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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Genotoxicity assessment on presence of metal(loid)s in drinking water source and tap water

İçme suyu kaynağı ve musluk suyunda metal(loid)lerin varlığına bağlı genotoksisite değerlendirmesi

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Abstract: It is important to quantify the concentrations of metalloids and heavy metals (metal(oid)s) in drinking water sources and tap water due to contamination of drinking water sources by natural processes and anthropogenic activities. In this study, the genotoxicity of drinking water sources (Kacalı River) and tap water (Perşembe district) was mainly investigated by comet assay. The effect of metal(loid)s was monitored *in vivo* using erythrocyte cells of *Cyprinus carpio*. The eight heavy metals (aluminium, nickel, cadmium, lead, manganese, iron, copper, zinc) and one metalloid (arsenic) were found in the water samples and the total content of metal(loid)s was determined seasonally. In general, the total metal(loid)s content of the Perşembe tap water was higher than that of the Kacalı River in all seasons. Especially in summer, tap water causes higher DNA damage in *C. carpio* erythrocytes. Water samples from the Kacalı River showed significantly higher genotoxicity compared to control groups in all seasons. Careful management of water supplies is needed to reduce the health risks associated with genotoxicity in drinking water.

Keywords: Comet assay, drinking water, DNA damage, metal(loid) content, tap water, *Cyprinus carpio*

Öz: İçme suyu kaynaklarının doğal süreçler ve antropojenik aktivitelerle kirlenmesi nedeniyle içme suyu kaynakları ve musluk sularındaki metaloid ve ağır metal (metal(oid)lerin) konsantrasyonlarının ölçülmesi önem kazanmıştır. Bu çalışmada esas olarak içme suyu kaynağı (Kacalı Deresi) ve musluk suyunun (Perşembe İlçesi) genotoksisitesi comet analizi yöntemi ile araştırılmıştır. Metal(oid)lerin etkisi *Cyprinus carpio*'nun eritrosit hücreleri kullanılarak *in vivo* izlenmiştir. Su örneklerinde sekiz ağır metal (alüminyum, nikel, kadmiyum, kurşun, manganez, demir, bakır, çinko) ve bir metaloid (arsenik) bulunmuş ve toplam metal(oid) içerikleri mevsimsel olarak belirlenmiştir. Genel olarak Perşembe musluk suyunun toplam metal(oid) içeriği tüm mevsimlerde Kacalı Deresi'nden daha yüksek bulunmuştur. Özellikle yaz aylarında musluk suyu *C. carpio* eritrositlerinde daha fazla DNA hasarına neden olmuştur. Kacalı Nehri'nden alınan su örnekleri, tüm mevsimlerde kontrol gruplarıyla karşılaştırıldığında önemli ölçüde daha yüksek genotoksisite göstermiştir. İçme suyundaki genotoksisite ile ilişkili sağlık risklerini azaltmak için su kaynaklarının dikkatli bir şekilde yönetilmesi gerekmektedir.

Anahtar kelimeler: Comet analizi, içme suyu, DNA hasarı, metal(loid) içeriği, musluk suyu, *Cyprinus carpio*

INTRODUCTION

Chemical elements whose properties are classified between those of non-metals and metals are called metalloids (Batley, 1998). Heavy metals, such as mercury, lead, and cadmium, are metals with a density greater than 4.5 g/cm^3 (Mao et al., 2022). In the earth's crust, atmosphere, and water, heavy metals occur naturally (Jannetto and Cowl, 2023). Heavy metals lead to pollution in water resources (Kılıç, 2021). Water pollution is one of the most important environmental problems today and pesticides, fertilizers, mining, sewage waste, domestic wastewater, eutrophication, thermal pollution, oil spills, acid rain, and radioactive waste are among the main factors of water pollution (Hussain et al., 2018; Kontaş, 2022; Altunkaynak et al., 2023). These pollutants in water bodies can come from both natural sources such as erosion, corrosion and precipitation, and anthropogenic activities such as mining, agricultural spraying, industrial and domestic wastewater (Zeng et al., 2019).

