of Gazi, Ankara, 06500, Turkey

Received: 3 April 2005/Accepted: 14 August 2006

Intense activity in industrial and agricultural sectors has inevitably increased the levels of heavy metals in natural waters (Nimmo et al. 1998; Gümgüm et al. 1994; Akif et al. 2002; Jordao et al. 2002). Heavy metals may exert either beneficial or harmful effects on plant, animal and human life depending on their concentration. They find their way into aquatic systems like rivers, lakes, or oceans through atmospheric fallout, dumping wastes, accidental leaks, run-off from terrestrial systems and by geological weathering. Extensive use of heavy metals, which indirectly affects the aquatic organism, leads to accumulation of toxicants on land and water bodies, (Karuppasamy 1999; Reinschuessel et al. 1989). For instance the discharge of potentially toxic heavy metals into the marine environment has become a global problem. In addition, food is a constant source of toxic heavy metals that accumulate in different parts of the human body and cause damage in many of its basic systems (renal, cardiovascular, gastrointestinal, endocrine, nervous, etc.) (Reilly 1991; WHO 1987). Non-essential metals (e.g. Pb, Cd, Hg) are held to be the most dangerous, since continuous exposure of marine organisms even to low concentrations may result in bioaccumulation and subsequent transfer to man through the food web (Förstner et al. 1983). The concentrations of heavy metals in the organs of fish are determined primarily by the pollution level of water and food and, thus, are indicators of the environmental pollution level. The concentrations themselves are the result of uptake and release processes with characteristic kinetics for elements and their biological half time, influenced by the age (size) of individuals, the feeding habits of the species and also the season. Moreover, various tissues accumulate heavy metals to different degrees, depending on their biochemical characteristics (Farkas et al. 2000; Olsson 1998). In addition to inhibiting the impulse conductivity by inhibiting the activities of monamine oxidase and acetylcholine esterase (Katti and Sathyanesan 1986), lead was found to impair the embryonic and larval development of fish species (Dave and Xiu 1991) and cause pathological changes in tissue and organs (Rubio et al. 1991).

This investigation aims at demonstrating the pathological effects of lead in certain concentrations and the accumulation of the metal in structurally different and metabolically active tissues like gills and liver in *Oncorhynchus mykiss* for 1, 2 and 3 day periods.

Correspondence to: M. Suiçmez

MATERIALS AND METHODS

Specimens (18-22 cm length; 120-150 g weight, 12 month old) of *Oncorhynchus mykiss* (Total animal, 90) were acclimatized and maintained in water tanks of 250 liter. We used $Pb(CH_3COO)_2$ (Merck) for the exposure. Fishes that were exposed to various heavy metal concentrations {0.1 mg/l (n=10), 1.0 mg/l (n=10) and 10.0 mg/l (n=10)} for 24, 48 and 72 hours, were not fed for 12 hours before treatment. Physicochemical conditions of water during the experimental period were as follows: temperature was 11.0±0.5 °C; pH was 7.56±0.06; DO was 8.20±0.72µg/g and $CaCO_3$ was 167.5±5.18 µg/g.

After the dissection of fish, liver and gills were rapidly removed, fixed in 10% neutral buffered formaldehyde, embedded in wax, 4-6µm sections were deparaffinized and stained with Haematoxylin&Eosin and viewed under binocular microscope.

