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Kars Çayı (Kars) Sediment ve Su Örneklerinin Mutajenik Etkilerinin Ames Testi ile Araştırılması

Investigation of the Mutagenic Effects of Sediment and Water Samples from the Kars River (Kars) Using the Ames Test

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Öz

Bu çalışmada, Kars Çayı'nın merkeze yakın olan bölgelerinden 3 ayrı istasyon belirlendi. Bu istasyonlar; 1. istasyon; Yolaçan Köyü mevkisi, 2. istasyon; Kafkas Üniversitesi kampüs mevkisi, 3. istasyon; aktif yerleşimin bittiği yer olan Kars müze mevkisidir. İstasyonlar arasında mesafe gözetilerek, belirlenen noktalardan 2022 Eylül ayında su ve sediment örnekleri alındı. Kısa zamanlı mutajenite test sistemlerinden biri olan Ames testi ile su ve sediment örneklerinin olası mutajenik özellikleri belirlendi. 3 ayrı istasyondan alınan su örneklerinden ekstraktlar (hekzan ve kloroform) ve sediment örnekleri hazırlandı. Çalışmada su ve sediment örneklerinin 10^0, 10^{-1}, 10^{-2} ve 10^{-3} seri dilüsyonları kullanıldı. Deneyler, *Salmonella typhimurium* TA98 ve TA100 mutant suşları ile metabolik aktivasyon (S9) yokluğunda gerçekleştirildi. Deney sonuçları; spontan kontrol, negatif kontrol dimetil sülfoksit (DMSO) ve pozitif kontrol grupları ile birlikte değerlendirildi. Pozitif kontrol olarak, metabolik aktivasyon (S9) yokluğunda TA98 suşu için 4-Nitro-o-fenilendiamin ve TA100 suşu için ise sodyum azid kullanıldı. Mutajenite testleri sonucunda, su örneklerinin hekzan ve kloroform ekstraktları ve sediment örneklerinde, çevresel kirliliğin daha az olduğu 1. istasyondan alınan örneklerde potansiyel mutajenik etki gözlemlenmezken, 2. istasyonda yalnızca çerçeve kayması değişimine yol açan mutajenik etki gözlemlendi. Çevresel kirlenmenin en fazla olduğu düşünülen 3. istasyonda ise hem çerçeve kayması, hemde baz çifti değişimine neden olan mutajenik etki görüldü.

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Abstract

In this study, three different stations were determined from the regions of Kars River that are close to the center. These stations are; 1st station; Yolaçan Village location, 2nd station; Kafkas University campus location, 3rd Station; Kars museum location where the active settlement ends. Water and sediment samples were taken from the determined points by considering the distance between the stations in September 2022. Possible mutagenic properties of water and sediment samples were determined with the Ames test, which is one of the short-term mutagenicity test systems. Extracts (hexane and chloroform) of water samples and sediment samples were prepared from samples taken from three different stations. Serial dilutions of 10^0, 10^{-1}, 10^{-2} and 10^{-3} of water and sediment samples were used in the study. Experiments were performed with TA98 and TA100 mutant strains of *Salmonella typhimurium* in the absence of metabolic activation (S9). Experiment results were evaluated together with spontaneous control, negative control dimethyl sulfoxide (DMSO) and positive control groups. 4-Nitro-ophenylenediamine for strain TA98 in the absence of metabolic activation (S9) and sodium azide for strain TA100 were used as positive controls. As a

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result of the mutagenicity tests of the hexane and chloroform extracts of the water samples and sediment samples, no potential mutagenic effect was observed in the samples taken from the 1st station where environmental pollution was less, while a mutagenic effect caused to only a frameshift change was observed in the 2nd station. Both frameshift and mutagenic effect causing base pair change were observed in the 3rd station, which is thought to have the highest environmental pollution.

