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Investigation of Possible Genotoxicity of Surface and Tap Waters Using the Comet Assay

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ABSTRACT

We tested the hypothesis that chronic exposure to total heavy metal loads in Cevizdere surface water and Ünye District tap water of Ordu Province (Türkiye) may cause a genetic toxic effect in erythrocyte cells of carp (*Cyprinus carpio*) individuals. For this purpose, total heavy metal (loid)s contents in the surface and tap waters were determined seasonally. It was determined that both Cevizdere surface water and Ünye District tap water caused DNA damage in erythrocyte cells of carp fish, and this damage was statistically significant when compared to control groups (P<0.05). As a result of the comet analysis, the DNA damage detected in the erythrocyte cells of *C. carpio* showed the presence of genotoxic potential of Cevizdere Stream surface water and Ünye District tap water. The genotoxicity of the surface waters of the Cevizdere Stream, which is the main source of drinking water for the Ünye District in Ordu City, as well as the Ünye District tap waters, was evaluated *in vivo* for the first time using the Comet assay. This study revealed genotoxic damage in *C. carpio* due to total heavy metal(loid) pollution in the surface water of Cevizdere Stream and tap water of Ünye District, contributing to a better understanding of the relationship between genotoxicity and heavy metal(loid) pollution.

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ÖZET

Ordu İli (Türkiye) Cevizdere yüzey suyu ve Ünye İlçesi musluk suyundaki toplam ağır metal yüklerine kronik maruz kalmanın sazan (*Cyprinus carpio*) bireylerinin eritrosit hücrelerinde genetik toksik etkiye neden olabilme hipotezini test ettik. Bu amaç doğrultusunda yüzey ve musluk sularındaki toplam ağır metal(loid) içerikleri mevsimsel olarak belirlenmiştir. Hem Cevizdere yüzey suyunun hem de Ünye İlçesi musluk suyunun sazan balıklarının eritrosit hücrelerinde DNA hasarına neden olduğu ve bu hasarın kontrol gruplarıyla karşılaştırıldığında istatistiksel olarak anlamlı olduğu belirlenmiştir (P<0,05). Comet analizi sonucunda *C. carpio*'nun eritrosit hücrelerinde tespit edilen DNA hasarı, Cevizdere yüzey suyu ve Ünye İlçesi musluk suyunun genotoksik potansiyelinin varlığını göstermiştir. Ordu İli, Ünye İlçesi'nin içme suyunun ana kaynağı olan Cevizdere'nin yüzey sularının ve Ünye İlçesi musluk sularının genotoksisitesi Comet yöntemi kullanılarak ilk kez *in vivo* olarak değerlendirilmiştir. Bu çalışma, Cevizdere yüzey suyu ve Ünye İlçesi musluk suyunda toplam ağır metal(loid) kirliliğine bağlı olarak *C. carpio*'da genotoksik hasar olduğunu ortaya koyarak, genotoksisite ile ağır metal(loid)ler arasındaki ilişkinin daha iyi anlaşılmasına katkı sağlamıştır.

Genetik

Araştırma Makalesi

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INTRODUCTION

Water pollution, caused by various types of pollutants, is mostly the result of human activities like industry, agriculture, and urbanization. This is one of the most critical problems for all organisms, including humans, in terms of protecting and sustaining water resources. In addition, another important concern about water pollution is the potential negative effects of genotoxic pollutants on humans and native biota. Different chemicals, such as heavy metals and metalloids, directly contribute to water pollution. These substances can cause DNA damage and mutations in living organisms. Even chronic exposure to low doses of these chemicals can negatively affect biodiversity and increase the risk of developing cancer in humans (Mitra et al., 2022). Heavy metals, which are among the leading pollutants in aquatic systems, can be found everywhere because they are formed because of atmospheric and geological processes as well as human activities. Water bodies around the world have long been affected by heavy metal pollution, making it a global issue. Heavy metals can also be used as environmental monitoring factors and are often preferred in monitoring the state of the environment and organisms. Heavy metals and metalloids (heavy metal(loid)s) receive significant attention due to their toxicity, accumulation, and their harmful effects on aquatic ecosystems, fish, and human health (Fikirdesici Ergen et al., 2023; Khan et al, 2023).

Heavy metal(loid)s can cause DNA damage in organisms in different ways, such as breaking DNA strands and DNA-protein cross-links (Gebel et al., 1998; Kousar & Javed, 2015; Karaismailoğlu, 2022). Therefore, it is vital to determine the genotoxic effects of these pollutants for environmental impact assessment and organism monitoring studies. Comet assay has found wide application in the literature as a sensitive method used to evaluate DNA damage in organisms in terrestrial and aquatic systems exposed to various pollutants (Mokhamer et al., 2019; Kontaş, 2022). Since genotoxic effects can be detected in fish species, as in many organisms, even in cases of exposure to low concentrations of pollutants, with the help of this method, it is among the effective indicators in monitoring environmental health (Bolognesi & Cirillo, 2014; Kontaş, 2022).

Ordu is an important province in the Black Sea Region of Türkiye, which attracts tourists due to its natural, historical places, sea, rivers, lakes, ponds, waterfalls, and the opportunity for many cultural and touristic activities. Cevizdere valley is one of the most important valleys of Ordu city, and Ünye district is a distinguished district located in this valley. Cevizdere Stream, located within the borders of Ünye district, is the most important stream source providing drinking water to this district. Ünye Drinking Water Treatment Plant, one of the main drinking water sources of the city, is located in the Cevizdere Stream basin. Agricultural land is cultivated in the basin, and animal husbandry and beekeeping activities are carried out. Additionally, the Ünye wastewater treatment plant is located on the east side of the downstream part of the study area (Oy, 2018; Beden & Ulke Keskin, 2021; Yedier et al., 2022). Cevizdere Stream and the basin in which it is located are also important for the economy of Ordu city. Ünye Cement Factory, located on the southern slopes of the valley where the Cevizdere Stream is located, is an important industrial facility for both the region and Türkiye (Ünye Municipality (MU), 2018). Ünye district's water resources are exposed to different pollutants such as agriculture, cement, textile, mining, and domestic waste (Kurucu & Bostancı, 2022; Bostancı et al., 2024). There are a limited number of studies in the literature investigating the genotoxicity of surface and tap water (Vazquez Boucard et al., 2017; Feretti et al., 2020). In addition, no detailed study was reported investigating the genotoxicity of surface water used as a drinking water source, as well as tap water in cities in the Black Sea region. Therefore, within the scope of this study, we tested the hypothesis that chronic exposure to total heavy metal loads in Cevizdere surface water and Ünye District tap water in Ordu Province (Türkiye) may cause genetic toxic effects on erythrocyte cells of carp (*Cyprinus carpio*) individuals.