A Perkin Elmer Analyst 700 AAS (Atomic Absorbtion Spectrophotometre) with deuterium background corrector was used in this study. Detection limit value of Pb in graphite furnace AAS was found to be 0.21µg/l. We used DORM-2 as certified reference material for fish (Table 1). Lead was determined by HGA graphite furnace using argon as inert gas. Pyrolytic-coated graphite tubes with a platform were used and signals were measured as peak area for lead. Operating conditions and instrumental parameters were set as recommended by the manufacturer. Milestone Ethos D microwave closed system was used for sample digestion. All reagents were analytical grade. Deionized water (Milli-Q Millipore 18.2 M Ω cm^{-1} resistivity) was used to prepare all aqueous solutions. All mineral acids and oxidants (HNO_3 and H_2O_2) were in highest quality grade (Suprapure, Merck). The stock solutions of metals (1000µg. g^{-1}) were obtained by dissolving the appropriate salts or the corresponding metals (E. Merck) and further diluted prior to use. Aldrich Matric Modifiers like $NH_4H_2PO_4$ used were purchased from Sigma High Purity. 0.5 g of sample was dissolved with 4 mL HNO_3 (65%) and 2 mL H_2O_2 (30%) in microwave digestion system. The conditions for microwave system were 2 min for 250 W followed by 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, vent: 8 min, respectively and dilued with 10 ml deionized water. A blank digestive was carried in the same way. This procedure was preferred because it is more accurate with respect to both time and recovers values. The recover values were nearly quantitative (> 95%) for the above digestion method.

Data obtained were analysed by using SPSSWIN (Spsswin 1994). When the F-test indicated significant ($P<0.01$) differences between treatments, means were separated by using Ducan's Multiple Range Test (Duncan 1955).

RESULTS AND DISCUSSION

In our study, different tissues generally showed different capacities for accumulating heavy metals. In Table 2, the Pb value of Liver in control group is different from the others, statistically ($P<0.01$). In Table 3, the control group is

Table 1. Results of analysis (μg/g) of standard certified material (Dogfish).

Element	Meterial	Certified level	Result
Pb	DORM-2	0.065 ± 0.007	0.061

Table 2. Accumulation of lead in fish tissues for 0.1mgPb/l.

Tissues	Control X ± sx	First day X ± sx	Second day X ± sx	Third day X ± sx
mg Pb/g Muscle	ND [d]	ND [d]	ND [d]	ND [d]
mg Pb/g Gill	0.00021 ± 0.000015 [a]	0.00024 ± 0.000020 [a]	0.00026 ± 0.000023 [a]	0.00028 ± 0.000025 [a]
mg Pb/g Kidney	0.00018 ± 0.000012 [b]	0.00019 ± 0.000013 [b]	0.00023 ± 0.000020 [b]	0.00026 ± 0.000023 [b]
mg Pb/g Liver	0.00017 ± 0.000012 [c]	0.00018 ± 0.000012 [b]	0.00022 ± 0.000020 [b]	0.00025 ± 0.000023 [b]
The final water level of Pb (mg/l)		0.0998	0.0997	0.0995

a,b,c,d: Mean values followed with different letters in the rows are significantly different P<0.01, X ± sx: Mean ± standard error, ND: Not detectable.

Table 3. Accumulation of lead in fish tissues for 1.0 mgPb/l.

Tissues	Control	First day	Second day	Third day
mg Pb/g Muscle	ND [e]	0.00025 ± 0.000023 [c]	0.00033 ± 0.000030 [c]	0.00039 ± 0.000035 [d]
mg Pb/g Gill	0.00021 ± 0.000015 [a]	0.00048 ± 0.000037 [a]	0.00056 ± 0.000052 [a]	0.00080 ± 0.000073 [a]
mg Pb/g Kidney	0.00018 ± 0.000012 [b]	0.00043 ± 0.000035 [b]	0.00054 ± 0.000052 [a]	0.00062 ± 0.000060 [b]
mg Pb/g Liver	0.00017 ± 0.000012 [c]	0.00042 ± 0.000040 [b]	0.00052 ± 0.000050 [b]	0.00059 ± 0.000060 [c]
The final water level of Pb (mg/l)		0.9987	0.9982	0.9976

a,b,c,d,e: Mean values followed with different letters in the rows are significantly different P<0.01, X ± sx: Mean ± standard error, ND: Not detectable.

Table 4. Accumulation of lead in fish tissues for 10.0mgPb/l.