Heavy metals can enter organisms directly from water or indirectly via the food chain. Heavy metals are known for their many negative properties such as persistence, bioaccumulation, and cellular, structural and environmental toxicity potential in aquatic systems and organisms (Zhang et al., 2014). Heavy metals, which have a high dispersal potential in aquatic systems due to such different factors, can maintain their presence in tap water as well as surface water and even drinking water (Zhang et al., 2014).

The detection of pollutants in aquatic systems and their possible genotoxic effects on organisms is important to study the effects on organisms and especially on fish (de Lapuente et al., 2015). Heavy metals are potentially genotoxic and carcinogenic and are known to induce oxidative stress in organisms. These genotoxic contaminants cause DNA damage in organisms and can even cause cell death by stimulating the production of reactive oxygen species (ROS) in cells (Lushchak, 2011). The complexity of contaminants in water samples leads to the preference of different genotoxicity

tests in studies. In this sense, the use of the comet assay test on different model organisms such as animal cells (*Onchorhynchus mykiss*, *Danio rerio*) for sensitive assessment of genotoxic effects of surface water samples is a very popular and accepted approach (Žegura and Filipič, 2019). The comet assay is a genotoxicity test that is generally applicable to all cell types and can be used on both prokaryotic and eukaryotic organisms. The comet assay can be used to assess water quality both *in vitro* and *in situ*. It is a reliable, rapid and cost-effective test for DNA damage susceptibility (Glei et al., 2016; Kontaş, 2022). The comet test allows the damaged DNA to be seen in the 'comet' structure and the damage in the cell increases as the calculated percentage of DNA in the tail increases and decreases as it decreases. (Doğan et al., 2022). Heavy metals are often found in trace amounts in aquatic environments, but human activities have the potential to release large amounts of these substances into water sources (Matos et al., 2017). Water is used for many human activities, and the quality of water needed for life is important (Mawari et al., 2022). Contamination of freshwater sources can result from human activities. The use of herbicides and pesticides causes an increase in metals in the water of Kacalı Stream. The differences in total metal content between seasons may be an indicator that surface waters are more polluted by different means in the respective seasons.

There is also the potential for contamination from corrosion of pipes used in plumbing fixtures and water distribution systems. The soft, acidic water can cause some complications in pipelines due to its contact with pipes, taps, and water fittings. In addition, if the source and drinking water contain high concentrations of sulfate and/or chloride, these corrosive chemicals can dissolve the lime in the water transmission system and cause the release of some undesirable heavy metals. These situations can 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; U.S.EPA, 2011). Many studies have reported that lead is often used for soldering purposes in water distribution and plumbing systems. As a result, Pb concentrations in tap water may increase (Sorlini et al., 2014; Ghoochani et al., 2023). Drinking water sources and tap water containing metals and other contaminants are a major environmental problem that threatens human health.

With increasing interest in the genotoxicity of contaminants in water, sensitive biological assays such as the comet assay have become an important tool for determining genotoxicity (Scalon et al., 2010). Understandably, given the current water shortage and the need for clean water for human consumption, there is growing concern about the genotoxicity of contaminants. For this reason, the genotoxicity of contaminated rivers, surface water resources and tap water can be more effectively revealed by combining the use of more sensitive biological assays with other supporting techniques.

There are many studies on this subject (Boucard et al., 2017; Turan et al., 2020; Altunkaynak et al., 2023). No detailed study was found investigating the genotoxicity of both surface and tap water in the city of Ordu. This study aims to provide reliable data for the future safe use of the drinking water resource (Kacalı River) in Ordu province and to help implement the necessary policies to improve the aquatic system. In this study, the total metal content (eight heavy metals (aluminium (Al), nickel (Ni), cadmium (Cd), lead (Pb), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn)) and a metalloid (arsenic (As)) in river and tap water were determined. In addition, we used the comet assay to evaluate the genotoxicity of tap water (Perşembe district) and surface water of the Kacalı River, an important water source that supplies drinking water to the local community of Ordu City.