1. INTRODUCTION

The increasing environmental pollution worldwide has been negatively affecting ecosystems, particularly aquatic ecosystems. This has led to significant social and economic challenges while contributing to the gradual disruption of the ecological balance. Environmental pollution in aquatic environments, unplanned population growth, and unregulated agricultural activities, particularly contamination caused by mutagenic and carcinogenic substances, have become significant issues affecting human health. While the technology required to meet the needs of the growing population brings innovations and conveniences to human life, it has also exacerbated the problem of environmental pollution. Moreover, interventions such as transferring water through closed pipelines for residential use or the misuse of water resources have contributed to the emergence of water pollution issues. Additionally, with technological advancements, the number of chemical substances used by humans has been steadily increasing over time (Öncül, 2009; Tomatis, 1979).

Numerous substances contribute to environmental pollution, such as various pharmaceuticals used by living organisms, industrial waste, food additives, and a wide range of chemicals employed in pest control. Investigating the mutagenic and carcinogenic effects of these natural or synthetic substances, which may cause environmental pollution, is crucial for the health of living organisms. Furthermore, assessing the impact of these substances on all living organisms and the importance of implementing preventive measures is a significant concern. The detection of organic chemical substances in aquatic environments is particularly challenging. Investigating these pollutants, especially those that have already been identified, in specific environments helps develop practical methods for determining the toxicity, pollution levels, and other characteristics of the studied areas. Therefore, combining molecular chemical analysis methods with short-term biological research techniques enables the development of a rapid, reliable, and practical method for identifying toxic substances in various environmental samples (Schuetzle & Lewtas, 1986; Akyıl, 2006; Uysal, 2006; Yüksel, 2005).

Water pollution occurs when the bacteriological, chemical, physical, and ecological characteristics of a water source are adversely affected, either directly or indirectly (Uslu & Türkman, 1987). It can take various forms, including inorganic substance pollution, organic substance pollution, solid waste pollution, thermal pollution, and radioactive contamination (Göksu, 2003). The chemicals and pollutants mentioned above can cause mutations in the genetic material of plants, animals, and humans, thereby exhibiting genotoxic effects (Gesamp, 1991; Galli & Schiestl, 1996).

Since there is a connection between mutagenic and carcinogenic effects, mutagenic tests play a crucial role in the examination of various substances with carcinogenic effects (Temizkan, 1994; Mortelmans & Zeiger, 2000). The Ames test, developed by Dr. Bruce Ames in 1972, is a reliable bacterial test that provides rapid results and is widely used to detect the presence or absence of mutagenic effects in various substances consumed or used by humans, such as drugs, extracts, cosmetic products, and food additives (Ames et al., 1975; Choy, 2001). In the Ames test, bacterial mutants are used to examine the mutagenic effects of various substances. Several strains of *Salmonella typhimurium*, such as TA98, TA100, TA1535, and TA1537, are used. When the necessary amino acid (histidine) is not synthesized, bacterial growth does not occur, and no colony formation takes place. However, after exposure to the tested chemical, the strains may regain the ability to synthesize histidine and form colonies (Maron & Ames, 1983; Mortelmans & Zeiger, 2000). The Ames test is widely used to evaluate chemical substances and environmental samples. In many studies, the Ames test has been extensively employed to determine mutagenic effects in samples such as surface waters (37%), sediments (41%), and soils (38%) (Beceren et al., 2017).

This study determined that no prior mutagenicity studies had been conducted in the Kars River, located in Kars province. For the first time, this study aims to investigate the presence or absence of mutagenicity in water and sediment samples from the Kars River using the Ames mutagenicity test and to emphasize the importance of taking preventive measures against water pollution.

2. MATERIALS AND METHODS

In this study, three separate stations were established in the central and near-central areas of the Kars River. Water and sediment samples were collected in September 2022, with careful consideration given to the distances between the stations.

