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The *Salmonella* Mutagenicity of Sediments from Gediz River (Western Turkey)

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Özet: *Gediz Nehri sedimentlerinde* Salmonella *mutajenite testi.* Bu çalışmada, Ramsar sitesi olarak koruma altında olan, büyük öneme sahip kuş cennetinin bulunduğu Gediz Nehri deltası sedimentinin, Salmonella typhimurium TA98 ve TA100 suşları kullanılarak yapılan Ames testi ile mutajenik potansiyeli araştırılmıştır. Her iki suşla yapılan mutajenisite testleri sonucunda sediment örneklerinin hiçbirinde mutajenik madde içermediği buna rağmen gözlenen bakteri büyümesinde ki azalmaya bağlı olarak tüm örneklerin toksik olduğu belirlenmiştir.

Anahtar Kelimeler: *Salmonella typhimurium*, Gediz Nehri, Mutajenite testi.

Abstract: In this study, sediments from the delta of Gediz River which is one of the most important bird areas protected as Ramsar site were investigated for their potential mutagenicity in TA98 and TA100 strains of *Salmonella typhimurium* by performing Ames test (plate incorporation assay) without metabolic activation. Mutagenicity results for both strains showed that the sediment samples contained no mutagenic substance but most of them had toxic effects which decreased the growth of bacteria in all sampling sites.

Key Words: *Salmonella typhimurium*, Gediz River, Mutagenicity.

Introduction

With its length of 401 km, Gediz River is the second largest river flowing into Aegean Sea from Western Anatolia of Turkey. Gediz Delta is an extensive wetland consisting of bays, salty marshes, freshwater marshes, large saltpans and four lagoons at the former mouth of Gediz River. The WWF-Turkey office declared that the site qualifies as an IBA (Important Bird Area) for its breeding populations of many bird species. Gediz Delta is one of Turkey's nine Ramsar sites (site No.945) protected by the Ramsar with the agreement number 7TR009 since 1998 and the Bern Convention (Ermert 2003).

Gediz River is heavily polluted due to agricultural drainage water, industrial wastewater and virtually all domestic wastewater from the entire area (Usak, Manisa and Izmir) (Elmaci et al., 2002). Industrial development, intensive agricultural activities and rapid increase in population of the region has led to impairment of water quality of superficial and underground water bodies in the region. Solid and liquid wastes are transferred into rivers with mostly no treatment. Heavy metals, toxic materials and other pollutant factors exist in these wastes. They are responsible for detrimental effects on soil, water and air and have reached critical levels (Parlak et al., 2006., Delibacak et.al.2002)

Although there have been many attempts to improve the water quality and protect the natural environment, the most recent one was established in early 1998 as a co-ordinating commitee named "Environmental Protection Service Association of Gediz Basin Provinces" with association of three provincial offices of the Ministry of Environment. However, since its establishment this service has achieved little due to lack of resources and inefficiency in enforcing existing standards and regulations. It also includes a reduction in support for utilisation of fertilizers and agricultural chemicals as non-point source pollution problems.

Determining the pollution, especially if caused by organic chemicals is economicaly and practically a difficult task due to complex molecular structure of organic chemicals in the aquatic environment. Among these pollutants, detection of mutagenes in aquatic environments is of great importance owing to their ability to induce cancer and their potential to damage the germ line, which may lead to fertility problems and to genetic damages in the future generations (Kutlu et al., 2004). Therefore, short-term bioassays coupled to chemical analysis is a valuable technique for screening toxic components in environmental samples (Schuetzle and Lewtas, 1986). Of these bioassays, *Salmonella typhimurium*/microsome test (Maron and Ames, 1983) is one of the most important one with proven accuracy. *Salmonella* mutagenicity assay (Ames test) was specially designed to detect chemically induced mutagenesis and a number of reports exist indicating the mutagenicity of complex mixtures in river water and sediments, lakes, industrial effluents and drinking waters using *Salmonella* mutagenicity test systems (Filipic and Toman, 1996; Kataoka et al., 2000; Hollert et al., 1999; Mamber et al., 1993; Cerna et al.,1998).