MATERIAL and METHOD

Sampling Areas and Collection of Water Samples

The sampling areas in the study were selected as the local consumers in the Ünye district for tap water samples and the water intake area of the drinking water treatment plant on the Cevizdere Stream, which provides drinking water to the Ünye district for surface water samples (Figure 1). Within the scope of the study, water samples were collected seasonally from several random points at the stations, representing these sampling points in 2020, and quickly transported to the laboratory in sterile polyethylene jerricans, where they were mixed in the laboratory to form a representative sample and transferred to the control and experimental tanks.

Water Chemical Analysis

In order to prepare the water samples for heavy metal analysis, firstly, the water sample representing each group was filtered with the help of a 0.45 µm nitrocellulose membrane filter, and nitric acid was added to keep the pH of the samples below 2. Then, the water samples were taken into Falcon tubes, labeled, and stored at +4 C° until analysis. The heavy metal(loid) concentrations (µg/L) in these water samples were measured on Inductively

Coupled Plasma Mass Spectrometry (ICP-MS) at Sinop University Scientific and Technological Research Application and Research Center (SUBITAM). Since the toxic effects will be evaluated based on the total heavy metal load within the scope of the study, the heavy metals in the relevant water samples were evaluated not individually but by taking into account the total heavy metal (loid) content.

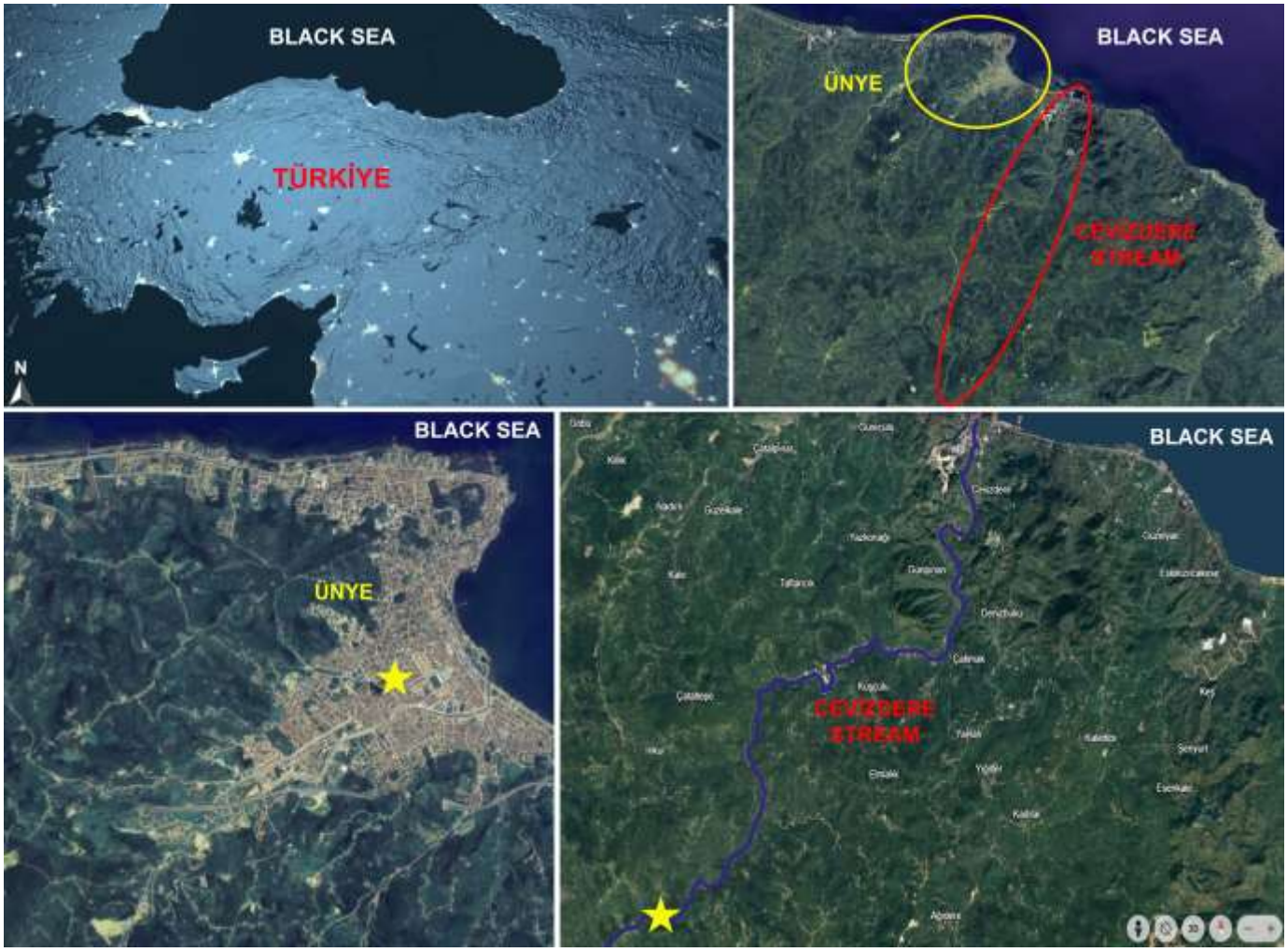

Figure 1. Sampling areas.
Şekil 1. Örnekleme alanları.

Test Organisms

Fish samples, *Cyprinus carpio*, were obtained from Suluova Yedikır Aquaculture Production and Research Station, Amasya, Türkiye. The experimental procedures of the study were approved by the Animal Experiments Local Ethics Committee, Ordu University (approval number: 82678388/5). The length and weight of *C. carpio* individuals were selected to be between 4.5 - 5.5 cm and 1.00 - 1.70 g, respectively, and the fish were transferred to 80 L aquariums in the laboratory where they were acclimated to the environmental conditions for a month. Aquariums were aerated with air stone diffusers and sponge air pumps. Oxygen concentration, pH, and temperature in aquarium water are between 80-90%, 7-8, and 24-26°C during the day and night periods, respectively. Fish samples were fed with commercial feed without additives twice a day, at 8 am and 5 pm, throughout the experiment. In this study, the experimental setup was designed into three main groups: Group I: Cevizdere Stream (Surface water), Group II: Ünye (Tap water), and Group III: Control groups (dechlorinated clean water). Fish samples were placed in the relevant tanks of these three groups, 20 fish in each tank. All experiments were carried out in three replicates. Water samples in the tanks were renewed every ten days. Three fish samples were randomly taken from each tank for comet analysis on the 10^{th}, 20^{th}, and 30^{th} days after the start of the experiment.

In vivo Comet Assay

At the end of the 10^{th}, 20^{th}, and 30^{th} days, three fish samples from each tank were anesthetized with clove oil, and blood samples were immediately collected from the hearts of these samples with heparinized syringes, and the

procedure of Comet assay was immediately performed (Sing et al., 1988; Kontaş, 2022). After the erythrocyte cells were stained, comet images were captured using a fluorescence microscope (Leica DM2500, Leica Microsystems, Germany) at 20X objective. Quantification of the DNA single-strand breaks in the stored images was analyzed using Comet Score 2.0 software (Tritek Corp, Sumerduck, VA, USA). A hundred erythrocyte cells were randomly counted for each fish sample. The % DNA in the tail (tDNA%) is a reliable parameter of DNA damage because both the extent and amount of DNA in the tail are considered (Hartmann et al., 2003; Kumaravel & Jha, 2006; Tung et al., 2024). Therefore, % tail DNA, which is a valid and reliable reflection of genetic damage, is used to assess DNA damage in erythrocyte cells of fish samples.

