Environ Monit Assess (2010) 165:55–66
DOI 10.1007/s10661-009-0926-5

Detecting micronuclei frequency in some aquatic organisms for monitoring pollution of Izmir Bay (Western Turkey)

Özlem Çakal Arslan · Hatice Parlak · Selma Katalay · Meltem Boyacioglu · Muhammet Ali Karaaslan · Hale Guner

Received: 26 November 2008 / Accepted: 18 April 2009 / Published online: 15 May 2009

Abstract Micronuclei tests is a system of mutagenicity testing used for determining the pollution and chemicals causing changes in DNA fragments such as micronuclei in the cytoplasm of interphase cells. Damage caused on the DNA by genotoxic pollutants is the first consequence occurring in the aquatic organisms. Thus, it was attempted to determine whether pollution affected the erythrocytes and gills of fish *Gobius niger* and haemolymph and gills of mussels *Mytilus galloprovincialis* living in Izmir Bay at the level of DNA by the means of micronuclei (MN) test. Organisms used in the MN test were collected from seven locations (Alsancak, Alaybey Shipyard, Karsiyaka, Bostanli, Göztepe, Konak and Pasaport) which are known as the most polluted part of inner Bay of Izmir (Western Coast of Turkey). According to the results of the present study, frequency of MN was found at high level in Alaybey Shipyard and Pasaport where wastes from existing dockyard contributed to high level of pollution. In conclusion, this study indicates that the micronuclei test gives sensitive results in monitoring the pollution, especially the pollution of harbor, and thus it might be used as standard method in regular monitoring of pollution of coastal ecosystem.

Keywords Micronuclei test · *Mytilus galloprovincialis* · *Gobius niger* · Pollution · Genotoxicity

Ö. Çakal Arslan (✉) · H. Parlak · M. Boyacioglu · M. A. Karaaslan
Faculty of Fisheries, Department of Hydrobiology, Ege University, Bornova 35100, Izmir, Turkey
e-mail: ozlem.cakal@ege.edu.tr

S. Katalay · H. Guner
Faculty of Arts & Sciences, Biology Department, Celal Bayar University, Manisa, Turkey

Introduction

There are numberless pollutants in the surface waters and sediment that are compromising survival of the organisms, altering their physiologies or giving rise to carcinogenesis. Consequences caused by these pollutants may remain recessive for several generations or may exhibit major effects in the population. Pollution in the aquatic environment causes multiple damages in the organisms, at the level of population and ecosystem, as in organ function, reproductive stages and biological diversity.

Aquatic organisms are exposed to many xenobiotics during their lifespan both from the water and through aquatic food chain. Studies reveal

the fact that a number of chemicals contaminating the environment have carcinogenic or mutagenic effects. The major sources for the mutagenic and carcinogenic substances are industrial and agricultural activities. Xenobiotics from these sources ultimately contact the aquatic ecosystems. Damage on the DNA by genotoxic pollutants is the first consequence in the aquatic organisms and thus, aquatic organisms are used in most of genotoxicity studies. Although many hazardous substances exist in the water and sediment and they are accumulated by aquatic organisms and triggers DNA or cellular damage and even affects the ecosystem by passing through the tropic chain (Izquierdo et al. 2003), it is time-consuming, not possible and not economic to analytically determine the concentration of such substances in the tissues with the available chemical methods. Thus, the biological methods and those based on screening for carcinogenic and mutagenic substances in the tissues of indicator organisms have gained importance.

Recently, the studies to determine the effects of pollutants on the gene structure of organisms have increased. Genotoxicity of contaminated waters has been studied well using standard in vitro genotoxicity experiments (Vahl et al. 1997). Additionally, an effect of the genotoxins on aquatic species affected by the contaminated environment has been determined by in situ studies (Harvey et al. 1999). Studying DNA damage at the level of chromosome constitute a necessary part of genetic toxicology (Fenech 2000) because chromosomal mutation plays the most important role in cancer formation. Thus, biomarkers have been intensively used in research programs and their protocols as routine tests are being performed (Bolognesi et al. 2006). Of these test systems, micronuclei (MN) test is one of the most reliable techniques used to determine genetic changes in the organisms. MN test gives reliable results for complex mixtures. In recent years, this test has been improved using many aquatic organisms. MN experiments is a fast method in detecting the chromosomal damage because it make it possible to determine the remaining chromosomes and broken chromosomes due to its several advantages such as (a) giving more objective results than other tests in detecting chromosomal impairments, (b) being easy to learn, (c) it does not require to count the chromosomes to investigate the chromatids and chromosomal damage hard to detect and see in the metaphase stage, (d) its preparation stage is fast and (e) it makes it possible to count thousands of cells, not hundreds of cells in each experiment (OECD 2004).

MN test was originally developed in the mammalian cells (Schmid 1975) and then, it was applied to many different organisms including mussels in order to detect cytogenetic damage (Schmid 1975; Venier et al. 1997; Bolognesi et al. 1999). The fact that many organisms living in the water (i.e. bivalves, crustacean, polychaeta) depend directly or indirectly on food chain and that these organisms expose to carcinogenic or mutagenic agents has led to such experiments in marine organisms. Marine crustaceans may biologically accumulate a number of chemically diverse chemicals that are mutagenic or carcinogenic for man. Mussels, biological indicators in determining genotoxic pollution are preferred in most ecotoxicological studies as they are filter-feeding, live as sessile and are of economical interest. Mutagenicity tests make it possible to detect such chemicals causing pollution in aquatic ecosystem (Mitchell and Kennedy 1992; Park et al. 1993). Erythrocyte micronuclei test in fish is a method used in monitoring aquatic pollutants of mutagenic character by using a number of different species (De Flora et al. 1993). Kligerman (1982) reported that many micronuclei existed in fish subjected to pollution. Micronuclei frequency varies depending on the season, type of pollution and fish species. Fish are the most preferred organisms in MN tests because they are the main biomonitor affected by the changing environment where pollutants discharged. Furthermore, they are usually preferred for testing possible genotoxic characteristics of physical and chemical agents because they expose to very diverse chemical substances either directly via water or indirectly via food chain in the ecosystem and because they response to xenobiotics in similar way with mammalians.