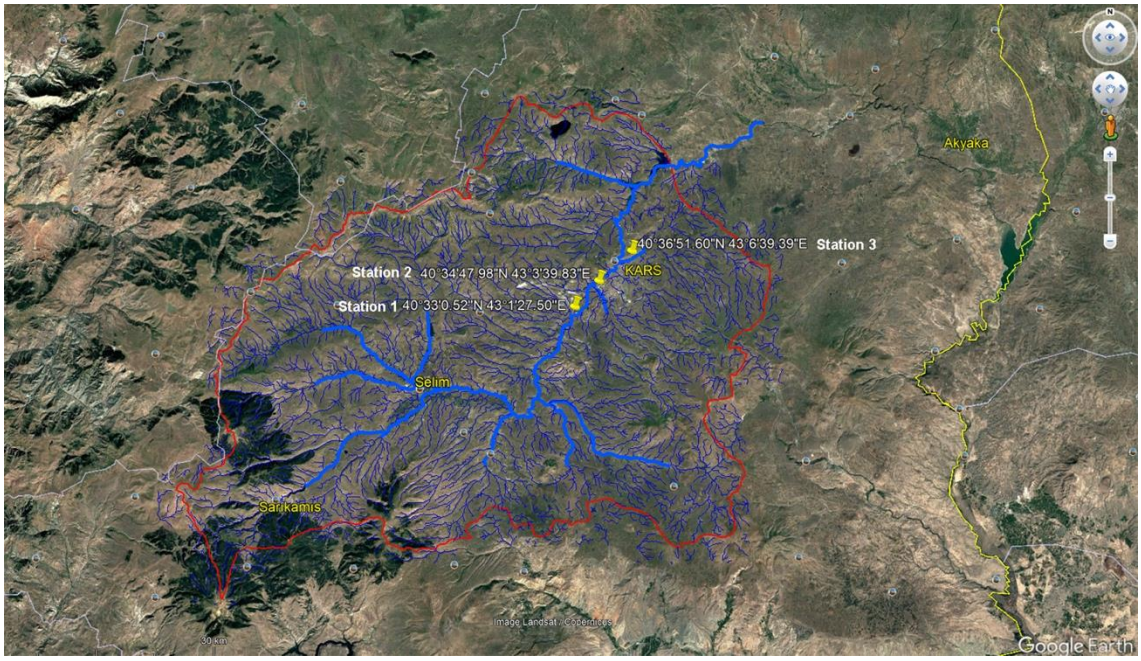


Figure 1. Stations of the Water and Sediment Samples on the General Map

The samples were collected from three specific locations along the Kars River. The coordinates of the sampling stations are as follows: Station 1 (40°33'0.52"N, 43°1'27.50"E), Station 2 (40°34'47.98"N, 43°3'39.83"E), located within the campus of Kafkas University, and Station 3 (40°36'51.60"N, 43°6'39.39"E). The stations where water and sediment samples were taken are shown in Figure 1.

Station 1, where water and sediment samples were collected, is located in the Yolaçan Village area along the Kars-Erzurum road. This station is at the confluence of the Kars River tributaries originating from Sarıkamış and Selim. The region surrounding this station includes residential areas, livestock farming activities, and agricultural lands. Station 2 is situated near the underpass within the campus of Kafkas University. This station is primarily influenced by nearby residential areas. Station 3 is located near the Kars Museum, marking the endpoint of active settlement. At this station, the Kars River, which flows through the city center, is contaminated by substantial amounts of domestic and industrial waste, along with other pollutants. Additionally, livestock farming activities are also observed in this area.

2.1. Extraction of Water and Sediment Samples

In this study, 5 liters of water samples collected from the Kars River were filtered using filter paper. Subsequently, 50 mL of hexane was added to 1000 mL of the filtered water sample, which was then stirred vigorously for a specific period. After the addition of hexane, phase separation was observed, and the samples were transferred into separate bottles. Water samples collected from the designated stations were initially extracted with hexane, followed by three successive extractions using chloroform. The solvents were removed using a rotary evaporator. The obtained water extracts were transferred into 1.5 mL Eppendorf tubes and prepared by dissolving them in dimethyl sulfoxide (DMSO). These extracts were stored at +4 °C (Singh et al., 1987; Güzey, 2013).