MATERIALS AND METHODS

Water sampling areas

The Middle Black Sea region of Turkey includes the coastal district of Perşembe, which is located in the province of Ordu (Figure 1).

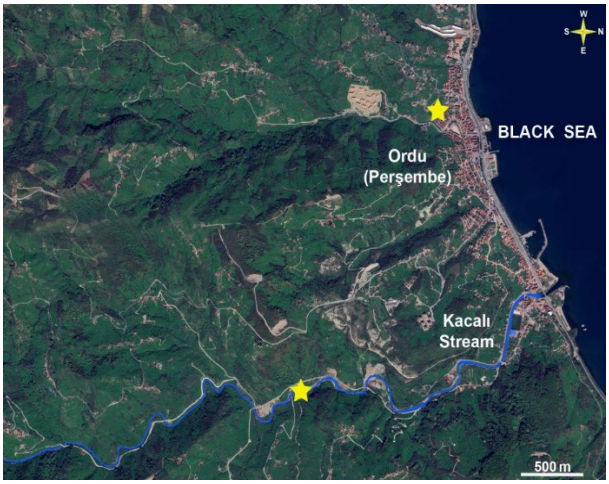

Figure 1. The sampling area (Kacalı Stream-Perşembe)

Perşembe was designated as a "Cittaslow" town, which is an international movement that is in favour of a high quality and peaceful way of life on 21 October 2012 (Şengür and Atabeyoğlu, 2018; Matcar, 2025). This study was conducted in the Kacalı stream, which flows from the Perşembe district of Ordu (Turkey) into the Black Sea, and the Perşembe tap water. Water samples were collected seasonally (winter, spring, summer and autumn seasons) from January 2020 to December 2020 from the entrance to the water intake points (in Kacalı stream) and tap water (Perşembe district). Samples were collected in three replicates to represent the study area (for surface and tap water). The water samples were collected in 10 L sterile bottles and taken to the laboratory.

Experimental design

Cyprinus carpio individuals were obtained from the Suluova Yedikır Aquaculture Production and Research Station (Amasya, Turkey). The experimental procedures of this study were approved by the Local Ethics Committee for Animal

Experiments, Ordu University (approval number: 82678388/5). The length and weight of *C. carpio* samples were selected between 4.5 - 5.5 cm and 1.00 - 1.70 g, respectively. Fish were acclimated to laboratory conditions for one month. The aquariums were aerated with air stone diffusers and sponge air pumps. The temperature, pH, and oxygen concentration of the aquarium water were maintained at 24-26°C, 7-8 and 80-90% during the day and night, respectively. Fish samples were fed commercial feed without additives twice a day during the experimental period. In this study, the experimental setup was designed as three main groups: (a) Kacalı stream (surface water), (b) Perşembe district (tap water) and (c) control groups (containing dechlorinated clean water). Fish samples (n=20) were placed in each tank. Five fish samples were randomly collected from each tank for comet assay on day 10, 20 and 30 of the experiments. All experiments were performed in three replicates.

Heavy metal analysis

The water in the tanks did not change throughout the experiment. Water samples (50 mL) were taken from each tank at the end of the 10th, 20th, and 30th day. The water samples (three replicates) were taken into 50 mL of Falcon tubes and acidified with 2M HCl (pH 2). Before the analysis, the water samples were filtered with Whatman GF/C type membrane filter (0.45 µm) (Alam et al., 2001). The heavy metals (Al, Cd, Cu, Fe, Mn, Ni, Pb, and Zn) and metalloid (As) concentrations (µg/L) of the water samples were determined by inductively coupled plasma mass spectrometry (ICP-MS) at the Scientific and Technological Research Application and Research Centre of Sinop University (SUBITAM). Since heavy metals were found as a mixture in the water samples taken from the stations, the toxicological effect of heavy metals was made on the total metal concentration (As, Al, Cd, Cu, Fe, Mn, Ni, Pb, and Zn).