In environmental mutagenesis, MN tests yield quite practical results in monitoring clastogenic and genotoxic effects of the pollutants. In order to obtain these results, aquatic organisms are usually used such as bivalvia *Mytilus*

galloprovincialis, *Crassotrea gigas* and *Chamelea galina* and fish rainbow trout *Oncorhynchus mykiss* and *Oreochromis niloticus* (Hooftman and Raat 1982; Manna et al. 1985; Metcalfe 1988; Rodriguez-Ariza et al. 1992; Al-Sabti et al. 1994; Cavas and Ergene-Gözükara 2003). In the previous studies, aquatic organisms exposed to contaminated waters were studied in order to determine effects of the genotoxins in their natural environments (Baršienė 1994; Baršienė and Baršytė 2000; Dixon et al. 2002). Fish and mussels are main indicators of health of the aquatic environment.

Izmir Bay, as study area, has extensive domestic and industrial pollution load. Variety of chemical wastes have been discharged into the Bay without treatment and in uncontrolled way because of rapidly increasing population of the city, extensive activities and capacities of several industries a growing exportation harbor since the year of 2000 when the municipal treatment system was introduced. Over the last 20 years, several research institutions studied impairment of ecosystem in Izmir Bay for both biological aspects such as decreasing species diversity and change in content of the pollutants such as heavy metals and pesticides in the tissues of several marine organisms as well as impairment in nutrient balance (Kucuksezgin et al. 2008a, b). Weak mutagenicity was found in a study by Boyacıoğlu (2004) on sediment samples to detect mutagenic substances in Izmir Bay. Following the municipality treatment plant introduced the Bay became cleaner day by day. As the most important agent is persistent organic pollutants (POPs) in marine ecosystem, the environment may not be considered being cleaned in a few years. So that some of the biological and reliable test must be applied to decide that the water or sediments include the residues of POPs.

Micronuclei test is a marker of cytogenetic damage caused by clastogenic or mutagenic compounds. Utilization of micronuclei test in determining genetoxic status of the aquatic environment is increasing rapidly (Al-Sabti and Metcalfe 1995; Hayashi et al. 1998) Due to present situation of Izmir Bay, the aim of the present study was to determine the level of the mutagenic effect by the means of micronuclei test using mussel, *M. galloprovincialis* and *black goby*, *Gobius Niger* Species.

Materials and methods

Study area

In order to determine whether pollution of mutagenic/carcinogenic origin existed at the cellular level in Izmir Bay, tests were carried out using gills, haemolymph and blood samples of the aquatic organisms (mussel and fish) obtained in winter and summer seasons from 1—Alsancak Harbor, 2—Alaybey Shipyard, 3—Karsiyaka, 4—Bostanli, 5—Göztepe, 6—Konak and 7—Pasaport stations.

For the MN test, 15 individuals of *M. galloprovincialis* 6 ± 3 cm in size obtained from harbor feet in Alsancak Harbor, Alaybey Shipyard, Karsiyaka, Bostanli, Göztepe, Konak and Pasaport locations and 20 individuals of *G. niger* (coal dealer goby fish) in 10 ± 3 cm caught by hooking were used (Fig. 1).

Mussel micronuclei test

Gill

Enzymatic digestion is required to obtain interphase cells when tissues are to be used in the micronuclei test. For this purpose, gills of the mussels opened by cutting the adductor muscle (Fig. 2) were kept for low enzymatic activity at 4°C in 0.25% trypsin solution and then trypsin was removed by centrifuging at 1,000 rpm. This process was repeated twice and the pellet was fixed with ethanol–acetic acid (3:1) (modified from Hayashi et al. 1998).

The fixed pellet was smeared on slide and three preparations were prepared for each individual. Then, dried samples were stained with Giemsa (5%, Sigma, Aldrich) and then kept in ethanol for 10 min. The cells with MN and BN were counted at ×100 magnification (immersion oil) by light microscopy. Five hundred gill cells from each preparation and 1,500 from each individual were inspected (22,500 cells per location).

Haemolymph

For the MN test, haemolymph taken with thin-tipped syringe is mixed with a fixative