For sediment samples, surface-layer sediments were collected using a Van Veen Grab sampler and a metal spatula to a depth of approximately 2 cm. The samples were placed in single-use petri dishes and dried in an oven at 60 °C. The dried sediment samples were ground into a fine powder using a porcelain mortar. From the powdered sediment, 0.1 g was weighed and transferred to Eppendorf tubes. Each tube was then treated with 1 mL of chloroform and hexane, followed by vortexing. The tubes were centrifuged at 7000 rpm for 10 minutes. The supernatant obtained during centrifugation was transferred to separate tubes, and this process was repeated three times. The collected supernatants were evaporated at +40 °C to remove the organic solvents. The resulting extracts were dissolved in DMSO (100%) and stored at +4 °C for further use in the study (Keijzer et al., 2000; Güzey, 2013).

The study utilized the TA98 and TA100 strains of *Salmonella typhimurium*, which were developed from the ancestral strain through in vitro mutagenesis studies conducted by Prof. Dr. Ames and Dr. Maron in 1971. The preparation of master plates and stock cultures of the *Salmonella typhimurium* TA98 and TA100 strains, verification of their genetic properties, and mutagenicity studies were performed using the plate incorporation method developed by Maron and Ames (1983).

When calculating the mutagenic effect, the number of revertant colonies was compared to the revertant colonies in the negative control. If the observed value was at least twice as high, the sample was considered mutagenic. Additionally, if an increase in the number of revertant colonies was proportional to the concentration, the sample was classified as

weakly mutagenic (Mortelmans & Zeiger, 2000). The control of histidine amino acid requirements, *uvrB* mutations, *rfa* mutations, R-resistance factor (RF), spontaneous reverse mutation frequencies, and negative controls were also carried out as part of the study.

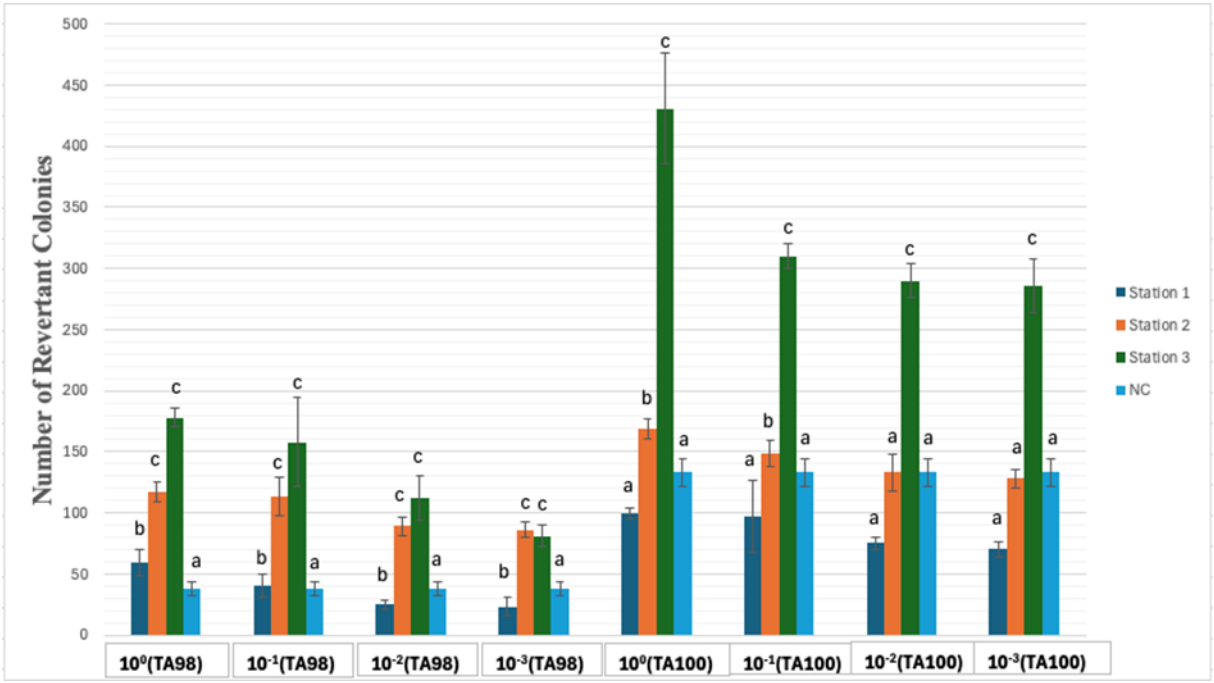
Hexane and chloroform extracts were prepared from the water and sediment samples collected from the Kars River. Four different dilutions (10^0, 10^{-1}, 10^{-2}, and 10^{-3}) were prepared for both water and sediment extracts. These dilutions were tested separately at four different concentrations, with each concentration repeated independently three times.