Tissues	Control	First day	Second day	Third day
mg Pb/g Muscle	ND [d]	0.00135 ± 0.00012 [c]	0.00167 ± 0.00014 [c]	0.00194 ± 0.00014 [c]
mg Pb/g Gill	0.00021 ± 0.000015 [a]	0.00350 ± 0.00032 [a]	0.00675 ± 0.00062 [a]	0.00863 ± 0.00073 [a]
mg Pb/g Kidney	0.00018 ± 0.000012 [b]	0.00215 ± 0.00018 [b]	0.00520 ± 0.00053 [b]	0.00737 ± 0.00068 [b]
mg Pb/g Liver	0.00017 ± 0.000012 [c]	0.00213 ± 0.00019 [b]	0.00512 ± 0.00043 [b]	0.00730 ± 0.00069 [b]
The final water level of Pb (mg/l)		9.9879	9.9740	9.9671

a,b,c,d: Mean values followed with different letters in the rows are significantly different P<0.01, X ± sx: Mean ± standard error, ND: Not detectable.

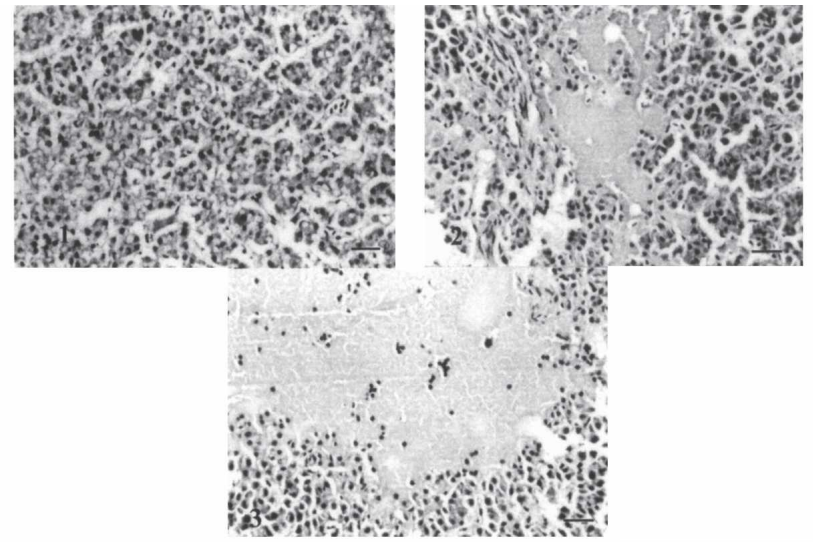

Figure 1–3. Liver, 1) Exposed to Pb-Vacuolation in the first day of 10,0 mg/l. Bar: 500μm (H&E).2) Exposed to Pb-Tubular degeneration and necrosis in the second day of 10.0 mg/l. Bar: 500μm (H&E). 3) Exposed to Pb-Necrosis in the third day of 10.0 mg/l. Bar: 500μm (H&E).

similar to the third day and the first day is similar to the second day in muscle and liver, statistically ($P<0.01$). In Table 4, there is statistical significant difference in value of Pb accumulation of muscle and liver with regard to control group. In muscle tissues (control and 0.1 mg/l groups-all days) however, they were below the limits of detection (Table 2). The highest concentration of lead (Pb) was detected in gills (Table 2-4). Since gills are the main sites for the uptake of oxygen, accumulation of metals through water filtering in gill tissues, which is in direct contact with medium, could be one explanation to this phenomenon. Metal accumulation was also effected by the metal concentrations and exposure time.

Histological analysis demonstrated degeneration of hepatic tissue that appeared as vacuolation (Fig. 1), tubular degeneration and necrosis (Fig. 2). The vacuolation appeared in the second day of 1.0 mg/l and in the first and the second day of 10.0 mg/l metal treatment. Vacuolation and necrotic area were also seen in second day of 10.0 mg/l. These necrotic areas increased more in the third day of 10.0 mg/l (Fig. 3).