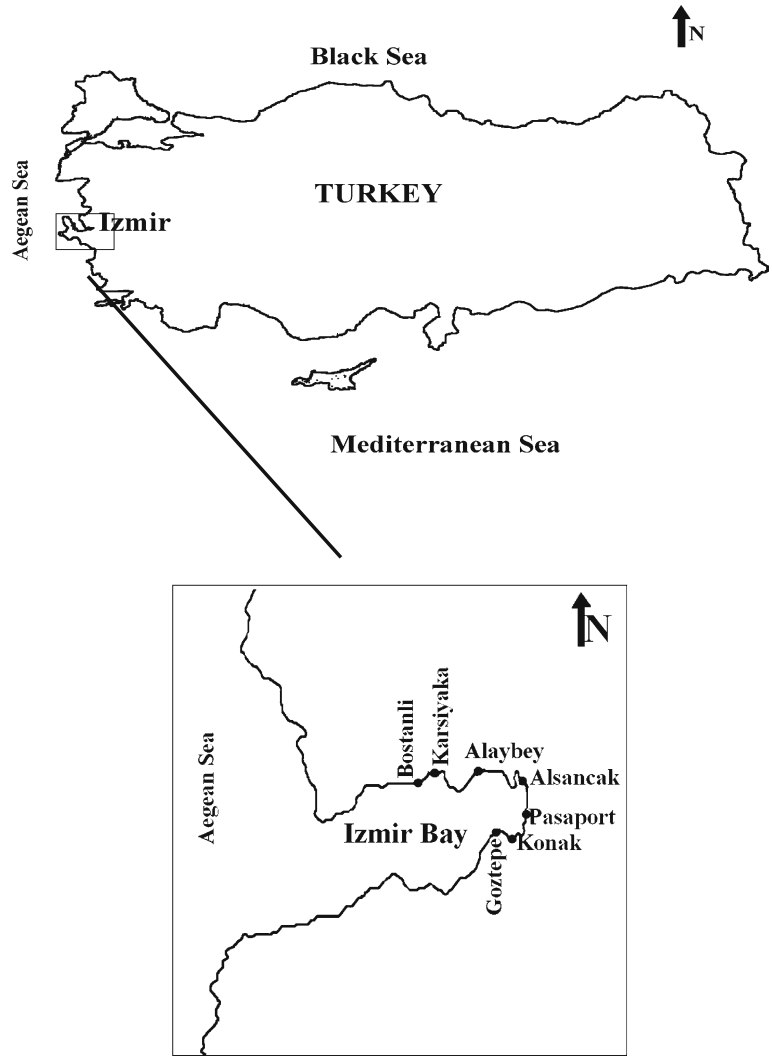

Fig. 1 Sampling site and stations of Izmir Bay (1—Alsancak Harbour, 2—Alaybey Shipyard, 3—Karsiyaka, 4—Bostanli, 5—Göztepe, 6—Konak, 7—Pasaport)

(3:1 methanol–acetic acid) and centrifuged at 1,000 rpm. The supernatant is removed after the fixation. The pellet is smeared on the slide then fixed with methanol for 10 min. It is allowed to dry, then stained with 5% Giemsa followed by rinsing with distilled water and closed with a cover slide. The preparation is covered by entelan to avoid exposure to the air (Woźnicki et al. 2004). No less than 500 cells are examined for each individuals and MN/BN formation and frequency is counted. A total of 1,500 cells were counted for each individual and MN/BN frequency was calculated as ‰ number of MN and BN.

Fish (*G. niger*) micronuclei test

Blood

Goby fish *G. niger* used in this study is considered as one of the indicator organisms for polluted environments in Mediterranean. The samples were collected from Bostanli and Pasaport coasts using hand hooks. No tests were performed for Alsancak Harbor and Alaybey Shipyard stations because no fish could be hooked at these locations. During the study, a total of 50 fish were examined of which mean total length was 10.3 ±

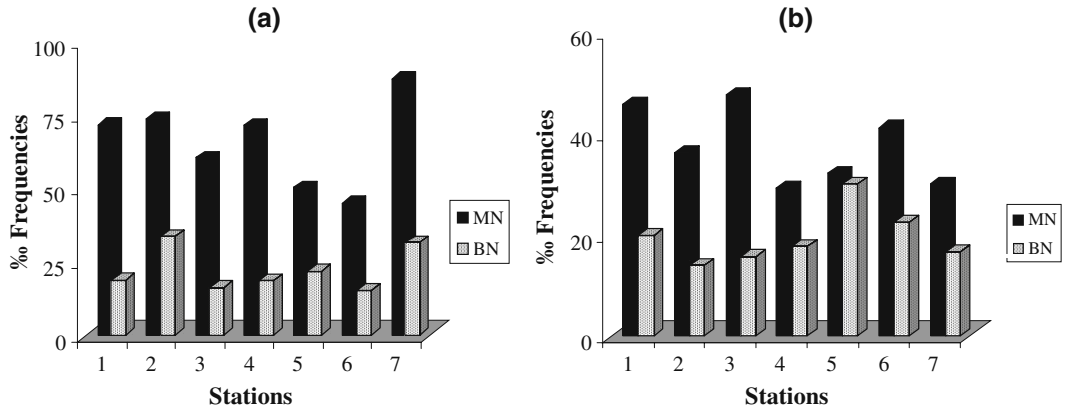

Fig. 2 Nuclear abnormalities observed in **a** gill and **b** haemolymph cells of *M. galloprovincialis* (mean ± standard error). *1* Alsancak Harbour, *2* Alaybey Shipyard, *3* Karsiyaka, *4* Bostanli, *5* Göztepe, *6* Konak, *7* Pasaport

1.65 cm. Periferic blood, 0.5 ml, sample was taken by caudal vein of the fish into 75-µl microhematocrit tubes by dissecting their caudal fins.

The preparations was made by smearing one drop of blood on the slide and kept in May–Grünwalt fixative for 15 min. They were dried out for 1 h and then they were rinsed with distilled water. As mentioned before, three preparations were made for each individual and 500 cells were examined for each preparation.

The preparations rinsed by keeping for 20 min in Giemsa stain diluted to 1/10 with distilled water were examined with light microscope after they were dried and formation of micronuclei and binuclei were examined in the blood cells.

Gill

For the micronuclei test, fish gills dissected by scissor were fixed in acetic acid. The samples were centrifuged at 2,000 rpm for 10 min in order to obtain epithelial cells shed from gill tissue by using acetic acid and pipette. The pellet obtained by this procedure is smeared on the slide and fixed by methanol. It is allowed to dry. Then, it is stained with 5% Giemsa and the slide is covered with entelan after.

Statistical analysis

Frequency of micronuclei and binuclei in the samples obtained from several locations in Izmir Bay during the winter and summer seasons were calculated based on 1,000 cells. Mann–Whitney and Student's *t* test were used to compare nuclear abnormalities (BN and MN frequencies) between the sampling locations. All statistical analyses were performed by Statistica 6.0 statistics software.

Results

The present study attempted to determine whether genotoxic potential existed in the environment of Izmir Bay using micronuclei test of indicator organisms. Frequency of BN and MN was determined. During the examinations, other nuclear abnormalities were observed such as nuclear bats but they were not included in the calculations since they were of statistically unimportant numbers.