Comet assay

Five fish samples from each tank were anesthetized with clove oil and blood samples were immediately taken from the hearts of these samples with heparinized syringes and the comet assay procedure was immediately performed at the end of the 10th, 20th, and 30th day. The comet assay was performed under alkaline conditions with some modifications (Kontaş, 2022; Chatha et al., 2024). For each fish, 300 µL of 1% low melting point agarose and 1 µL of blood were mixed and the slides were coated with 150 µL of this mixture. The slides were placed on a cooled layer for 30 minutes and then carefully placed in cold lysis buffer (pH 10) for one hour at 4°C. The stock solution for the lysis solution was prepared with 10 mM Tris, 2.5 M NaCl and 100 mM EDTA. Lysis buffer was prepared with 10 mL DMSO, 1 mL Triton and 89 mL stock solution. After one hour, the slides were placed in a cold electrophoresis buffer (pH> 13, prepared with 200 mm EDTA and 10 N NaOH). Electrophoresis was performed at 1 V/cm for 20 min at 4°C. After the electrophoresis phase, the slides were placed in a neutralization buffer (pH 7) for 15 min. Each slide was stained with ethidium bromide and examined under a fluorescence microscope attached to a TXR filter. One hundred erythrocytes were randomly counted for each fish sample. In genotoxicity studies, the tail DNA value (tDNA%), tail moment (TM), and olive tail moment (OTM) are the most reliable comet assay parameters used to determine DNA strand breaks (Kumaravel and Jha, 2006; Jiang et al., 2023). Therefore, in this study, tail DNA% (tDNA%), tail moment (TM), and olive tail moment (OTM) values were evaluated using CometScore software (Tritec Corp, Sumerduck, VA, USA).

Statistical analysis

Descriptive statistics of tDNA%, TM, and OTM parameters used to determine DNA damage in fish erythrocytes were calculated for each group. Groups (surface water, tap water, control), exposure periods (10th, 20th, 30th day), and seasons (spring, summer, autumn, winter) were compared by one-way ANOVA. A statistically significant P value is one less than 0.05. All tests were performed using the statistical analysis program MINITAB 17.0 (Minitab, Inc., State College, PA).

RESULTS

The total metal content is presented seasonally for the surface and tap water samples in Figure 2. In this study, the total metal content in the water of fish exposed to surface and tap water decreased during the exposure periods (10th, 20th, and 30th day).

The average total heavy metal content in Kacalı stream surface water and Perşembe tap water on days 10, 20, and 30 in the spring season were 112.71±8.25, 40.73±5.2, 19.92±3.23 and 166.77±8.57, 48.09±5.12, 18.34±2.47, respectively (Figure 2A). The average total heavy metal content in Kacalı stream surface water and Perşembe tap water on days 10, 20, and 30 in summer season were 136.65±7.35, 110.57± 6.27, 29.61±4.12 and 202.27±9.24, 63.68±4.89, 45.85±4.32, respectively (Figure 2B). The average total heavy metal content in Kacalı stream surface water and Perşembe tap water on days 10, 20, and 30 in autumn season were 35.31±3.14, 21.62±2.75, 16.27±2.13 and 85.65±5.24, 45.70±4.47, 11.26±1.16, respectively (Figure 2C). The average total heavy metal content in Kacalı stream surface water and Perşembe tap water on days 10, 20, and 30 in winter season were 47.93±5.78, 22.21±2.86, 13.37±1.47 and 79.58±5.11, 30.54±2.95, 11.82± 1.24, respectively (Figure 2D). In general, the total metal content of the Perşembe tap water was higher than that of the Kacalı Stream surface water in all seasons. The Kacalı stream had the highest total heavy metal concentration in the summer season ($P<0.05$). The water of the Kacalı River showed a high concentration of metal content especially in summer on the 30th day (Figure 2).

