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Investigation of Histopathological and Cytogenetic Effects of Heavy Metals Pollution on *Cyprinus carpio* (Linneaus, 1758) in the Gölmarmara Lake, Turkey

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Abstract: Concentration of heavy metals were measured in the surface water, sediments and three different organs of *Cyprinus carpio* from the Gölmarmara lake (Manisa, Turkey). Four sampling sites located at different parts of the lake were pre-defined. The accumulation of copper, zinc, cadmium, cobalt, lead, chromium, iron, mangane in gills, liver and muscle of *C. carpio* were determined. Also, histopathological changes in gill, liver and muscle tissue were examined at light microscopical level. The order of accumulation of heavy metals was found to be Zn>Pb>Fe>Co>Mn>Cr>Ni>Cu>Cd in water, Zn>Cr>Pb>Fe>Co>Mn>Ni>Cu>Cd in sediment, Zn>Cr>Pb>Ni>Cu>Fe>Co>Cd in muscle and Zn>Cr>Pb>Cd>Ni>Cu>Fe>Co in liver and Zn>Cr>Ni>Pb>Cu>Cd>Co>Fe in gills. As a result of histopathological examinations, a significant decrease in mean length of primary and secondary lamellae was observed. Cellular proliferation caused secondary lamellae fusion, ballooning degenerations of secondary lamellae as well as distribution of necrotic and clavate secondary lamellae. In the liver, altered staining, swollen and ruptured parenchymal cells reduce of glycogen in hepatocytes and vacuolar structure filled with cellular debris were seen. In muscle tissue, focal necrosis, cellular dissolution and a decline or loss of striatation in muscle fibres were found. The frequency of micronucleus formation did not show significant differences in fish samples caught from the Gölmarmara lake.

Key words: Bioaccumulation, water, sediment, fish, histopathology, heavy metals

INTRODUCTION

In the past century, increased pace of the industrialization, advances in technology and increased population of the world led to environmental pollution. Fresh water is indispensable for life. As lakes are still waters their ecology is deteriorated more rapidly by human activities compared to rivers. The most important threats to lakes by human activities are degradation of food-chain (eutrophication), acidification, salination, pollution by heavy metals and other toxic substances and alteration of natural water regime (water usage-agriculture-energy-domestic and global warming).

In the last decade, this issue has become a focus of attention as fresh waters contaminated by pollutants are not only a threat to water procurement but also a damage to the aquatic life. River systems can be severely contaminated by heavy metals as a result of domestic, industrial, mining-related and agricultural effluents (Salomons and Forstner, 1984; Langston, 1990; Lee and Stuebing, 1990; Gumgum *et al*., 1994; Yigit and Altindag, 2002). Contamination of the rivers by heavy metals may have catastrophic effects on the ecological balance of the aquatic environment and the diversity of the aquatic organisms can be limited by the extent of the contamination (Suzuki *et al*., 1988). Heavy metals discharged to the environment enter the aquatic environment predominantly directly or as a result of erosion of the rocks, geochemical erosion, atmospheric precipitations or rain-induced erosion (Förstner and Wittmann, 1983; Salomons and Forstner, 1984; Lee and Stuebing, 1990; Pardo *et al*.,1990; Boughriet *et al*., 1992; Gumgum *et al*., 1994; Veena *et al*., 1997; Klavins *et al*., 2000; Yu *et al*., 2001).

These are considered significant pollutants of aquatic ecosystems due to their environmental continuity and tendency to accumulate in aquatic organisms (Veena *et al*., 1997; Kalay and Canli, 2000). For instance, Cd accumulates generally in the kidneys and to lesser extents in the liver and gills of fish (Kumada *et al*., 1980; Hassan *et al*., 1993; Mengchang *et al*., 1998). Moreover, since heavy metals

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show biological accumulation they can exert toxic effects even at locations far away from the source of pollution (Barlas, 1997). Toxic substances can disrupt the physiology of the animal by causing stress. This stressors act at tissue and cellular levels and can cause the death of the organism. Fish are sensitive to acute and chronic environmental changes and elicit a classical stress response. This stress response involves alterations in plasma glucocorticoids and catecholamines. Environmental changes can induce hypoxia, metabolic acidosis and alkalosis, hypotension and hypoglycemia (Wendelaar, 1997; Fabbri *et al*., 1998).

With its wide external surface area, gill is an organ that separates blood from water in fish and interacts directly with environmental parameters. It is very sensitive to the changes in concentrations of environmental parameters (pH, salinity, temperature, ammonia, heavy metals, etc.). These changes cause morphological alterations by disrupting the structural integrity of the gill. Therefore, gills are considered as indicators of water pollution (Randi *et al*., 1996; Ortiz *et al*., 1999; Bhagwant and Elahee, 2002; Wood *et al*., 2002; Koca *et al*., 2005).

Liver plays a key role in maintaining the internal homeostasis in vertebrates. Having a rather dynamic structure, it is a good study model since it regulates many metabolic and physiological processes and is involved especially in detoxification mechanisms (Segner, 1998).