Micronuclei test with *M. galloprovincialis*

BN and MN frequencies were calculated in 1,500 cells from gills and haemolymphs of the mussels

Table 1 Nuclear abnormalities observed in gill and haemolymph cells of mussels (mean ± standard error)

Gill cells				Haemolymph cells		
Station	N ($n = 15$)	MN ($n = 15$)	BN ($n = 15$)	N ($n = 15$)	MN ($n = 15$)	BN ($n = 15$)
1	909.98 ± 32	71.40 ± 27.72	18.62 ± 7.01	934.67 ± 20.13	45.64 ± 16	19.69 ± 8.8
2	893.20 ± 48	73.40 ± 32.26	33.40 ± 21.0	950.31 ± 20.94	35.96 ± 17.2	13.73 ± 11.9
3	914.22 ± 25.7	62.22 ± 18.54	18.54 ± 23.6	936.9 ± 19.4	47.6 ± 19.2	15.6 ± 6.4
4	910.58 ± 23.8	71.06 ± 20.9	18.35 ± 14.6	975.58 ± 140.2	29.13 ± 14.5	17.51 ± 11.9
5	928.4 ± 20.2	49.95 ± 14.4	21.55 ± 9.1	938 ± 30.5	32.1 ± 19.3	29.9 ± 21.2
6	940.22 ± 19.9	44.7 ± 13.3	15.06 ± 10.1	936.5 ± 35.2	40.98 ± 18	22.5 ± 23.1
7	881.82 ± 31.7	86.76 ± 27.3	31.42 ± 13.2	953.78 ± 24.2	29.82 ± 16.7	16.40 ± 12.4

1 Alsancak Harbour, *2* Alaybey Shipyard, *3* Karsiyaka, *4* Bostanli, *5* Göztepe, *6* Konak, *7* Pasaport

taken from Izmir Bay. Table 1 shows the nuclear abnormalities in the gills of mussels from Izmir Bay. According to examinations of mussel gills, MN frequency detected from 1,500 gill cells ranged between 30.56–89.76‰ and BN frequency between 6.19‰ and 33.40‰.

As can be seen in Table 1 and Fig. 2, higher MN and BN frequencies were found in mussel gills taken from station 2 and 7 than those taken from others. As mentioned previously, considering that micronuclei and binuclei frequencies vary depending on pollution one may argue that these two locations are more polluted than others. Determined MN frequencies showed statistically significant differences when micronuclei and binuclei frequencies were compared statistically between locations ($p < 0.005$) (Fig. 3). Significant difference was found when BN frequencies obtained by observing the gill cells (1,500 cells) were compared between the locations ($p < 0.0001$).

Micronuclei and their frequency found as a consequence of microscopic examinations in haemolymph cells of 15 individual of *M. galloprovincialis* from each location was shown in Table 2. As seen in the Table 2, MN frequency ranged between 29.13‰ and 47.55‰ and BN frequency between 13.73‰ and 29.9‰. When the frequencies were compared with those of the gill cells, higher BN and MN frequencies in the gill cells indicate that gills are better marker in micronuclei test comparing on mussels.

According to microscopic counting on haemolymph cells, as can be understood from Table 1 and Fig. 2, station 2 seems to have highest numbers in terms of both BN and MN frequencies. Statistically significant difference was found between MN and BN values when micronuclei and binuclei frequencies of the locations ($p < 0.05$) (Fig. 3). Although significant difference was found between MN values when micronuclei and binuclei frequencies were compared statistically between the locations, no significant difference was found as a result of statistical comparison between other locations.

Micronuclei test with *G. niger*

MN and BN frequencies were calculated based on microscopic examinations of slides with blood and gill cells of goby fish *G. niger*. Resultant data were compared statistically.

Two types of nuclear abnormality were found in the present study on epithelial gill cells of *G. niger* as in the samples of mussel gills and haemolymph. Furthermore, abnormalities in lobulated and devised forms were also observed in the nuclear structure but they were neglected in the calculations because they were few in number.

Table 2 shows averages and standard errors of nuclear abnormalities in epithelial gill cells of fish from two sampling locations (station 4 and 7). Micronuclei averages ranged between 24‰ and 29‰ and binuclei averages between 31‰ and 58‰. MN and BN frequencies were found at higher level in fish from station 7. No significant difference was found when frequencies of micronuclei and binuclei found in gill cells of G. *niger* caught from Izmir Bay were statistically compared between the locations (Fig. 4).

Table 2 gives the averages and standard errors of nuclear abnormalities in blood cells of fish from two sampling locations (station 4 and 7). Mean

