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INVESTIGATION OF MUTAGENIC POTENTIAL OF WATER AND SEDIMENT FROM KARAMENDERES RIVER (ÇANAKKALE, TURKEY) USING THE AMES TEST

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ABSTRACT

In this study, the mutagenic effects of water and sediment samples taken from 5 stations between Kumkale and Karaköy locations on the Karamenderes River were investigated with the short time mutagenicity test system of the Ames test. Different extracts (hexane, chloroform and dichloromethane) and five different concentrations (10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) of water and sediment samples were prepared for testing in the study. Experiments were performed in the presence and absence of S9, which contains the TA98 and TA100 mutant strains of Salmonella typhimurium bacteria and microsomal enzymes. As a result of the mutagenicity studies, it was determined that the water samples had mutagenic effect causing base pair changes at all the stations for the chloroform extract. Mutagenicity was determined at the first station for the hexane extract. It was determined that there are weak mutagenic effects in the dichloromethane extract and in sediment samples from different stations.

1. INTRODUCTION

Today, environmental pollution has become an important problem because of rapid population growth, unplanned industrialization and ignorant agricultural practices. Domestic and industrial wastes and pesticides are discharged into water sources without proper treatment and this causes pollution of already limited water resources. Some of these substances cannot be broken down, sedimented, adsorbed or destroyed in any way. Therefore, these chemicals accumulate in water resources and threaten the health of living organisms in the environment (Ayan, 2005).

Many of the chemicals involved in aqueous ecosystems have mutagenic and carcinogenic effects, causing changes in living DNA (Anonim, 1991). These chemicals can be effective even at very low concentrations. It is not possible to analytically determine the chemical structures of these substances, which accumulate in tissues, with existing chemical methods. For this reason, biological methods and indicators based on carcinogenic and mutagenic substance scanning in tissues have gained importance. Since carcinogens and

mutagenic substances can be found in aquatic organisms with an important place in human nutrition, monitoring of the genotoxic effects of these substances has great importance for human health (Kotelevtsev and Stepanova, 1995).

The Ames test was specifically designed to detect the mutagenesis of chemical substances (Ames *et al.*, 1975). With this testing system, mutagenesis of more than 5000 chemicals and bioactive components isolated from plants and plants, soil, water and air can be identified in a short time; as well as the antimutagenic effect of phytochemicals such as plants, artificial or natural chemicals and vitamins, carotenoids, flavonoids and terpenoids (Maron and Ames, 1983; Hong and Lyu, 2011; Vu *et al*., 2012). The Ames test is one of the most commonly used test systems for bacterial mutagenicity because of its easy of application and sensitivity, with details well known and characterized (Gatehouse *et al*., 1998).

Certain chemical substances become active after being metabolized in vivo. The cytochrome P450 metabolic oxidation system in the liver of humans and lower-level creatures has the ability to metabolize these chemicals to DNA-reactive, electrophilic forms. Since there is no such enzyme system in bacteria, microsomal enzymes are added to the test system. This enzyme mixture (cytochrome P-450 enzyme system and various metabolic enzymes) is referred to in brief as S9 and is usually obtained from the rat liver (Maron and Ames, 1983, Mortelmans and Zeiger, 2000; Sakura *et al*., 2004).

The Karamenderes River, which is our study area, is contaminated with pesticides used in agricultural activities in the vicinity. In addition, many leather and olive oil plants operating in the districts of Bayramic and Ezine, along with the effluent from dairies and slaughterhouses, are sources of pollution due to inadequate refinement (Anonim, 2007).