Muscle tissue on the other hand compared to the other vertebrates, comprise a major percentage of the body weight in fish (Fabbri *et al*., 1998) and is valuable economically.

Histological examination of the tissues is a useful tool in assessing the effects of environmental parameters. The results of numerous studies have showed that animals exposed to trace amounts of heavy metals and pesticides even if they do not die, sustain serious damage to their viscera. It is a widely known fact that metals show biological accumulation at hazardous levels on the surface of the sediments in benthic and planktonic organisms and other organisms by entering the food-chain and as a result, aquatic organisms and humans are adversely affected. Therefore, toxicological research on metals discharged to aquatic environments is essential for the biological life to exist and the nature to be protected (Davies *et al*., 1991; Srivastava *et al*., 1994; Ankley *et al*., 1996; Klavins *et al*., 2000; Gonzales *et al*., 2000; Singh, 2001).

Besides their toxic effects, these pollutants also exert genotoxic effects. Micronucleus test is one of the very useful methods to assess the genotoxicity in aquatic environment. This test is being successfully used in numerous organisms, especially in fish. Fresh-water fish species such as *Barbus plebjus* (Minissi *et al*., 1996), *Oncorhyncus mykiss* (De Flora *et al*., 1993), *Zacco platypus*, *Leiognathus nuchalis*, *Ditrema temmincki* (Hayashi *et al*., 1998), *Salmo trutta* (Sanchez-Galan *et al*., 1998) and *Lepomis gibbosus* (Koca *et al*., 2005) have been reported to be good targets for *in-situ* examination of rivers and lakes using micronucleus test as a genotoxicity indicator. However, its usefulness in other species such as *Phoxinux phoxinus* (Sanchez-Galan *et al*., 1998), *Genyonemus lineatus* (Carrasco *et al*., 1990) is questionable due to lack of sensitivity or low sensitivity to certain heavy metals and pollutants.

The objective of the present study was to determine the trace elements present in the water and sediments of Lake Golmarmara, which has a considerable economic value and in *C. carpio* farmed in this lake and the histopathological and genotoxic effects induced by these elements in the tissues of *C. carpio*.

MATERIALS AND METHODS

Study area: Gölmarmara Lake is in west Anatolia, Turkey (38°31'N, 28°05'E). The Lake is located approximately 78 km away from the city of Manisa. It is used for irrigation and fishery. In this study surface water, sediment and fish samples were collected from four diffeerent sampling sites at Gölmarmara Lake (Fig. 1).

1st station: The area has a wetland characteristic. It was heavily populated by water fowls.

2nd station: In this area the characteristic of sediment is soft and there were plants and detritus.

3rd station: The area most densily populated by water fowls. In the summer months because of the submers and emers type plants, there were detritus in the sediment.

4th station: The depth of the area was measured 1, 2 m in maximum (October) and 6 m in minimum (April). The area was heavily populated by water fowls. The color of sediments was close to grayish black.

Analytical procedures

Sampling and sample preparation: Water samples were collected from a depth of 0.5 m below the surface into clean 1-l polyethylene bottles by means of a Nansen Sampler. Then, 1 mL of 0.5% HNO_3 was added to acidify the water samples (Bernhard, 1976). Sediment samples were collected using grab sampler from four sites. Samples obtained from the study site were brought to laboratory in freezer in polyethlene bags and kept until time

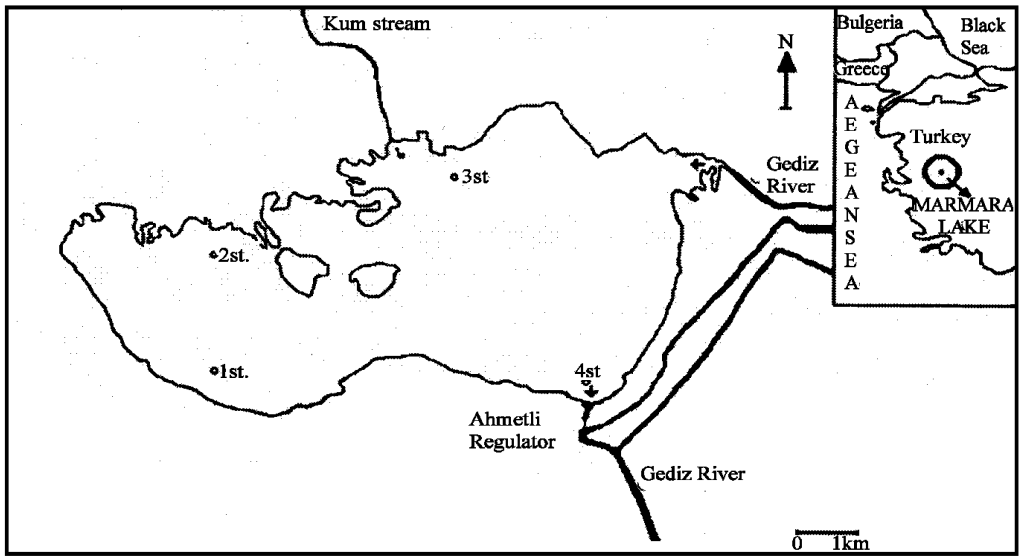

Fig. 1: Map of the Gölmarmara Lake, showing the samples collection sites identified by a number

of analyses at -21°C in sterile polyethylene bags after acidification with HNO_3. On the day of analyses, samples were initially thawed to room temperature followed by drying in an incubator at 100°C and grinding to powder that would be filtered through 100 µm. One gram of the filtered sample was then mixed with HF: HCIO: HCI, respectively at 1:1:6 placed on the hot plate and vaporized gradually for mineralization. Then n/10 HCI was added on the samples to get a final solution of 50 mL. Finally, the samples were filtered through (blue tape) filters kept at +4°C until analyses (Bernhard, 1976).