Histopathological lesions of *O. mykiss* gill under lead (Pb) exposure showed dilation of blood vessels (Fig. 4), ruptured epithelial cells (Fig. 5), fusion (Fig. 6) and shortening of secondary lamellae (Fig. 7). The dilation of blood vessels and hypertrophy of epithelial cells appeared in the first day (10.0 mg/l dose group) and in the second day (1.0 mg/l dose group). The thickness of secondary lamellae had increased due to widespread hypertrophy of epithelial cells. Thus, fusions were also observed between secondary lamellae. The damage after Pb exposure would lead to a reduction in effective surface area, and an increase in diffusion distance

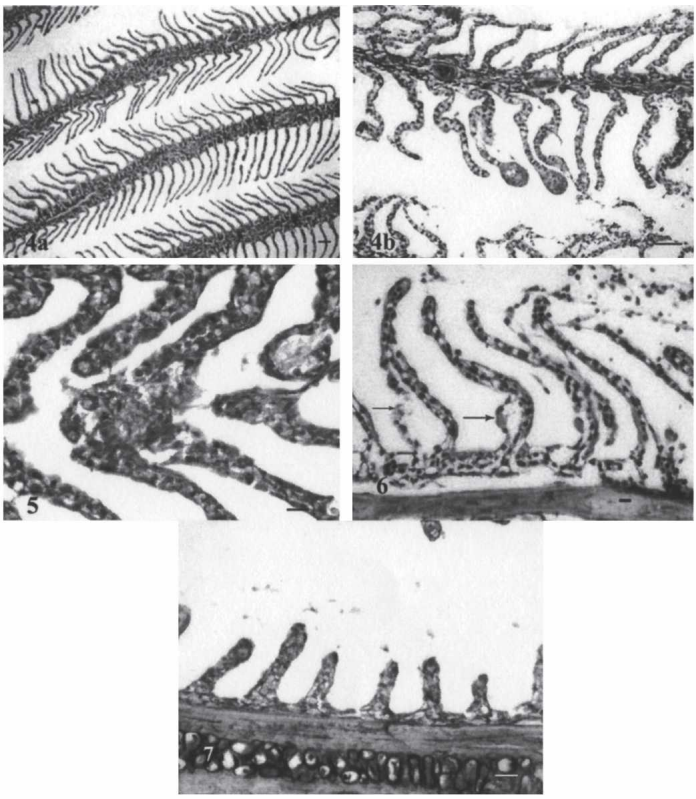

Figure 4–7. Gill, 4a) Controle Bar: 600µm, 4b)Exposed to Pb-Dilatation of blood vessel in the first day of 10.0 mg/l. Bar: 500µm (H&E). 5) Exposed to Pb-Fusion in the second day of 10,0 mg/l. Bar: Bar: 100µm (H&E). 6) Exposed to Pb-Epithelial rupture in the third day of 1,0 mg/l. Bar: 100µm (H&E). 7) Exposed to Pb-Shortening of Secondary lamellae in the third day of 10,0 mg/l. Bar: 100µm (H&E).

for respiratory exchange. Secondary lamellae were observed to shorten in high Pb concentrations (in second and third day of 10 mg/l dose groups). These shortenings reduce respiratory surface of gills and this causes animals to die.

The highest metal concentrations were found in the liver and gill (liver>gill>muscle for Cu, Fe, Mn and Zn in mullet, gill>liver>muscle for Ni, Mn and Zn in catfish) (Karadede et al. 2004). The following relationship was found regarding the Pb accumulations among tissues and organs: kidney>gill>liver>spleen>muscle (Cicik et al. 2004). Median lead levels ranged from 0.002 to 0.027 µg/g in the muscle, 0.012 to 0.093 µg/g in the gills and 0.026 to 0.048 µg/g in the liver (Mormede and Davies 2001). The highest Pb accumulation in tissues of *O. mykiss* that exposed to different concentrations of Pb was found in gills (gill>kidney > liver > muscle) as shown Table 1-3. Similar results have been reported in *Capoeta capoeta umbla* (Canpolat and Çalta 2003). Tulasi et al. (1992), also reported the more Pb accumulation in gills than in liver. Another investigation with *Tilapia zilli* has demonstrated that gill could

accumulate more Pb than liver (Karataş and Kalay 2002). Compared with liver, the highest level of Pb accumulation was found in spleen and gills in *Gillichthys mirabilis*, whereas the lowest accumulation was in muscle tissue of this species (Somero et al. 1977). The accumulation of Pb in muscle tissue of *O. mykiss*, however, reached significant levels only after 1.0 mg/l dose.