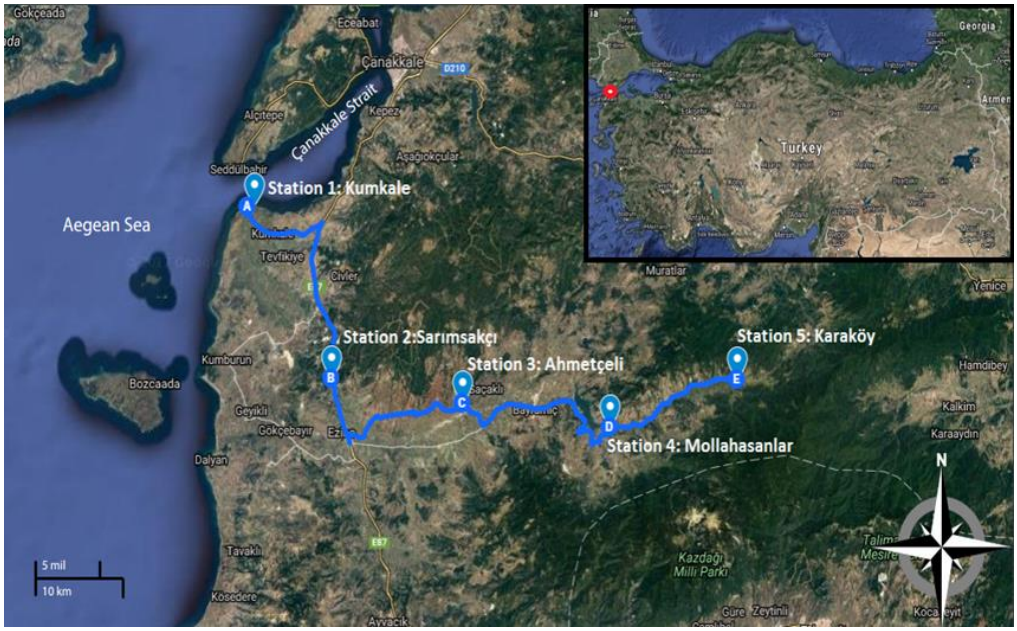
As a result of a literature review, it was determined that there were no previous mutagenic studies about Karamenderes River. In this study, the aim was to investigate the potential mutagenic effects of water and sediment samples taken from Karamenderes River.

2. MATERIAL AND METHODS

2.1. Study Area and Sampling Stations

The Karamenderes River rises in Kazdağı and passes near the Bayramic and Ezine districts of Çanakkale and flows into the Dardanelles near Kumkale. Karamenderes River is the only source of water that constantly flows into the region (Kayacan, 2008). Water and sediment samples were taken from 5 stations (1. Kumkale, 2. Sarımsaklı, 3. Ahmetçeli, 4. Mollahasan, 5. Karaköy) along Karamenderes River in November 2012 (Figure 1). The water samples were taken from the area where the current is not very high in amounts of 5 lt and sediment samples were obtained from the surface of the river bed in amounts of 250 g. The water samples were placed in polyethylene bottles (500 mL) and immediately transported to the laboratory.

Figure 1 . The stations samples are taken at Karamenderes River



2.2. Chemicals and Test Strains

4-Nitro-o-phenylenediamine (NPD), glucose, L-histidine, D-biotin, glucose-6-phosphate and rat liver S9 fraction were purchased from Sigma-Aldrich; sodium azide (SA, NaN_3),2-aminofluorene (2AF), magnesium sulfate ($MgSO_4.7H_2O$), citric acid monohydrate ($C_6H_8O_7.H_2O$), potassium phosphate (K_2HPO_4), sodium hydroxide (NaOH), sodium chloride (NaCl), potassium chloride (KCl), magnesium chloride ($MgCl_2.6H_2O$), sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$), disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$) and dimethyl sulfoxide (DMSO) from Merck; bacto agar and nutrient broth were purchased from Oxoid.

The mutagenicity assay was performed with the standard plate incorporation method with and without metabolic activation (S9 mixture) as described by Maron and Ames (1983). *Salmonella typhimurium* TA98 strains were used for frame shifts and TA100 strains were used to identify changes that caused base pair mutations. *S. typhimurium* strains were kindly provided by Prof. Dr. Hülya Sivas (Anadolu University, Faculty of Science, Department of Biology) and maintained as described by Maron and Ames (1983).

2.3. Extraction of The Water and Sediment Samples

Extraction of the water samples was carried out with the liquid-liquid extraction method. A 5-liter sample of water from each station had 500 mL used each time, and 25 mL of solvent was added to quickly flush the balloon in the flask and then left for a while for the phase to form. The solvent was then removed by separation of the solvent and placed in separate glass bottles and this process was repeated twice with fresh extraction solvent. n-Hexane ($CH_3(CH_2)_4CH_3$), chloroform ($CHCl_3$) and dichloromethane (CH_2Cl_2) solvents were used respectively for extraction. The extracts were then evaporated at 45 ºC using an evaporator (Spectral, Heidolph, Laborator 4001) and water bath, and dimethyl sulfoxide (DMSO) was added before the test to dissolve the remaining precipitate (Singh *et al*., 1987).

