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Research Article

Evaluation of River Water Genotoxicity Using the Piscine Micronucleus Test

Serap Ergene,[1] Tolga Çavaş,[1]* Ayla Çelik,[1] Nurcan Köleli,[2] and Cemil Aymak[1]

[1]*Department of Biology, Faculty of Sciences and Letters, Mersin University, 33342 Mersin, Turkey*

[2]*Department of Environmental Engineering, Faculty of Engineering, Mersin University, 33342 Mersin, Turkey*

The Berdan River, which empties into the Mediterranean Sea on the east coast of Turkey, receives discharges of industrial and municipal waste. In the present study, the in vivo piscine micronucleus (MN) test was used to evaluate the genotoxicity of water samples collected from different locations along the Berdan River. Nile tilapia (*Oreochromis niloticus*) were exposed in the laboratory for 2, 4, and 6 days, and micronuclei were evaluated in peripheral blood erythrocytes, gill cells, and caudal fin epithelial cells. A single dose of 5 mg/L cyclophosphamide was used as a positive control. In addition to micronuclei, nuclear abnormalities (NAs), such as binucleated cells and blebbed, notched, and lobed nuclei, were assessed in the erythrocytes, and chemical analyses were carried out to determine the amount of heavy metals in the water samples. MN and NA frequencies were significantly elevated (up to 2- to 3-fold) in fish exposed to river water samples taken downstream of potential discharges, and the elevated responses in gill and fin cells were related to the concentration of heavy metals in the water. MN frequencies (expressed as micronucleated cells/1,000 cells), in both treated and untreated fish, were greatest in gill cells (range: 0.80–3.70), and generally lower in erythrocytes (range: 0.50–2.80), and fin cells (range: 0.45–1.70). The results of this study indicate that the Berdan River is contaminated with genotoxic pollutants and that the genotoxicity is related to the discharge of wastes into the river water. Environ. Mol. Mutagen. 48:421–429, 2007.

Key words: river water; genotoxicity; fish; in vivo micronucleus test; nuclear abnormalities

INTRODUCTION

The pollution of water resources is a serious and growing problem. Industrial effluents, agricultural runoff, and municipal wastewater contain unknown substances and complex mixtures that are released into the environment and can lead to the contamination of water resources [Claxton et al., 1998]. The continuous release of these substances, despite the existence of relevant legislation, has increased interest in evaluating the genotoxicity associated with polluted aquatic environments [Chen and White, 2004; Porto et al., 2005; Cavas and Ergene-Gozukara, 2005a]. The presence of heavy metals in the aquatic environment is of major concern because of their toxicity and genotoxicity to many life forms [Valko et al., 2005; Florea and Busselberg, 2006]. Numerous industrial processes produce aqueous effluents that contain heavy metals and trace metals, including chromium (Cr), cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), zinc (Zn), magnesium (Mg), iron (Fe) and arsenic (As) [Ali et al., 1996]. Furthermore, farming and cities can contribute to the pollution of water resources with heavy metals through contamination by domestic effluents (Cd, Cu, Cr, Pb, and Zn), mineral fertilizers (Cd, Cr, Mo, Pb, U, V, and Zn), and pesticides (Cu, As, Pb, Mn, and Zn) [Alloway, 1995; Aonhgusa and Gray, 2002].

The flow of the Berdan River in southern Turkey is controlled by the Berdan Dam (Fig. 1). The water impounded by the Berdan Dam and water in the downstream portions of the Berdan River are used for irrigation and as sources of drinking water for the cities of Tarsus and Mersin. Tarsus, with some 200,000 inhabitants, is

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*Correspondence to: Tolga Çavaş, Department of Biology, Faculty of Sciences and Letters, Mersin University, Mersin, Turkey.
E-mail: tcavas@mersin.edu.tr

