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Monitoring of Genotoxic Pollution in Coastal Areas of the Iskenderun Bay, North-Eastern Mediterranean

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Abstract

This research examines genetic damage induced by metal pollution, using the golden grey mullet (L. aurata) as a bioindicator in the coastal region where Sarıseki Stream meet with Iskenderun Bay. The COMET assay and Micronucleus (MN) test were used to collect samples of L. aurata and coastal saltwater once a season for a year from both the sampling location and a reference site. The analysis showed that the concentrations of metals in water samples from both areas were below national and international standards. However, seawater from the sampling site had higher levels of chromium (Cr), lead (Pb), iron (Fe), and zinc (Zn) than the reference site in all seasons and on an annual basis. Notably, L. aurata from the sampling site exhibited a higher incidence of DNA damage and nuclear anomalies compared to specimens from the reference site, with significantly elevated micronucleus (MNi) values detected in the erythrocytes of L. aurata collected from the affected site throughout all seasons ($P < 0.01$). This study elucidates the genotoxic effects of pollution on L. aurata in this specific coastal area of the Northeastern Mediterranean. The results highlight the need for more study, including in vivo and in situ investigations, to assess the impacts of metals and other contaminants more thoroughly.

Keywords:

Sarıseki stream, metal, comet assay, micronucleus test, dna damage.

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Introduction

Pollution of marine ecosystem is a growing and significant environmental problem. Despite legislation enacted to address this issue, toxic pollutants discharged into rivers, seas, and lakes, along with local and industrial waste, are the primary sources of aquatic pollution (Yücel & Çam, 2021). The aquatic environment and its inhabitants are significantly affected by environmental pollution and serve as indicators for detecting pollution. In recent years, there has been a significant increase in studies investigating the genotoxic potential of chemicals in the aquatic environment and their effects on aquatic organisms, as well as the identification of genotoxic agents (Ergenler & Turan, 2023). That's as harmful chemicals that go into the air and soil ultimately make their way into the water and build up there. The ongoing discharge of these compounds results in their buildup within the benthic and pelagic food chains, hence heightening interest in toxicity research concerning contaminated aquatic habitats (Turan et al., 2023; Ergenler & Turan, 2024). Toxic contaminants discharged into aquatic locations threaten marine life, the overall ecosystem, and human health (Yazıcı & Şişman, 2015). In ecotoxicological studies, investigating the toxic response of native fauna as an indicator of environmental pollution is important. Fish are often used as bioindicator species because they play a variety of roles within the ecosystem, accumulate toxic substances, and respond at a cellular level to even low concentrations of contaminants More importantly, because fish species occupy the top of the aquatic food chain and directly impact human health through the food chain, their use in biomonitoring studies is crucial (Marcon et al., 2010; Viana et al., 2018). Grey mullet's wide distribution in lagoons, downstream rivers, and coastal areas, its tolerance to temperature, salinity, and oxygen fluctuations, and its omnivorous but also detrivorous feeding habits make it a species that is in direct contact with xenobiotics within its ecosystem (sediments and other aquatic organisms). These characteristics make this species a preferred model species for ecotoxicological and biochemical studies. Furthermore, its ability to react to ecological pollution and, more importantly, its potential for food consumption by local people in the regions where it is found, have led to the selection of the grey mullet (Liza aurata) as a bioindicator species (Bouzenda et al., 2017; Turan et al., 2023).