Sediment samples taken from the designated stations were dried at 60 °C in a petri dish. The dried sediments were poured into a porcelain mortar and powdered, weighed to the amount

of 0.1 g and then placed in sterile tubes. They were vortexed by adding 1 mL of hexane, chloroform, and dichloromethane (1:1:1,v:v:v). The supernatant was then sterilized by centrifugation in a refrigerated centrifuge (Hettich, Universal 320R) at +4 °C and 5600 g for 10 minutes and the same procedure was repeated three times. Solvents of the obtained extracts were evaporated with a rotary evaporator in a water bath at 40 °C. Extracts were stored in the refrigerator at +4 °C. When the test was performed, the remaining precipitate was dissolved by adding DMSO (Keijzer et al., 2000).

2.4 Methods

Amounts of 0.1 mL of an overnight bacterial culture and 0.5 mL of sodium phosphate buffer (0.2 M, pH 7.4 for assay) were added to 2 mL aliquots of top agar containing different concentrations of each extract. The resulting complete mixture was poured onto minimal agar plates prepared as described by Maron and Ames (1983). The plates were incubated at 37 ºC for 48 h and the revertant bacterial colonies on each plate were counted.

For the positive control, 4-Nitro-o-phenylenediamine (NPD) for *S. typhimurium* TA98 strain and sodium azide (SA, NaN_3) for TA100 strain were used in the absence of S9. In the presence of S9, 2-aminofluorene (2AF) was used for both strains. In the negative control, dimethyl sulfoxide (DMSO) was used for both strains. All samples were tested on triplicate plates for each concentration and the studies were applied in two independent experiments. An extract was considered mutagenic if the number of revertants per plate was at least doubled compared to the solvent controls (Maron and Ames, 1983). Data were collected as the mean ± standard deviation of three experiments.

3. RESULT and DISCUSSION

In the absence of S9, with the concentration of 10^{-2} *S. typhimurium* TA100 strain was found to be mutagenic in hexane extracts obtained from water samples, while at higher concentrations the number of revertant colonies decreased in parallel to the toxic effect. At station 4, both strains were found to have poor mutagenic activity at all concentrations except 10^{-4} concentration. In the S9 experiment, there was no significant increase compared to the negative control (Table 1).

In chloroform extracts, *S. typhimurium* TA98 strain in the absence of S9 had weak mutagenicity at 10^{-1} concentration, and cytotoxic effect at 10^0 concentration from station 4. In TA100 strains, except for station 4, the highest concentration (10^0) of mutagenic activity was detected at each station. Although there were higher numbers of colonies at the 4th station in the 10^{-1} dose than in the other stations, there was no increase in the number of colonies due to cytotoxic effect at 10^0 doses. In study with S9, the TA98 strain had the highest concentration of mutagenic response at the 4th station. In the TA100 strains, mutagenicity at the 3rd station was determined at the highest concentration, and mutagenicity at the 4th and 5th stations was high (Table 2).

In dichloromethane extracts, in the absence of S9, the highest concentration of *S. typhimurium* TA100 strains showed weak mutagenicity at stations 2 and 3, while at stations 1, 4 and 5, concentration-dependent revertant colony counts were increased. In the TA98 strain, mutagenic results were not found at any station. In the presence of S9 it was determined that the number of colonies due to concentration at the 3rd station increased in the TA100 strain. There was no significant increase in colony number in TA98 strains (Table 3).