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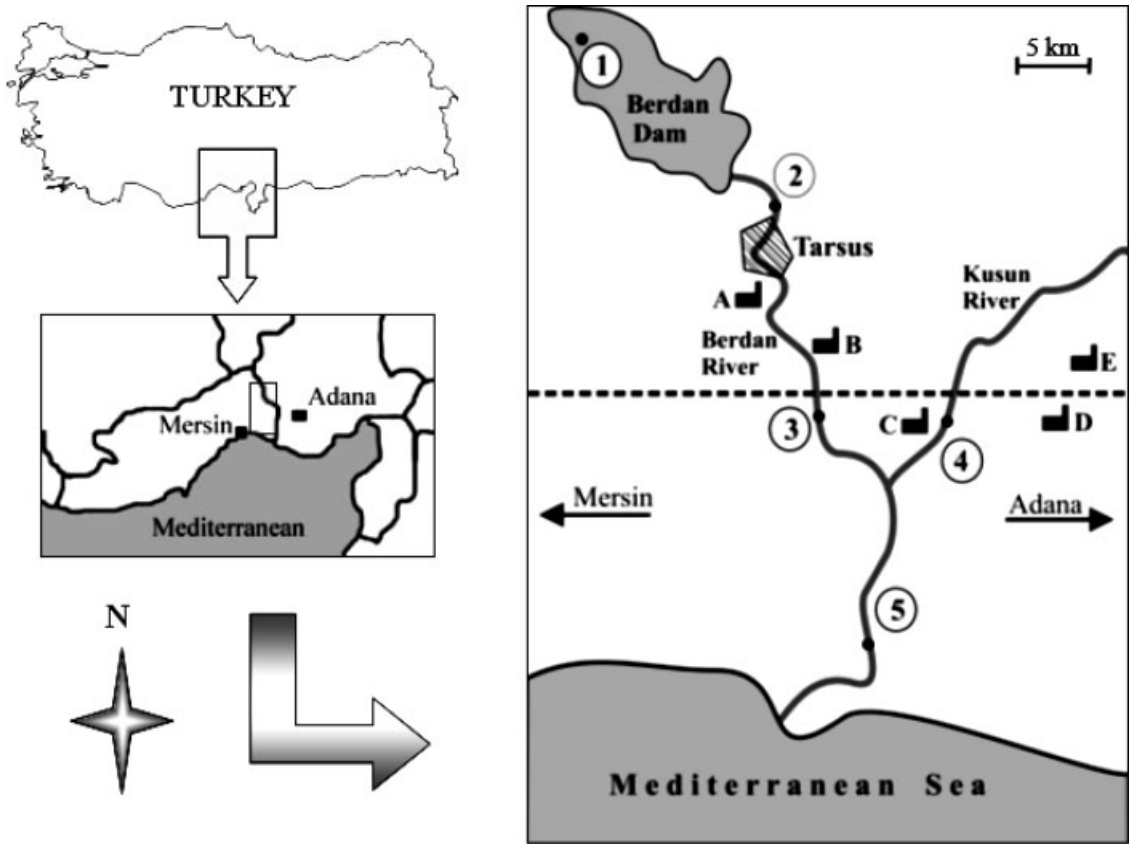

Fig. 1. Map of the study area. The circled numbers (1–5) indicate water sampling stations. Capital letters indicate the location of industrial companies. **A**: Oil factory, **B**: Dye plant, **C**: Industrial machine factory, **D**: Glasswool plant, **E**: Textile plant.

located on the Berdan River 15-km upstream of the eastern Turkish Mediterranean coast. The majority of the industrial and domestic waste waters generated in this city are discharged into the Berdan River via the State Hydraulic Works (SHW) channels, the Kusun River, and other waterways. Previous studies have detected elevated levels of Pb, Cu, Cd, Ni, and Cr in the Berdan River [Kumbur and Vural, 1989; Ozer, 2001; Demirel and Kulege, 2005]. According to Ozer [2001], the main sources of pollution in the Berdan River are the discharge of inadequately treated or untreated industrial and domestic effluents and the drainage of areas of high pesticide and manure usage by the SHW channels.

The micronucleus (MN) test is considered one of the most useful methods for evaluating genotoxicity in aquatic systems. Micronuclei are formed by both clastogenic and aneugenic compounds. Micronuclei are chromosome fragments or whole chromosomes that lag at cell division due to the lack of a centromere, damage, or a defect in cytokinesis [Heddle et al., 1991]. Genotoxicity has been measured in the marine and freshwater environments by scoring MN induction in plants [Duan et al., 1999; Yang, 1999; Monarca et al., 2003], amphibians [Fernandez et al., 1989; Djomo et al., 2000; Wirz et al., 2005], mussels [Klobucar et al., 2003; Barsiene et al., 2004; Nigro et al., 2006], and fish [Ayllon and Garcia-Vazquez, 2000; De Andrade et al., 2004; Cavas and Ergene-Gozukara, 2005b; Porto et al., 2005; Da Silva Souza and Fontanetti, 2006]. Fish are extremely valuable in toxicity monitoring as they accumulate hazardous compounds in their tissues, are directly or indirectly consumed by humans, and are capable of transforming xenobiotic compounds into carcinogenic and mutagenic metabolites [Law, 2001; Van Der Oost et al., 2003]. Therefore, the piscine MN test has become popular in the recent years [Al-Sabti and Metcalfe, 1995; Cavas and Ergene-Gozukara, 2005b; Da Silva Souza and Fontanetti, 2006]. Erythrocytes are the most commonly used cells in the piscine MN test; however, hepatocytes [Pietrapiana et al., 2002; Cavas et al., 2005], gill epithelial cells [Takai et al., 2004; Cavas and Ergene-Gozukara, 2005a,b], and fin cells [Arkhipchuk and Garanko, 2005] also have been used.

The formation of morphological nuclear alterations (NAs) was first described in fish erythrocytes by Carrasco et al. [1991]. NAs, including blebbed, lobed, and notched nuclei and binucleated cells, have been used by several

investigators as possible indicators of genotoxicity [Ayllon and Garcia-Vazquez, 2000; Cavas and Ergene-Gozukara, 2003, 2005a,b; Da Silva Souza and Fontanetti, 2006]. Although the mechanisms responsible for NAs have not been fully explained, these abnormalities are considered to be indicators of genotoxic damage and, therefore, they may complement the scoring of micronuclei in routine genotoxicity surveys.

