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Study of Mutagenicity of Metabolized Pollutants in Some Tissues of Black Gobby (*Gobius niger*) and Black Mussels (*Mytilus galloprovincialis*) from Izmir Bay (Western Turkey)

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Abstract: In the present study, fish and mussel samples from Izmir bay of Agean sea were studied for their mutagenic potential in TA98 and TA100 strains of *Salmonella typhimurium* using Ames test (plate incorporation assay) without metabolic activation. Black gobby (*Gobius niger*) samples were collected from Bostanli and Pasaport locations and mussel (*Mytilus galloprovincialis*) samples were collected from Alsancak, Alsancak harbour, Alaybey Shipyard, Karsiyaka, Bostanli, Goztepe, Konak and Pasaport locations on Izmir bay. Extracts were obtained from hepatic and muscular tissues of black goby and whole soft parts of the mussels. Obtained extracts were studied for mutagenicity using the Ames test with *S. typhimurium* TA98 and TA100 strains. According to results of the present study, mutagenicity was observed in liver extracts of the fish from Pasaport location whereas no mutagenicity was observed in liver and muscle extracts of fish from Bostanli location. Among the extracts of mussel samples collected from 8 locations only those from Alsancak Harbour were observed to possess weakly and directly mutagenic effects on *S. typhimurium* TA98 (frame shift mutations).

Key words: *Salmonella typhimurium*, Ames test, *Gobius niger*, *Mytilus galloprovincialis*, Izmir bay, Turkey

INTRODUCTION

The study area, Izmir bay has higly impaired environment due to the rapid increase of the population and development of industry. The sources of pollution are the untreated domestic and industrial wastes, atmospheric and agricultural pollution, shipping, dredging activities in the harbor and the disposal of the dredged material to the outer bay. Domestic and industrial wastewater is discharged to the bay from over 100 major raw sewage outfalls located either around the inner bay until 2000 when the municipality wastewater treatment plant were established (Aksu *et al*., 1998; Balci and Kucuksezgin, 1994). The harbor is still affecting the inner bay through heavy marine transportation activities.

Boyacioglu (2004) determined weak mutagenicity of sediment samples in Izmir bay. Kucuksezgin *et al.* (2008) have studied the effects of metal pollution on the activity of SOD and GPx in the mussel (*Mytilus galloprovincialis*) and showed that there was an increase in SOD enzyme activity and a decrease of GPx enzyme activity due to metal pollution at the inner part of bay. All information above indicates that sediment of Izmir bay has been contaminated and it is possible that several pollutants have been accumulated in the tissues of biota especially inhabiting on benthic zone.

It is possible to study (by means of a genetic test-system) the accumulation of mutagenic and carcinogenic substances in tissues of marine animals, algae and sediments. In this case it is necessary to extract and isolate the mutagenic compounds from water animals tissues.

Now, mutagenic and carcinogenic compounds are being increasingly found in the tissues of aquatic organisms. The mutual action of these harmful agents may be especially dangerous for the aquatic organisms (Kotelevtsev and Stepanova, 1995). On the other hand, it is well known that metabolic products of some chemicals may show toxic or genotoxic properties directly or following metabolization in digestive glands of the organisms.

Therefore, identification of specific chemicals responsible for impacts on human health or ecological effects is a major research subject in the environmental and health sciences (Grifoll *et al*., 1990). One of the features of mutagenic and carcinogenic compounds is

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that they can display biological effects even at very low concentrations which therefore makes it difficult to identify them particularly in the biological tissues. However, using chemical tecniques, it is not possible to determine whether a given substance has carcinogenic or mutagenic characteristics. Therefore, biological testing and monitoring of carcinogenic and mutagenic compounds is particularly important (Shugart, 1995) Besides, short-term bioassays such as the Ames test have gained acceptance as useful tools in mass screening of potentially carcinogenic compounds in the environmental samples (Schuetzle and Lewtas, 1986). Among them, the *Salmonella typhimurium* assay is still of value (Maron and Ames, 1983) despite a recent criticism (Zeiger, 1987; Tennat *et al*., 1987). Other short-term bioassays may have better sensivity but they are less specific than the Ames test (Tennat *et al*., 1987). In addition, a variety of compounds have been tested by the Ames assay (Zeiger, 1987).

In the present study, researchers aimed to determine existence of accumulated or metabolized mutagenic compounds in the hepatic and muscular tissues of black gobby (*Gobius niger*) and whole soft parts of mussel (*M. galloprovincialis*) collected from Izmir bay using the Ames's *S. typhimurium* test (Maron and Ames, 1983).