Statistical Analysis

The DNA damage, as recorded by the alkaline comet assay, was analyzed considering the mean (± standard error) of the % tail DNA measured. The data were normally distributed (Kolmogorov–Smirnov normality test) and therefore Student's t test was performed for comparison of means between groups (control and treated groups). Then, comparisons according to the groups (Group I, Group II, Group III), the exposure times (10^{th}, 20^{th}, and 30^{th} days), and the seasons (autumn, winter, spring, and summer) were tested using the Minitab 18.0 Statistical Software. P-values lower than 0.05 were considered statistically significant.

RESULTS and DISCUSSION

Total Heavy Metal(loid)s Contents of Water Samples

When the heavy metal data in tap water and surface water samples were evaluated according to the relevant water quality criteria based on each heavy metal data (except for copper in winter and spring seasons), it was determined that they were generally within the range suitable for human use (Official Gazette of the Republic of Türkiye, 2005; 2019; 2022). However, since heavy metals together will create more toxic effects in the water environment, it was deemed more appropriate to compare the total heavy metal loads for water samples in each season for the purpose of the study. Total heavy metal(loid)s contents in the Cevizdere Stream surface water and the Ünye District tap water of Ordu province were determined as 26.703 ± 4.785 µg / L for Cevizdere Stream and 134.135 ± 7.128 µg / L for Ünye District tap water in spring, 47.955 ± 3.942 µg/L for Cevizdere Stream and 57.521 ± 5.412 µg / L for Ünye District tap water in summer, 27.684 ± 4.842 µg / L for Cevizdere Stream and 31.212 ± 4.587 µg / L for Ünye District tap water in Autumn, 40.052 ± 4.235 µg / L for Cevizdere Stream and 96.101 ± 6.053 µg / L for Ünye District tap water in winter.

When the total heavy metal(loid)s contents of the surface waters of the Cevizdere Stream and the tap waters of Ünye District were compared, it was determined that the total load contents of the tap waters were higher than the surface waters. A similar situation in terms of total heavy metal load was reported in a study conducted in the Black Sea region, which covers the sample area of the current study and is consistent with the data in the current study (Bostancı et al., 2024). It was stated that parameters such as temperature, pH, DO, and water salinity can affect the concentration of heavy metals in water (Wisha et al., 2018). Increasing temperature values in water may have different effects on heavy metals. Depending on the chemical properties of the heavy metal, it may show a parallel to the temperature increase or the opposite may be the case (Gati et al., 2016; Lazăr et al., 2024). This situation may also lead to their seasonal toxicological effects being different. When the total heavy metal(loid)s contents were evaluated seasonally, the total heavy metal(loid)s contents in the surface waters of the Cevizdere stream were determined to be lower than the value in the tap water in their respective groups in all seasons. The differences between the total heavy metal(loid)s amounts in the Cevizdere Stream surface water and Ünye District tap water were statistically significant in spring, summer, and winter seasons ($P < 0.05$), and it was determined that there was no statistical difference in the autumn season ($P > 0.05$).

Heavy metalloids are significant pollutants due to their high potential to spread from various sources. In addition, heavy metalloids pose a particular concern in aquatic systems and humans because they can show high toxicity to organisms even at low concentrations (Marcovecchio et al., 2007). Therefore, heavy metalloid pollution in water is not only a worldwide environmental problem but also a critical issue for human health (Sekabira et al., 2010). For this reason, it is crucial to continuously assess the quality of water sources. Therefore, analyzing and quantifying these toxic agents in water resources is of great importance. In order to protect human health globally, various international organizations such as USEPA, WHO, EPA, and the European Union Commission have established guidelines for the presence of heavy metals in drinking and surface waters (Mohod & Dhote, 2013; Eissa et al., 2023). The coastal districts of Ordu city, like Ünye, are heavily polluted with metalloid-based pollutants. These pollutants, which can have bioaccumulative and toxic effects on organisms, come from natural sources like rock erosion and human activities such as domestic and industrial wastewater discharge, as well as the use of herbicides and pesticides (Kurucu & Bostancı, 2022; Bostancı et al., 2024). The variation in the total load of heavy metals (loids) in surface water samples in the Ünye district between seasons could be due to changes in climate as well as

an increase in the district's population because of tourism activities. Additionally, surface waters are more affected by factors such as agricultural drainage, domestic sewage, and municipal wastewater. In addition to population growth, the concentration of heavy metal(loid)s in surface waters has increased due to industrialization in the coastal regions of Ünye District. Similar to this ur study, many studies have stated that domestic and industrial wastewaters are the primary contributors to heavy metal pollution in coastal cities and districts, posing a significant genotoxic risk to aquatic systems (Bashar & Fung, 2020; Liu et al., 2022). Sunjog et al. (2012) reported that industrial and domestic wastewaters in Serbia have genotoxicity potential because it is not treated before being released into waterways, and that they can be effectively monitored with comet analysis in aquatic ecosystems in Serbia. Silva et al. (2020) found that elevated levels of certain metals in urban water can lead to genetic damage in *Astyanax lacustris*, consistent with this study's findings.

It was reported that contaminants in tap and drinking waters used for consumption are related to the presence of disinfection by-products and corrosion in pipes used in distribution systems, and genotoxic damage may occur in organisms exposed to these pollutants (Richardson et al., 2003; Zegura et al., 2009; Cortés & Marcos, 2018). A similar situation may also be the case in terms of heavy metal pollution in the tap water of Ünye District. Ünye District's main water is provided by long-distance water transportation through pipes. The district's water distribution system is old and worn, making it more susceptible to corrosion, and as a result, contaminants from pipes in the distribution system can contaminate tap water. In this study, it is thought that this may be one of the main reasons for heavy metal(loid) pollution in tap water in the Ünye district of Ordu province. In addition, after many connection points in plumbing fixtures and pipes in water distribution systems are joined by welding processes, heavy metals in these parts can migrate into water due to aging in these parts over time. Moreover, contamination in the water may result from potential issues such as breakage and cracking at these connection points. Many studies have found that lead is commonly used for soldering in water distribution systems and plumbing pipes, potentially leading to increased levels of heavy metals in tap water (Jaishankar et al., 2014; Sorlini et al., 2014; Ghoochani et al., 2023). In addition, it was reported that in residences using plastic pipes, possible breakage and leakage of plastic pipes in close contact with domestic garbage dumps may cause heavy metal-containing leachate to mix with tap water and may be responsible for the increase in heavy metal concentration in tap water (Duplay et al., 2013; Chowdhury et al., 2021). Moreover, soft acidic water may cause some complications in pipelines due to its contact with plumbing, taps, and water fittings in residences. These situations may cause corrosion in water transmission and installation pipes and increase the concentration of some heavy metals such as Fe, Cu, Pb, and Ni in tap water (WHO, 2005; USEPA, 2011; WHO, 2022). In addition, high levels of heavy metals such as Cu in tap water may have a corrosive effect on the increased dissolved oxygen level in tap water and heavy metal(loid)s leakage from pipes in buildings. The pollution of heavy metals (loid)s in the surface water of Cevizdere Stream and tap water in the Ünye district can be attributed to various reasons.