Experiments were performed with *S. typhimurium* TA98 and TA100 mutant strains in the absence of metabolic activation (S9). Spontaneous control, negative control, and positive control groups were included. In the absence of S9, 4-nitro-o-phenylenediamine (NPD) was used as the positive control for the TA98 strain, while sodium azide (SA) was used for the TA100 strain. NPD was applied at 10 µg/petri, and SA was applied at 1 µg/petri in the positive control group. Dimethyl sulfoxide (DMSO), which was used to dissolve the water and sediment extracts, was applied to the negative control group.

3. RESULTS

Water and sediment samples were collected from three designated stations along the Kars River. In the experiments, the averages and standard deviation values of revertant colony counts for the negative control, spontaneous control, and positive control groups were calculated for water and sediment extracts tested on *S. typhimurium* TA98 and TA100 strains. The results are presented graphically in Figures 2, 3 and 4.

When calculating the mutagenic effect value, the number of revertant colonies was compared to the number of revertant colonies in the negative control (NC). If the result was twice as high, it was considered mutagenic. Additionally, when an increase in the number of revertant colonies occurred in a concentration-dependent manner, it was evaluated as weak mutagenicity.

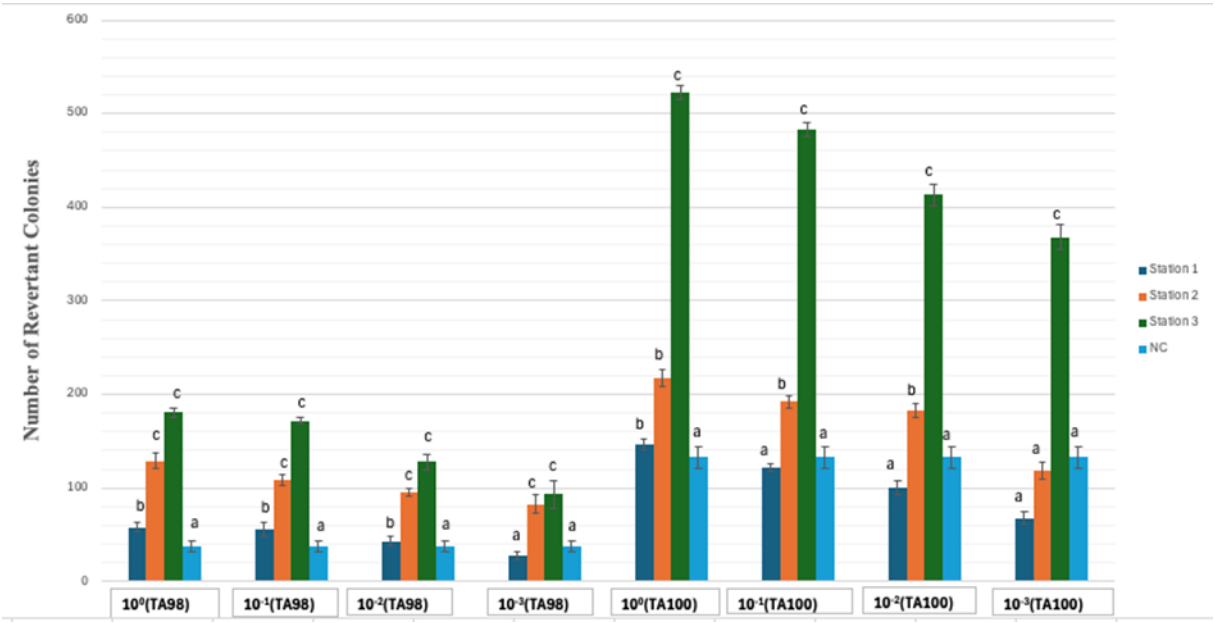


*(a: no mutagenic effect, b: weak mutagen, c: mutagen)