Histological analysis demonstrated an evident degeneration of hepatic tissue that appeared as hypertrophy and irregularity of hepatic cells with a nuclear pycnosis (Campana et al. 2003). Livercord disarray, necrosis, pycnotic nuclei in hepatic cell, accumulated cytoplasmic granules and empty blood vessels were found in the Liver of Phenyl Mercuric Acetate (PMA) treated *Channa punctatus* (Karuppasamy 2000). In our study, however, the highest degeneration in the liver was seen in the third day of 10 mg/l in *O. mykiss*. Rubio et al. (1991) reported that lead causes to pathological changes in tissue and organs of *Procambarus clarkii.* We also observed that the similar changes in liver and gills of *O. mykiss.* In adition, it was determined that lead accumulation increased in gill, kidney, liver and muscle with long-term. Degeneration was generally in the form of vacuolation, tubular degeneration and necrosis. Toxicants induced in different amounts in the liver of fish can be recorded as an index of the identification of pollutional stress in fish.

Gills exposed to lead showed widespread damages. These included epithelial lifting with the formation of subepithelial spaces, hypertrophy and necrosis of epithelial cells, lamellar rupture and fusion; symptoms consistent with exposure to gill irritants (Mallatt 1985). Dalzell and Macfarlane (1999) reported that after 14 days of exposure to commercial iron sulphate, the thickness of secondary lamellae had increased due to widespread epithelial cell hypertrophy. The epithelium covering the secondary lamellae lifts away in a continuous sheet from the pillar cell system thus increases the diffusion distance from water to blood (Skidmore and Tovell 1972). Karuppasamy (2000) reported that histopathological lesions of gill under PMA exposure of *C. punctatus* shows vacuolation and rupture epithelial cell, degeneration of pillar and chloride cells, dilation of blood vessel, fusion and shortening of secondary lamellae. Besides the dilation of blood vessels and fusion, we have mostly observed rupture of epithelial cells and shortening of secondary lamellae in our study. These degenerations increase with the time of exposure and metal concentration. Shortening of secondary lamellae were the last stage of deformations that result in the death of fish. This experimental study clearly demostration one fact. Even in very low concentrations, heavy metals, which cause environmental pollution, accumulate in different tissues (especially in gills, kidney and liver) of living fish in the long time.

Acknowledgments. We are indebted to the department of biology (Gaziosmanpaşa Universty) for financial support of this work (Grant Nr. 2003/24 Gaziosmanpaşa University Science Research Found). Furthermore, we thank to Osman Öncü for checking the English.

REFERENCES

Akif M, Khan AR, Sok K, Min KS, Hassan Z, Maal-Abrar M (2002) Textile effluents and their contribution towards aquatic pollution in yhe Kabul River (Pakistan). J Chem Soc Pakistan 24:106-111

Campana O, Sarasquete C, Blasco J (2003) Effect of lead on ALA-D activity, metallothionein levels, and lipid peroxidation in blood, kidney, and liver of the toadfish *Halobatrachus didactylus*. Ecotoxicol Environ Safety 55:116-125

Canpolat Ö, Çalta M (2003) Heavy metals in some tissues and organs of *Capoeta capoeta umbla* (HECKEL, 1843) fish species in relation to body size, age, sex and seasons. Fresenius Environ Bull 12:961-966

Cicik B, Ay Ö, Karayakar F (2004) Effects of lead and cadmium interaction on the metal accumulation in tissue and organs of the Nile Tilapia (*Oreochromis niloticus*).Bull Environ Contam Toxicol 72:141-148

Dalzell DJB, Macfarlane AA (1999) The toxity of iron to Brown trout and effects on the gills: a comparison of two grades of iron sulphate. J Fish Biol 55:301-315