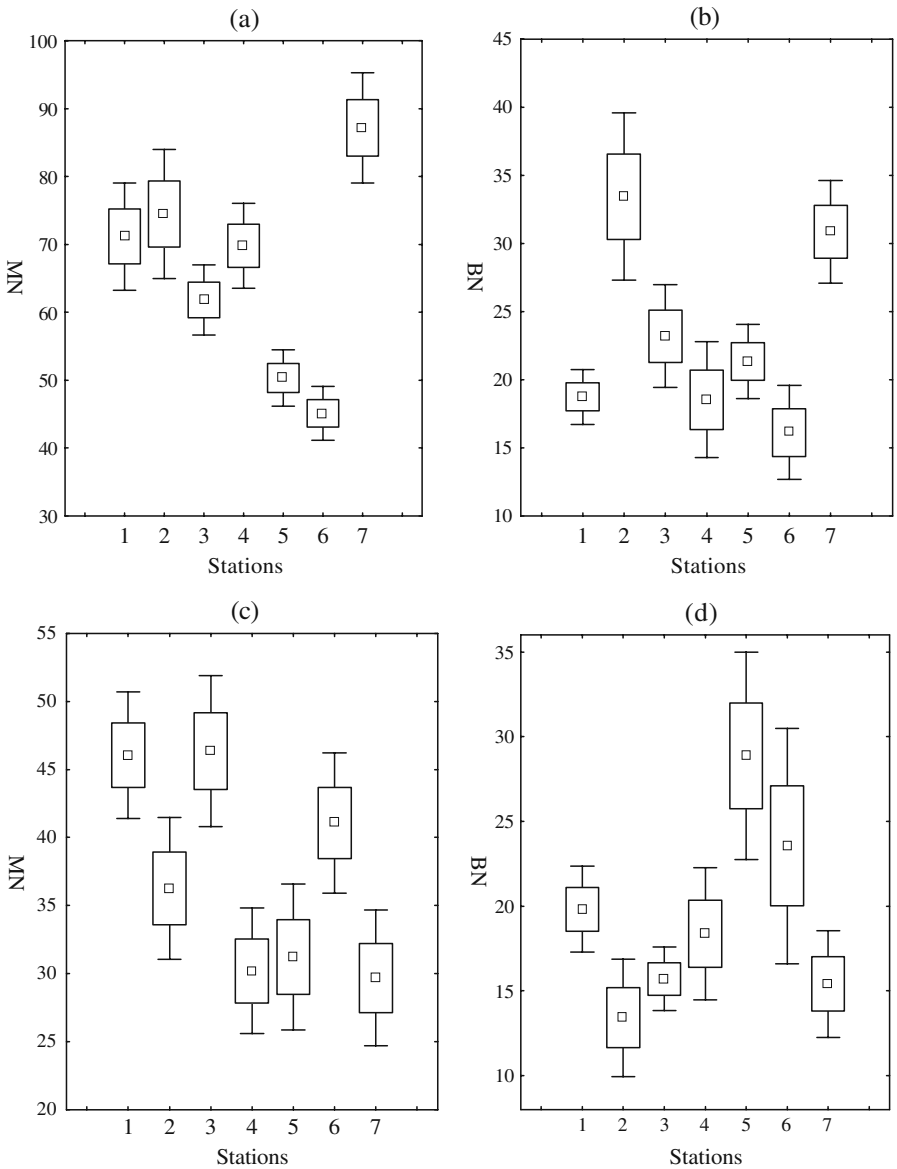

Fig. 3 Distribution of MN and BN frequencies in **a–b** gill and **c–d** haemolymph of *M. galloprovincialis* by stations and statistical comparison. *1* Alsancak Harbour, *2* Alaybey Shipyard, *3* Karsiyaka, *4* Bostanli, *5* Göztepe, *6* Konak, *7* Pasaport

micronuclei frequencies ranged between 24‰ and 26‰ and mean binuclei frequencies between 4.9‰ and 13‰. Although MN frequency was found to be higher at station 4 (26.9‰), BN frequency in blood cells of fish from station 7 was found to be much higher (13‰) than in those from 4.

With effect of density of pollution, the presence of MN in a cell increases or formation of

Table 2 Nuclear abnormalities observed in gill and blood cells of *G. niger* (mean ± standard error)

Gill				Blood		
Station	N ($n = 25$)	MN ($n = 25$)	BN ($n = 25$)	N ($n = 25$)	MN ($n = 25$)	BN ($n = 25$)
4	940.60 ± 74	24.00 ± 36.4	31.38 ± 31.1	968.2 ± 4.2	26.90 ± 12.7	4.90 ± 6.2
7	923.50 ± 65.9	29.00 ± 22.5	58.67 ± 53.2	963 ± 7.74	24.00 ± 13.3	13.0 ± 17.1

4 Bostanli, *7* Pasaport

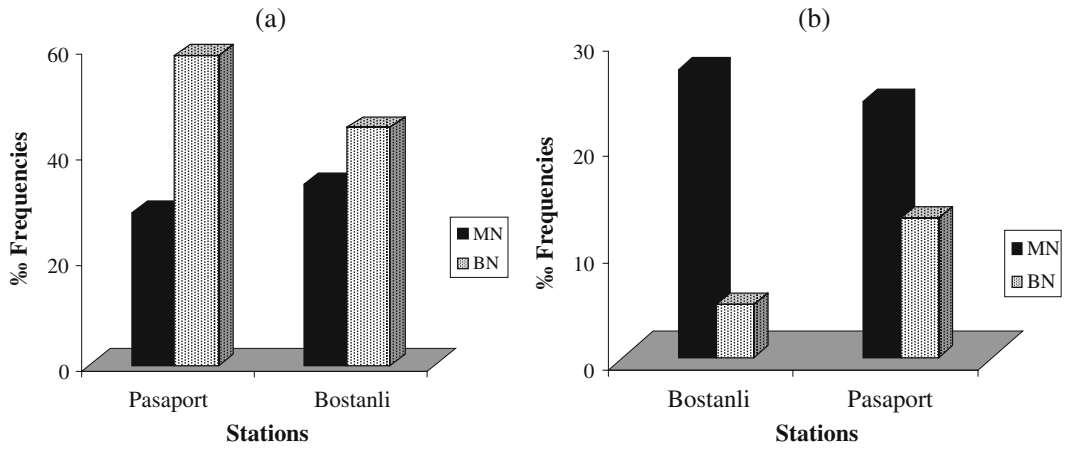

Fig. 4 Nuclear abnormalities observed in **a** gill and **b** blood cells of *G. niger* (mean ± standard error). 4—Bostanli, 7—Pasaport

BN became observed. Formation of BN has been found at high level where pollution is extensive. Thus, station 7 was observed to be more polluted (Fig. 4). Based on observations on erythrocytes, there was no statistically important difference was found between the locations in frequencies of both micronuclei and binuclei.

Discussion

Genotoxic effects of the pollutants were examined in micronuclei (MN) tests carried out with haemolymph, erythrocyte and gill cells of *M. galloprovincialis* and *G. niger* in Izmir Bay. These tests were used as biomarker because MN test gave reliable results and due to its easy applicability. The present study indicates environmental hazard of pollutants present in Izmir Bay although type and amount of these pollutants remain unknown.

Based on the results of BN and MN counting on erythrocyte and epithelial gill cells from the fish, it was determined that mutagenic potential of station 7 was more than that of station 4 although the difference between the two locations was statistically insignificant.

MN and BN frequencies were found to be highest in the samples from station 1 according to the tests on mussel haemolymph cells. It was found that statistically significant differences existed between BN and MN frequencies when the locations were compared statistically ($p < 0.05$). Based on the microscopic observations on epithelial gill cells of the mussels to determine abnormalities in the cell nucleus, the highest MN frequency was found in Pasaport station (station 7) whereas the lowest one was found at station 2.

We do not have reference values because we are not sure about the cleanness; thus results from the fish and mussel samples were evaluated to make comparisons with those from the study with the same species by Bolognesi et al. (1999, 2006; MN frequency in the control group, ‰2.8 to 30.8‰).

Erythrocytes are widely used in the micronuclei experiments (Arkhipchuk and Garanko 2005). Such studies are recommended because gill cells as well exhibit high sensitivity to the agents promoting formation of micronuclei (Hayashi et al. 1998). Generally, gills are metabolically active tissues because their cells are under the influence of aquatic circulation system (Arkhipchuk and Garanko 2005).