The piscine MN test has been used for genotoxicity monitoring of river systems affected by different pollutant sources [de Andrade et al., 2004; Da Silva Souza and Fontanetti, 2006; de Lemos et al., 2007]. In using MN analysis to evaluate the genotoxicity of aquatic systems, three different approaches have been used: (1) measuring micronuclei in wild fish caught in situ at study stations; (2) measuring micronuclei in caged fish exposed in situ at study sites; and (3) measuring micronuclei in fish exposed to water samples under controlled laboratory conditions. An inherent weakness of in situ studies using wild fish is the lack of a true negative control. This problem usually is addressed by the selection of a clean reference area. Also, when studying a river system, it is not always possible to find the same species in the upstream and the downstream regions of the stream.

In the present study, we have evaluated the genotoxicity of water collected at different sites along the Berdan River in the in vivo piscine MN test. Nile tilapia (*Oreochromis niloticus*) were exposed to water samples in the laboratory, and MN analyses were carried out on erythrocytes and gill epithelial cells. In this study, we also evaluated MN frequencies in caudal fin cells to assess the suitability of this tissue for MN analysis. NAs were measured in peripheral erythrocytes to evaluate their induction in comparison to micronuclei. Finally, the water samples were assayed for the presence of heavy metals and some other pollutants.

MATERIALS AND METHODS

Study Area

The study area is situated in a region with a typical Mediterranean climate; wet and mild winters, combined with dry and hot summers, are typical for the coastal zone around the Mediterranean Sea. The Berdan River flow is strongly dependent on the seasonal rains. The average discharge at the Berdan Dam in Tarsus is 38 m^3/s. The average yearly rainfall is 600–650 mm. Showers start in October and continue until mid-April; the maximum rainfall is in December [Demirel and Kulege, 2005]. The concentrations of pollutants in water depend upon enrichment and dilution phenomena caused by rainfall. Low rainfall and low river flows can have an adverse effect through reduced dilution of pollutants, while high rainfall also can increase pollution through greater leaching of pollutants from the soil into rivers, or overflows from sewage systems. Thus, water samplings were carried out in mid-May 2004 following the rainy winter and spring seasons when the total rainfall amounts were recorded as 435 and 49 mm, respectively [MTSO, 2004]. The total amount of rainfall received by the study area in 2004 was 538 mm [MTSO, 2004].

Water samples were collected from the Berdan River system at five locations. The sampling sites are referred as Sites 1, 2, 3, 4, and 5 from upstream to downstream. Figure 1 is a map of study area, showing the sampling sites and the location of industrial plants. Site 1 is located in the lake formed by the Berdan Dam; this water is the main source of drinking water for the cities of Mersin and Tarsus. Site 2 is located in the river upstream of Tarsus, while Site 3 is located downstream of Tarsus. Site 4 is in the Kusun River, before it joins the Berdan River. Finally, Site 5 is located in the downstream portion of the Berdan River, just before it reaches the Mediterranean. The factories located around the study area are referred to as A (an oil factory), B (a dye plant), C (an industrial machine factory), D (a glasswool plant), and E (a textile mill).

Experimental Design

Oreochromis niloticus (the Nile tilapia; Family: Cichlidae) was chosen for this study because it is commonly availabile in most fish farms in Turkey. Specimens of *O. niloticus* with an average weight of 20 ± 5.5 g and length of 6.5 ± 1.5 cm were supplied by the Cukurova University fish farm (Adana, Turkey). Its relatively low chromosome number ($2n = 46$), as well as its proven sensitivity to toxic chemicals, makes this species a suitable model organism for the MN test [Ergene et al., 1998; Cavas and Ergene-Gozukara, 2003, 2005a]. Before beginning the experiments, the fish were acclimated under laboratory conditions for two weeks at a population density of 15 specimens per 90 l aquarium, at a water temperature of 25°C, and using a 12-hr light:12-hr dark photoperiod.

The assays were carried out in aquaria each containing 5 fish and 15 l of test water. Five fish were used for each water sample at each sampling time. Fish were exposed to the test water for 2, 4, or 6 days. Each sampling time had a parallel negative control that received dechlorinated tap water. A further set of fish was treated with 5 mg/L cyclophosphamide (CP) (Sigma, Germany) as a positive control. The test water was renewed every 48 hr to minimize changes due to metabolism by the fish, volatilization of less stable substances, and organism catabolites. At the end of the exposure periods, blood, gill, and fin tissue were collected and processed for MN analyses. A total of 105 fish were used for the experiments.