After killing, the fish samples were kept on ice for processing in the laboratory. Approximately, 4 g of the epaxial muscle on the dorsal surface of the fish, the entire liver and two gill racers from each sample were dissected, washed with ice-cold distilled water, dried in filter paper, weighed, packed in polyethylene bags and frozen at -30°C until needed for analysis. After digestion, the samples were cooled down to room temperature and diluted to 25 mL with 2.5% HNO_3. The mean length and weight of the fish were 318.3±7.4 mm and 549±10.5 g for *C. carpio* (n: 87). The age of caught fish samples of 80.5% on carp population was determined 2-4 years old. The Cd, Co, Cu, Ni, Pb and Zn in the samples were determined by means of a Varian Terra Liberty II inductively coupled plasma atomic emission spectrometer. The concentrations of heavy metals were expressed as micrograms per gram of wet weight tissue.

Histological procedures: For light microscope analyses, the gill, liver and muscle tissues from *C. carpio* were fixed in both buffered neutral formalin and Saint-Marie fixative (+4°C) (Tuckett and Morris-Kay, 1988), dehydrated in graded ethanol series, cleared in xylene and embedded in paraffin. Five-micrometer-thick gill, liver and muscle tissue slices cut by means of a rotary microtome (Leica RM 2145) were dehydrated and stained with Mayer's Haematoxylin Eosin (H and E), Gomori trichrom, Masson trichrom and Periodic Acid Shiff-Haematoxylin (PAS-H) stain (Bancroft and Cook, 1994). The sections were examined and photographed using an Olympus BX 51 microscope.

Cytogenetic procedures: The fish were killed by a blow to the head followed by decerebration. A drop of blood from the caudal vessels of each fish specimen was smeared on slides and air dried. After fixation in absolute methanol for 20 min, the slides were stained with a 10% Giemsa solution rinsed in distiled water and mounted with entellan. The frequency (%) of MN was determined by examining an average of 1000 erythrocytes per slide at x1000 magnification. Coded and randomized slides were scored using a blind review by a single observer.

RESULTS AND DISCUSSION

Analytical results: Heavy metal analyses of water samples taken periodically from four stations over a period of 10 months showed that the levels of Cadmium, Cobalt, Chromium, Copper, Iron, Manganese, Nickel, Lead and Zinc varied between 0.0035-0.0667, 0.0078-0.0748, 0.01-0.0723, 0.0003-0.0681, 0.0651-0.8771, 0.0212-0.0715, 0.0001-0.0666, 0.0465-0.8617 and 0.5617-4.3361 ppm, respectively. Analyses of the sediment samples for heavy metals on the other hand showed that the levels of

Cadmium, Cobalt, Chromium, Copper, Iron, Manganese, Nickel, Lead and Zinc were in the range of 0.0857-4.3756, 0.1004-11.6786, 0.0067-10.6861, 0.0047-7.5656, 0.3471-3.6071, 0.1165-2.8616, 0.0407-3.8566, 1.6671-5.6875 and 5.5861-21.6881 ppm, respectively.

Measurements obtained at heavy metals analyses of water samples from the four stations selected for the study showed variation with respect to stations. According to the analyses, mean measurements in water samples were 0.0047-0.0341 ppm for Cadmium, 0.0292-0.0569 ppm for Cobalt, 0.0143-0.0566 ppm for Chromium, 0.0066-0.0327 ppm for Copper, 0.1957-0.5446 ppm for Iron, 0.0387-0.0547 ppm for Manganese, 0.0048-0.0421 ppm for Nickel, 0.3171-0.4210 ppm for Lead and 2.2450-3.2750 ppm for Zinc.

The order of heavy metal concentrations in water samples measured in each station were Zn>Fe>Pb>Co>Cr>Mn>Ni>Cu>Cd in Station 1, Zn>Fe>Pb>Mn>Co>Cr>Cd>Mn>Ni>Cu in Station 2, Zn>Pb>Fe>Co>Mn>Cr>Ni>Cu>Cd in Station 3 and Zn>Pb>Fe>Mn>Cu>Co>Cr>Ni>Cd in Station 4. The order of the means of h eavy metals in water samples from all stations was Fe>Pb>Mn>Co>Zn>Ni>Cr>Cu>Cd (Table 1). In the sediment samples, cadmium measurements were in the range of 0.2289-1.0056 ppm, Cobalt varied between 0.6812-5.8670 ppm, Chromium between 0.5961-7.2374 ppm, Copper between 0.5068-1.9419 ppm, Iron between 1.1103-3.2199 ppm, Manganese between 0.6957-1.6411 ppm, Nickel between 0.2929-1.7990 ppm, Lead between 2.5154-3.1780 ppm and Zinc between 8.9167-15.2640 ppm (Table 2).