Chemical pollutants with genotoxic and carcinogenic potential in the aquatic ecosystem are a serious concern because they pose a threat to both aquatic and terrestrial life. These concerns have increased interest in developing techniques and bioindicators to monitor genomic damage from hazardous pollutants in the marine environment (Çavaş & Ergene-Gözükara, 2005). Among the available genotoxicity test systems, Micronucleus (MN) analysis and Comet Assay are widely used to evaluate structural and numerical chromosomal aberrations and DNA damage induced by clastogenic and aneugenic agents (Araldi et al., 2015; Luan & Honma, 2022; Carrasco et al., 1990). Various studies shown in coastal bays, estuaries, and harbors around the world including İzmir Bay and Çandarlı Bay (ın Turkey), as well as coastal lagoons such as Mãe-Bá Lagoon (Brazil) and Goa (India) have documented genotoxic damage in aquatic organisms attributable to anthropogenic pollution. (Arslan et al., 2010) researched using the micronucleus test on mussels (*Mytilus galloprovincialis*) and fish (*Gobius niger*) revealed elevated MN and other nuclear anomalies in erythrocytes and gill cells from harbor-impacted sites indicating the assay's high sensitivity for detecting harbor-derived genotoxicant in İzmir Bay. Egüz & Boyacıoğlu, 2023 reported that same gulf (İzmir Inner Bay) was contaminated by mutagenic and toxic substances. Similarly, in Mãe-Bá Lagoon, Brasil, both MN and Comet assessments revealed genotoxic damage in ichthyofauna, although statistical associations across species or sites were less distinct (Camargo Filho et al., 2022). D'Costa et al., 2017 noticed the genetic damage and the concentrations of trace metals and total petroleum hydrocarbons in natural populations of Arius arius in along the coast of Goa, India as an indicator of the pollution status of coastal water.

Iskenderun Bay, located in the northeastern Mediterranean, is a highly industrialized coastal region subjected to significant anthropogenic pressure, including trace metal pollution from industrial discharges

(iron-steel plants, cement plants, fertilizer plants, liquefied petroleum gas plants, oil transfer docks, and other industrial plants) (Yılmaz et al., 2010; Duysak, 2019; Eken & Akman, 2017), shipping activities. In this region, the iron and steel industries are the most important (Yücel & Çam 2021). The first genotoxicity studies due to metal pollution in Iskenderun Bay, Northeastern Mediterranean, and in the rivers flowing into the gulf was conducted by Turan ve ark., (2020, 2022, 2023) in our laboratory. Overall, the findings highlight the genotoxic potential of the coastal environment of Iskenderun Bay and support the continued use of fish-based biomonitoring strategies to detect sublethal contaminant effects at the cellular level.

Although establishing the relationships between metal accumulation and DNA damage is a very important challenge in terms of environmental security, there are not enough studies covering different regions of the bay and using different techniques in environmental monitoring (Ergenler & Turan, 2023). Therefore, this study was investigated genetic damage due to metal pollution using L. aurata as a bioindicator species in the coastal area where the Sarıseki Stream flows into the Iskenderun Bay by COMET assay and Micronucleus (MN) test.

Materıal And Methods

Sampling Area and Sampling Procedure

The study area included the site where Konacık region was selected as the unpolluted (reference) site and Sarıseki Stream, one of the freshwater sources flowing into the Iskenderun Bay, flows into the Northeastern Mediterranean (Dural Eken & Akman, 2018) (Figure 1).

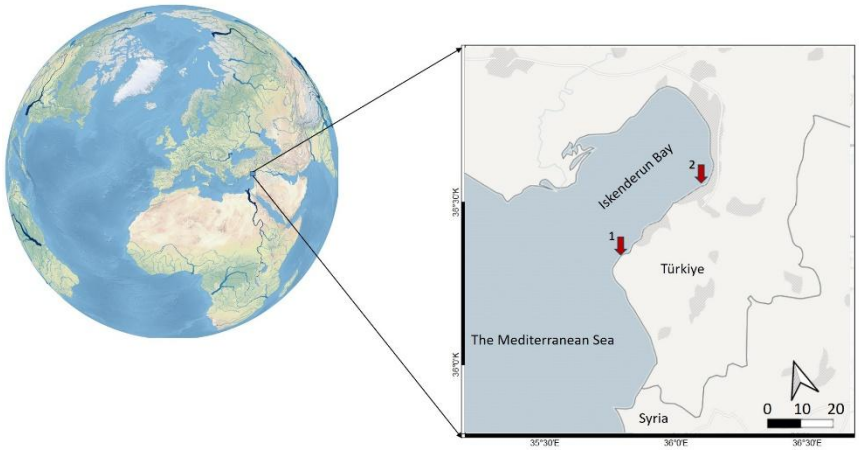

Figure 1. Map of reference site (1) and sampling site (2) in the iskenderun bay, northeastern mediterranean