Sediment samples were found to have poor mutagenic activity at the 2nd, 3rd and 5th stations at the highest concentration in *S. typhimurium* TA100 strains in the absence of S9. No mutagenic activity was observed in the TA98 strain in the experiment without S9 and in the S9 experiment for both strains (Table 4)

According to the results of the research, it was determined that there is mutagenic activity in the water samples taken from Karamenderes River. In the absence of S9 in *S. typhimurium* TA100 strain, mutagenicity was found at all the stations in the chloroform extract obtained from the water samples. The highest mutagenic response was found in the chloroform extract at stations 4 and 5 in the presence of S9. This result shows that at these two stations, metabolic activation results in metabolites in the living body may cause mutagenic effects. It was also found that weak mutagenic activity was observed in hexane extract at station 4 (Table 1). It is possible that such a result would occur due to the residues of the pesticides used in the apple orchards in this region. The presence of mutagenicity in hexane and chloroform extracts from water samples suggests that pesticide residues may be present in the samples. Organochlorine and organophosphate pesticides in water can be trapped by these two solvents (Aleem and Malik, 2005).

As a result of the studies done, many pesticides have been shown to cause mutagenic effect (Aleem and Malik, 2005; Zhao *et al*., 2010). In a study in India, mutagenic potential of water samples from three stations on the Ganges River were investigated. Mutagenicity was detected even at low concentrations in strains TA98 and TA100. Some pesticides such as dichlorodiphenyltrichloroethane (DDT), β-hexachlorocyclohexane (BHC), aldrin, and dieldrin have been identified in water samples with High Performance Liquid Chromatography (HPLC) analyses (Rehana *et al*., 1995).

In a study carried out on the Karamenderes River and near the soil, the results of the analyses revealed that many pesticides, especially α-endosulfan and hexachlorohexane, can cause pollution (Yıldırım and Özcan, 2007).

At the third station, mutagenicity in the absence of S9 in the *S. typhimurium* TA100 strain in the chloroform extract and weak mutagenicity in the presence and absence of S9 in the dichloromethane extract were determined. It is possible that the observed mutagenic effect is due to the pollution of the wastes from settlements in the surrounding area and the consequent drainage of inadequately treated leather and olive oil production wastes from Bayramiç town. The mutagenic activity, especially in the dichloromethane extract, demonstrates the presence of complex mixtures in this region. This is because in the Ames test, it has been reported that dichloromethane is a suitable solvent for basic-neutral compounds for the extraction of water samples from complex environmental mixtures (Nielsen, 1992; Lippincott *et al*., 1990).

At the second station, the mutagenic response was determined in the chloroform extract. In the dichloromethane extract, the mutagenic effect was weak. The station with the lowest mutagenic activity was determined to be the second station. Although there is agricultural activity in this area, the mutagenic potential is diminished due to the dilution of the pollution load from the third station.

At the first station, mutagenicity was also observed in hexane and chloroform extracts. In the dichloromethane extract, a weak mutagenic effect was found. In this region where the river enters the sea, the accumulation of pollutants can result in mutagenic activity.

In a study in Slovenia, samples from the Sora River contaminated with water from wastewater treatment plants, domestic waste and landfills were examined with the Ames test system and 11 samples were found to have mutagenic activity ((Filipic, 1995).

Strong mutagenic activity was not observed in any sediment samples collected from the Karamenderes River. It was determined that there are poor mutagenic effects at stations 2, 3 and 5. This shows that the amount of mutagenic substances in the river is low in water and insoluble in sediment (Table 4).

Maccubbin *et al*. (1991) tested sediment samples from the Detroit River in Michigan and Lake Michigan using Ames in the United States of America, where industrial, domestic and agricultural wastes are heavily involved. High mutagenic activity was detected at 16 sites resulting in metabolic activation of chemicals containing microsomal enzymes (S9). Strong mutagenic activity was not detected in any of the samples when S9 was not applied.

It is not accurate to combine the results of this study and the mutagenicity obtained in previous studies to a single cause. This is because some chemicals do not have genotoxic effects on their own, but they are known to create this effect when they are combined. For this reason, the analysis of the chemicals in water and sediment samples is important for the correct interpretation of mutagenic results.