In fish specimens, the concentrations were highest in the liver followed by the gill and muscle indicating that heavy metal accumulation was highest in the hepatic tissues. The higehst concentration measured was of zinc in the liver (15,6450 ppm) and the lowest concentration was of cadmium in the muscle (0.0048 ppm) (Table 3).

Histological results:

Gills: In gill tissue, shortening of the primary and secondary lamellae (Fig. 2a), loss of secondary lamellae (Fig. 2b), separation of the secondary lamellar epithelium, clavate lamellae formation (Fig. 2c-d) and aggregation of blood cells as a result of circulation disorder induced by enlargement of the capillaries in the secondary lamellae (Fig. 2e) were observed. While integrity of secondary lamellae was impaired as a result of the necrosis and exfoliation observed in secondary

Table 1: The heavy metal concentrations in the Gölmarmara Lake's water (Mean±SD, ppm Mg L^{-1})

Heavy metals	Station 1	Station 2	Station 3	Station 4
Cd	0.0049±0.0008	0.0341±0.0195	0.0321±0.0120	0.0047±0.0009
Co	0.0569±0.0129	0.0412±0.0224	0.0496±0.0215	0.0292±0.0049
Cr	0.0566±0.0130	0.0345±0.0068	0.0306±0.0003	0.0143±0.0054
Cu	0.0327±0.0230	0.0066±0.0066	0.0283±0.0218	0.0320±0.0090
Fe	0.4421±0.2088	0.5446±0.2059	0.1952±0.0741	0.2490±0.0873
Mn	0.0547±0.0097	0.0521±0.0101	0.0387±0.0134	0.0409±0.0134
Ni	0.0421±0.0216	0.0079±0.0167	0.0048±0.0016	0.0087±0.0259
Pb	0.4160±0.1783	0.3171±0.2897	0.3399±0.2572	0.4210±0.1864
Zn	3.2750±0.7750	2.2879±0.7128	2.2450±0.8638	2.3936±1.0328

Table 2: The heavy metal concentrations in the Gölmarmara Lake's sediment (Mean±SD, ppm mg kg^{-1} dry weight)

Heavy metals	Station 1	Station 2	Station 3	Station 4
Cd	1.0056±1.1702	1.0018±0.4894	0.2289±0.1532	0.5917±0.2596
Co	5.867±2.601	0.9435±0.4299	1.447±0.8595	0.6812±0.3683
Cr	3.6987±3.4859	7.2374±0.9707	1.6729±0.9497	0.5961±0.8134
Cu	1.9419±2.4439	0.5455±0.1611	0.5523±0.1798	0.5068±0.1794
Fe	3.2199±1.9828	1.2718±1.3243	1.4573±1.3346	1.1103±1.1471
Mn	0.6957±0.6417	0.9570±0.8016	1.6411±0.7592	0.9724±0.5241
Ni	1.799±1.0092	0.5524±0.4995	0.5066±0.0686	0.2929±0.2178
Pb	3.178±1.1027	2.6407±0.5843	2.5154±0.1902	2.7488±0.7686
Zn	15.264±5.0199	10.79±4.1779	8.9167±3.4198	10.488±3.2358

Table 3: The heavy metal concentrations of fish samples from the Gölmarmara Lake (Mean±SD, ppm mg kg^{-1} wet weight)

	Heavy metal concentrations N = 15		
Heavy metals	Muscle	Liver	Gills
Cd	0.0048±0.0040	0.0721±0.0060	0.0655±0.0010
Co	0.0124±0.0078	0.038±0.0176	0.0362±0.0106
Cu	0.0745±0.0261	0.3080±0.0749	0.3557±0.0049
Ni	0.0990±0.0280	0.6010±0.0212	1.4552±1.2367
Pb	0.3120±0.3450	0.9620±0.0026	1.0205±1.1165
Zn	4.8585±2.6820	15.6450±14.0920	5.432±0.3606
Cr	0.5200±0.070	2.2600±1.150	1.9601±0.450
Fe	0.0525±0.4994	0.0567±0.0667	0.0224±0.0021