Genotoxicity of Cevizdere Stream surface water and Ünye tap water

In the present study, the comet test was successfully performed in *Cyprinus carpio* erythrocyte cells. The values of DNA in the tail (%) used to determine DNA damage in fish exposed to Cevizdere Stream and Ünye tap water, are summarized in detail according to the experimental groups (tap water, surface water, and control), and seasons (spring, summer, autumn, and winter), and exposure times (10^{th}, 20^{th}, and 30^{th} days) (Figure 2). In the present study, it was found that the surface waters of Cevizdere Stream and tap waters of Ünye District exhibited significant genotoxic effects in *C. carpio* samples compared to the negative control, as determined by comet analysis. It was determined that genotoxic damage increased in direct proportion to the total heavy metal content in water samples and exposure times, and showed a positive correlation. Comet analysis data of the fish individuals exposed to Cevizdere Stream surface waters in the spring revealed that tail DNA% values were 13.31% on the 10^{th} day, 13.96% on the 20^{th} day, and 15.70% on the 30^{th} day (Figure 2A). These results also show that the genotoxic effect of Cevizdere Stream surface water in the spring season on the fish increases depending on the exposure time. There are statistical differences in tail DNA% between exposure times in the Cevizdere Stream surface water group in the spring season ($P<0.05$). According to the comet analysis results of *C. carpio* individuals exposed to Ünye tap waters in spring, tail DNA% values were 13.43% on the 10^{th} day, 14.55% on the 20^{th} day, and 15.94% on the 30^{th} day (Figure 2A). These results show that the genotoxic effect of Ünye tap waters in the spring season on the fish increases depending on the exposure time. There are statistical differences in tail DNA% between exposure times in the Ünye tap water group in the spring season ($P<0.05$). It was also determined that Ünye tap water causes more DNA damage than Cevizdere surface water in the spring season. In addition, it was determined that there were statistical differences in tDNA% when both Cevizdere Stream surface water and Ünye tap water were compared with the negative control group at all exposure periods (10^{th}, 20^{th}, and 30^{th} days) in the spring season (Figure 2A).

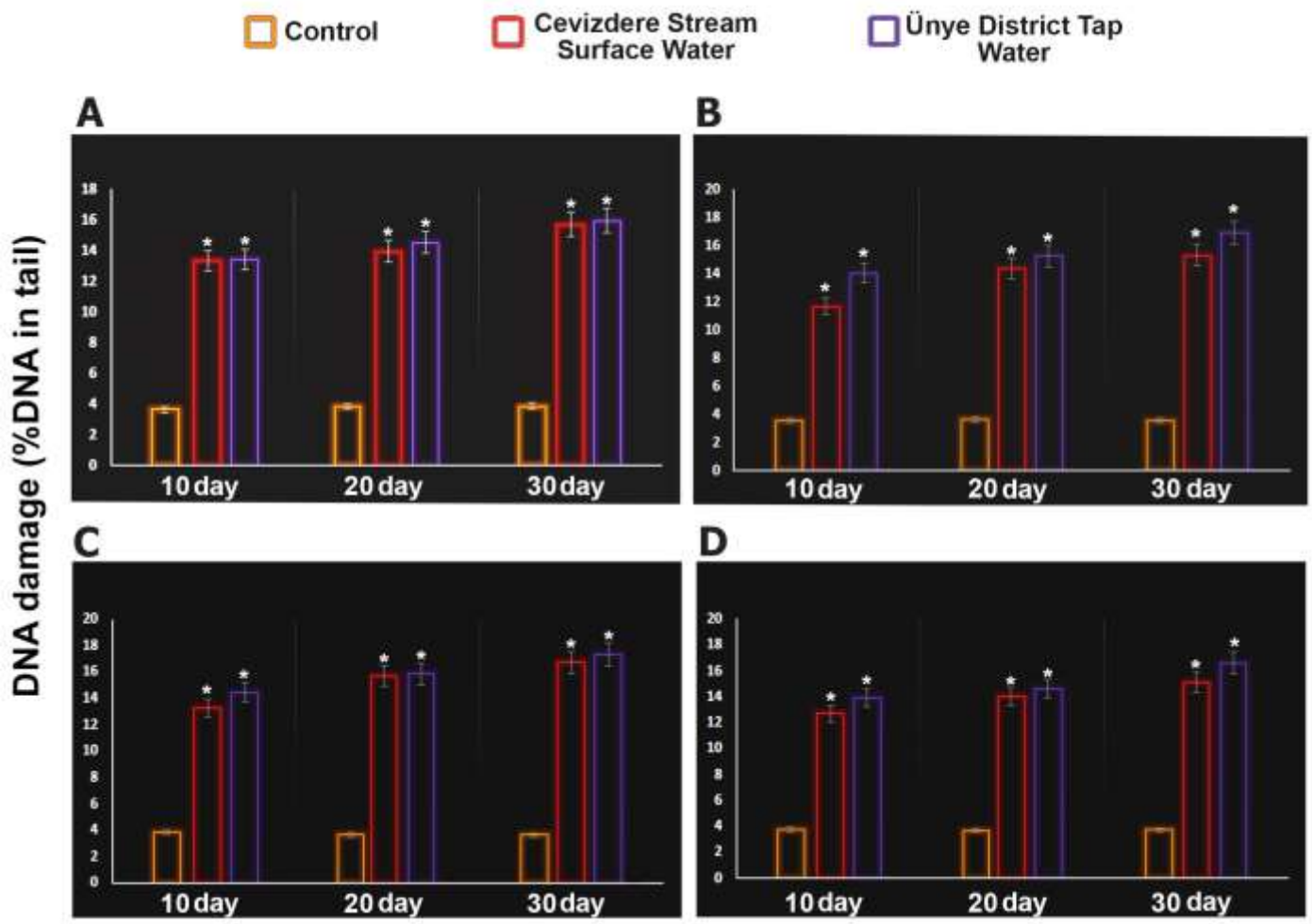

Figure 2. DNA percentages in the tail in erythrocyte cells of Cyprinus carpio subjected to increasing exposure time to water extracts in Cevizdere Stream surface water, Ünye District tap water, and control groups in spring (A), summer (B), autumn (C), and winter (D) seasons.