Physical and Chemical Analysis of Water Samples

Water samples from the Berdan River were collected in polyethylene bottles from the five stations indicated in Figure 1. The pH, dissolved oxygen, electric conductivity (EC), and temperature of the samples were measured in the field using a portable pH-EC meter (HI 9812; HANNA Instruments, Padova, Italy) and oxygen meter (SyberScan DO 100; Eutech Instruments, Singapore). All the samples were placed in a cool box (4°C) and were transported to the laboratory on the same day. For dissolved metals determination, the samples were vacuum filtered through a 0.45 μm membrane filter. 100-ml samples of the filtered water were acidified with 5 ml of concentrated HNO_3 and 0.5 ml HCl, and then concentrated fivefold by evaporation. All the samples were stored at room temperature until analysis. Dissolved Cd, Cu, Pb, and Zn concentrations were determined by atomic absorption spectrometry (Perkin Elmer AAS 700; Überlingen, Germany). The concentrations of Cr(VI), Cl^-, F^-, NO^{3-}, and SO_4^{2-} were measured by photometry (Hanna C 200; HANNA Instruments). In addition, suspended solid material (SSM) was measured using standard methods [APHA, 1998]. All reagents were of analytical grade and obtained from Merck (Darmstadt, Germany). The AAS was calibrated with standard solutions that were prepared from commercial materials (1,000 mg/l, Merck Titrisol solution). The standard addition method was used to correct for matrix effects. The absorption wavelength and the detection limits of the metals were as follows: 228.8 nm and 0.0006 mg/l for Cd; 324.8 nm and 0.003 mg/l for Cu; 232.0 nm and 0.008 mg/l for Ni; and 217.0 nm and 0.02 mg/l for Pb.

Data were assessed with standard solutions for accuracy and precision using a quality control system integral to the analytical procedure.

Analysis of Micronuclei and Nuclear Abnormalities

Five fish were used for each test group. Peripheral blood samples were obtained from the caudal vein of the specimens and smeared onto precleaned slides. After fixation in pure ethanol for 20 min, the slides were allowed to air-dry, and then the smears were stained with 10% Giemsa solution for 25 min. All slides were coded and scored blind. Five slides were prepared for each fish, and 1,500 cells were scored from each slide under 100 × magnification. Small, nonrefractive, circular, or ovoid chromatin bodies, displaying the same staining and focusing pattern as the main nucleus, were scored as mironuclei [Al-Sabti and Metcalfe, 1995].

NAs were classified in erythrocytes according to Carrasco et al. [1990]. Briefly, cells with two nuclei were considered as binucleated. Blebbed nuclei had a relatively small evagination of the nuclear membrane and contained euchromatin. Nuclei with evaginations larger than those in the blebbed nuclei, including those with several lobes, were classified as lobed nuclei. Nuclei with vacuoles or voids with appreciable depth into the nucleus were recorded as notched nuclei.

The MN test also was performed on gill cells, according to Cavas and Ergene-Gozukara [2003]. Briefly, cells were isolated from the gill arches, fixed, smeared onto clean slides, and stained with a 5% Giemsa solution for 30 min. Five slides were prepared for each fish, and 1,500 cells were scored from each slide under 100 × magnification. Micronuclei were nonrefractive, no more than 1/3 the size of the main nucleus, and lying near the main nucleus.

Micronuclei were analyzed in fin cells according to the methods described by Arkhipchuk and Garanko [2005]. Six days prior to the sampling time, the edges of the caudal fins were cut at a depth of 2–3 mm to stimulate regeneration and the fish were returned into the water samples. Using this protocol, tissue regeneration occurs at the end of caudal fins of fish exposed to the test water. The newly regenerating parts of caudal fin tissue were used for MN analysis. Because of the necessity for this tissue regeneration, only 6-day exposure samples were analyzed. The regenerating fin tissue was fixed in a 1:1:13 acetic acid-glyceric acid-water mixture for at least 1 day; slides then were prepared, air-dried, and stained with 5% Giemsa for 30 min.

Statistical Analysis

Data were tested for normality using the Kolmogorov-Smirnov test. Since the data had a normal distribution, parametric tests were used to determine differences at the 0.05 level of significance. Multiple comparisons were performed using one-way analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) test. Student's t-test was used to compare differences between two groups when necessary. Linear regression analysis was used to test correlations between the total heavy metal content of water samples and frequencies micronuclei and NAs. The correlations were made with the sum of all metal concentrations. The method of least squares was used for comparison and Pearson correlation coefficients were calculated. All statistical analyses were performed using SPSS 9 for the PC (SPSS, Chicago, IL).

RESULTS

Chemical analysis of water samples indicated that water from the downstream sampling sites of the river system (Sites 3, 4, and 5) had higher concentrations of heavy metals (Cd, Cr, Cu, Ni, and Pb) than sites from the upper river (Sites 1 and 2) (Table I). For comparison, Table I also shows the pollutant limits established by the Turkish Water Pollution Control Regulation [2001]. Several downstream sampling sites had Cr, Cd, and Ni concentrations at or in excess of these limits.