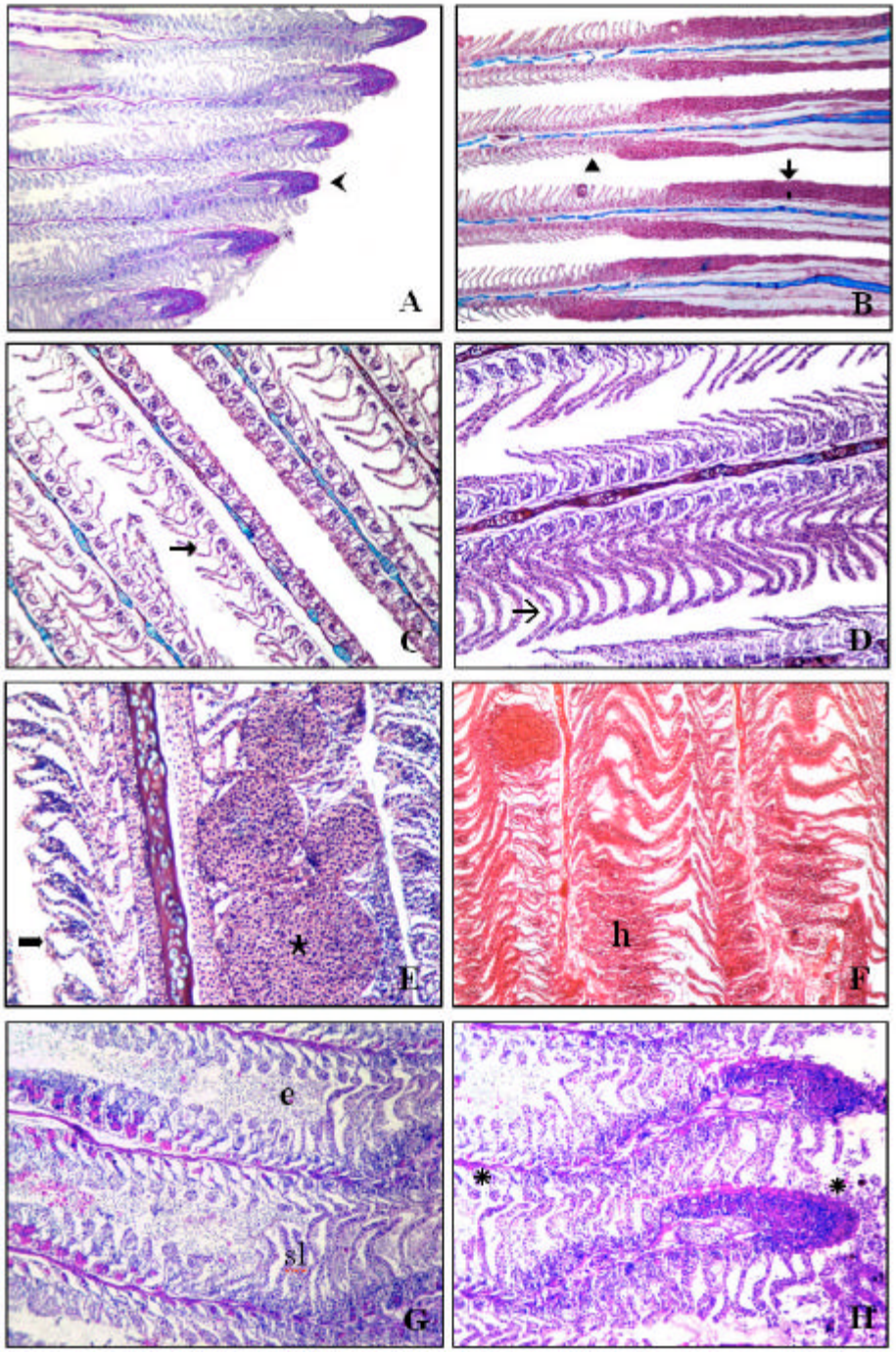

Fig. 2: The observed changes in gills of *C. carpio*. (a) ➤: Decrease of the mean length of primary lamellae, (b) ▸: Decrease of the mean length of secondary lamellae, (c) →: The loss of secondary lamellae and epithelial separating in secondary lamellae, (d) →: Clavate lamellae formation, (e) ➡: Epithelial separating, ✶: Accumulation of blood cells, (f) h: Hyperplasia, (g) sl: secondary lamellae epithelium, e: Erythrocyte releases and (h) Mucous cells in tip of the primary lamellae and in the secondary lamellae epithelium (✱). a: PAS + H, x4, b: Masson trichrome, x4, c-d: Gomori Trichrome, x10, e: Gomori Trichrome, x20, f: Masson Trichrome, x10, g-h: PAS+H, x10