The aim of this study was to investigate the potential for contamination by mutagenic subtances in the sediments from delta of Gediz River which may have a role as a depository

compartment in ecosystems. For this purpose two strains of *Salmonella typhimurium* with frameshift mutation (TA 98) and base-pair substitution mutation (TA 100) were used in plate incorporation assay in the absence of metabolic activation.

Materials and Methods

Sediment samples were taken from 8 stations over 6 time periods between May 1998 and May 1999 (Figure 1). St1, 2, and 3 are located on delta of Gediz River.

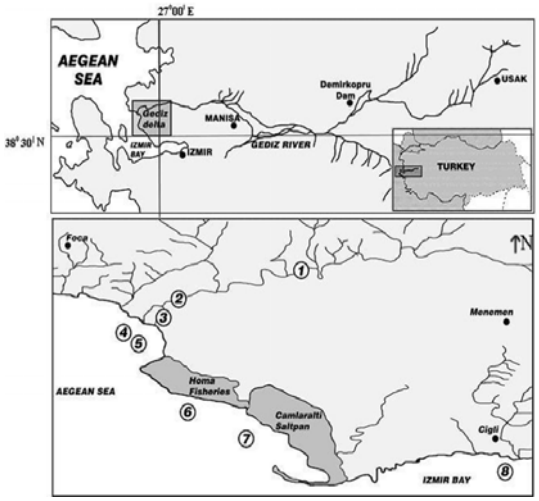

Figure 1. Sampling sites on the study area of Gediz River Basin.

The other stations St 4, 5, 6, 7, 8 were on marine environment in close vicinity where was admitted to be affected by the Gediz River. Van-Veen grap sampler was used to obtain sediment samples. The samples were taken from surface layer (about 2 cm thick) with metal spatula and stored under cold-chain up to laboratory. The samples were dried in room temperature and after extraction they stored in deep freeze at -20ºC.

Nutrient broth and bacto agar were purchased from Oxoid (Hardy Diagnostics). Dimethylsulfoxide (DMSO), histidine, biotin and sodium azide were purchased from Merck. Ampiciline trihydrate was purchased from Sigma.

Sediment samples from Gediz River were crash to powder and sifted in the laboratory and then placed in portions of 1 g into sterile teflon tubes and mixed with 1 ml hexane/choloroform/acetone (1:1:1 v:v:v) using a vortex mixer. Subsequently, the samples were centrifuged for 10 minutes at +4°C at 5600 g^- (Sigma K3) and supernatants were transferred into sterile tubes. This procedure was repeated for 3 times and pooled supernatants was evaporated and then dissolved by adding 1 ml of dimethylsulfoxide (DMSO). Each sample underwent Ames test in three replicates with both TA98 and TA100 strains of *S. typhimurium* (Kotelevtsev and Stepanova ,1995).

Salmonella mutagenicity tests were performed using the standard plate incorporation method (Maron and Ames, 1983) with the TA98 and TA100 strains of *Salmonella typhimurium*, and without S9-derived metabolic activation in this study.

Mutant TA98 and TA100 strains of *S. typhimurium* used in Ames test were obtained from Dr. Bruce Ames (UCLA/Berkeley-USA). Genetic control of bacterial strains used in Ames/Salmonella test was performed prior to the experiments. 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 µl of melted agar containing 0.5mM histidine and biotin. The molten top agar was then poured onto a minimal glucose agar base plate and incubated at 37°C for 2 days. NaN_3 (1.5µg/plate) and Mitomycin-C (0.5µg/plate) were used as positive controls. Each dilution of extracts and controls were assayed in triplicate. Following incubation, number of the revertant colonies was counted (His^- revertants) (Maron and Ames, 1983).