Table 1. Mutagenicity results of *S. typhimurium* TA98 and TA100 strains of hexane extracts obtained from water samples

Treatment		Concentration	Number of his^+ revertant colony/Plate			
			TA98		TA100	
			S9 (-)	S9 (+)	S9 (-)	S9 (+)
			mean±SD	mean±SD	mean±SD	mean±SD
Positive control	NPD	10^{-2}	811±94,55			
	SA	10^{-3}			1189±147,33	
	2AF	$5x10^{-3}$		957±107,29		1295±156,70
Station 1		10^{0}	25±4,36	26±1,53	180±38,42	137±15,39
		10^{-1}	19±2,08	26±4,73	179±24,06	135±10,39
		10^{-2}	24±6,24	35±8,18	306±7,09	141±9,85
		10^{-3}	15±4,93	29±3,06	248±32,19	160±26,23
		10^{-4}	13±4,16	31±7,23	179±3,61	192±28,00
Station 2		10^{0}	16±5,03	32±1,53	128±9,24	154±12,77
		10^{-1}	22±4,58	34±1,53	115±9,29	194±17,78
		10^{-2}	19±4,58	38±6,56	167±31,01	160±9,29
		10^{-3}	20±3,79	29±3,46	160±2,08	162±15,31
		10^{-4}	15±4,04	29±6,00	132±4,16	139±17,95
Station 3		10^{0}	33±4,58	38±5,13	168±4,73	130±14,57
		10^{-1}	23±1,53	23±5,13	150±10,60	159±10,97
		10^{-2}	25±2,65	26±6,56	210±15,95	180±15,10
		10^{-3}	26±4,58	30±7,64	148±7,00	139±5,29
		10^{-4}	26±1,73	27±3,21	169±26,54	190±15,57
Station 4		10^{0}	38±2,08	29±5,51	213±34,78	163±7,02
		10^{-1}	41±1,53	38±1,73	285±22,19	149±7,81
		10^{-2}	44±7,21	27±6,81	214±31,09	183±14,05
		10^{-3}	37±6,81	31±7,21	266±11,93	137±19,43
		10^{-4}	26±5,51	34±1,53	162±22,68	135±13,50
Station 5		10^{0}	17±3,79	42±6,56	179±27,23	162±21,08
		10^{-1}	24±4,04	28±4,36	134±13,61	125±13,05
		10^{-2}	26±5,29	30±2,65	143±27,39	145±6,56
		10^{-3}	18±3,06	34±3,21	156±17,39	142±5,29
		10^{-4}	34±5,00	31±4,51	143±29,50	165±10,26
Negative control	DMSO		24±3,50	28±5,98	135±21,06	144±34,76
Spontaneous control			24±6,94	25±6,59	157±27,52	152±18,64

*NPD: 4-Nitro-*o*-phenylenediamine, SA: Sodyum azide, 2AF: 2-aminoanthracene, DMSO: Dimethyl sulphoxide

Table 2. Mutagenicity results of *S. typhimurium* TA98 and TA100 strains of chloroform extracts obtained from water samples

Treatment		Concentration	Number of revertant colony/Plate			
			TA98		TA100	
			S9 (-)	S9 (+)	S9 (-)	S9 (+)
			mean±SD	mean±SD	mean±SD	mean±SD
Positive control	NPD	10^{-2}	811±94,55			
	SA	10^{-3}			1189±147,33	
	2AF	$5x10^{-3}$		957±107,29		1295±156,70
Station 1		10^{0}	36±4,51	36±7,55	359±47,15	222±13,89
		10^{-1}	28±4,36	30±8,50	144±12,66	197±5,51
		10^{-2}	20±4,16	21±1,73	127±5,51	115±15,82
		10^{-3}	28±6,08	22±3,51	116±11,55	138±9,00
		10^{-4}	25±6,42	24±2,65	114±7,77	142±24,38
Station 2		10^{0}	20±3,51	36±4,58	277±23,86	199±17,21
		10^{-1}	20±3,21	26±1,53	120±2,08	121±13,65
		10^{-2}	18±3,51	23±6,24	125±8,39	143±14,74
		10^{-3}	15±2,08	28±2,65	147±24,33	129±5,51
		10^{-4}	19±5,13	28±5,29	139±21,07	126±23,52
Station 3		10^{0}	35±6,51	40±2,52	375±26,21	324±20,42
		10^{-1}	23±5,13	32±6,81	153±11,36	163±32,08
		10^{-2}	18±3,21	30±5,69	151±12,42	118±20,98
		10^{-3}	17±2,08	28±7,09	128±4,58	119±25,03
		10^{-4}	19±4,04	28±3,06	124±27,07	151±18,15
Station 4		10^{0}	17±5,51	64±10,69	219±10,58	498±29,82
		10^{-1}	40±5,69	35±4,04	216±14,53	203±22,85
		10^{-2}	26±5,03	35±1,53	135±16,50	184±29,46
		10^{-3}	22±3,79	32±2,31	94±7,09	125±7,09
		10^{-4}	19±3,21	26±3,06	115±10,82	165±9,29
Station 5		10^{0}	21±1,53	38±6,51	302±7,09	514±13,53
		10^{-1}	22±3,79	19±4,58	216±11,00	202±19,14
		10^{-2}	14±1,53	28±0,58	151±16,44	152±25,16
		10^{-3}	18±4,51	28±4,93	152±15,50	129±5,13
		10^{-4}	19±4,93	19±2,08	171±7,55	132±3,79
Negative control	DMSO		24±3,50	28±5,98	135±21,06	144±34,76
Spontaneous control			24±6,94	25±6,59	157±27,52	152±18,64