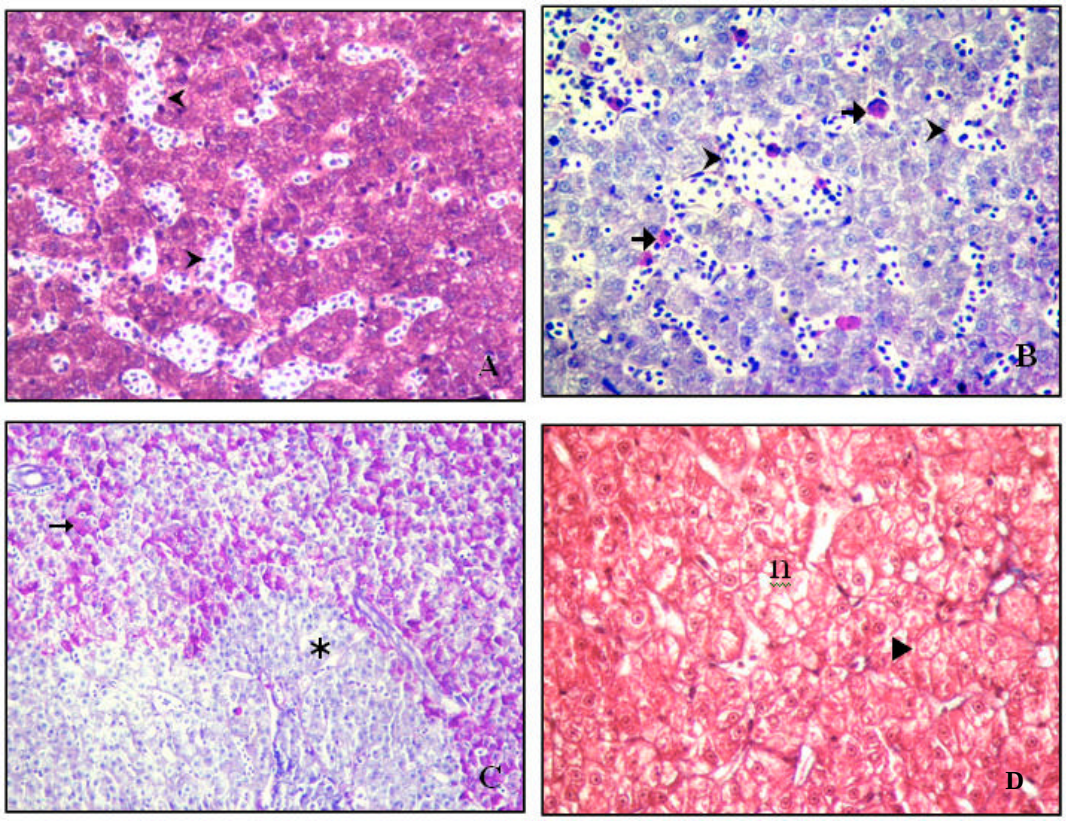

Fig. 3: The observed changes in liver of *C. carpio*. (a) ➤: Enlarged and Erythrocyte-filled capillaries, (b)→: Eosinophil cells, (c) →: Intracellular glycogen, ✶: Decrease in glycogen and (d) ▸: Intracellular vacuolization, n: necrosis. a: Gomori Trichrome, x40, b: PAS+H, x40, C: PAS+H, x20, d: Masson Trichrome, x40

lamellar epithelium, adhesion was observed due to hyperplasia (Fig. 2f). Moreover, capillary degeneration of the secondary lamellae and erythrocyte release were observed (Fig. 2g). There were excessive amount of mucus-secreting cells in the primary lamellae, in regions where the secondary lamellae shed and in the secondary lamellae epithelium (Fig. 2h).

Liver: String of cells formed by hepatocytes with occasional enlarged and erythrocyte-filled sinusoids between them and increased eosinophilia was observed (Fig. 3a, b). Within the hepatocytes, intracytoplasmic glycogen deposits in the form of particles and of various sizes, vacuolization and disruption of the cellular integrity were evident (Fig. 3c, d). There were also regional glycogen losses within the hepatic tissue (Fig. 3c).

Muscle: Disorganization of the intermyofibrillary network (Fig. 4a), releases of myocyte nucleus and necrosis with loss of striation in muscle fibrils were observed in the muscle tissue (Fig. 4b, c). Loss of endomysium layer surrounding the muscle fibers was noted (Fig. 4d). There were also very few glycogen particles in the myocyte cytoplasm (Fig. 4d).

Cytogenetic results: As a result of examining 1000 cells in each fish, we did not observe any micronucleus in erythrocytes.

In the present study, we measured the concentrations of heavy metals Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in water, sediment and fish samples taken from 4 stations in Lake Gölmarmara and we determined the differences in concentrations between stations.

Analyses of water, sediment and fish samples were carried out using ICP-AES and the results are given in Table 1-3. Between September 2004 and August 2005, the lowest Cd was 0.0035 ppm measured in January in water samples from Station 4 and the highest Cd concentration was 4.3756 ppm, measured in December in sediment samples from Station 1. Cd concentration was