Exposure of *O. niloticus* to the positive control, the negative control water, and each of the river water samples produced higher MN frequencies in gill cells than in erythrocytes at all the sampling times (Table II; $P < 0.05$). Significant increases in MN frequencies were detected in fish exposed to the water samples from the downstream sites (3, 4, and 5) for as little as 2 days ($P < 0.05$). MN frequencies were not increased in fish exposed to water samples from Berdan Dam (Site 1) or the upstream Site 2. Six days of exposure to river water samples from Sites 3, 4, and 5 also significantly increased the MN frequencies in fin epithelial cells ($P < 0.01$) (Fig. 2). The frequencies of NAs in peripheral blood erythrocytes are summarized in Table III. Similar to MN frequencies, the NA frequencies in fish exposed to river water samples from the downstream sampling sites were significantly higher ($P < 0.01$) than the negative control, while exposure to water from Sites 1 and 2 had no effect on NA frequencies. The frequency of NAs also was increased significantly in the fish expososed to the positive control, CP ($P < 0.001$).

According to least-square analysis, there was a significant linear relationship between total heavy metal concentrations and MN frequencies in gill ($R^2 = 0.795$) and fin ($R^2 = 0.695$) epithelial cells ($P < 0.05$). Pearson correlation coefficients for these relationships were $r = 0.900$ and $r = 0.850$ in gill and fin cells, respectively. However, no significant correlations were found between the metal concentrations and the genotoxicity endpoints in erythrocytes, with the exception of the frequency of binuclei ($P < 0.05$).

DISCUSSION

In the present study, analysis of water samples revealed elevated levels of Pb, Cu, Cd, Cr, and Ni in the downstream portion of the Berdan River system. Our results are in agreement with the results of previous studies. For example, chemical analysis of water samples taken from the Berdan River in 1999 revealed elevated levels of Pb (1.12 μg/l), Cu (4.20 μg/l), Cd (11.8 μg/l), Ni (36 μg/l), and total Cr (74.8 μg/l) in the downstream portion of the river in comparison with the concentrations of Pb (0.15 μg/l), Cu (1.1 μg/l), Cd (3 μg/l), Ni (6.8 μg/l), and total Cr (0.7 μg/l) in the upstream region [Ozer, 2001]. Similarly, Kumbur and Vural [1989] reported that the Berdan River was contaminated with Cd, Ni, Fe, Zn, Mn, and Pb.

As shown in Figure 1, there are several factories located in the study area which may contribute to the elevated amounts of heavy metals detected in the down-

TABLE I. Physical and Chemical Properties of Water Samples Collected From Berdan River and Their Limit Values According to Turkish Water Pollution Control Regulation [2004]

	Sampling stations					
Measured parameters	1	2	3	4	5	Limit values
pH	8.2	8.1	8.2	8.2	7.9	6.5–8.5
Dissolved O_2 (mg/l)	6.8	7.8	7.1	6.9	4.3	8
Electric Cond. (μs/cm)	350	342	353	685	631	–
Suspended solids (mg/l)	71.0	87.8	4.6	15.1	64.7	30
SO_4^{-2} (mg/l)	31	40	43	109	111	200
NH_4^+–N (mg/l)	<0.01	<0.01	0.10	0.42	0.11	0.2
NO_2^-–N (mg/l)	<0.01	<0.01	<0.01	0.01	0.04	0.02
PO_4^{3-} (mg/l)	<0.1	<0.1	<0.1	<0.1	1.45	0.02
F^- (μg/l)	<10	<10	<10	<10	<10	1,000
Br^- (μg/l)	10	20	<10	20	10	–
Cr^{6+} (μg/l)	<0.05	<0.05	<0.05	<0.05	<0.05	0
Total Cr (μg/l)	<3	<3	<3	100	130	20
Cd (μg/l)	1	1	2	2	3	3
Cu (μg/l)	<1.5	<1.5	<1.5	4	3	20
Ni (μg/l)	60	120	116	140	160	20
Pb (μg/l)	<0.015	<0.015	<0.015	<0.015	0.08	10

TABLE II. Micronucleus Frequencies in Peripheral Blood Erythrocytes and Gill Epithelial Cells of *O. niloticus* Exposed to River Water Samples

Exposure		Control groups		Water samples				
duration	Cell type	Negative	Positive	1	2	3	4	5
2 days	Erythrocyte	0.50 ± 0.02[a]	4.50 ± 0.85[b]	0.60 ± 0.08	0.60 ± 0.03	1.40 ± 0.33[c]	1.30 ± 0.20[c]	1.60 ± 0.17[c]
	Gill	1.10 ± 0.09	5.30 ± 0.70[b]	0.95 ± 0.10	1.00 ± 0.10	1.60 ± 0.25[c]	3.20 ± 0.90[d]	3.10 ± 0.40[d]
4 days	Erythrocyte	0.50 ± 0.05	5.40 ± 0.65[b]	0.50 ± 0.03	0.50 ± 0.05	1.90 ± 0.20[d]	1.80 ± 0.43[d]	2.20 ± 0.20[d]
	Gill	0.80 ± 0.05	6.20 ± 0.50[b]	0.90 ± 0.16	1.10 ± 0.07	2.20 ± 0.15[d]	3.25 ± 0.35[b]	3.50 ± 0.70[b]
6 days	Erythrocyte	0.60 ± 0.10	5.10 ± 0.60[b]	0.40 ± 0.03	0.40 ± 0.06	2.70 ± 0.47[b]	2.80 ± 0.84[b]	2.60 ± 0.60[b]
	Gill	1.00 ± 0.15	6.40 ± 0.85[b]	1.30 ± 0.28	1.10 ± 0.13	2.90 ± 0.43[d]	3.55 ± 0.60[b]	3.70 ± 0.85[b]