The Konacık region (36°21'45.1"N 35°49'16.2"E), off the coast of the Gulf of Iskenderun, which is the reference region, is generally in the open area of the Gulf, far from industry and settlements. Sarıseki Stream (36°39'50.4"N 36°12'50.3"E), the innermost point of the bay and the chosen study area, and the coastal area where it flows into the bay are densely populated with industrial facilities. The high cumulative impact of the Sarıseki organized industrial zones, where large and small-scale industrial facilities, along with heavy industrial facilities, are located, is a primary cause of pollution (Turan et al., 2022; Turan et al., 2020).

Subjects of golden grey mullet (L. aurata) and coastal seawater samples were collected annually using the sampling location and the reference site through a duration of a year. Water samples were collected from sampling sites in duplicate, from 30 cm below the water surface. 1000 ml dark-colored polyethylene (PE)

bottles, previously cleaned with HNO3 and deionized water, were rinsed with a small amount of water sample and then filled with running water. These were stored in refrigerated containers (+4°C) and transported to the laboratory. Water temperature (0°C), pH, and dissolved O2 (mg/L) were measured at the sampling sites using YSI brand oxygen meters and pH meters. The salinity, electrical conductivity, and total dissolved solids of the samples were taken by the portable YSI- type salinity/conductivity meter. Mullets were collected using a spreading net with local fishermen and, sacrificed for tissue sampling by anesthetizing with 5 mg L-1 quinaldine sulfate (Sigma Chemical Company, Germany) (Bircan-Yildirim et al., 2010). The sampling process was carried out very quickly and sensitively with the least exposure to stress.

Trace metal analysis

Water samples brought to the laboratory were pretreated within approximately 72 hours by filtering them with blue band filter paper. If analysis was not to be performed, the pH was adjusted to 2-3 with a 1+1 HNO3 solution for longer storage. 100 ml of the water samples to be analyzed were placed in 250 ml containers, and 5 ml of HNO3 (55%) was added. The acidified samples were then placed on a hot plate and evaporated until the concentration dropped to 20 ml. 5 ml of HNO3 (55%) and 10 ml of HClO4 (70%) were added, and this process was continued until the brown smoke turned white. The water samples, cooled to room temperature, were diluted to 100 ml with distilled water and made ready for analysis (Cabrera et al., 1995). Metal measurements in water were performed using ICP-MS. The heavy metal content of the samples was calculated mathematically in µg L-1. Standards were prepared daily from the metal-free Merck brand ICP Standard (Multielement-IV), and analyses were conducted in triplicate. Detection Limits by Elements in ICP-MS Elite used for water samples; Cd:0.0008; Pb: 0.0016; Cr: 0.0097; Fe: 1.4756; Zn: 0.1331

Genotoxicty tests

The comet experiment was performed by the cellular dissociation approach of (Cavalcante et al., 2008) the slides were analyzed with a fluorescence microscope. One hundred gill cells were visually assessed and analyzed according to Collins' categorization approach. Damage percentages, arbitrary unit values, and the genetic damage index were computed (Collins, 2004). The Micronucleus test was conducted on blood smears, and 10 fish were examined for micronuclei from various locations and seasons. Morphological anomalies of the nucleus were assessed using peripheral smear and classified into six primary categories: Micronucleus, Kidney, Binucleus, Notched, Budded, and Lobed nucleus.

Statistical Analysis

ANOVA and Duncan's multiple comparison test were used to look at differences between stations for all parameters. SPSS Statistics 21 was used for the studies.