In this study using single dose, the sample was considered to be mutagenic when the number of revertant colonies in the test plates was doubled the number of revertants in solvent control (Kutlu et al., 2004).The toxicity of the samples on the bacteria was evaluated on the basis of significantly reduced number of revertants compared to solvent control (Zeytinoglu et al.,2000). The results were given by means of nine plates from three replicates and ± S. D. values.

Results

The results of the mutagenicity of solvent extracts of sediment samples from delta of Gediz River and the marine environment in close vicinity were given in Table 1, 3. And figures 2-3. According to the results the number of revertants increased but none of them doubled the number of colonies in solvent control.

Thus, the mutagenicity of sediment samples from the delta of Gediz River can be categorized as non-mutagenic. In some periods, the numbers of revertants were decreased comparing to solvent control which means the toxic effect occurred to decrease the growth of colonies.

In conclusion, the tests using TA98 strains of *S. typhimurium* showed that the sediment samples from delta stations had toxic effect which was clearly understood from low MIs.

However the sediments from marine stations caused increase in revertant colony numbers nearly 1.5 times of the MI of the solvent control (Table 2, 4).

On the other hand, the tests using TA100 strain showed no mutagenic but strong toxic effect on bacterial colonies at St 1 while the number of the revertants increased up to 1.2 in MI at the St 2 and St 3.

The tested sediment samples from the St 4, 5, and 6 from marine environment caused growth of the colonies to stop since the MI ranged between 0.5-0.9 but the samples from St7 and St8 were found to be almost mutagenic (Table 4).

Discussion

The aim of the present study was to investigate the potential for contamination by mutagenic substances in delta of Gediz River using *Salmonella* mutagenicity systems (Ames test). Plate incorporation assay was performed without metabolic activation. It should be pointed out that the Ames test was performed for screening analysis in order to identify the most significant sites for an initial assessment of the basin as to presence of mutagenic compounds (Vargas et al., 2001).

In the present study, all stations exhibited toxic properties in at least one sampling period and with at least one of two strains in mutagenicity study. In the related studies, toxic effects of the sediment samples on bacteria were observed on the basis of significantly reduced number of the revertants compared to the solvent controls. Kutlu et al., (2004) performed Ames test on water and sediment samples from Porsuk River without metabolic activation using TA98 and TA100 strains and found mutagenic and toxic effects with both strains at 5 different concentrations and in different sampling sites. Sediments from both the Porsuk River possessed weak direct frameshift mutagenicity and a weak base-pair substitution mutation on the contrary of the Delta of Gediz River.

Table 1. Mutagenicity analysis of sediment samples using *S. typhimurium* assay with TA98 strain in the absence of metabolic activation. (*) ($p<0.05$). Negative Control: DMSO, NC: 22-33; Positive control: Mitomicine-C (0.5µg/plate): No growth.

TA98	NC[a]	May98	Aug98	Nov98	Feb98	Mar99	May99
St1	25.6±4.7	15.5±2.1	20.5±4.9	17.5± 4.9	31± 0	20.5± 4.9	12.5± 2.1
St2	42±6.2	-	-	-	26.6± 1.5	47 ±4.9	48.6± 1.1
St3	25.6±4.7	13± 0	31.5± 0.7	27.5± 3.5	24.5±4.9	25.5± 3.5	21± 1.4
St4	25.6±4.7	20.5±0.7	23± 0.7	30± 1.4	31± 0	20± 5.6	31±0
St5	32±2.8	27±6	42± 0	16± 5	48± 4.2	28.3± 4.1	51± 0.7
St6	32±2.8	34±10.8	36.3± 4.5	34± 8.4	51± 0.7	31.3± 2.3	-
St7	32±2.8	37.6± 9	-	33± 2.1	43.6± 5.5	24± 1.4	35.3±6.6
St8	42±6.2	36.3±5.5	33.6± 4.5	33.6± 6	43± 4.3	44± 7	42.3± 3

Table 2. Mutagenic index: number of his^+induced in the sample per number of spontaneous his^+ in the negative control (NC).