Dave G, Xiu R (1991) Toxicity of mercury, copper, nickel, lead and cobalt to embryos and larvae of Zebrafish *Brachydanio rerio*. Arch Environ Contam Toxicol 21:126-134

Duncan DB (1955) Multiple range test and multiple F tests. Biometrics 11:1-42

Farkas A, Salanki J, Varanka I (2000) Heavy metal concentrations in fish of Lake Balaton. Lake and Reservoirs. Res Manage 5:271-279

Förstner U, Wittmann GTW (1983) Metal pollution in the aquatic environment 2nd ed.Berlin: Springer. p:486

Gümgüm B, Ünlü E, Tez Z, Gülsün Z (1994) Heavy metal pollution in water, sediment and fish from the Tigris River in Turkey. Chemosphere 29:111-116

Jordao CP, Pereira MG, Bellato CR, Pereira JL, Matos AT (2002) Assesment of water systems for contaminants from domestic and industrial sewages. Environ Monit Assess79:75-100

Karadede H, Oymak SA, Ünlü E (2004) Heavy metals in mullet, *Liza abu*, and catfish, *Silurus triostegus*, from the Atatürk Dam Lake (Euphrates), Turkey. Environ Int 30:183-188

Karataş S, Kalay M (2002) Accumulation of lead in the gill, liver, kidney and brain tissues of *Tilapia zilli*. Turkish J Vet Anim Sci 26:471-477

Karuppasamy R (2000) Tissue histopathology of *Channa punctatus* (Block) under phenyl mercuric acetate toxicity. Bull of Pure Applied Sci 19A:109-116

Katti SR, Sathyanesan AG (1986) Lead nitrate induced changes in the brain constituents of the freshwater fish *Clarias batrachus*. Neurotoxicology 3:47-52

Mallatt J (1985) Fish gill structural changes induced by toxicants and other irritants: A statistical Review. Canadian J Fish Aquat Sci 42:630-648

Mormede S, Davies IM (2001) Trace elements in deep-water fish species from the Rockall Trough. Fish Res 15:197-206

Nimmo DR, Willox MJ, Lafrancois TD, Chapman PL, Brinkman SF, Greene JC (1998) Effects of metal mining and milling on boundary waters of Yellowstone National Park, USA. Environ Manage 22:913-926

Olsson PE (1998) Disorders associated with heavy metal pollution. In: J.F.

Leatherland and P.T.K. Woo (eds), Fish Diseases and Disorders, CABI Publishing, Newyork, USA.105-131
Reily C (1991) Metal contamination of food. London. Elsevier Science.
Reinschuessel R, Bennett RO, May EB, Lipsky MM (1989) Renal histopathological changes in the goldfish, (*Cerassius auratus*) after sublethal exposure to hexachlorobutodiene. Aquat Toxicol 15:169-180
Rubio R, Tineo P, Terreblanca A, Del-Ramo J, Mayans JD (1991) Histological and electron microscopical observations on the effects of lead on gills and midgut gland of *Procambarus clarkii.* Toxicol Environ Chem 31:347-352
Skidmore JF, Tovell WA (1972) Toxic effects of zinc sulphate on the gills of Raibow Trout. Wat Res 6:217-230
Somero GN, Chow TJ, Yancey PH, Snyder CB (1977) Lead accumulation rates in tissues of the estuarine teleost fish *Gillichthys mirabilis*; salinity and temperature effects. Arch Environ Contam Toxicol 6:337-348
Spsswin (1994) SPSS for Windows 6.1.3. Copyright Q SPSS Inc., v 1989-1994, Istanbul, Turkey
Tulasi SJ, Reddy PUM, Romana-Rao JV (1992) Accumulation of lead andeffects on total lipids on lipid derivates in the freshwater fish *Anabas testudineus* (Bloch). Exotoxicol Environ Safe 23:33-38
WHO (1987) Toxicological evaluation of certain food additives and contaminants. Cambridge, UK: Cambridge University Press