[a]Data expressed as micronucleated cells/1,000 cells ± SD.
[b]$P < 0.001$; [c]$P < 0.05$; [d]$P < 0.01$; compared to negative control group. Positive control received cyclophosphamide; numbering of sampling stations is as shown in Figure 1.

stream portion of the Berdan River system. Glass factory and machine-building factory effluents may contain Pb, Ni, and Cr [Turkish Water Pollution Control Regulation, 2004]. Effluents from dye and pigment production plants as well as textile mills are known to contain Cu, Cr, Ni, and Cd [Cavas and Ergene-Gozukara, 2003; Sultan and Hasnain, 2005; Manzoor et al., 2006]. Also, domestic effluents can contain elevated amounts of heavy metals, such as Cd, Cu, Cr, Pb, and Zn [Chino et al., 1991; Aonhgusa and Gray, 2002].

In our study, treatment of fish with river water samples collected at downstream sites produced significant increases in the frequencies of micronucleated cells in three tissues. Induction of micronuclei was reported previously for fish exposed to Cd [Ayllon and Garcia Vazquez, 2000; Sanchez-Galan et al., 2001; Zhu et al., 2004; Arkhipchuk and Garanko, 2005; Cavas et al., 2005], Cu [Zhu et al., 2004; Arkhipchuk and Garanko, 2005; Cavas et al., 2005], Cr [Al-Sabti et al., 1994; de Lemos et al., 2001; Zhu et al., 2004], and Pb [Cestari et al., 2004; Ferraro et al., 2004]. Therefore, it is plausible that elevated concentrations of heavy metals could have contributed to the elevated MN and NA frequencies produced by the downstream Berdan River water samples.

Among its several oxidation states, trivalent chromium, Cr(III), together with hexavalent chromium, Cr(VI), are the main forms of Cr present in aquatic environments [ATSDR, 1998]. Chromate is the prevalent species of Cr(VI) in natural aqueous environments, and chromate is the major pollutant from Cr-related industries [EPA, 1998]. Once it comes in contact with cells, the chromate anion easily crosses cell membranes and is reduced in the cell. Reduction of Cr(VI) results in the formation of Cr(III), a stable reduced form that binds DNA more efficiently than Cr(VI) [EPA, 1998]. Although Cr(IV) was undetectable in water from the sampling stations, measurements indicated that total Cr levels in downstream Sites 4 and 5 were 5- to 6.5-fold higher than the maximum

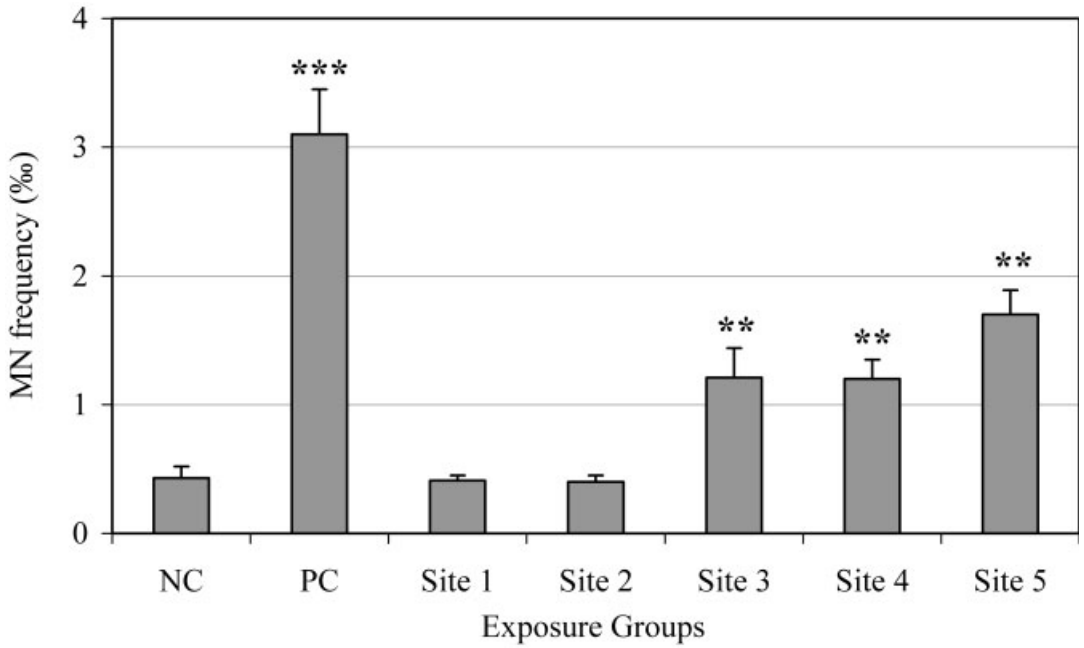

Fig. 2. Micronucleus frequencies in fin cells of *O. niloticus* exposed to river water samples. NC, tap-water control; PC, positive control (cyclophosphamide); the numbers refer to sampling sites (Fig. 1). Frequencies given in micronucleated cells/1,000 cells (‰) ± SD. $^{**}P < 0.01$ vs. NC; $^{***}P < 0.001$ vs. NC.