Şekil 2. Cevizdere Deresi yüzey suyu, Ünye İlçesi musluk suyu ve kontrol gruplarında ilkbahar (A), yaz (B), sonbahar (C) ve kış (D) mevsimlerinde su ekstraktlarına artan sürelerle maruz bırakılan Cyprinus carpio'nun eritrosit hücrelerindeki kuyruktaki DNA yüzdeleri.

According to the comet analysis results of *C. carpio* individuals exposed to Cevizdere Stream surface water samples in summer, tail DNA% values were 11.68% on the 10th day, 14.41% on the 20th day, and 15.31% on the 30th day (Figure 2B). These results also show that the genotoxic effect of Cevizdere Stream surface water in the summer season on the fish increases depending on the exposure time. It was determined that Cevizdere Stream surface water samples in the summer season caused statistical differences in tDNA% examined in the study, according to the exposure time in *C. carpio* ($P<0.05$). Comet analysis data of fish individuals exposed to Ünye tap water in the summer revealed that tail DNA% values were 14.06% on the 10th day, 15.27% on the 20th day, and 16.93% on the 30th day (Figure 2B). These results show that the genotoxic effect of Ünye tap waters in the summer season on the fish increases depending on the exposure time. There are statistical differences in tail DNA% between exposure times in the Ünye tap water group in the summer season ($P<0.05$). Ünye tap water causes more DNA damage than Cevizdere Stream surface water in the summer season, similar to the spring season. Moreover, statistical differences in tDNA% values were found when comparing both Ünye tap water and Cevizdere surface water with the negative control group at all exposure times in the summer (Figure 2B).

As a result of Comet analysis of *C. carpio* individuals exposed to Cevizdere Stream surface water samples in autumn, tail DNA% values were determined as 13.25% on the 10th day, 15.70% on the 20th day, and 16.71% on the 30th day (Figure 2C). These results show that the genotoxic effect of Cevizdere Stream surface water in the autumn season on the fish increases depending on the exposure time. Cevizdere Stream surface water samples in the autumn season caused statistical differences in tDNA% examined in the study, according to the exposure time in

C. carpio (P<0.05). Tail DNA% values in erythrocyte cells of *C. carpio* individuals exposed to Ünye tap water samples in the autumn were 14.45% on the 10th day, 15.84% on the 20th day, and 17.35% on the 30th day (Figure 2C). These results show that the genotoxic effect of Ünye tap waters in the autumn season on the fish increases depending on the exposure time. There are statistical differences in tail DNA% between exposure times in the Ünye tap water group in the autumn season (P<0.05). It was found that in autumn, Ünye tap water causes more DNA damage than Cevizdere Stream surface water, similar to the other seasons. Furthermore, it was determined that there were statistical differences in tDNA% values when both Ünye tap water and Cevizdere Stream surface water were compared with the negative control group at all exposure times in the autumn (Figure 2C).

Tail DNA% values in erythrocyte cells of *C. carpio* individuals exposed to Cevizdere Stream surface water samples in the winter were 12.73% on the 10th day, 14.02% on the 20th day, and 15.10% on the 30th day (Figure 2D). These results show that the genotoxic effect of Cevizdere Stream surface water in the winter season on the fish increases depending on the exposure time. There are statistical differences in tail DNA% between exposure times in the Cevizdere Stream surface water group in the winter season (P<0.05). According to the comet analysis results of erythrocyte cells of fish individuals exposed to Ünye tap water in winter, tail DNA% values were determined as 13.92% on the 10th day, 14.62% on the 20th day, and 16.63% on the 30th day (Figure 2D). These results show that the genotoxic effect of Ünye tap waters in the winter season on the fish increases depending on the exposure time. There are statistical differences in tail DNA% between exposure times in the Ünye tap water group in the winter season (P<0.05). It was also determined that Ünye tap water causes more DNA damage than Cevizdere Stream surface water in winter, similar to other seasons. Moreover, it was determined that there were statistical differences in tDNA% values when both Ünye tap water and Cevizdere surface water were compared with the negative control group at all exposure times in the winter (Figure 2D). In this study, the genotoxic effects of tap and surface waters were successfully carried out using the Comet assay. Like this study, the comet assay was used in many studies on the genotoxicity of water and has proven to be a sensitive and rapid method to detect DNA damage in organisms (Yuan et al., 2005; Zani et al., 2005; Buschini et al., 2008).

DNA damage in the erythrocyte cells of carp fish exposed to the surface waters of the Cevizdere Stream, which is the main drinking water source of Ünye District of Ordu province, and the tap water of Ünye district, may be caused by organic and inorganic pollutants. In addition, in monitoring studies in surface water and tap water, it is difficult to identify the compounds that may be responsible for possible negative effects associated with exposure to environmental pollutants in aquatic environments in mixed form and to directly attribute the genotoxic effect to a single type of pollutant. Heavy metals are among the most important pollutants affecting organisms and ecosystem health in aquatic systems. Moreover, the interactions of the relevant heavy metals (loid) with each other are quite complex, and these substances can show different effects when they are mixed in the same environment, as well as their effects when they are found alone in the environment (Kocadal et al., 2020; Kontaş, 2022; Mitra et al., 2022; Kontaş Yalçınkaya et al., 2025). In the current study, chemical analysis results of water samples showed the presence of metals in Cevizdere surface waters and Ünye district tap water. Even if they are not at very high levels, these pollutants have the potential to cause DNA damage to erythrocyte cells when evaluated as total heavy metal(loid)s. It is widely reported in the literature that exposure to these heavy metals may cause toxicogenic damage and carcinogenesis in organisms. When the DNA in the tail is evaluated, it is clear that these water samples cause DNA damage in the erythrocyte cells of carp fish. Similar to the current study, DNA damage caused by heavy metals (such as Cu, Fe, Mn, and Cd) was reported in *Clarias gariepinus* sampled from the Orontes River (Turan et al., 2020). There are many studies in the literature that mainly use the average value of tail length and tDNA% to evaluate genotoxicity (Alink et al., 2007; Pereira et al., 2012; Osman et al., 2012; Nwani et al., 2013). The mean tail length and tDNA% values were reported as 35.17 ± 1.370 % and 45.14 ± 5.610% in *Alburnus chalcoides* from Melet River (Kontaş & Bostancı, 2020). Moreover, the mean tDNA% value in *Clarias gariepinus* exposed to the surface waters of the Orontes River was calculated as 17.746 ± 1.072 % (Turan et al., 2020). In this study, this value was calculated as 14.323 ± 1.236 % for spring, 13.800 ± 1.890 % for summer, 15.220 ± 1.779 % for autumn, and 13.950 ± 1.187 % for winter in the samples exposed to Cevizdere Stream surface water seasonally. In the *Channa punctatus*, it was determined that the percentage of tail DNA% in individuals in the control group was 1.6 ± 0.32, while it was 16.5 ± 0.61 in the treatment group, and this increase was reported to indicate significant DNA damage in this species (Mehra & Chadha, 2021).