*NPD: 4-Nitro-*o*-phenylenediamine, SA: Sodyum azide, 2AF: 2-aminoanthracene, DMSO: Dimethyl sulphoxide

Table 3. Mutagenicity results *of S. typhimurium* TA98 and TA100 strains of dichloromethane extracts obtained from water samples

Treatment		Concentration	Number of revertant colony/Plate			
			TA98		TA100	
			S9 (-)	S9 (+)	S9 (-)	S9 (+)
			mean±SD	mean±SD	mean±SD	mean±SD
Positive control	NPD	10^{-2}	811±94,55			
	SA	10^{-3}			1189±147,33	
	2AF	$5x10^{-3}$		957±107,29		1295±156,70
Station 1		10^{0}	23±2,65	19±2,65	191±17,58	150±14,57
		10^{-1}	28±3,00	24±6,11	166±27,43	171±3,61
		10^{-2}	28±6,24	26±5,69	155±6,00	146±14,74
		10^{-3}	21±4,16	21±4,35	141±4,58	139±15,50
		10^{-4}	19±3,51	22±3,79	120±15,72	132±10,82
Station 2		10^{0}	24±1,53	32±4,93	254±17,06	184±13,05
		10^{-1}	21±3,46	24±4,93	139±9,45	150±15,10
		10^{-2}	22±2,08	27±1,73	108±22,37	182±11,50
		10^{-3}	29±2,65	19±2,52	164±7,64	169±28,57
		10^{-4}	16±3,79	19±3,79	121±5,03	132±3,79
Station 3		10^{0}	29±2,31	17±4,16	227±25,16	206±11,14
		10^{-1}	30±4,04	17±1,15	140±20,03	164±15,52
		10^{-2}	16±2,00	23±4,00	162±30,83	129±6,03
		10^{-3}	30±2,52	20±3,46	143±16,52	132±14,04
		10^{-4}	27±4,73	17±5,86	150±26,91	118±7,55
Station 4		10^{0}	26±2,89	21±3,05	206±26,69	203±9,45
		10^{-1}	18±4,58	22±4,16	157±12,74	123±14,73
		10^{-2}	19±5,51	24±3,21	144±21,45	200±25,94
		10^{-3}	18±3,06	23±4,93	155±4,04	163±11,37
		10^{-4}	21±3,79	23±6,08	140±15,10	153±24,38
Station 5		10^{0}	36±6,11	28±1,53	186±3,79	205±30,02
		10^{-1}	39±3,46	26±3,46	143±12,22	143±7,09
		10^{-2}	25±3,79	21±3,06	137±11,59	161±20,30
		10^{-3}	16±4,93	26±6,08	130±13,58	167±6,66
		10^{-4}	18±3,51	22±3,79	120±12,34	156±16,44
Negative control	DMSO		24±3,50	28±5,98	135±21,06	144±34,76
Spontaneous control			24±6,94	25±6,59	157±27,52	152±18,64

*NPD: 4-Nitro-*o*-phenylenediamine, SA: Sodyum azide, 2AF: 2-aminoanthracene, DMSO: Dimethyl sulphoxide