Some intra-species factors that may influence the results in the MN test include age, gender, feeding status, health, reproductive status and genetic ancestors. Fish may exhibit differences for developing MN due to gender. Extent of cytogenetic damage may vary depending on hormonal status (Virgano et al. 1993).

Increase in number of MN is indirect marker of numeric and structural chromosomal irregularities cause in the cells by many agents. With increasing

environmental pollution, the organisms are increasingly exposed to negative effects of toxic, carcinogenic and mutagenic agents. In the present study, thus, the genetic damage in the organisms at locations where extensive pollution exists has been determined using MN test.

Al-Sabti et al. (1994) observed that frequency of forming MN was the same both in situ groups and those subjected to chromium in the laboratory when they examined the fish they collected from Ljubljanica River and those subjected to 50 ng/ml of chromium in the laboratory (Bryan 1976). Buschini et al. (2004) determined micronuclei frequency by subjecting *Cyprinus carpio* species to lake water in order to determine genotoxic potential of surface water of Castiglione del Lago Lake, Italy. The investigators kept young individuals of *C. carpio* to the lake water for 20 days and then determined MN frequency and other nuclear abnormalities in the erythrocytes from blood taken from the fish. As a result, they reported that MN frequency increased in the fish population as exposure time increased, there were no differences in MN frequency among the species and substantially higher MN frequency was observed in the fish exposed to sodium hypochloride compared to those from clean areas.

In order to determine genotoxic potential of Cai River which was contaminated by petrochemical pollution of Brazil, Lemos et al. (2007) determined micronuclei frequency in the blood samples of *Pimaphales promelas* fish species in that region. MN frequency was found to be 1.6 ± 1.8‰ in samples from control group of the study. MN frequency in the blood samples from the fish exposed to river water in different periods was found to be similar to that of the control group (7.88 ± 5.99‰). This study when compared with our investigations Izmir Bay was found a very polluted area by MN frequencies in fish gill and blood (ranging between 24‰ and 29‰).

There are many studies both on organisms collected from their natural environment and those exposed to chemicals. In previous studies, Cavas and Ergene-Gözükara (2003) determined MN frequencies and other nuclear abnormalities in both gill cells and blood from *Oreochromis niloticus* species subjected to wastes of textile industry. The investigators reported that MN frequency increased parallel to the increase in exposure time and concentration in the gill cells although both micronuclei and nuclear abnormalities decreased as subjection time and concentration in the erythrocytes increased. They noted that difference between two types of cell was related to cellular kinetics and cellular renewal and as a second option they noted that the difference was due to the fact that gill cells were affected by directly and continuously contaminated waters.

Klobucar et al. (2003) performed genotoxicity assessment via MN test in hemocytes of *Dreissena polymorpha*. For this purpose, samples collected from Drava River were transferred to four areas with different pollutant density (Drava, Zagreb, Oborovo, Sisak and Lukavec) and subjected to complex pollutants. The lowest level of MN frequency in obtained mussel haemolymph was observed in Drava River, the reference area (5‰). Higher MN frequencies were observed in Lukavec contaminated by chemical wastes from Sisak region (27‰), in Sisak contaminated by waste water from petroleum refinery (52‰), in Oborovo which was closest to the intermediately polluted area and thus almost similarly affected by contaminants as Zagreb (3.1%). No increase was observed in MN frequency in mussels from intermediately polluted Zagreb area containing wastewater from pharmacological and food industries. Results we obtained from field studies are compatible with those from the study above.

In a study by Venier and Zampieron (2005) to determine genetic damage in *M. galloprovincialis* and *Zosterisessor ophiocephalus*, two species in Venice Lagoon in Italy, it was reported that genetic damage existed by examining MN and cellular abnormalities in haemolymph and gill tissues (five individuals from each location). MN frequency was reported to range between 33‰ and 37‰ in that study. As a result of the study, it was reported that the species naturally existing in the Lagoon were subjected to pollution causing genetic damage. Three different species of fish were used for micronuclei tests in the monitoring study to determine environmental mutagens in different areas of Baltic Sea (Baršienė et al. 2006). As a result of the study, it was reported

that the pollutants causing genetic damage existed in the environment as understood from observation of MN formations. Meanwhile, where more mutagenic-carcinogenic pollutants existed was determined by finding changes in MN frequencies among the locations. In the study by Barsiene et al. on Baltic Sea in 2006, it was reported that increase was observed in micronuclei formation due to increase in pollution.

In a study by Dolcetti and Venier (2002), MN frequencies in Mediterranean mussel *M. galloprovincialis* was examined to determine genetic damage in both the individuals collected from its natural environment and those subjected to benzopyrene in laboratory setting. In that study, it was reported that MN formation was observed in the mussels (12 alive) collected from different areas in different periods and that micronuclei frequency found in the gills (about 8.5‰) was higher than that found in the haemolymph (about 48). And it was noted that MN frequency increased parallel to the increase in pollution for long years.

Micronuclei test was performed in brachial cells of *Mytilus edulis* in order to determine pollution on two different coastal areas discharging domestic waste (Gijon and Pueto Madryn) and on locations of Puerto Madryn discharging industrial waste. For the mussels collected from three different locations, MN test was shown to be sensitive in monitoring domestic pollution and proposed as bioindicator in routine pollution investigations in coastal ecosystems (Izquierdo et al. 2003).

Izquierdo et al. (2003) performed MN test in the branchial cells of *M. edulis* from Gijon containing domestic waste and Madryn containing domestic and industrial waste. For the three locations, no effect was observed in micronuclei assessment in the samples taken while removing from the effect. Mean micronuclei frequency ranged between 1.42 ± 1.38‰ and 17.5 ± 2.61‰ when all samples from Gijon and Argentine were compared. MN frequency was 5.75 ± 1.42‰ in the samples from the location closest to Gijon area whereas it was calculated to be 1.42 ± 1.38‰ in the samples from the location outer most from the effect. MN frequency was 11.58 ± 2.93‰ in the samples from the location of Argentine closest to domestic discharge whereas it was calculated as 4.5 ± 1.62‰ in the samples from the location farthest from the effect. MN frequency was calculated to be 17.5 ± 2.61‰ in the samples from the location of Argentine closest to the industrially polluted whereas it was found to be 8.17 ± 1.34 in the samples from the location farthest from the effect. In the view of these results, it was observed that micronuclei frequency decreased in the mussel samples as the distance from the effect increased. In the current study it was observed that micronuclei frequency was highest in the mussel samples from station 2 known to be the most polluted area while it decreased as the distance from the effect increased (4, 5, 6 and 7). This conclusion is conformable to our study.