MATERIALS AND METHODS

Samples were taken in March 2007 in the area of Izmir bay (38°42'N, 29°25'E). Izmir bay is located on the eastern Agean sea. The bay is usually considered to consist of three sections: The inner, middle and outer bays. The port of Izmir city and several industries are located on the inner bay. The sampling sites on the inner bay of Izmir are shown in Fig. 1.

Mussel and fish samples were taken from 8 sites on the area of Izmir bay on the coast of Aegean sea. Four to six mussels were collected from each site so that average weight of mussels collected from each site be 6 g and they were kept in acetone (1 mL g^{-1}). Length of black gobby ranged between 10 and 13 cm. Averagely 5 g of muscle tissue was taken from 5 samples from pasaport (site 8). Three fish were catch from Bostanli (site 5) and averagely 3 g of muscle tissue was taken from them and placed in acetone (1 mL g^{-1}). Muscular and hepatic tissues of the fish and mussels were homogenized in acetone (1 mL g^{-1}) and hexane mixture (1:1) using a Polytron-type tissue homogenizer and extracted with equal volumes of solvent mixture until the extract became colorless and all of the extracted fractions for the same sample were combined and dried in a rotary evaporator before dissolving in 1 mL Dimethylsulphoxide (DMSO) ready for use in the Ames test. Consistency of the extraction method was throughly evaluated earlier (Kotelevtsev *et al*., 1994).

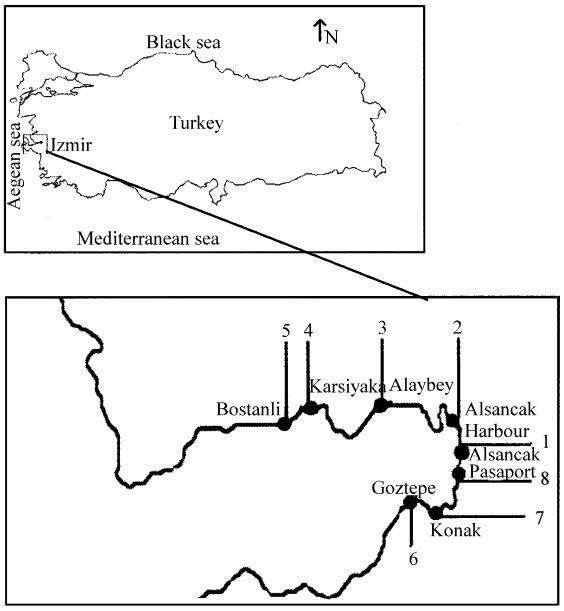

Fig. 1: Sampling sites on the study area of Izmir bay (site 1-Alsancak port; site 2-Alsancak Harbour; site 3-Alaybey; site 4-Karsiyaka; site 5-Bostanli; site 6-Goztepe; site 7-Konak; site 8-Pasaport)

In the present study, Salmonella mutagenicity tests were performed using the standard plate incorporation method (Maron and Ames, 1983) with the TA98 and TA100 strains of *S. typhimurium* and without S9-derived metabolic activation. In order to test mutagenicity without metabolic activation, 100 μL of organic extract was mixed with 100 μL of an overnight culture of bacteria and 2 mL of melted agar containing 0.5 mM histidine and biotin. Each dilution of extracts and controls were studied in triplicate. Following incubation, number of the revertant colonies was counted (His^- revertants).

Statistical analyses were performed using Student's t-test and significant differences were determined between data. The Statistica-6.0 software was used for statistical analyses (Hocking, 1996).

RESULTS AND DISCUSSION

The present study aimed to determine if the tissue of mussel and gobies contain direct or metabolized chemicals which has mutagenic potential from Izmir bay. In this mutagenicity study on liver tissue specimens of black gobby from Pasaport (site 8), number of revertant colonies at 50 and 100 μL was found to be lower than control and toxic effect was observed. In the experiment with TA100 strain, weak mutagenicity was observed at 50 μL with a value 2 times the negative control and toxic effect was

observed at 100 μL with a value being lower than negative control (Table 1) (Fig. 2). No mutagenicity was observed on muscle and liver tissue specimens of the black gobby collected from Bostanli (Table 1 and 2) (Fig. 2). According to AMES criteria, weak mutagenic effect was found in the mussel samples from Alsancak Harbor (site 2) in the mutagenicity study carried out with TA98 strain of *S. typhimurium* (Table 3) (Fig. 3) but no mutagenic effect was found in either strains on the mussel samples from other sites i.e., Alsancak port (site 1), Alaybey Shipyard (site 3), Karsiyaka (site 4), Bostanli (site 5), Goztepe (site 6), Konak (site 7) and Pasaport (site 8), (Table 3 and 4) (Fig. 3).