Figure 2. Effects of Hexane Extracts at Concentrations of 10^0, 10^{-1}, 10^{-2}, and 10^{-3} from Water Samples Collected from Stations 1, 2, and 3 of Kars River on *S. Typhimurium* TA98-TA100 Strain in the Absence of S9.

The effects of hexane extract obtained from water samples collected from the 1., 2., and 3. stations of the Kars River on *S. Typhimurium* TA98 strain were analyzed, and the results are presented in Figure 2. At the 1. station, all concentrations of the hexane extract exhibited weak mutagenic activity (b) compared to the negative control values. However, at the 2. and 3. stations, mutagenic activity (c) was observed at all concentrations.

In the TA100 strain, no mutagenic activity was observed at the 1. station. In contrast, the 3. station showed mutagenic activity (c) across all concentrations. At the 2. station, weak mutagenic activity (b) was detected only at 10^0 and 10^{-1} concentrations.

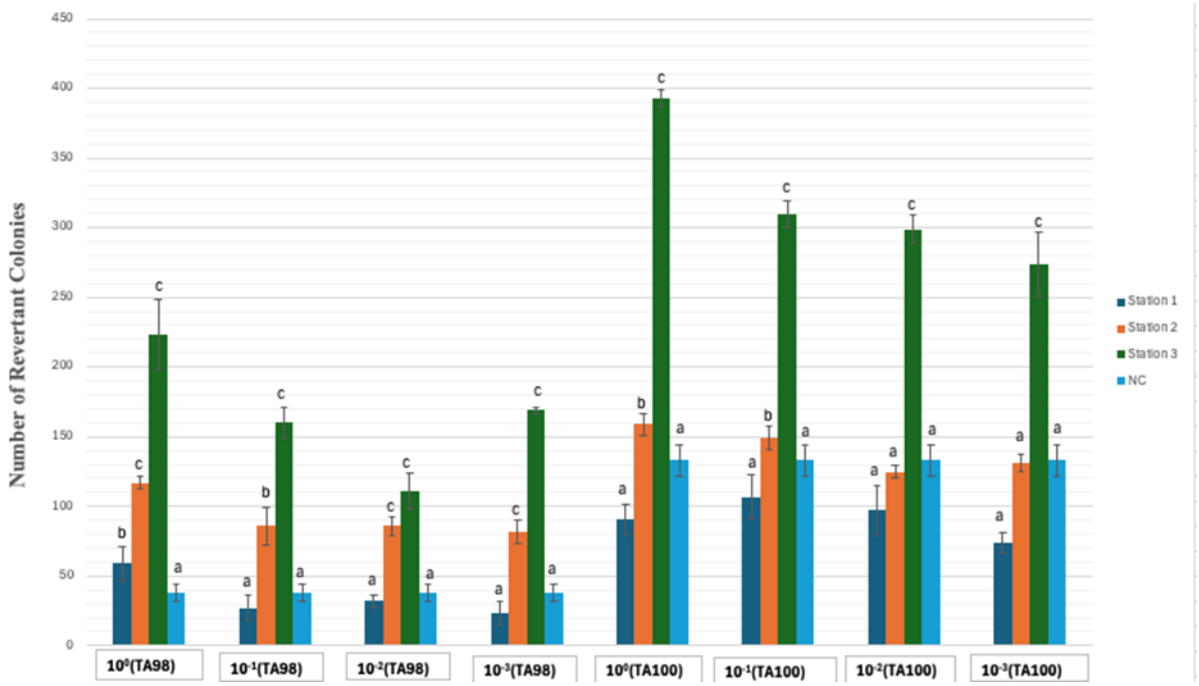


*(a: no mutagenic effect, b: weak mutagen, c: mutagen)

Figure 3. The Effects of Chloroform Extracts from Water Samples Collected at Stations 1, 2, and 3 of the Kars River at Concentrations of 10^0, 10^{-1}, 10^{-2}, and 10^{-3} on *S. Typhimurium* TA98-TA100 Strain in the Absence of S9.