The present study investigated the genotoxicity of surface and tap water samples from the drinking water source of the Perşembe district. The comet assay was used to measure DNA

damage. The results of the comet assay are presented in Table 1. In summer, samples from Kacalı Stream caused a significant increase in DNA damage in erythrocytes on day 30, and water samples showed marked toxicity on day 10 (P<0.05). Regarding surface water, water samples from Kacalı River showed significantly higher genotoxicity in all seasons (P<0.05) compared to controls. In addition, significantly higher levels of DNA damage were found in the summer, autumn, and winter seasons in the tap water (P<0.05).

Tap water samples from the Perşembe district showed a statistically significant increase in genotoxicity compared to controls (P<0.05). Tap water samples also showed genotoxic activity in the spring season, but the toxicity was lower than that of surface water on all exposure days (P<0.05). Tap water showed significantly higher levels of DNA damage on all exposure days than surface water in the other seasons except spring (P<0.05) (Table 1).

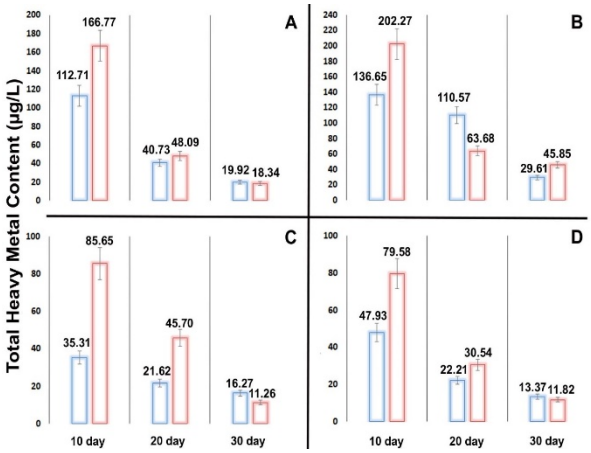

Figure 2. Seasonal total heavy metal content of Kacalı Stream water (Blue) and Perşembe tap water (Red). A: Spring, B: Summer, C: Autumn, D: Winter

Table 1. The comet assay on erythrocyte cells in *C. carpio* by surface and tap water effect in different seasons (tDNA%: tail DNA%, TM: tail moment, and OTM: olive tail moment) (Mean±S.D.) (Sp: spring, S: summer, A: autumn, W: winter, KSSW: Kacalı Stream Surface Water, PTW: Perşembe Tap Water)