Table 1: Mutagenicity analysis of *G. niger* samples using *S. typhimurium* assay with TA 100 strain in the absence of metabolic activation (site 8-Pasaport, site 5-Bostanli)

TA100 (*G. niger*)	NC[a]	50 μL	100 μL	A-criteria
Site 8 Pas. (Muscle)	106±3.5	117.5±16.2	131.0±2.80*	NM
Site 8 Pas. (Liver)	106±3.5	215.0±1.40*	72.0±7.00*	WM
Site 5 Bos. (Muscle)	106±3.5	98.5±2.10*	118.0±6.30	NM
Site 5 Bos. (Liver)	106±3.5	120.5±3.50*	96.5±10.6*	NM

[a]Negative control: DMSO; Spontan revertant colonies: 110-112; Number of the revertant colonies of positive control with NaN_3 (1.5 μg plate^{-1}): 840-1320. *Analysis of mutagenic activity of fish samples as the number of His$^+$revertants in Ames test without metabolic activation system; Student's t-test, significant data are shown in (*) ($p<0.005$); A-Cr: Ames Criteria, SM; Strongly Mutagenic; MM: Moderate Mutagenic; WM: Weakly Mutagenic; NM: Non-Mutagenic

Table 2: Mutagenicity analysis of *G. niger* samples using *S. typhimurium* assay with TA 98 strain in the absence of metabolic activation (site 8-Pasaport, site 5-Bostanli)

TA98 (*G. niger*)	NC[a]	50	100 μL	A-criteria
Site 8 Pas. (Muscle)	20.6±2.0	16.5±0.0*	19.5±10.6	NM
Site 8 Pas. (Lliver)	20.6±2.0	20.6±2.1*	18.0±1.40	NM
Site 5 Bos. (Muscle)	20.6±2.0	20.5±6.3	15.5±3.50*	NM
Site 5 Bos. (Liver)	20.6±2.0	24.0±2.8	15.0±0.00	NM

[a]Negative control: DMSO; Spontan revertant colonies: 23-31; Mitomycin-C (0.5 μg plate^{-1}): No growth; *Analysis of mutagenic activity of fish samples as the number of His$^+$revertants in Ames test without metabolic activation system; Student's t-test, significant data are shown in (*) ($p<0.005$); A-Cr: Ames Criteria, SM; Strongly Mutagenic; MM: Moderate Mutagenic; WM: Weakly Mutagenic; NM: Non-Mutagenic

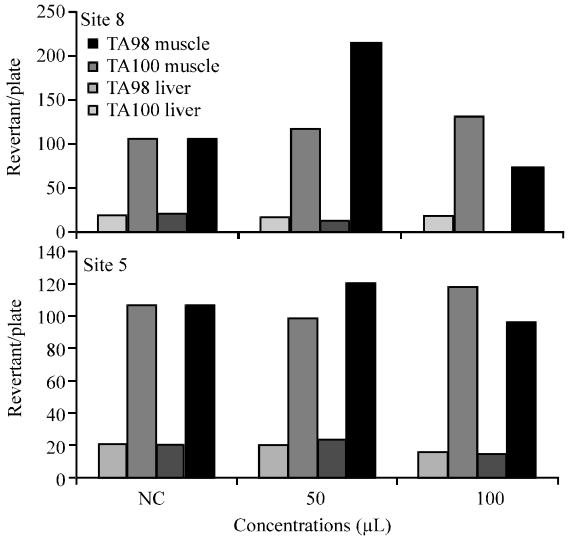

Fig. 2: Mutagenicity of *G. niger* samples from Izmir Bay (site 8-Pasaport; site 5-Bostanli). (NC: Values of solvent control (DMSO)

According to the criteria for Ames test system, a sample can be considered to be mutagenic in two situations: when number of the histidine back-revertant prototrophs at least doubles that of the spontaneous revertants and when the number of back-revertant colonies is less than the 2 times of that of spontaneous revertants but the number should show a dose-dependent increase (Maron and Ames, 1983).