The effects of chloroform extract obtained from water samples collected from the 1., 2., and 3. stations of the Kars River on *S. Typhimurium* TA98 strain were analyzed, and the results are presented in Figure 3. At the 1. station, weak mutagenic activity (b) was observed at the concentrations of 10^0, 10^{-1}, and 10^{-2}. However, at the 2. and 3. stations, mutagenic activity (c) was detected at all concentrations.

In the TA100 strain, weak mutagenic activity (b) was observed only at the 10^0 concentration at the 1. station, while no mutagenic activity (a) was detected at other concentrations. At the 3. station, mutagenic activity (c) was observed across all concentrations. At the 2. station, weak mutagenic activity (b) was detected only at the concentrations of 10^0, 10^{-1}, and 10^{-2}.



*(a: no mutagenic effect, b: weak mutagen, c: mutagen)

Figure 4. The Effects of Extracts from Sediment Samples Collected at Stations 1, 2, and 3 of the Kars River at Concentrations of 10^0, 10^{-1}, 10^{-2}, and 10^{-3} on *S. Typhimurium* TA98-TA100 Strain in the Absence of S9.

The effects of sediment samples collected from the 1., 2. and 3. stations on the *S. Typhimurium* TA98 strain were analyzed, and the results are presented in Figure 4. At the 1. station, weak mutagenic activity (b) was observed only at the 10^0 concentration. At the 2. station, mutagenic activity (c) was detected at concentrations of 10^0, 10^{-2}, and 10^{-3}. At the 3. station, mutagenic activity (c) was observed at all concentrations.

In the TA100 strain, no mutagenic activity was observed at the 1. station. At the 3. station, mutagenic activity (c) was reported at all concentrations, while at the 2. station, weak mutagenic activity (b) was detected only at the concentrations of 10^0 and 10^{-1}.

Sediment samples, particularly those from the 3. station, demonstrated strong mutagenic activity at all concentrations in both strains. This finding indicates that the contaminants accumulated in the sediment create a more intense mutagenic effect compared to water samples.

In conclusion, there is significant variation in environmental pollution levels among the stations, and this pollution plays a critical role in influencing mutagenic effects.

As the dilution factor decreased, a reduction in the number of revertant colonies was noted. This observation clearly demonstrates that the mutagenic effect is concentration-dependent.

Substances exhibiting concentration-dependent mutagenicity are commonly observed among environmental pollutants or chemicals.

The mutagenicity tests revealed no potential mutagenic effects in the hexane and chloroform extracts of water or sediment samples from the 1. Station. However, at the 2. station, mutagenic effects leading to frameshift mutations were detected. At Station 3, mutagenic effects causing both frameshift mutations and base pair substitutions were observed.

4. DISCUSSION AND CONCLUSION

This study aimed to assess the pollution level of the Kars River by determining the presence of mutagenic materials stored in sediments and dissolved in water. The investigation of mutagenicity was conducted using the Salmonella/Ames test with TA98 and TA100 strains in an environment lacking metabolic enzymes (without S9). Water and sediment samples collected from three different stations were subjected to doses of 10^0, 10^{-1}, 10^{-2}, and 10^{-3}. The experiments were independently repeated three times for each of the four concentration doses. As a result of the mutagenicity tests, no potential mutagenic effects were observed in the water samples (hexane and chloroform extracts) and sediment samples collected from Station 1. However, at Station 2, a mutagenic effect causing only frameshift mutations was detected. At Station 3, mutagenic effects causing both frameshift mutations and base pair substitutions were observed.