	Groups (n=15)	Exposure Times (day)	tDNA (%)	TM	OTM
Sp	KSSW	10	13.72 ± 0.370^{xBa}	0.202 ± 0.011^{xAb}	0.505 ± 0.027^{xAd}
		20	14.69 ± 0.387^{xABa}	0.217 ± 0.013^{xAab}	0.498 ± 0.026^{xAb}
		30	15.94 ± 0.398^{xAab}	0.239 ± 0.013^{xAab}	0.469 ± 0.027^{xAb}
	PTW	10	12.51 ± 0.331^{yCb}	0.177 ± 0.009^{xBa}	0.529 ± 0.027^{xAc}
		20	14.31 ± 0.342^{xBa}	0.221 ± 0.011^{xAa}	0.511 ± 0.026^{xAb}
		30	15.71 ± 0.431^{xAb}	0.257 ± 0.015^{xAab}	0.470 ± 0.032^{xAb}
	Control	10	3.60 ± 0.132^{zAa}	0.042 ± 0.001^{yAa}	0.236 ± 0.013^{yAa}
		20	3.44 ± 0.131^{yAa}	0.040 ± 0.002^{yAa}	0.214 ± 0.012^{yAa}
		30	3.58 ± 0.136^{yAa}	0.041 ± 0.001^{yAa}	0.235 ± 0.013^{yAa}
S	KSSW	10	13.57 ± 0.417^{xBa}	0.264 ± 0.015^{xAa}	1.091 ± 0.035^{xAa}
		20	13.85 ± 0.435^{xBab}	0.242 ± 0.015^{xAa}	0.924 ± 0.031^{yBa}
		30	16.33 ± 0.465^{yAa}	0.269 ± 0.016^{xAa}	0.914 ± 0.028^{xBa}
	PTW	10	14.01 ± 0.380^{xBa}	0.218 ± 0.013^{yAa}	0.958 ± 0.026^{yAa}
		20	14.79 ± 0.422^{xBa}	0.247 ± 0.016^{xAa}	1.025 ± 0.031^{xAa}
		30	17.95 ± 0.429^{xAa}	0.279 ± 0.015^{xAa}	0.940 ± 0.023^{xAa}
	Control	10	3.90 ± 0.136^{yAa}	0.045 ± 0.001^{zAa}	0.260 ± 0.012^{zAa}
		20	3.83 ± 0.161^{yAa}	0.046 ± 0.001^{yAa}	0.267 ± 0.013^{zAa}
		30	3.77 ± 0.131^{zAa}	0.044 ± 0.001^{yAa}	0.238 ± 0.012^{yAa}
A	KSSW	10	10.14 ± 0.388^{yCc}	0.161 ± 0.012^{xBb}	0.795 ± 0.030^{xBc}
		20	12.63 ± 0.374^{yBb}	0.194 ± 0.011^{yABb}	0.910 ± 0.027^{yAa}
		30	13.99 ± 0.393^{xAc}	0.209 ± 0.014^{xAb}	0.870 ± 0.026^{xABa}
	PTW	10	12.49 ± 0.354^{xBb}	0.183 ± 0.012^{xBa}	0.852 ± 0.029^{xBb}
		20	13.66 ± 0.321^{xABa}	0.229 ± 0.012^{xAa}	1.020 ± 0.022^{xAa}
		30	14.58 ± 0.399^{xAb}	0.212 ± 0.011^{xABb}	0.859 ± 0.023^{xBa}
	Control	10	3.80 ± 0.164^{zAa}	0.046 ± 0.001^{yAa}	0.265 ± 0.013^{yAa}
		20	3.77 ± 0.132^{zAa}	0.044 ± 0.001^{zAa}	0.240 ± 0.012^{zAa}
		30	3.88 ± 0.145^{yAa}	0.046 ± 0.001^{yAa}	0.261 ± 0.013^{yAa}
W	KSSW	10	12.02 ± 0.331^{yBb}	0.211 ± 0.012^{xAb}	0.967 ± 0.027^{xAb}
		20	13.77 ± 0.379^{xAab}	0.208 ± 0.011^{yAab}	0.888 ± 0.025^{yAa}
		30	14.78 ± 0.371^{xAbc}	0.240 ± 0.014^{xAab}	0.932 ± 0.024^{xAa}
	PTW	10	13.04 ± 0.335^{xBab}	0.200 ± 0.011^{xBa}	0.907 ± 0.024^{xABab}
		20	14.11 ± 0.367^{xBa}	0.265 ± 0.015^{xAa}	0.963 ± 0.024^{xAa}
		30	15.74 ± 0.384^{xAb}	0.243 ± 0.014^{xABab}	0.884 ± 0.023^{xBa}
	Control	10	3.78 ± 0.130^{zAa}	0.044 ± 0.001^{yAa}	0.240 ± 0.012^{yAa}
		20	3.82 ± 0.160^{yAa}	0.046 ± 0.001^{zAa}	0.266 ± 0.013^{zAa}
		30	3.89 ± 0.141^{yAa}	0.045 ± 0.001^{yAa}	0.262 ± 0.012^{yAa}

Different A, B, C superscripts indicate statistical differences between exposure times (P<0.05), different x, y, z superscripts in a column indicate statistical differences between groups (P<0.05), and different a, b, c, d superscripts indicate statistical differences between seasons (P<0.05).

DISCUSSION

In the current study, the heavy metal concentration of tap water detected an unexpected positive genotoxic effect on *C. carpio* erythrocytes. However, there was no previous study on the genotoxicity of tap water in Ordu province. The genotoxic potential of metalloids and heavy metals has been reported in *Oreochromis niloticus* erythrocytes (Barbosa et al., 2010), and *Clarias gariepinus* gill and liver cells (Turan et al., 2020). The comet assay has also been proposed to monitor the genotoxicity and toxicity of surface water samples in many countries (Žegura et al., 2009; Chakrabarty and Sarma, 2011; Kontaş and Bostancı, 2020; Lovinskaya et al., 2022; Picinini et al., 2022).