The evaluation according to the criteria above makes basis of confirmation of low numbers for mutagenicity. For example:

- For *Salmonella typhimurium* TA98
- 20.6±2 (22.6-18.6)×2 = 41.2±4 (37.2-45.2)
- For *Salmonella typhimurium* TA100
- 106±3.5 (109.5-102.5)×2 = 212±7 (219-205)

Assessment of mutagenicity in marine organisms provides a reliable index of exposure to mutagenic and

Table 3: Mutagenicity analysis of *M. galloprovincialis* samples using *S. typhimurium* assay with TA 98 strain in the absence of metabolic activation

TA98-Mussel	NC[a]	25 μL	50 μL	100 μL	A-criteria
Site 1	27.3±3.2	25.6±3.00	28.0±6.9	25.0±1.7	NM
Site 2	27.3±3.2	31.0±4.50	40.5±0.7*	23.6±3.7	WM
Site 3	27.3±3.2	30.0±5.50	28.6±4.1	22.3±7.5	NM
Site 4	27.3±3.2	22.0±3.60	29.0±6.1	24.3±7.2	NM
Site 5	27.3±3.2	28.3±10.2	29.0±6.0	17.3±4.5*	NM
Site 6	27.3±3.2	28.0±1.70	27.3±2.0	18.6±2.3*	NM
Site 7	27.3±3.2	27.0±0.00	23.6±4.0	18.6±1.5*	NM
Site 8	27.3±3.2	17.5±4.90*	20.3±1.1*	15.6±2.5*	NM

[a]Negative control: DMSO; Spontan revertant colonies: 28-32; Mitomycin-C (0.5 μg plate^{-1}): No growth. *Analysis of mutagenic activity of fish samples as the number of His$^+$revertants in Ames test without metabolic activation system; Student's t-test, significant data are shown in (*) ($p<0.005$); A-Cr: Ames Criteria, SM; Strongly Mutagenic; MM: Moderate Mutagenic; WM: Weakly Mutagenic NM: Non-Mutagenic

Table 4: Mutagenicity analysis of *M. galloprovincialis* samples using *S. typhimurium* assay with TA 100 strain in the absence of metabolic activation

TA100-Mussel	NC[a]	25 μL	50 μL	100 μL	A-criteria
Site 1	122.5±12.5	121.0±9.80	157.0±12.7	139.0±2.00*	NM
Site 2	122.5±12.5	142.5±17.6	136.5±0.70*	136.0±5.60*	NM
Site 3	122.5±12.5	118.6±7.60*	119.0±7.90*	120.0±10.3*	NM
Site 4	122.5±12.5	130.0±4.00*	136.0±6.20	143.6±2.00	NM
Site 5	122.5±12.5	131.5±0.70*	119.0±12.5*	129.0±8.90*	NM
Site 6	122.5±12.5	128.5±2.12*	118.5±7.70*	132.0±5.00*	NM
Site 7	122.5±12.5	123.0±15.5*	132.0±7.20	155.0±7.00	NM
Site 8	122.5±12.5	117.5±2.10	145.0±7.00	144.0±12.7	NM

[a]Negative control: DMSO; Spontan revertant colonies: 110-135; Number of the revertant colonies of positive control with NaN_3 (1.5 μg plate^{-1}): 1920-2100. *Analysis of mutagenic activity of fish samples as the number of His$^+$revertants in Ames test without metabolic activation system; Student's t-test, significant data are shown in (*) ($p<0.005$); A-Cr: Ames Criteria, SM; Strongly Mutagenic; MM: Moderate Mutagenic; WM: Weakly Mutagenic NM: Non-Mutagenic