Results

Physico-chemical parameters

Table 1 showed the seasonal physicochemical parameters at sample and reference sites across one year. The sampling site's coastal saltwater samples met Turkish Environmental Guidelines 2015 Coastal Waters Quality Criteria for temperature, pH, salinity, electrical conductivity, and total dissolved solids.

Table 1. The seasonally physicochemical parameters in the sampling and reference sites (mean ± standard deviation)

SAMPLING SITE				
	Winter	**Spring**	**Summer**	**Autumn**
Temperature (°C)	18.00±0.50	22.00±1,00	30.50±1,00	30.00±0.50
D.O (mg L^{-1})	6.00±1.50	6.50±1.00	5.50±2.50	7.25±1.00
pH	7.00±1.00	8.00±0.10	8.20±1.70	7.80±1.00
Salinity (‰)	35.00±1.00	36.10±1.00	38.00±1.00	37.00±1.00
Electrical Conductivity (µS cm^{-1})	230.00±0.15	330.00±2.50	435.00±2.00	400.00±2.50
Total Dissolved solid (g L^{-1})	49.44±0.002	48.66±0.002	50.00±0.002	45.50±0.001
REFERANCE SITE				
	Winter	**Spring**	**Summer**	**Autumn**
Temperature (°C)	16.50±0.50	21.00±1.00	28.00±0.50	29.30±1.00
D.O (mg L^{-1})	8.30±1.00	7,00±1,01	6,50±1,22	7,50±0,4
pH	8.32±1.05	7.10±1.04	8.00±1.33	7.90±1.14
Salinity (‰)	36.00±1.02	36.88±1.04	38.00±1.00	36.6±1.00
Electrical Conductivity (µS cm^{-1})	280.00±1.13	330.00±1.50	435.30±3.00	260.00±1.70
Total Dissolved solid (g L^{-1})	44.56±0.005	45.70±0.006	49.80±0.001	47.36±0.02

Trace metals

Trace metal concentrations such as Cd, Cr, Pb, Fe, and Zn in water collected from the sampling and reference points are presented in Table 2. In general, the metal concentrations examined in water samples taken from both sampling areas were found to be below both national (TEG, 2016) and international standards (EPA, 2006,2007; US EPA, 2004) and within acceptable limits.

Table 2. Seasonal trace metal concentrations in coastal seawater samples from sampling and reference sites (µg L-1)

Season/metal	**REGIONS**			Kutlu & Kocar,(2017).	**US EPA (2004)**		**EPA (2007, 2016)**	
WINTER	**Sampling site**	**Referance site**	**CMC**	**CCC**	**CMC**	**CCC**	**CMC**	**CCC**
Cd***	0.031±0.002	0.002±0.001	<0.45(Class 1)	0.2	40	8.8	33	7.9
Cr***	4.821±0.026	1.262±0.301	88	4.2	-	-	1.1	50.0
Pb***	1.723±0.144	0.094±0.022	14	1.3	210	8.1	-	5.6
Fe***	56.431±2.187	25.270±6.147	101	36	-	-	-	-
Zn**	30.123±4.467	12.423±3.753	76	5.33	90	81	90	81
SPRING								
CdND	0.036±0.008	0.030±0.002	<0.45(Class 1)	0.2	40	8.8	33	7.9
Cr***	7.214±1.101	1.171±0.035	88	4.2	-	-	1.1	50.0
Pb***	0.989±0.009	0.807±0.002	14	1.3	210	8.1	-	5.6
Fe**	65.075±12.037	30.831±9.775	101	36	-	-	-	-
Zn**	35.196±4.951	15.577±6.741	76	5.33	90	81	90	81
SUMMER								
CdND	0.121±0.004	0.107±0.005	<0.45(Class 1)	0.2	40	8.8	33	7.9
Cr***	11.732±7.141	5.088±0.178	88	4.2	-	-	1.1	50.0
Pb***	0.852±0.047	0.423±0.077	14	1.3	210	8.1	-	5.6
Fe***	125.741±14.002	45.178±7.275	101	36	-	-	-	-
Zn**	45.178±7.275	15.631±4.157	76	5.33	90	81	90	81
AUTUMN								