TA98	May98	Aug98	Nov98	Feb98	Mar99	May99	M[b]
St1	0.6	0.8	0.6	1.2	0.8	0.4	-
St2				0.6	1.1	1.1	-
St3	0.5	1.2	1	0.9	0.9	0.8	-
St4	0.8	0.9	1.1	1.2	0.8	1.2	-
St5	0.8	1.3	0.5	1.5	0.8	1.5	-
St6	1	1.1	1	1.5	0.9		-
St7	1.1		1	1.3	0.7	1.1	-
St8	0.8	0.8	0.8	1	1	1	-

[b] *Mutagenicity*

Table 3. Mutagenicity analysis of sediment samples using *S .typhimurium* assay with TA100 strain in the absence of metabolic activation. Student's *t*-test, significant data are shown in (*) ($p<0.05$).[a] Negative Control: DMSO, NC:123-126; Positive control: (NaN_3)(1,5µg/plate) :1200-1360.

TA100	NC[a]	May98	Aug98	Nov98	Feb98	Mar99	May99
St1	126± 9.5	78.5± 4.9	107.5±17.6	114 ±15.5	74± 11.3	87± 0	81.5±10.6
St2	126 ±1.7	-	-	-	150 ±19*	140± 32	126±14.1
St3	95.5± 13.4	103.5± 0.7	115.5±4.9	125± 0	121.5±16.2	102±16.9	124± 0
St4	126.5±13.4	102 ±16.9	99.5 ±7.7	115± 0	87± 0	108.5± 0	111± 0
St5	127 ±1.7	111.5± 2.1*	118±12.7	96.5±13.4	123± 0	113.5±19	114± 0
St6	127± 1.7	100.5± 4.2*	91.5± 12	116 ±1.46	105.5± 0	125±1.48	-
St7	127 ±1.7	80± 0	-	142.5±6.3	75± 11.3*	115± 0	118 ±8.48
St8	127 ±1.7	170± 19	140.5± 0.7*	148±11.3	187± 9.8*	132± 2.8	200± 1.4

Table 4. Mutagenic index: number of his^+ induced in the sample per number of spontaneous his^+in the negative controls(NC). [b] Mutagenicity.

TA100	May98	Aug98	Nov98	Feb98	Mar99	May99	M[b]
St1	0.6	0.8	0.9	0.5	0.6	0.6	-
St2	-	-	-	1.1	1.1	1	-
St3	1	1.2	0.9	1.2	1	1.2	-
St4	0.8	0.7	0.9	0.6	0.8	0.8	-
St5	0.8	0.9	0.7	0.9	0.8	0.8	-
St6	0.7	0.7	0.9	0.8	0.9	-	-
St7	0.6	-	1.1	0.5	0.9	0.9	-
St8	1.3	1.1	1.1	1.4	1	1.5	-