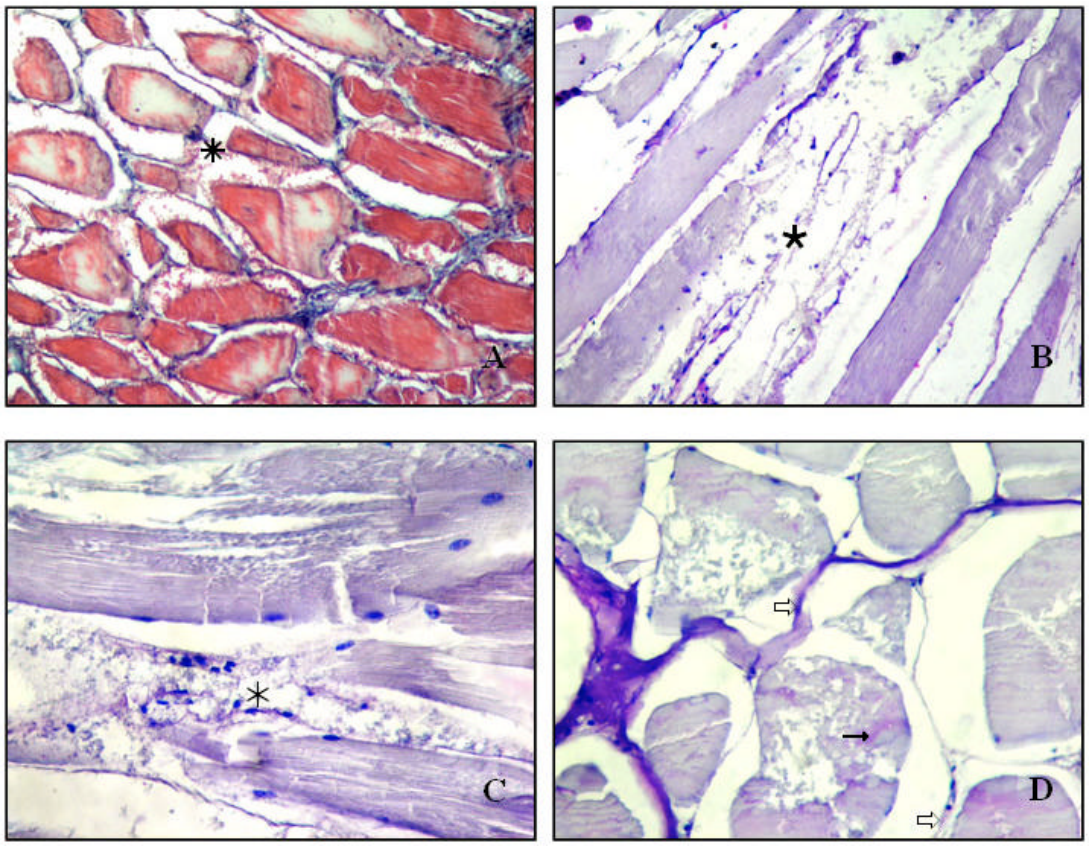

Fig. 4: The observed changes in muscle of *C. carpio*. (a) ✷: Disorganization of the intermyofibrillary network, (b) ✶: Focal necrosis and cellular dissolution, (c) ✳: Dissolution in sarcoplasm and releases nuclei of myocyte and (d) ➞ Glycogen particles in myocytes, a: Loss of endomysium layer. a: Gomori trichrome, x20, b: PAS+H, x10, c: PAS+H, x40, d: PAS+H, x40

higher than that recommended for drinking water by WHO (1993), Commission of European Communities (EU, 1978), USA (Gray, 1994), Russia (Committee for Fisheries 1993) and Turkey (Drinking water standard TS-226 of Turkish Institute of Standards, 1984). The lowest Co was 0.0078 ppm, measured in September in water samples from Station 3 and the highest Co concentration was 11.6786 ppm, measured in November, in sediment samples from Station 1. Co concentration measured in Lake Gölmarmara is lower than the drinking water standard of Russia. The lowest Cr was 0.0067 ppm, measured in December in sediment samples from Station 4 and the highest Cr concentration was 10.6861 ppm, measured in November in sediment samples from Station 1. Cr concentration is lower than the drinking water standard stipulated in the USA but higher than WHO, EU and TS-226 standards. Water was classified as 1st quality in terms of Cr. The lowest Cu was 0.0003 ppm, measured in December in water samples from Station 1 and the highest Cu concentration was 7.5656 ppm measured in October in sediment samples from Station 1. Copper concentration we measured was less than all drinking water limits. The lowest Fe was 0.0651 ppm measured in December in water samples from Station 1 and the highest Fe concentration was 6.8751 ppm, measured in December in sediment samples from Station 1. Measured Fe levels were lower than TS-226 standards but higher than the standards of WHO, EU, USA and Russia. The lowest Mn was 0.0212 ppm, measured in May in water samples from Station 3 and the highest Mn concentration was 2.8616 ppm, measured in July and September in sediment samples from Stations 1, 2 and 3. Mn concentration was lower than WHO, Russia and TS-226 but higher than EU and USA drinking water standards. The lowest Ni was 0.0001 ppm, measured in September in sediment samples from Station 1 and the highest Ni concentration was 3.8566 ppm, measured in May in sediment samples from Station 1. When Ni contents of dinking water standards were compared the measurements were higher than WHO and Russia and lower than EU standards. The lowest Pb

was 0.0465 ppm, measured in September in water samples from Station 1 and the highest Pb concentration was 5.6875 ppm, measured in March in sediment samples from Station 1. Pb concentration measured was higher than all standards, making it a 4th quality water. The lowest Zn was 0.5617 ppm, measured in September in Station 4 and the highest Zn concentration was 21.6881 ppm, measured in January in Station 1 (Gumgum *et al*., 1994; Yigit and Altindag, 2002; Pardo *et al*., 1990; Yu *et al*., 2001). It is widely accepted that heavy metal is taken through water, food and sediments (bottom sediments). However, sufficiency of metal taken from contaminated waters and food depends on salinity, temperature, ecological needs and metabolism (Yigit and Altindag, 2002; Suzuki *et al*., 1988; Yu *et al*., 2001; Avila-Perez *et al*., 1999).