Cd**	0.187±0.006	0.091±0.011	<0.45(Class 1)	0.2	40	8.8	33	7.9
Cr***	7.588±1.841	2.002±0.007	88	4.2	-	-	1.1	50.0
Pb***	1.008±0.034	0.541±0.044	14	1.3	210	8.1	-	5.6
Fe***	70.254±17.321	33.124±9.573	101	36	-	-	-	-
Zn***	25.963±7.321	10.345±2.987	76	5.33	90	81	90	81
ANNUAL								
CdND	0.094±0.070	0.057±0.045	<0.45(Class 1)	0.2	40	8.8	33	7.9
Cr**	7.838±4.104	2.380±1.673	88	4.2	-	-	1.1	50.0
Pb**	1.143±0.361	0.466±0.270	14	1.3	210	8.1	-	5.6
Fe**	79.375±30.426	31.075±9.289	101	36	-	-	-	-
Zn**	34.115±9.144	13.493±4.586	76	5.33	90	81	90	81

The data is provided as arithmetic mean ± standard deviation, with significance levels comparing water samples from the sampling and reference areas. The Turkey Environmental Guide (TEG) (2016) lists surface water contaminants and environmental quality criteria. US and EPA determine maximum and continuous amounts in aquatic habitats.

Water collected from the sampling point had higher Cr, Pb, Fe, and Zn concentrations than the reference point, for all seasons and annually. This difference was also found to be statistically significant. Only Cadmium concentration was found to be similar, with no statistically significant difference between the sampling sites. The highest metal concentration in water samples taken from the sampling area was found to be iron. The highest iron concentration was found in summer (125.741±14.002 µg L-1), and the lowest (56.431±2.187 µg L-1) in winter. Seasonal and annual evaluations revealed that metal concentrations in water, from highest to lowest, were as follows: Fe>Zn>Cr>Pb>Cd.

Comet Assay

Table 3. The Comet Assay was used to assess DNA damage in the gill cells of L. aurata, indicating both sampling and reference locations.

	SAMPLING SITE	**REFERENCE SITE**
DAMAGE FREQUENCY (%)		
WinterND	32.67±6.42	27.00±1.01
Spring**	40.00±1.01	27.67±0.57
Summer***	46.33±1.15	27.00±1.57
Autumn***	36.00±4.58	26.67±0.57
ANNUAL***	38.75±6.326	27.08±0.79
ARBITRARY UNIT (AU)		
WinterND	77.33±16.65	57.00±6.55
Spring**	104.67±5.51	61.33±4.62
Summer***	108.33±3.05	57.05±4.55
Autumn***	98.33±15.82	55.00±3.61
ANNUAL***	97.17±16.12	57.58±5.26
DAMAGE INDEX (DI) (%)		
WinterND	0.77±0.16	0.57±0.06
Spring**	1.04±0.05	0.61±0.04
Summer***	1.08±0.03	0.57±0.04
Autumn***	0.98±0.15	0.55±0.03
ANNUAL***	0.97±0.16	0.57±0.05

The frequency of DNA damage (%DF), arbitrary units of damage (AU), and the genetic damage index (%GDI) assessed using comet assay in L. aurata obtained from the sampling location and reference site are presented in Table 3.

The data are shown as arithmetic mean ± standard deviation. Indicate significance level between DNA damage frequency (%) in gill cells of fish from the sampling and reference station (*, P<0.05; **, P<0.01, ND: P>0.05).