Dailianis et al. (2003) performed MN assessment in haemolymph and gills of *M. galloprovincialis* collected from Thermaikos and Strymonikos Bays (South Greece) between July and October, 2001. As a result of MN assessment either gill or haemolymph tissues, it was observed that no significant difference existed between seasons when samplings in June and October were compared.

Based on this literature information, the current study did not consider the relationship of seasonal factors mentioned to MN frequency since no significant effect of the mentioned seasonal factors was observed on MN frequency. In our study when micronuclei frequencies at gill and blood cells of *G. niger* and gill and haemolymph cells of *M. galloprovincialis* compared with previous studies, Izmir Bay was found polluted by mutagenic and genotoxic compound.

In conclusion, the current study indicates that MN test in the mussels and fish yields sensitive results in monitoring pollution; especially the harbor pollution and thus, it might be used as a standard method in regularly monitoring the pollution of coastal ecosystem.

Acknowledgement The present study was conducted in the context of Scientific Research Project of Ege University Faculty of Fisheries, Hydrobiology Department (Project No.: 2006/SÜF/008).

References

Al-Sabti, K., Franko, M., Andrijanic, B., Knez, S., & Stegnar, P. (1994). Chromium induced micronuclei

in fish. *Journal of Applied Toxicology, 14*, 333–336. doi:10.1002/jat.2550140503.

Al-Sabti, K., & Metcalfe, C. D. (1995). Fish micronuclei for assessing genotoxicity in water. *Mutation Research, 343*, 121–135. doi:10.1016/0165-1218(95)90078-0.

Arkhipchuk, V. V., & Garanko, N. N. (2005). Using the nucleolar biomarker and the micronucleus test on in vivo fish fin cells. *Ecotoxicology and Environmental Safety, 62*, 42–52. doi:10.1016/j.ecoenv.2005.01.001.

Baršienė, J. (1994). Chromosome set changes in mollusks from highly polluted habitats. In A. Beaumont (Ed.), *Genetics and evolution of aquatic organisms* (pp. 434–446). London: Chapman, Hall.

Baršienė, J., & Baršytė, L. D. (2000). Environmental genotoxicity in Klaipeda port area. *International Review of Hydrobiology, 85*, 663–672. doi:10.1002/1522-2632(200011)85:5/6<663::AID-IROH663>3.0.CO;2-S.

Baršienė, J., Schiedek, D., Rybakovas, A., Šyvokienė, J., Kopecka, J., & Förlin, L. (2006). Cytogenetic and cytotoxic effects in gill cells of the blue mussel *Mytilus* spp. from different zones of the Baltic Sea. *Marine Pollution Bulletin, 53*(8–9), 469–478. doi:10.1016/j.marpolbul.2005.11.015.

Bolognesi, C., Landini, E., Roggieri, P., Fabbri, R., & Viarengo, A. (1999). Genotoxicity biomarkers in the assessment of heavy metal effects in mussels: Experimental studies. *Environmental and Molecular Mutagenesis, 33*, 287–292. doi:10.1002/(SICI)1098-2280(1999)33:4<287::AID-EM5>3.0.CO;2-G.

Bolognesi, C., Perrone, E., Roggieri, P., & Sciutto, A. (2006). Bioindicators in monitoring long term genotoxic impact of oil spill: Haven case study. *Marine Environmental Research, 62*, 287–291. doi:10.1016/j.marenvres.2006.04.047.

Boyacıoğlu, M. (2004). İzmir Körfezi sedimentlerinde direkt mutajenlerin belirlenmesi. *E.U. Su ürünleri Dergisi, 21*(1–2), 23–27

Bryan, G. W. (1976). Some aspects of heavy metal tolerance in aquatic organisms. In A. P. M. Lockwood (Ed.), *Effects of pollutants on aquatic organisms* (pp. 7–34). London: Cambridge University Pres.

Buschini, A., Martino, A., Gustavino, B., Monfrinotti, M., Poli, P., & Rossi, C. (2004). Comet assay and micronucleus test in circulating erythrocytes of *Cyprinus carpio* specimens exposed in situ to lake waters treated with disinfectants for potabilization. *Mutation Research, 557*, 119–129.

Cavas, T., & Ergene-Gözükara, S. (2003). Micronuclei, nuclear lesions and interphase silver stained nucleolar organizer regions (AgNORs) as cyto-genotoxicity indicators in Oreochromis niloticus exposed to textile mill effluent. *Mutation Research, 534*, 93–99.

Dailianis, S., Domouhtsidou, G. P., Raftopoulou, E., Kalayianni, M., & Dimitriadis, V. K. (2003). Evaluation of neutral red retention assay, micronucleus test, acetylcholinesterase activity and a signal transduction molecule (cAMP) in tissues of *Mytilus galloprovincialis* (L.), in pollution monitoring. *Marine Environmental Research, 56*, 443–470. doi:10.1016/S0141-1136(03)00005-9.

De Flora, S., Vigario, L., D'Agostini, F., Camoirano, A., Bagnasco, M., Bennecelli, C., et al. (1993). Multiple biomarkers in fish exposed in situ to polluted river water. *Mutation Research, 319*, 167–177. doi:10.1016/0165-1218(93)90076-P.

Dixon, D. R., Pruski, A. M., Dixon, L. R. J., & Jha, A. N. (2002). Marine invertebrate eco-genotoxicology: A methodological overview. *Mutagenesis, 17*, 495–507. doi:10.1093/mutage/17.6.495.

Dolcetti, L., & Venier, P. (2002). Susceptibility to genetic damage and cell types in Mediterranean mussels. *Marine Environmental Research, 54*, 487–491. doi:10.1016/S0141-1136(02)00142-3.