The order of concentration of metals in the tissues of *C. carpio* were Zn>Cr>Pb>Ni>Cu>Co>Cd>Fe in muscle, Zn>Cr>Pb>Cd>Ni>Cu>Fe>Co in the liver and Zn>Cr>Pb>Cd>Ni>Cu>Co>Fe in the gills. Barlas (1997) found high concentrations of Pb and Cd in the tissues of *C. carpio*. Ayaş and Kolonkaya on the other hand reported that Hg and Pb accumulated in high concentrations in the muscle tissue of *C. carpio* (Avila-Perez *et al*., 1999).

Toxic environment induces two types of structural alterations in living organisms: degeneration and direct effects that can cause necrosis and immune responses elicited by the host (Hughes *et al*., 1979; Bhagwant and Elahee, 2002).

Tissues such as liver and gill are metabolically active tissues and studies have showed that large amounts of heavy metals accumulate in these tissues (Tulasi *et al*., 1992; Allen, 1995).

Thophon *et al*. (2003) demonstrated that acute toxic effects of Cd targeted the gill lamellae and renal tubules first and that toxic effects on the gills were less severe than those in the kidneys and liver.

Gill serves as a good model to study the effects of toxicants in water on tissues. Furthermore, it is the biggest surface of contact of the fish with the acoustic environment and therefore, toxic substances cause injuries in gills. These injuries include edema, excessive hypertrophy and hyperplasia of lamellar epithelia and chloride cells. Changes observed in tissues occur in an effort to downsize gill, i.e., respiration surface area to reduce the intake of toxicants (Mallatt, 1985; Koca *et al*., 2005) and increase diffusion distance. Hence, there would be a decline in the respiratory function of the gills. This in turn, affects the health of the fish (Skidmore and Tovell, 1972) and can even lead to death (Thophon *et al*., 2003). The results of the present study are in agreement with the literature and gills emerge as the mostly affected tissue.

Aneurism is a circulation disorder characterized by pooling of copious amount of blood in secondary lamellae. It is caused by the collapse of the pillar cell system (Alazemi *et al*., 1996). It has also been argued that it occurs not only to protect the gill epithelia against mechanical wear but also against infection (Olson and Fromm, 1973; Bhagwant and Elahee, 2002). Experimental studies have shown that Cd is responsible of this pathological state (Alazemi *et al*., 1996).

In the study, mucous cells were observed at the tips of the primary lamellae and between the secondary lamellae. It has been reported that these cells, a kind of connective tissue cell, proliferate in response to parasitic infections (Reite, 2005). Meanwhile, their mucous secretion prevents the entry of chemical toxicants and harmful microorganisms by increasing the diffusion distance in the gills. On the other hand, they hinder oxygen intake, causing hypoxia (Nero *et al*., 2006), consequent decrease in swimming of the fish and behavioral imbalance.

Changes observed in the liver of *C. carpio* were hepatocellular necrosis, widenening of the sinusoids and pooling of red blood cells, findings similar observed in the livers of fish exposed to Cu (Arellano *et al*., 1999). Histopathological changes of various degrees cytoplasmic lysis, pyknotic nucleus and necrosis cause high metabolic activity in hepatocytes in response to an increase in various pollutants (Cooley *et al*., 2000; Ptashynski and Klaverkamp, 2002; Thophon *et al*., 2003; Gul *et al*., 2004). It has been reported that these degenerative changes are parallel to the changes caused by petroleum hydrocarbons (Myers *et al*., 1998).

In a TEM study by Tayal *et al*. (2000), the researchers demonstrated that exogenous Cd deposited as loosely bound insoluble metal complexes in gill, liver and muscle cells.

Micronuclei occur as a short-term response to cytogenetic injury and disappear from the organism in a few days. Therefore, they serve as a useful tool to investigate the fresh-water ecosystems. The results of some studies on polluted and un-polluted rivers indicate that micronucleus test can be used as an indicator of genotoxicity in certain fish species such as *Salmo turutta* and *Oncorhyncus mykiss*.

It was interesting not to observe micronucleus in *C. carpio* despite the contamination in Lake Gölmarmara. This can be either due to certain fish species such as *Genyonemus lineatus* (Carrasco *et al*., 1990), *Phoxinux phoxinus* (Sanchez-Galan *et al*., 1998) and *Anguilla anguilla* (Rodriguez-Cea *et al*., 2003) being less susceptible to pollutants or to having a very effective system that prevents the increase of micronuclei in the

peripheral blood and eliminates the micronuclei (Zuniga *et al*., 1996; Zuniga-Gonzalez *et al*., 2000). It is possible that *C. carpio,* which we examined in the present study, possesses one or both of these systems.

CONCLUSION

In this research, water, sediment and tissue (gill, liver and muscle) analyses depicted the presence of toxicants in the waters of Lake Gölmarmara even though micronucleus was not observed in *C. carpio*.

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