Increasing exposure time in carp fish in all seasons caused an increase in DNA damage, and in this study, a positive relationship was observed between exposure time and DNA damage in all groups except the control group. A similar positive relationship between DNA damage and exposure time has been reported previously (Ahmed et al., 2005). Moreover, we observed an increase in DNA damage in erythrocyte cells of the *C. carpio*, depending on the total metalloid amount. It revealed a similar trend in *Labeo rohita* after exposure to organophosphate pesticides in blood samples (Mohanty et al., 2011) and in *Channa punctatus* after exposure to tetrabromobisphenol A (Sharma et al., 2019) and profenophos (Pandey et al., 2011). The increases in DNA damage observed in fish, depending on

the exposure time as a result of metalloid accumulation in their environment, are quite understandable. In high-temperature seasons (spring and summer), the concentration of pollutants and especially heavy metal(loid)s in water increases due to the decrease in the amount of surface water. This was also confirmed by the total heavy metal(loid)s load determined in the surface and tap water samples in this study. Therefore, this situation can be associated with the DNA damage detected in *C. carpio* in warm seasons. In low-temperature seasons (winter and autumn), especially as a result of rainfall, pollutants from agricultural areas and environmental wastes enter surface water resources in the form of oil. In this study, DNA damage caused by surface water in the erythrocyte cells of *C. carpio* during low-temperature seasons may be associated with excessive rainfall in these seasons. This study is also consistent with previous data reporting an increase in DNA damage in fish from waters contaminated with different pollutants, such as industrial, agricultural, and domestic wastes. In addition, it also supports the results emphasized in many genotoxic studies that the amount of DNA damage may vary depending on exposure time and seasonal differences (Dhawan et al., 2009; Andem et al., 2013; Kontaş, 2022).

CONCLUSION

This study revealed that the surface and tap waters of Ünye district, one of the important towns of Ordu province in the Black Sea Region, were polluted with heavy metal(loid)s and that the total heavy metal load in these waters caused genotoxic effects on carp fish. This study is the first to use comet analysis to evaluate the possible genotoxicity of the surface water of Cevizdere Stream, used as a drinking water source in Ünye district of Ordu province, and the tap water in these regions on carp fish. Concentrations of such contaminants in drinking water can often vary from nanograms per liter to micrograms per liter, often resulting in such contaminants falling below detection limits in analysis and their genetic toxic effects remaining undetected. In addition, although heavy metals do not cause genotoxic effects individually and in low amounts, these heavy metals have the potential to come together and create genotoxic effects as a total heavy metal load. Therefore, instead of evaluating the pollutants individually in such aquatic environments, it is necessary to evaluate them based on total accumulation, which can provide more detailed information about the relevant environment. In addition, it is important to analyze the pollutants in water with alternative analyses, such as comet analysis, to obtain detailed information about the current conditions of these environments. For this reason, local consumers should also be sure that the distributed water is of good quality until it reaches users, even if the surface water is thoroughly purified in treatment plants and offered for use as tap water. It is recommended that users use this water by taking the necessary precautions instead of using it directly in order to prevent negative health effects.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors declare no conflicts of interest.

REFERENCES

Ahmed, Z., Dent, R.G., Suggate, E.L., Barrett, L.B., Seabright, R.J., Berry, M., Logan, A. (2005). Disinhibition of neurotrophin-induced dorsal root ganglion cell neurite outgrowth on CNS myelin by siRNA-mediated knockdown of NgR, p75NTR, and Rho-A. *Molecular and Cellular Neuroscience, 28*(3), 509-523.

Alink, G.M., Quik, J.T.K., Penders, E.J.M., Spenkelink, A., Rotteveel, S.G.P., Maas, J.L., Hoogenboezem, W. (2007) Genotoxic effects in the Eastern mudminnow (*Umbra pygmaea* L.) after exposure to Rhine water, as assessed by use of the SCE and Comet assays: a comparison between 1978 and 2005. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 631*(2), 93-100.

Andem, A.B., Agbor, R.B., Ekpo, I.A. (2013). Review on comet assay: a reliable tool for assessing DNA damage in animal models. *Journal of Current Research in Science, 1*(6), 405-427.

Bashar, T., Fung, I.W.H. (2020). Water pollution in a densely populated megapolis, Dhaka. *Water, 12*(8), 2124.

Beden, N., Ulke Keskin, A. (2021). Estimation of the local financial costs of flood damage with different methodologies in Ünye (Ordu), Turkey. *Natural Hazards, 108*, 2835-2854.

Bolognesi, C., Cirillo, S. (2014). Genotoxicity biomarkers in aquatic bioindicators. *Current Zoology, 60*(2), 273-284.

Bostancı, D., Kontaş Yalçınkaya, S., Yedier, S., Tunca, E., Alkan, S. (2024). Determination of metal(loid) levels in drinking water resources and tap waters in Ordu Province. *The Black Sea Journal of Sciences*, *14*(2), 742-759.

Buschini, A., Carboni, P., Pellacani, C., Rossi, C., Poliü, P. (2008). Cytotoxic and genotoxic potential of drinking water: A comparison between two different concentration methods. *Water Research, 42*(8-9), 1999-2006.

Chowdhury, S., Kabir, F., Mazumder, M.A.J., Alhooshani, K., Al-Ahmed, A., Al-Suwaiyan, M.S. (2021). Models for predicting heavy metal concentrations in residential plumbing pipes and hot water tanks. *Aqua, 70*(7), 1038-1052.

Cortés, C., Marcos, R. (2018). Genotoxicity of disinfection byproducts and disinfected waters: A review of recent literature. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 831*, 1-12.

Dhawan, A., Bajpayee, M., Parmar, D. (2009). Comet assay: a reliable tool for the assessment of DNA damage in different models. *Cell Biology and Toxicology, 25*(1), 5-32.

Duplay, J., Khedhiriz, S., Semhiy, K., Darragiz, F. (2013). Water quality in a protected natural wetland: sebkhet El Kelbia, Tunisia. *International Journal of Environmental Studies, 70*(1), 33-48.

Eissa, F., Elhawat N., Alshaal, T. (2023). Comparative study between the top six heavy metals involved in the EU RASFF notifications over the last 23 years. *Ecotoxicology and Environmental Safety, 265*, 115489.

Feretti, D., Acito, M., Dettori, M., Ceretti, E., Fatigoni, C., Posadino, S., Zerbini, I., Villarini, M., Moretti, M., Castiglia, P., Azara, A. (2020). Genotoxicity of source, treated and distributed water from four drinking water treatment plants supplied by surface water in Sardinia, Italy. *Environmental Research, 185*, 109385.