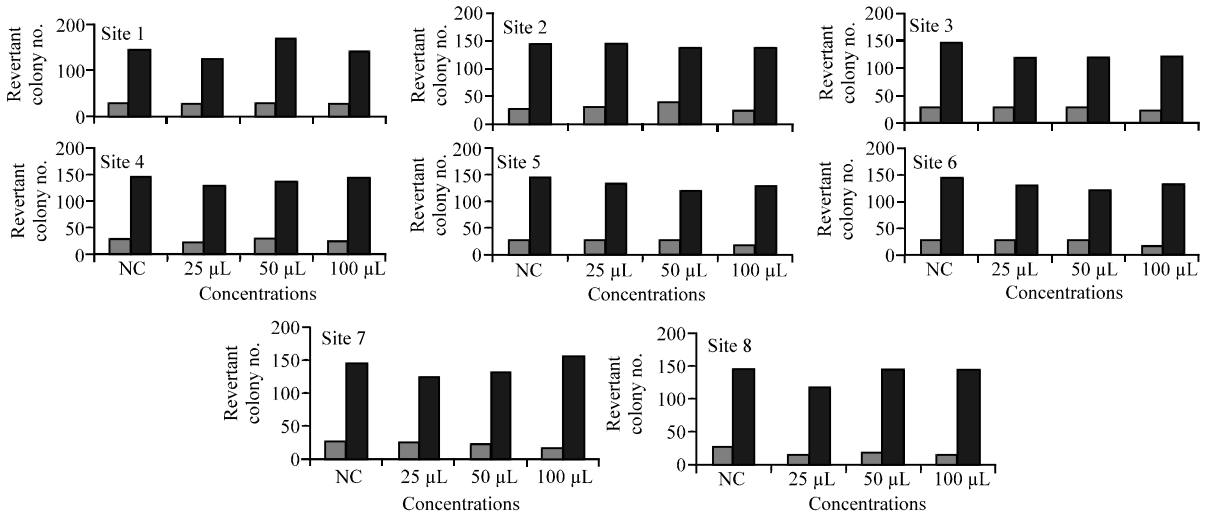

Fig. 3: Mutagenicity of *M. galloprovincialis* samples from Izmir bay (site 1-Alsancak port; site 2-Alsancak Harbour; site 3-Alaybey; site 4-Karsiyaka; site 5-Bostanli; site 6-Goztepe; site 7-Konak; site 8-Pasaport) (NC: Values of solvent control (DMSO)

potentially carcinogenic substances. Bivalve molluscs seem to represent ideal indicators of *in situ* exposure and have been proposed as biomonitors of the concentration of mutagens from polluted seawater (UNEP/WHO, 1995). Mussels provide an excellent system for monitoring marine pollutants (Goldberg, 1975). Arslan *et al.* (2010) examined genotoxic effects of the pollutants in Micronuclei (MN) tests carried out with erythrocyte and gill cells of *M. galloprovincialis* and *G. niger* from Izmir bay. That study indicated that MN test on the mussels and fish gave sensitive results in monitoring pollution especially in the harbours.

Similarly, Lopez-Barea and Pueyo (1998) used the extracts of mussels from polluted areas of Venice Lagoon and detected mutagenicity in strain TA 98 His^+ frameshift mutations but not in TA 100 His^+ base-pair substitutions of *S. thyphimurium* (Frezza *et al.*, 1982). Polycylic aromatic hydrocarbons containing benzo (a) pyrene were found in a study on oysters in Seto inland sea region and found to be mutagenic in Ames test (Hayatsu and Hayatsu, 1988). Kira *et al.* (1983) found mutagenicity in their study without S9 on mussel samples collected from a non-agricultural region of harbors surrounded by industrial parks. Stepanova *et al.* (1999) obtained extracts from tissue of a wide range of aquatic organisms from Lake Baikal and from the Selenga river estuary for mutagenicity tests of Ames Salmonella microsome test. According to that study, in some muscle samples of the extracts from these aquatic organisms showed weak mutagenicity on *S. thyphimurium* TA98 (i.e., frameshift mutations).

In a study by Arin and Sen (1999), the degree of induction of cytochrome P450 1A associated with EROD activity and immunochemical detection of cytochrome P450 1A in leaping mullet and common sole were used as biomarkers for assessment of PAH and/or PCB type organic pollutants along the Izmir bay on the Aegean sea coast. The fish from highly urbanized and industrial section of the bay, Pasaport showed highly elevated enzyme activities (1293.292, n = 208, pmol/min/mg) which were about 62 times higher than value at the reference site. Results of the study TCE-SV (1991) showed that concentrations of dissolved/dispersed petroleum hydrocarbons ranged from <1 mg L^{-1} in the outer bay to 12.45 mg L^{-1} in the surface waters of the inner bay. Izmir bay, today is one of the most notable pollution areas in Turkey. The average number of commercial ships visiting the harbor each year is approximately 2000. Municipality's treatment facility of Izmir bay discharges waste water into the bay after treating it but toxic substances remain in the sediment and living organisms.

The present study is of importance since it is the first research detecting the indirect (metabolized) mutagenicity of pollutants which are ingested and metabolized in the organisms and further studies should be performed with more diverse organisms to be screened mutagenically. Furthermore, the micronuclei test indicates environmental hazard of pollutants present in Izmir bay although, type and amount of these pollutants remain unknown (Arslan *et al.*, 2010).

CONCLUSION

In the study, the present study proves that substances of mutagenic characteristics still exist in Pasaport port (site 8) and Alsancak Harbor (site 2), supported by previous studies conducted independently on the same locations. Further studies are needed to adapt this method as aroutine biomonitoring tool in

parallel with many of the promising applications, supporting its applicability and importance (Cerna *et al*., 1991).

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