The % DNA damage frequency and other damage parameters were significantly higher at sampling site the gill cells of L. aurata in all the seasons (except winter) than reference site. The highest levels of DNA damage were detected at sampling site in summer with 46.33±1.15% DF, 108.33±3.05 AU, and 1.08±0.03% GDI in the gill cell. Average values over the year of % DF, AU and % GDI) were significantly different (P<0.001) from each other between L. aurata collected from sampling site and reference site.

Micronucleus test

Table 4 illustrated the occurrence of micronuclei (MNi) and erythrocytic nuclear anomalies in the erythrocytes of L. aurata. Significantly elevated MNi values (P < 0.01) were seen in the erythrocytes of L. aurata obtained from the sampling site across all seasons, excluding fall, in comparison to those from the reference site.

Table 4. Frequencies of micronucleus and erythrocytic nuclear abnormalities (%) in blood smears of l. aurata from sampling and reference sites

	SAMPLING SITE	REFERENCE SITE
MICRONUCLEUS		
Winter***	7.17±0.15	4.73±0.40
Spring***	11.33±0.29	6.40±1.04
Summer**	12.37±0.15	9.07±1.62
AutumnND	10.13±0.23	9.40±1.97
ANNUAL**	10.25±2.04	7.40±2.34
KIDNEY		
Winter***	7.50±0.20	4.67±0.15
Spring**	10.17±0.15	6.37±1.62
Summer**	11.17±0.15	8.57±0.92
AutumnND	9.20±0.10	8.87±1.27
ANNUAL**	9.51±1.42	7.12±2.03
BINUCLEUS		
Winter***	7.60±0.10	6.30±0.10
Spring***	10.70±0.20	7.13±0.63
Summer**	12.07±0.11	8.90±1.04
AutumnND	9.57±0.06	9.23±1.42
ANNUAL**	9.98±1.71	7.89±1.50
NOTCHED		
Winter***	6.93±0.11	9.33±0.21
Spring**	10.17±0.15	7.80±1.38
Summer**	11.40±0.26	8.33±1.33
AutumnND	9.20±0.10	8.70±1.73
ANNUALND	9.42±1.71	8.54±1.25
LOBBED		
Winter**	8.00±0.20	7.43±0.15
Spring***	10.30±0.26	7.87±0.23
Summer**	12.20±0.10	8.93±0.98
AutumnND	9.57±0.11	9.27±1.36

ANNUAL**	10.02±1.58	8.37±1.07
BUDDED		
Winter**	5.10±0.17	5.53±0.25
Spring***	8.70±0.20	5.27±0.46
Summer**	9.70±0.17	6.87±1.62
AutumnND	7.83±0.15	7.10±1.85
ANNUAL**	7.83±1.79	6.19±1.36

The significant levels of Micronucleus (%) frequencies in the blood samples of fish obtained from the sampling and reference stations are denoted as $P<0.05$ or $P<0.01$, respectively.

Discussion

This research assesses the genotoxic impacts of trace metal contamination in coastal marine ecosystems, using Liza aurata as a bioindicator. The study used the Comet Assay and Micronucleus assays to evaluate physicochemical properties and trace metal concentrations in coastal waters, as well as DNA damage indicators in fish from both affected and control locations. This study offers novel insights into the genotoxic reactions of L. aurata in the coastal region where the Sarıseki Stream settles with İskenderun Bay in the Northeastern Mediterranean.

The physicochemical parameters of coastal water quality, such as temperature, pH, salinity, electrical conductivity, and total dissolved solids, conformed to the Turkish Environmental Guidelines, exhibiting negligible deviation from reference values, indicating they did not interfere with genotoxicity outcomes. Trace metal tests indicated markedly higher concentrations of chromium, lead, iron, and zinc at the sample location relative to the reference, with iron levels peaking in summer. Cadmium concentrations were uniform across regions, suggesting either a constant distribution or distinct geochemical processes within the aquatic environment.