Fikirdesici Ergen, Ş., Tekatlı, Ç., Altındağ, A., Kamışlı, G., Kocatürk Döngel, K. & Tunca, E., (2023). Determination of Metal(loid)s in Mavi Dam Lake Sediment (Ankara) and Evaluation of Health Risks Level *Kahramanmaraş Sütçü İmam Üniversitesi Tarım Ve Doğa Dergisi, 26*(5), 1010-1020.

Gebel, T., Birkenkamp, P., Luthin, S., Dunkelberg, H. (1998). Arsenic (III), but not antimony (III), induces DNA-protein crosslinks. *Anticancer Research, 18*(6A), 4253-4257.

Ghoochani, S., Hadiuzzaman, M., Mirza, N., Brown, S.P., Salehi, M. (2023). Effects of water chemistry and flow on lead release from plastic pipes versus copper pipes, implications for plumbing decontamination. *Environmental Pollution, 337,* 122520.

Hartmann, A., Agurell, E., Beevers, C., Brendler-Schwaab, S., Burlinson, B., Clay, P., Collins, A., Smith, A., Speit, G., Thybaud, V., Tice, R.R. (2003). Recommendations for conducting the *in vivo* alkaline comet assay. *Mutagenesis, 18*(1), 45-51.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B., Beeregowda, K.N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology, 7*(2), 60-72.

Karaismailoğlu, C. (2022). Cytotoxic and genotoxic effects of oxyfluorfen on the somatic cells of *Allium cepa*. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 25*(2), 207-214.

Khan, B.N., Ullah, H., Ashfaq, Y., Hussain, N., Atique, U., Aziz, T., Alharbi, M., Albekairi, T.H., Alasmari, A.F. (2023). Elucidating the effects of heavy metals contamination on vital organ of fish and migratory birds found at freshwater ecosystem. *Heliyon*, *9*(11), e20968.

Kocadal, K., Alkas, F., Battal, D., Saygi, S. (2020). Cellular pathologies and genotoxic effects arising secondary to heavy metal exposure: A review. *Human & Experimental Toxicology, 39*(1), 3-13.

Kontaş, S. (2022). *In vivo* assessment of genotoxic effects in *Cyprinus carpio* L., 1758 (Teleostei: Cyprinidae) exposed to selected metal(oid)s. *Toxicology and Industrial Health, 38*(5), 277-286.

Kontaş, S., Bostancı, D. (2020). Genotoxic effects of environmental pollutant heavy metals on *Alburnus chalcoides* (Pisces: Cyprinidae) inhabiting lower Melet River (Ordu, Turkey). *Bulletin of Environmental Contamination and Toxicology, 104*(6), 763-769.

Kontaş Yalçınkaya, S., Bostancı, D., Yedier, S. (2025). Genotoxicity assessment on presence of metal(loid)s in drinking water source and tap water. *Ege Journal of Fisheries and Aquatic Sciences*, *42*(1), 33-40.

Kousar, S., Javed, M. (2015). Diagnosis of metals induced DNA damage in fish using comet assay. *Pakistan Veterinary Journal, 35*(2), 168-172.

Kumaravel, T.S., Jha, A.N. (2006). Reliable comet assay measurements for detecting DNA damage induced by ionising radiation and chemicals. *Mutation Research, 605*, 7-16.

Kurucu, G., Bostancı, D. (2022). Ordu'nun derelerindeki fiziko-kimyasal özelliklere yeni katkılar: Curi Deresi (Ünye-Ordu). *Balıkesir Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, *24*(2), 700-708.

Lazăr, N., Simionov, I., Petrea, Ş., Iticescu, C., Georgescu, P., Dima, F., Antache, A. (2024). The influence of climate changes on heavy metals accumulation in *Alosa immaculata* from the Danube River Basin. *Marine Pollution Bulletin, 200*, 116145.

Liu, J., Hu, L.X., Deng, W.J., Ying, G.G., Hong, H., Tsang, E.P.K., Barceló, D. (2022). Pilot study of pollution characteristics and ecological risk of disinfection byproducts in natural waters in Hong Kong. *Environmental Toxicology and Chemistry, 41*(10), 2613-2621.

Marcovecchio, J.E., Botte, S.E., Freije, R.H. (2007). Heavy metals, major metals, trace elements. in: Nollet, L.M., (Ed), Handbook of water analysis. CRC Press, London, pp. 275–311.

Mehra, S., Chadha, P. (2021). Naphthalene-2-sulfonate induced toxicity in blood cells of freshwater fish *Channa punctatus* using comet assay, micronucleus assay and ATIR-FTIR approach. *Chemosphere, 265*, 129147.

Mitra, S., Chakraborty, A.J., Tareq, A.M., Emran, T.B., Nainu, F., Khusro, A., Idris, A.M., Khandaker, M.U., Osman, H., Alhumaydhi, F.A., Simal-Gandara, J. (2022). Impact of Heavy metals on the environment and human health: novel therapeutic insights to counter the toxicity. *Journal of King Saud University – Science, 34*(3), 101865.

Mohanty, S., Mishra, S.K., Patnaik, R., Dutt, A.K., Pradhan, S., Das, B., Patnaik, J., Mohanty, A.K., Lee, S.J., Dondorp, A.M. (2011). Brain swelling and mannitol therapy in adult cerebral malaria: a randomized trial. *Clinical Infectious Diseases, 53*(4), 349-355.

Mohod, C.V., Dhote, J. (2013). Review of heavy metals in drinking water and their effect on human health. *International Journal of Innovative Research in Science, Engineering and Technology, 2*(7), 2992-2996.

Mokhamer, E.H., Radwan, E.H., Elsaka, M. (2019). Oxidative stress and DNA damage in Nile Tilapia (*Oreochromis niloticus*) as biomarkers of aquatic pollution. *Journal of Bioscience and Applied Research, 5*(1), 92-109.

MU (Municipality of Ünye), (2018). Research and explanation report of 1/1000 scaled Ünye-Ordu revision and additional zoning plan.https://docplayer.biz.tr/105006253-Unye-ordu-revizyon-ve-ilave-imar-plani-1-1000-arastirma-ve-plan-aciklama-raporu.html, (Accessed 14 February 2024).

Nwani, C.D., Nagpure, N.S., Kumar, R., Kushwaha, B., Lakra, W.S. (2013). DNA damage and oxidative stress modulatory effects of glyphosate-based herbicide in freshwater fish, *Channa punctatus*. *Environmental Toxicology and Pharmacology, 36*(2), 539-547.

Official Gazette of the Republic of Türkiye (2005). İnsani Tüketim Amaçlı Sular Hakkında Yönetmelik. T.C. Sağlik Bakanlığı Halk Sağlığı Genel Müdürlüğü, Ankara, Türkiye. Available at https://www.mevzuat.gov.tr/mevzuat?MevzuatNo=7510&MevzuatTur=7&MevzuatTertip=5 (in Turkish).

Official Gazette of the Republic of Türkiye (2019). İçme Suyu Temin Edilen Suların Kalitesi ve Arıtılması Hakkında Yönetmelik. T.C. Tarım ve Orman Bakanlığı Su Yönetimi Genel Müdürlüğü, Ankara, Türkiye. Available at https://www.resmigazete.gov.tr/ eskiler/2019/07/20190706-8.htm (in Turkish).