The potential genotoxic effects of these contaminants in fish are also not fully understood. According to the results, both surface water and tap water interacted with the erythrocyte cells of *C. carpio*. For the first time, the genotoxicity of surface water from the Kacalı stream, one of the main drinking water sources in Ordu province and tap water from Kacalı districts were evaluated *in vivo* using the comet assay. The present study revealed that heavy metals were present in surface water and tap water analysis results. Heavy metals in water systems may act alone or in combination with other heavy metals. Heavy metal may not be effective at low doses but may become effective when combined with other low doses of heavy metals (Dağ and Arıcı, 2021; Mitra et al., 2022). Since this possible effect was taken into account in our study, the effects of heavy metals were evaluated in a combined manner and it was found that although the metal concentrations were not very high, the total metal content in waters had the potential to cause genotoxic damage to *C. carpio*.

It is difficult to identify the compounds that may be responsible for the possible adverse effects associated with exposure to mixed forms of environmental contaminants in the aquatic environment and to attribute the genotoxic effect directly to another factor. In addition, the interactions of the relevant heavy metals with each other are quite complex. These substances can exhibit different effects when mixed together in the same environment, as well as when they are present alone (Kontaş, 2022; Mitra et al., 2022).

Previous studies have shown the presence of numerous heavy metals in drinking water (Yeo et al., 2021; Luo et al., 2022). These harmful compounds originate not only from pollution but also from disinfection processes, especially when surface water is chlorinated. The majority of chlorinated compounds found in drinking water are non-volatile and difficult to identify (Ceretti et al., 2016). In many studies, genotoxic effects in tap water are mostly associated with the presence of disinfection by-products (Richardson et al., 2003; Cortés and Marcos, 2018). Drinking water contamination may originate from point sources of drinking water and other non-point sources of pollution or materials used in distribution systems (Žegura et al., 2009; Bozzo et al., 2013). One of the main causes of heavy metals contamination in tap water was the pollutants released from pipe sediments, especially in Ordu. The various factors can cause microbial and chemical changes in water distribution systems. The increase in microorganisms can also lead to corrosion of water pipes in these systems (Song et al., 2023). In addition, the properties of the water (temperature, oxygen content, particulate matter, and pH), as well as the age, type, structure (deposits and corrosion) and quality of the pipe materials can contribute to biological degradation.

Untreated industrial and domestic wastewater had genotoxic potential in aquatic ecosystems in Serbia, which could be effectively monitored by comet assay (Sunjog et al., 2012). Similarly, da Silva et al. (2020) found that *Astyanax lacustris* may suffer genetic damage when urban water contains high concentrations of certain metals. According to the WHO (2022), disinfecting water from surface sources may produce hazardous substances. Contamination of tap water can result from both water distribution systems and these disinfection treatments. In general, the most effective way to manage a drinking water distribution system is to reduce the possibility of contamination and quality degradation during transport, particularly by safeguarding water quality. Previous study has reported waterborne disease outbreaks in the UK (Cairncross, 2003) as a result of water contamination in the distribution system.

The current study results were consistent with previous data showing an increase in DNA damage in fish exposed to different types of contaminants and were also supported by exposure time and season, which has been highlighted in many genotoxicity studies (de Flora et al., 1993; Rocco et al., 2012). As in the current study, the comet assay sensitively detected DNA damage induced by different genotoxic agents in a dose-dependent manner. The comet assay has been successfully used in many studies, including our study, to investigate the effects of genotoxic pollutants on DNA integrity (Ternjej et al., 2010; Kontaş and Bostancı, 2020; Jiang et al., 2023). Due to its sensitivity to water contamination, the comet assay has recently been recognized as an efficient test to be used in genotoxicity studies. The comet assay has been applied to several pollutants in different types of water, such as drinking water, industrial effluents, lake and river water (Jiang et al., 2023). The comet assay is based on the assessment of DNA damage in different cell types (Žegura and Filipič, 2019).