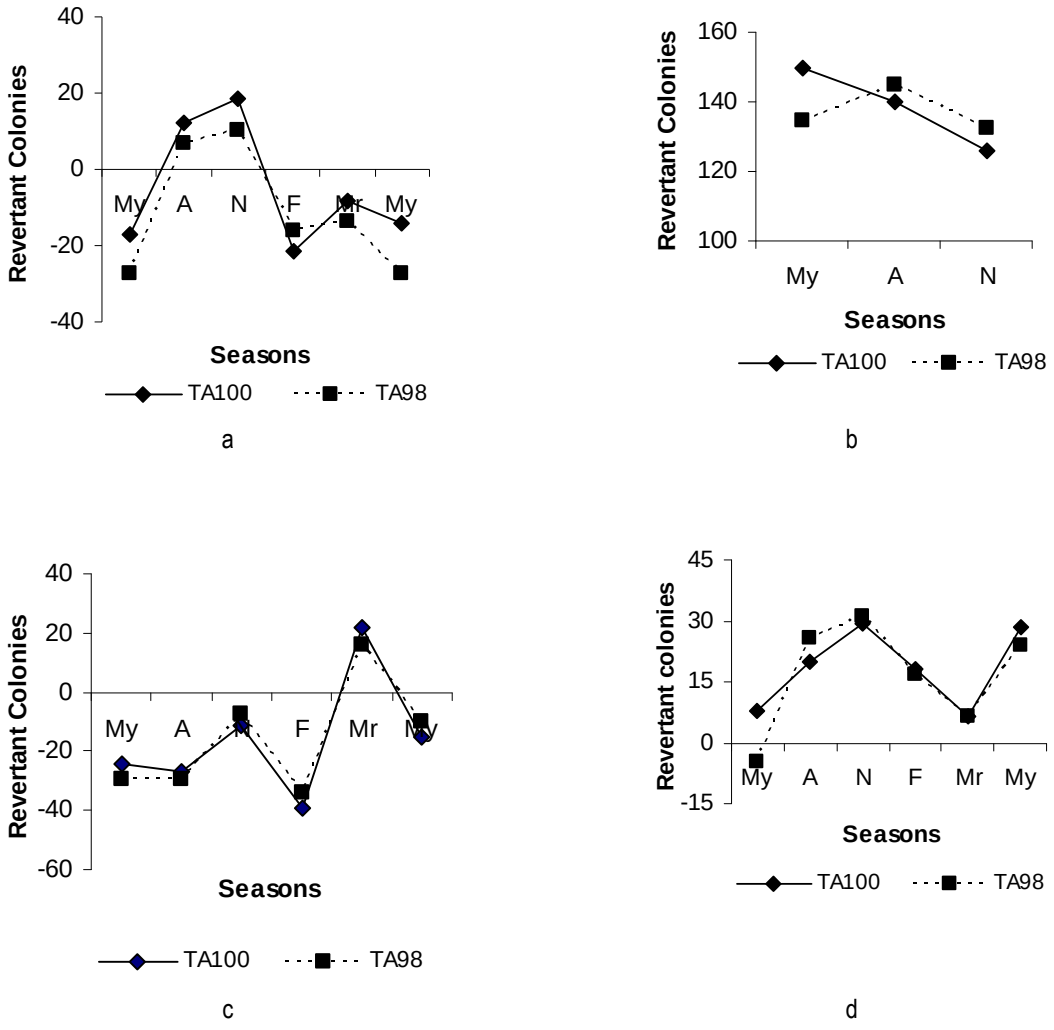

Figure 2. Mutagenicity of the sediment samples from Gediz River. (a) Station 1 ,(b) Station 2, (c) Station 3, (d) Station 4

Parlak et al., (2006) had reported elevated Cr concentrations in the sediment samples from the same stations. Chromium levels of the stations were found to be at high levels as following: St 1: 49.658, St 2: 1530.633; St 3: 671.88, St 4: 47.044; St 5: 26.905; St 6: 40.650; St7: 94.310; and St 8: 252.410 (mg/kg dry weight) .This can support up the result of this study which revealed the toxic effects of the sediments on growth of bacteria S. *typhimurium.* Also, Kucuksezgin et al., (2006) reported that elevated heavy metal and total petroleum haydrocarbon levels in the sediment of outer bay of Izmir and Gediz River were declared as the major source of anthropogenic input into the area.

This study is of importance in indicating that Gediz River is of toxic characteristics rather than being mutagenic because such a genotoxicity study with purpose of screening has not been performed on Gediz River so far. In the Gediz River no mutagenic activity was detected in any of the sediment samples tested, but genotoxins in the complex sediment may possibly be transported to the biological food chain via biomagnification and may contaminate water resources and soil system.

Considering that the water from the branches of Gediz River has been used for irrigation and stock-raising, one could easily understand the extent of the hazards of pollution in Gediz River. Therefore, the first step to eliminate the pollution in Gediz River should be to control polluting sources.