Biomarker studies showed that L. aurata living in the impacted region had sublethal but physiologically significant genotoxic effects, even while trace metal levels were within national and international safety limits (TEG, 2016; US EPA, 2004–2007). The Comet Assay findings clearly showed a statistically significant increase ($P < 0.001$) in DNA strand breakage in the gill cells of fish from the sample location across all seasons except winter. In the summer, when iron levels were higher, the highest levels of DNA damage were seen (46.33 ± 1.15; AU: 108.33 ± 3.05; GDI: 1.08 ± 0.03). The findings are consistent with recent studies that demonstrate seasonal fluctuations in metal bioavailability and oxidative stress potential, particularly during warmer months when metabolic activity and pollutant uptake in aquatic organisms intensify (Turan et al., 2023; Ali et al., 2024). The findings of this research are in close agreement with those of D'Costa et al., 2017, who investigated the genotoxic effects of trace metals and petroleum hydrocarbons on marine fish species along the Goa coast, India. In agreement with our results in Liza aurata from the impacted coastal location, D'Costa et al., 2017 reported markedly elevated levels of DNA damage and micronuclei frequency in fish obtained from polluted marine habitats compared to those from less contaminated regions. Both investigations demonstrated that greater concentrations of Fe, Pb, and Zn correspond with enhanced genotoxic reactions, notably in gill and erythrocyte cells. This indicates that even sub-threshold metal exposure, characterized as below regulatory limits, may cause quantifiable cytogenetic and genotoxic harm. D'Costa et al. also out that the amount of genotoxicity caused by metals changes with the seasons, with more DNA damage happening before the monsoon. This discovery corresponds with our results indicating maximum DNA damage and iron content during the summer season. Both results demonstrate that the Comet Assay and Micronucleus test are dependable complementary methods for

identifying early genotoxic stress in marine bioindicators. Our results corroborate D'Costa and colleagues' findings that trace metal bioaccumulation may induce significant genotoxicity in marine fish populations, underscoring the need for comprehensive biomonitoring within coastal pollution assessment frameworks.

The MN test, together with the Comet Assay, showed that there were a lot more micronuclei and other nuclear problems in the erythrocytes of L. aurata from the sample location in all seasons except for autumn ($P < 0.01$). This c damage shows that chromosomes have become unstable over time, maybe because of long-term exposure to metals. Micronuclei are established indicators of genotoxic stress and clastogenic or aneugenic events, while further anomalies such as lobed nuclei, notched nuclei, and binucleated cells further substantiate the presence of sublethal cellular disturbances. These biomarker findings clearly imply that trace metal concentrations, even when they are below regulatory limits, may have harmful effects on the DNA of marine animals. The discrepancy between metal concentrations and biological outcomes underscores the need of integrating biomonitoring tools, such as the Comet Assay and MN test, into coastal environmental assessments (Mitkovska et al., 2017). The heightened vulnerability of gill tissues to DNA damage, as seen in this work, correlates with their initial involvement with solvents and greater respiration, making them suitable target tissues for early-warning biomonitoring.

Conclusion

The research highlights the impact of pollution on the genotoxicology of L. aurata near the Sarıseki Stream and Iskenderun Bay. This fish species exhibited higher DNA damage and nuclear abnormalities at the polluted site compared to a reference site. The presence of trace metals is linked to this DNA damage, indicating that L. aurata could serve as a bioindicator for water quality assessment. The study emphasizes the need for further research to explore the effects of various pollutants on genotoxicity in these fish through in vivo and in situ investigations.

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Author Contributions

Sevim Cansu ÖZDEMİRKAN: Conceptualization, methodology, formal analysis, investigation, writing—original draft.

Funda TURAN: Conceptualization; project administration; methodology; writing, original draft; reviewing and editing.

All authors provided feedback on earlier drafts of the work. All authors have reviewed and endorsed the final article.

Conflict of Interest

Conflicting objectives the researchers open up any conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Compliance with Ethical Standards

Every necessary international, national, and institutional guideline for the treatment and handling of animals has been complied with.

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