There were significant seasonal variations in tail DNA percentage, tail moment, and olive tail moment. The variations of tDNA% were highly significant when comparing the values obtained in summer with those obtained in other seasons. Furthermore, the differences between surface and tap water samples were specified in all seasons. tDNA% values at three exposure times varied between 13.57-16.33 in Kacalı surface water and 14.01-17.95 in Perşembe tap water in summer. These values were between 12.02-14.78 in surface water and 13.04-15.74 in tap water in the same season. The differences in tDNA% values in the water samples may be affected by the significant temperature fluctuations in Ordu. DNA damage in

Mugil sp. and *Netuma* sp. was significantly higher in spring and summer compared to autumn and winter. Higher temperatures in surface waters were associated with higher levels of DNA damage than seasons with lower temperatures (Andrade et al., 2004). Exposure time caused an increase in DNA damage in erythrocyte cells of *C. carpio*. A positive relationship between DNA damage and exposure time has also been described previously (Kontaş and Bostancı, 2020; Kontaş, 2022).

The results of heavy metal analysis combined with the results of genotoxicity tests would provide a more robust basis for assessing the risks to human health associated with the use of drinking water. Low levels of genotoxicity, which are typical of drinking water samples, can be detected using genotoxicity tests. The present study did not attempt to directly identify which heavy metals in surface and tap water are responsible for genetic damage. These waters are likely to contain other pollutants as well as heavy metals. Aquatic organisms and humans are exposed to all heavy metals simultaneously. For this reason, it was considered more appropriate to calculate the genotoxic potential of surface and tap water based on the total load. Furthermore, heavy metals with complex chemical combinations can cause genotoxic effects in aquatic systems, even at very low concentrations (Hemachandra and Pathiratne, 2017).

In order to assess the mutagenic and/or genotoxic hazards of drinking water, a series of short-term in vitro analyses were performed on different drinking water samples before and after distribution. It has been reported that these waters can cause genetic damage in various organisms (Lan et al., 2018; Kontaş and Bostancı, 2020; Jiang et al., 2023). Although water is treated in drinking water facilities, tap water may have different metal concentrations due to contamination and corrosion during transport from the water facility through the supply system (Khan et al., 2015; Hossain et al., 2022). A similar case was observed in Perşembe tap water, where the total metal content in water samples was higher than in surface water samples. This situation shows the presence of genotoxic pollutant(s) in tap water, which still need to be identified and removed, even though they are purified in network treatment stations.

CONCLUSIONS

In this study, the toxic effects presumably caused by heavy metals in the surface water of Kacalı River and the tap water of Perşembe were observed. The present study showed that additional knowledge is needed about the potential toxicity of the drinking water source (Kacalı River) and the tap water (Perşembe district). The quality of the tap water consumed by humans needs to be investigated in relation to the dissolved metal composition and the possible health risks associated with it need to be assessed. The contamination of the drinking water in this area is likely to increase, with adverse consequences for organisms and humans. During the installation, operation and maintenance phases of the water treatment system, potential threats to the receiving environment and public health can be reduced by implementing an efficient drinking water management program. Strict enforcement of regulations and regular monitoring are also essential during the operational phase.

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AUTHOR CONTRIBUTIONS

Seda Kontaş Yalçınkaya, Derya Bostancı, Serdar Yedier: Conceptualisation, methodology; Derya Bostancı: Project management; Seda Kontaş Yalçınkaya, Serdar Yedier: Research, sample collection, observation; Seda Kontaş Yalçınkaya: Preparing an original draft; Seda Kontaş Yalçınkaya, Derya Bostancı, Serdar Yedier: Data analysis, writing, revising, reviewing and editing.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICS APPROVAL

All experimental procedures were approved by Animal Experiments Local Ethics Committee, Ordu University (Approval number: 82678388/5).

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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