When the results are evaluated, it is evident that a weak mutagenic effect was detected at Station 1. The mutagenic effect increased at Station 2 compared to Station 1, and the highest mutagenic effect was observed at Station 3. The mutagenic potential of water and sediment samples shows similarities across the stations. The low mutagenic effect at Station 1 and the highest mutagenic effect at Station 3 can likely be attributed to the passage of the Kars River through the city center of Kars Province. Active residential areas, shopping centers, and livestock waste contribute to the pollution of the Kars River. Since Station 3 is located on

the outskirts of the city, the high mutagenic effect observed there can be linked to these factors.

In the experiments conducted, the number of revertant colonies observed in the spontaneous, negative, and positive control groups was found to be consistent with previously reported studies in the literature (Maron & Ames, 1983; Mortelmans & Zeiger, 2000; Güzey, 2013; Çakmak, 2013).

In a study investigating the mutagenic effects of the Nagara River and its sediments using the Ames test system, samples were subjected to extractions with different solvents. The study focused on mutagenic activity using the *S. typhimurium* TA100 strain in the presence of S9 (+). The highest mutagenic effect was observed in the isooctane-benzene group (Sato et al., 1983).

Another study revealed that surface waters associated with industrial activities exhibited mutagenic effects. Additionally, the mutagenicity of water entering and exiting treatment plants was higher compared to surface waters, emphasizing the need to evaluate the efficiency of treatment plants (Vargas et al., 1995).

The mutagenicity of drinking water was tested in another study, and positive results were reported for both strains (TA98-TA100). This study indicated that chemical agents capable of causing both base pair substitutions and frameshift mutations might be present in drinking water (Tortora, 1992).

Sediment samples collected from the Adriatic Sea were extracted using petroleum ether and methanol and analyzed with the Ames test using the *S. typhimurium* TA98 strain in the presence of S9 (+). Mutagenic activity was reported in five out of the seven extracts tested (Picer et al., 2001).

A study conducted by Boyacıoğlu investigated sediment samples from İzmir Bay (inner, middle, and outer regions) using the Ames test system with TA98 and TA100 strains. The study reported various forms of pollution across the regions and compared their mutagenic effects (Boyacıoğlu, 2004).

Further studies on water and sediment samples from other rivers, including the Karasu Stream in Bilecik (Ateş, 2011), the Tunca, Meriç, Arda, and Ergene Rivers (Soylu, 2012), and

the Tunca River (Güzey, 2013), have shown varying levels of mutagenic effects, emphasizing the importance of studying pollution in aquatic ecosystems.

The increase in the human population, continuous industrial growth, urbanization, and agricultural activities are contributing to ecosystem degradation. Polluted wetlands directly threaten human and environmental health.

In this study, water and sediment samples from the Kars River revealed no potential mutagenic effect in water samples from Station 1, while a mutagenic effect causing frameshift mutations was observed at Station 2. At Station 3, both frameshift mutations and base pair substitutions were observed. In particular, the samples from Station 3 indicated a high level of pollution, suggesting a potential mutagenic risk.

Considering these findings, it is essential to implement measures to minimize aquatic pollution, which causes genotoxic effects in living organisms. Controlling the use of pesticides in agricultural areas, filtering domestic and industrial waste before it is released into water sources, reducing plastic usage, and avoiding products that cannot decompose naturally are critical steps toward preventing pollution.

The method used in this study (Ames test) is a preliminary step in determining mutagenic activity. To ensure the reliability of the findings, in vivo and in vitro genotoxicity tests should be conducted, and the results should corroborate those of the Ames test.

Statement

This article is derived from the Master's thesis of Beyza SEZEN.

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Conflict of Interest

The authors declare that there is no conflict of interest among them.

Author Contributions

The planning of the research was carried out by P. Aksu-Kılıçle, data collection was conducted by B. Sezen, and data analysis was performed by P. Aksu-Kılıçle and B. Sezen.

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