Official Gazette of the Republic of Türkiye (2022). Su Kirliliği Kontrolü Yönetmeliğinde Değişiklik Yapılmasına Dair Yönetmelik. T.C. Çevre, Şehircilik ve İklim Değişikliği Bakanlığı, Ankara, Türkiye. Available at https://www.resmigazete.gov.tr/eskiler/2022/12/20221217-8.htm (in Turkish).

Osman, A.G.M., Abuel-Fadl, K.Y., Kloas, W. (2012). In situ evaluation of the genotoxic potential of the river Nile: II. Detection of DNA strand-breakage and apoptosis in *Oreochromis niloticus niloticus* (Linnaeus, 1758) and *Clarias gariepinus* (Burchell, 1822). Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 747(1), 14-21.

Oy, H. (2018). The prehistoric settlement at Tozkoparan (Unye-Ordu-Turkey). *Karadeniz Araştırmaları, 15*(57), 33-41.

Pandey, A.K., Nagpure, N.S., Trivedi, S.P., Kumar, R., Kushwaha, B. (2011). Profenofos induced DNA damage in freshwater fish, *Channa punctatus* (Bloch) using alkaline single cell gel electrophoresis. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 726*(2), 209-214.

Pereira, S., Camilleri, V., Floriani, M., Cavalie, I., Garnier-Laplace, J., Adam-Guillermin, C. (2012). Genotoxicity of uranium contamination in embryonic zebrafish cells. *Aquatic Toxicology, 109,* 11-16.

Richardson, S.D., Thruston, A.D., Jr Rav-Acha, C., Groisman, L., Popilevsky, I., Juraev, O., Glezer, V., McKague, A.B., Plewa, M.J., Wagner, E.D. (2003). Tribromopyrrole, brominated acids, and other disinfection byproducts produced by disinfection of drinking water rich in bromide. *Environmental Science & Technology, 37*(17), 3782-3793.

Sekabira, K., Oryem Origa, H., Basamba, T.A., Mutumba, G., Kakudidi, E. (2010). Assessment of seasonal variation in water quality in River Rwizi using multivariate statistical techniques, Mbarara Municipality, Uganda. *International Journal of Environmental Science & Technolog, 7*(4), 759-770.

Sharma, P., Chadha, P., Saini, H.S. (2019). Tetrabromobisphenol A induced oxidative stress and genotoxicity in fish *Channa punctatus*. *Drug and Chemical Toxicology, 42*(6), 559-564.

Silva, E.P.D., Benvindo-Souza, M., Cotrim, C.F.C., Motta, A.G.C., Lucena, M.M., Antoniosi Filho, N.R., Pereira, J., Formiga, K.T.M., Melo, E., Silva, D. (2020). Genotoxic effect of heavy metals on *Astyanax lacustris* in an urban stream. *Heliyon, 6*(9), e05034.

Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research, 175*(1), 184-191.

Sorlini, S., Gialdini, F., Collivignarelli, C. (2014). Metal leaching in drinking water domestic distribution system: an Italian case study. *International Journal of Environmental Health Research, 24*(6), 497-514.

Sunjog, K., Gačić, Z., Kolarević, S., Višnjić-Jeftić, Ž., Jarić, I., Knežević-Vukčević, J., Vuković-Gačić, B., Lenhardt, M. (2012). Heavy metal accumulation and the genotoxicity in barbel (*Barbus barbus*) as indicators of the Danube river pollution. *The Scientific World Journal*, *2012*, 351074.

Tung, G.K., Gandhi, G. (2024). Baseline and oxidatively damaged DNA in end-stage renal disease patients on varied hemodialysis regimens: a comet assay assessment. *Molecular and Cellular Biochemistry, 479,* 199-211.

Turan, F., Eken, M., Ozyilmaz, G., Karan, S., Uluca, H. (2020). Heavy metal bioaccumulation, oxidative stress and genotoxicity in African catfish *Clarias gariepinus* from Orontes river. *Ecotoxicology, 29*(9), 1522-1537.

USEPA (United States Environmental Protection Agency), (2011). Exposure Factors Handbook, Final ed., (EPA/600/R-09/052F). Washington DC: U.S. http://cfpub.epa.gov/ (Accessed 14 February 2024)

Vazquez Boucard, C., Lee-Cruz, L., Mercier, L., Ramírez Orozco, M., Serrano Pinto, V., Anguiano, G., Cazares, L., Díaz, D. (2017). A study of DNA damage in buccal cells of consumers of well- and/or tap-water using the comet assay: Assessment of occupational exposure to genotoxicants. *Environmental and Molecular Mutagenesis, 58*(8), 619-627.

Wisha, U.J., Hariati, A., Ramadhan, M., Mustikasari, E., Mutmainah, H., Ilham, I. (2018). Spatial distribution of disolved heavy metals (Hg, Cd, Cu, Pb, Zn) on the surface waters of Pare Bay, South Sulawesi. *ILMU KELAUTAN: Indonesian Journal of Marine Sciences, 23*(4), 199-206.

WHO (World Health Organization), (2005). Nickel in Drinking Water. WHO/SDE/WSH/05.08/55. Background document for development of WHO Guidelines for Drinking Water Quality. Available online: http://www.who.int/water_sanitation_health/gdwqrevision/nickel2005.pdf, (Accessed 14 February 2024)

WHO (World Health Organization), (2022). Guidelines for drinking-water quality: Fourth edition incorporating the first and second addenda. Geneva: World Health Organization; 2022. https://www.who.int/publications/i/item/9789240045064, (Accessed 14 February 2024)

Yedier, S., Kontaş, S., Bostancı, D. (2022). Assessing of fluctuating asymmetry in otolith of the *Alburnus* spp. from Anatolian lotic and lentic systems. *Ege Journal of Fisheries and Aquatic Sciences, 39*(1), 32-38.

Yuan, J., Wu, X.J., Lu, W.Q., Cheng, X.L., Chen, D., Li, X.Y., Liu, A.L., Wu, J.J., Xie, H., Stahl, T., Mersch-Sundermann, V. (2005). Chlorinated river and lake water extract caused oxidative damage, DNA migration and cytotoxicity in human cells. *International Journal of Hygiene and Environmental Health, 208*(6), 481-488.

Zani, C., Feretti, D., Buschini, A., Poli, P., Rossi, C., Guzzella, L., Di Caterino, F., Monarca, S. (2005). Toxicity and genotoxicity of surface water before and after various potabilization steps. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 587*(1-2), 26-37.

Zegura, B., Heath, E., Cernosa, A., Filipic, M. (2009). Combination of in vitro bioassays for the determination of cytotoxic and genotoxic potential of wastewater, surface water and drinking water samples. *Chemosphere, 75*(11), 1453-1460.