In conclusion those studies of this type have importance in predicting the primary mutagenic effects of water and sediments contaminated by industrial and urban drainage on living organisms. Although the sediments from Gediz River were not found to be contaminated by mutagenic substances, additional experiments should be performed using rat-liver homogenate (S9) in order to determine the genotoxic potentials of metabolized pollutants in mammalian systems.

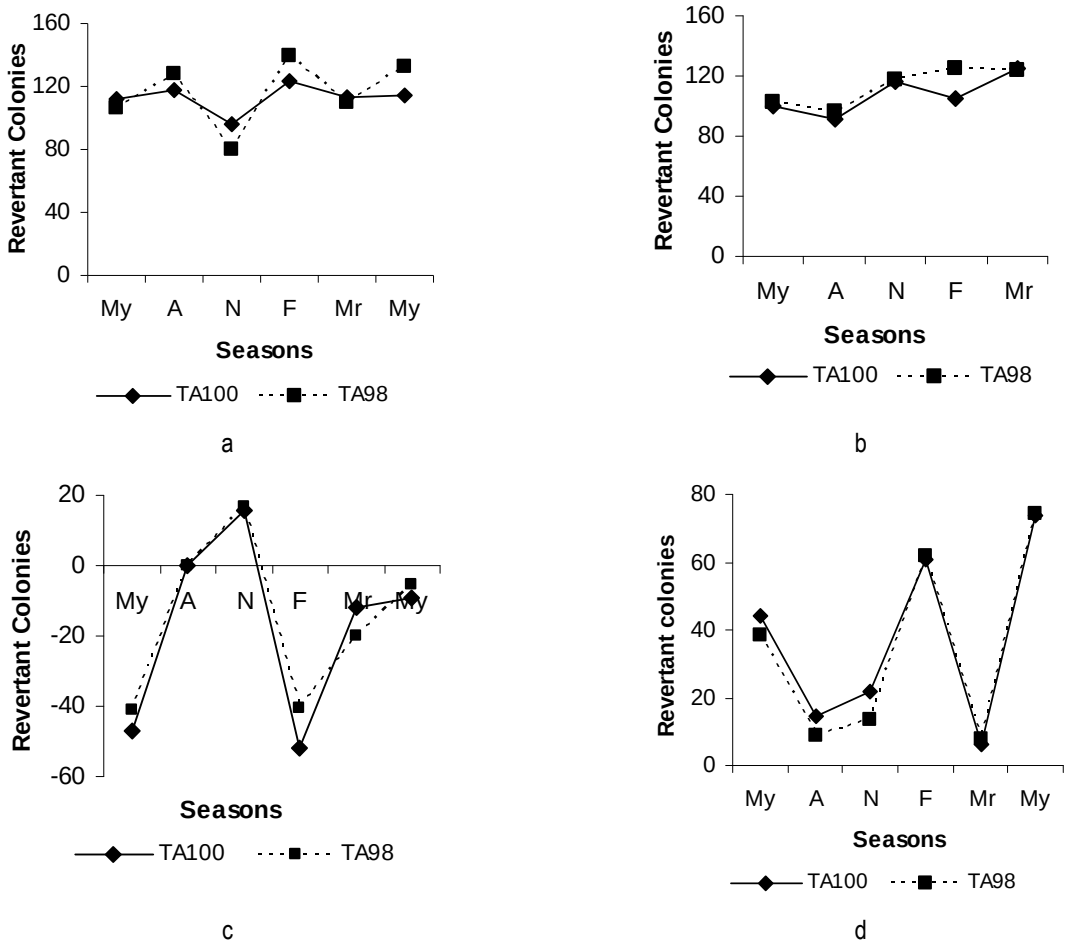

Figure 3. Mutagenicity of the sediment samples from Gediz River. (a) Station 5, (b) Station 6, (c) Station 7, (d) Station 8.

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