TABLE III. Frequencies of Erythrocyte Nuclear Abnormalities in *O. niloticus* Exposed to River Water Samples

Exposure duration	Nuclear abnormality	Control groups		Water samples				
		Negative	Positive	1	2	3	4	5
2 days	Lobbed	0.40 ± 0.03[a]	2.30 ± 0.35[b]	0.51 ± 0.07	0.40 ± 0.03	0.90 ± 0.13[c]	1.10 ± 0.25[c]	1.00 ± 0.15[c]
	Blebbed	0.60 ± 0.06	2.90 ± 0.65[b]	0.58 ± 0.05	0.55 ± 0.05	0.80 ± 0.26	1.20 ± 0.07[c]	0.90 ± 0.09[c]
	Notched	0.84 ± 0.08	4.13 ± 1.10[b]	0.90 ± 0.09	0.70 ± 0.08	1.50 ± 0.40[c]	1.55 ± 0.37[c]	1.60 ± 0.43[c]
	Binuclei	0.40 ± 0.05	3.10 ± 0.77[b]	0.55 ± 0.06	0.50 ± 0.06	0.94 ± 0.05[c]	1.05 ± 0.06[d]	1.07 ± 0.07[d]
4 days	Lobbed	0.40 ± 0.03	3.50 ± 0.75[b]	0.56 ± 0.09	0.45 ± 0.08	1.45 ± 0.11[d]	1.55 ± 0.50[d]	1.50 ± 0.18[d]
	Blebbed	0.50 ± 0.03	2.80 ± 0.45[b]	0.50 ± 0.04	0.60 ± 0.08	1.50 ± 0.35[d]	1.28 ± 0.18[d]	1.20 ± 0.15[d]
	Notched	0.74 ± 0.05	4.90 ± 1.30[b]	0.65 ± 0.06	0.57 ± 0.09	1.52 ± 0.28[c]	1.35 ± 0.23[c]	1.90 ± 0.52[d]
	Binuclei	0.38 ± 0.07	3.40 ± 0.55[b]	0.60 ± 0.08	0.40 ± 0.02	1.10 ± 0.35[d]	1.25 ± 0.27[d]	1.16 ± 0.37[d]
6 days	Lobbed	0.51 ± 0.05	5.37 ± 0.75[b]	0.50 ± 0.05	0.50 ± 0.02	1.46 ± 0.20[d]	1.53 ± 0.35[d]	1.49 ± 0.15[d]
	Blebbed	0.40 ± 0.03	3.30 ± 0.59[d]	0.55 ± 0.07	0.56 ± 0.05	1.45 ± 0.28[d]	1.50 ± 0.30[d]	1.38 ± 0.35[d]
	Notched	0.80 ± 0.06	7.15 ± 1.26[b]	0.57 ± 0.03	0.62 ± 0.07	2.05 ± 0.40[c]	1.95 ± 0.34[c]	1.75 ± 0.20[c]
	Binuclei	0.62 ± 0.04	3.28 ± 0.63[b]	0.45 ± 0.08	0.50 ± 0.05	1.30 ± 0.31[d]	2.15 ± 0.63[d]	1.80 ± 0.44[d]

[a]Data expressed as cells with NAs/1,000 cells ± SD.
[b]$P < 0.001$; [c]$P < 0.05$; [d]$P < 0.01$; compared to negative control group. Positive control received cyclophosphamide; numbering of sampling stations is as shown in Figure 1.

level permitted by Turkish regulation (20 μg/l). Previous reports indicate that Cr(III) produces genetic damage by its direct interaction with DNA [Blasiak and Kowalik, 2000]. Cells absorb Cr(III) by passive diffusion [Smith, 1970; Ashby et al., 1995], where it may be trapped inside the cells, allowing toxic levels to be reached [Stearns et al., 1995]. Thus, total Cr levels are relevant to the toxicity of this metal and total Cr in effluents is strictly regulated in many countries.