Fenech, M. (2000). The in vitro micronucleus technique. *Mutation Research, 455*, 81–95. doi:10.1016/S0027-5107(00)00065-8.

Harvey, J. S., Lyons, B. P., Page, T. S., Stewart, C., & Parry, J. M. (1999). An assessment of the genotoxic impact of the sea empress oil spill by the measurement of DNA adducts levels in selected invertebrate and vertebrate species. *Mutation Research, 441*, 103–114.

Hayashi, M., Ueda, T., Uyeno, K., Wada, K., Kinae, N., Saotome, K., et al. (1998). Development of genotoxicity assay systems that use aquatic organisms. *Mutation Research, 399*, 125–133. doi:10.1016/S0027-5107(97)00251-0.

Hooftman, R. N., & Raat, W. K. (1982). Induction of nuclear anomalies (micronuclei) in the peripheral blood erythrocytes of the eastern mudminow *Umbra pygmaea* by ethylmethanesulphonata. *Mutation Research, 104*, 147–152. doi:10.1016/0165-7992(82)90136-1.

Izquierdo, J. I., Machado, G., Ayllon, F., d'Amico, V. L., Bala, L. O., Vallarino, E., et al. (2003). Assessing pollution in coastal ecosystems: A preliminary survey using the micronucleus test in the mussel *Mytilus edulis*. *Ecotoxicology and Environmental Safety, 55*, 24–29. doi:10.1016/S0147-6513(02)00041-6.

Kligerman, D. (1982). Fishes as biological detectors of the effects of genotoxic agents. In J. Heddle (Ed.), *Mutagenicity: New horizons in genetic toxicology* (pp. 435–456). New York: Academic Press.

Klobucar, G. I. V., Pavlica, M., Erben, R., & Papes, D. (2003). Application of the micronucleus and comet assays to mussel *Dreissena polymorpha* haemocytes for genotoxicity monitoring of freshwater environments. *Aquatic Toxicology (Amsterdam, Netherlands), 64*, 15–23. doi:10.1016/S0166-445X(03)00009-2.

Kucuksezgin, F., Kayatekin, B. M., Uluturhan, E., Uysal, N., Acikgoz, O., & Gonenc, S. (2008a). Preliminary investigation of sensitive biomarkers of trace metal pollution in mussel (*Mytilus galloprovincialis*) from Izmir Bay (Turkey). *Environmental Monitoring and Assessment, 141*, 339–345. doi:10.1007/s10661-007-9900-2.

Kucuksezgin, F., Uluturhan, E., & Batki, H. (2008b). Distribution of heavy metals in water, particulate matter and sediments of Gediz River (Eastern Aegean). *Environmental Monitoring and Assessment, 141*, 213–225. doi:10.1007/s10661-007-9889-6.

Lemos, C. T., Rödel, P. M., Terra, N. R., D'Avila, O. N. C., & Erdtmann, B. (2007). River water genotoxicity

evaluation using micronucleus assay in fish erythrocytes. *Ecotoxicology and Environmental Safety, 66*(3), 391–401. doi:10.1016/j.ecoenv.2006.01.004.

Manna, G. K., Banerjee, G., & Gupta, S. (1985). Micronucleus test in the peripheral erythrocytes of the exotic fish. *The Nucleus, 23*, 176–179.

Metcalfe, C. D. (1988). Induction of micronuclei and nuclear abnormalities in the erythrocytes of mudminowa (*Umbra limi*) and brown bulheads (*Ictalurus nebulosus*). *Bulletin of Environmental Contamination and Toxicology, 40*, 489–495. doi:10.1007/BF01688371.

Mitchell, S., & Kennedy, S. (1992). Tissue concentrations of organochlorine compounds in common seals from the coast of Northern Ireland. *The Science of the Total Environment, 115*, 235–240. doi:10.1016/0048-9697(92)90040-Y.

OECD (2004). Oecd guideline for the testing of chemicals draft proposal for a new guideline 487: In Vitro micronucleus test, 487.

Park, E., Lee, J., Etoh, H., & Etoh, Y. I. A. (1993). Fish cell line (ULF-23HU) derived from the fin of the central mud minnow (*Umbra limi*): Suitable characteristics for clastogenicity assay. *In Vitro Cellular & Developmental Biology, 25*, 987–994. doi:10.1007/BF02624131.

Rodriguez-Ariza, A., Abril, N., Navas, J. I., Dorado, G., Lopez-Barea, J., & Pueyo, C. (1992). Metal mutagenicity and biochemical studies on bivalve mollusks from Spanish Coasts. *Environmental and Molecular Mutagenesis, 19*, 112–124. doi:10.1002/em.2850190205.

Schmid, W. (1975). The micronucleus test. *Mutation Research, 31*, 9–15.

Vahl, H. H., Karbe, L., & Westendorff, J. (1997). Genotoxicity assessment of suspended particulate matter in the Elbe River, comparison of Salmonella microsome test, arabinosine resistance test, and umu-test. *Mutation Research, 394*, 81–93.

Venier, P., Maron, S., & Canova, S. (1997). Detection of micronuclei in gill cells and haemocytes of mussels exposed to benzo[*a*]pyrene. *Mutation Research, 390*, 33–44.

Venier, P., & Zampieron, C. (2005). Evidence of genetic damage in grass gobies and mussels from the Venice lagoon. *Environment International, 31*, 1053–1064. doi:10.1016/j.envint.2005.05.016.

Virgano, L. A., Bagnasco, M., Bennicelli, C., & Melodia, F. (1993). Xenobiotic metabolizing enzymes in uninduced and induced rainbow trout (*O.mykiss*): Effects of diets and food deprivation. *Comparative Biochemistry and Physiology, 104*, 51–55.

Woźnicki, P., Lewandowska, R., Brzuzan, P., Ziomek, E., & Bardega, R. (2004). The level of DNA damage and the frequency of micronuclei in haemolymph of fresh water mussels *Anodonta woodiana* exposed to Benzo[a] pyrene. *Acta Toxicologa, 12*(1), 41–45.