Table 4. Mutagenicity results of *S. typhimurium* TA98 and TA100 strains of sediment samples

Treatment		Concentration	Number of revertant colony/Plate			
			TA98		TA100	
			S9 (-)	S9 (+)	S9 (-)	S9 (+)
			mean±SD	mean±SD	mean±SD	mean±SD
Positive control	NPD	10^{-2}	811±94,55			
	SA	10^{-3}			1189±147,33	
	2AF	$5x10^{-3}$		957±107,29		1295±156,70
Station 1		10^{0}	32±2,08	17±2,65	167±14,01	155±9,07
		10^{-1}	25±5,03	17±2,52	174±6,03	146±5,51
		10^{-2}	24±4,16	19±2,00	165±5,03	139±9,07
		10^{-3}	28±4,51	19±1,53	198±26,50	154±14,53
		10^{-4}	22±4,04	18±4,51	157±7,09	141±6,56
Station 2		10^{0}	18±2,65	21±2,00	227±37,17	126±12,06
		10^{-1}	17±2,52	17±2,08	165±21,73	121±10,82
		10^{-2}	22±3,51	21±1,53	146±17,50	120±9,17
		10^{-3}	18±3,51	22±1,73	187±20,52	121±6,56
		10^{-4}	22±6,03	20±2,00	201±13,75	111±16,09
Station 3		10^{0}	24±5,51	23±3,21	228±8,19	138±8,50
		10^{-1}	18±4,04	16±3,00	169±16,52	115±14,98
		10^{-2}	23±1,53	16±2,65	159±19,76	113±9,07
		10^{-3}	27±3,51	18±1,15	151±17,52	117±6,51
		10^{-4}	24±4,58	22±3,21	156±25,03	133±8,19
Station 4		10^{0}	32±4,04	32±8,14	174±31,94	128±9,07
		10^{-1}	17±2,08	24±3,51	148±12,70	139±10,54
		10^{-2}	24±5,51	17±4,16	113±10,02	114±9,54
		10^{-3}	27±6,56	23±4,16	165±24,03	134±8,50
		10^{-4}	27±3,61	20±3,06	114±19,97	114±7,77
Station 5		10^{0}	30±5,69	16±3,51	234±31,01	137±8,33
		10^{-1}	28±1,15	29±5,86	163±30,27	142±9,07
		10^{-2}	26±1,53	24±3,00	186±24,50	128±8,00
		10^{-3}	25±5,50	19±3,61	140±22,11	121±9,17
		10^{-4}	26±1,53	17±4,16	168±22,72	141±9,61
Negative control	DMSO		24±3,50	28±5,98	135±21,06	144±34,76
Spontaneous control			24±6,94	25±6,59	157±27,52	152±18,64

*NPD: 4-Nitro-*o*-phenylenediamine, SA: Sodyum azide, 2AF: 2-aminoanthracene, DMSO: Dimethyl sulphoxide

4. CONCLUSION

Today, pollution of water resources as a result of anthropogenic activities presents a great danger. With industrialization and urbanization, many wetlands are polluted, and this pollution directly or indirectly threatens the health of people and other living organisms. As a result of the studies and the different test methods used, it is clear that water and sediment are contaminated with many genotoxic compounds and that these compounds cause mutagenic effects on different organisms.

In this study, water samples and sediment samples taken from the Karamenderes River in Çanakkale were found to have mutagenic activity at all stations in the absence of S9 in the chloroform extract and at the highest concentration at the two stations in the presence of S9. The water samples were found to have a mutagenic response at one station in the absence of S9 in the hexane extract. It was also shown that there are weak mutagenic effects at different stations in dichloromethane extracts and sediment samples. With these results, it is necessary for farmers to be aware of the use of pesticides in agricultural activities carried out around the Karamenderes River. In addition, it is very important to purify and treat the waste from settlements and industry in the surrounding area in order to prevent pollution of the river.

There are more and more unknown mutagenic and carcinogenic compounds found in water sources. Conducting genotoxicity studies in conjunction with chemical analyses is also important in determining the harmfulness of these chemicals by accurately identifying mutagens in the water. Apart from this, other in vitro and in vivo tests should be performed and the results obtained should be supported so that the results can be confirmed.

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