Although the mechanism of Cu genotoxicity remains poory understood, Cu toxicity is believed to be due to nonspecific binding of the reactive metal cation, Cu^{2+}, to biologically important molecules [Gabbianelli et al., 2003]. In addition, high Cu concentrations lead to an increase in the rate of free radical formation [Gwozdzinski, 1995; Bogdanova et al., 2002], which may cause genotoxic effects. It was suggested that Cd genotoxicity is mainly manifested as single strand DNA breaks generated

by direct Cd-DNA interactions as well as by the action of incision nucleases and/or DNA-glycosylases during DNA repair [Privezentsev et al., 1996]. Ni compounds generate specific structural chromosomal damage as well as micronuclei [Sen et al., 1987; Arrouijal et al., 1990]. In addition to chromosomal damage, DNA-protein crosslinks and oxidative DNA base damage were observed in Ni(II)-exposed cells [Patierno et al., 1985; Kasprzak, 1991]. Also, Ni genotoxicity may be potentiated through the generation of DNA-damaging reactive oxygen species and the inhibition of DNA repair by this metal [Kazimierz et al., 2003]. The mechanisms of Pb genotoxicity may involve indirect damage to DNA affecting the stabilization of chromatin [Johansson and Pellicciari, 1998] or by the interaction of the metal with repair processes [Hartwig et al., 1990]. Also, Bonacker et al. [2005] reported that Pb salts may induce micronuclei by disturbing microtubule function.

In the recent years, considerable attention has been paid to the simultaneous expression of morphological NAs and micronuclei in the piscine MN test [Cavas and Ergene-Gozukara, 2005a,b; Da Silva Souza and Fontanetti, 2006; Matsumoto et al., 2006]. It has been suggested that problems in segregating tangled and attached chromosomes or gene amplification via the Breakage-Fusion-Bridge cycle could cause lobed nuclei or blebbed nuclei during the elimination of amplified DNA from the nucleus [Tolbert et al., 1992; Shimizu et al., 1998, 2000]. However, additional experimental evidence is needed to evaluate these hypotheses. We previously demonstrated the induction of NAs in the erythrocytes of *O. niloticus* exposed in the laboratory to a textile mill effluent as well as to CP [Cavas and Ergene-Gozukara, 2003]. In addition, Bombail et al. [2001] found increased erythrocyte MN and NA frequencies in *Pholis gunellus* specimens captured from polluted areas. We also observed elevated NA frequencies in peripheral erythrocytes of *Mugil cephalus* captured from polluted areas [Cavas and Ergene-Gozukara, 2005b]. In the present study NA frequencies were increased significantly in erythrocytes of tilapia exposed to water samples from the downstream regions of the Berdan River and the Kusun River.

Although significant increases in MN and NA frequencies were observed in all three tissues of fish exposed to water samples from downstream sites on the Berdan River system, regression analysis revealed that the correlation between heavy metal concentrations and MN frequencies was significant only in gill ($P < 0.05$) and fin ($P < 0.05$) epithelial cells, cells that are directly in contact with the water. No significant correlations were found between metal concentrations and the erythrocyte genotoxicity endpoints, with the exception of binucleus frequencies ($P < 0.05$). It is possible that the increases in erythrocyte MN and NA frequencies in fish exposed to downstream water samples could be due to other genotoxic chemicals, such as polycyclic aromatic hydrocarbons, that may present in the river water.

Our previous investigations on different fish species indicate that gill cells are more sensitive to MN induction than erythrocytes [Cavas and Ergene-Gozukara, 2003, Cavas and Ergene-Gozukara, 2005a,b]. Similarly, Hayashi et al. [1998] reported that MN frequencies in five different fish species captured from the field were significantly higher in gill cells than in erythrocytes. In the present study, comparative analysis in *O. niloticus* also revealed higher MN frequencies in gill cells than in erythrocytes for both control and treated fish. The use of gill cells may be superior to erythrocytes because they have higher mitotic indices, have higher background levels of micronuclei, and are directly exposed to environmental contaminants. This later property is shared by fin cells, which are in direct contact with the environment. The use of fish fin cells for in vivo analyses was first proposed by Arkhipchuk and Garanko [2005]. In this approach, fin cells with damaged DNA are forced to undergo division, during which micronuclei can be formed. Our preliminary results showed that 6 days of cell growth is necessary to obtain a large enough cell population from the regenerating fin tissue of *O. niloticus* to be able to perform the genotoxicity assays. As indicated by Arkhipchuk and Garanko [2005], the proposed approach solves the important bioethical task of keeping the fish under investigation alive. They also reported that MN frequencies in fin cells of *Carassius auratus gibelio* were increased following exposure to Cd, Cu, and chloral hydrate. Thus, our findings are in general agreement with those of Arkhipchuk and Garanko [2005].

In conclusion, water from the downstream stations on the Berdan River system was gentotoxic in the in vivo piscine MN test. The genotoxicity of the water samples generally correlated with their heavy metal content. These results highlight the genotoxicity of the pollution in this area, and suggest that industrial and domestic effluents discharged into the river may place local populations at risk of disease. Our results also indicate that fin cells maybe suitable for the evaluation of micronuclei in fish, and can be used in combination with blood and gill cells for piscine MN analysis and therefore deserve further testing for standardization. Finally, we recommend that Berdan River water be analyzed chemically for additional classes of toxicants to identify other contaminant(s) that may be responsible for the genotoxicity of river samples.

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D. G. Ennis