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Bioaccumulation of trace metals and genotoxicity responses in *Liza aurata* as an indicator of industrial pollution

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Abstract

Heavy metal contamination in the coastal and marine ecosystems is becoming a serious risk to aquatic organisms and humans. This study reports the effects, including genetic damage, of accumulations of trace metals on Liza aurata, which is used as a bio-indicator species, in the Payas coast of Iskenderun Bay, north-eastern Mediterranean by COMET Assay. *L. aurata* were seasonally collected from a sampling site and a reference site for one year. Physicochemical parameters in water and trace metals in the tissues of fish collected from these sites were determined by electrochemical techniques. High DNA damage frequency in *L. aurata* was observed along the Payas coast of Iskenderun Bay compared to the reference site because of pollutants. The detected high levels of Cd, Pb, Fe and Cu accumulation in L. aurata exceed the maximum levels allowed by the national and international limit values. Significant positive correlations between Cd, Pb, Hg, Cr, Fe, Zn, and Cu accumulations and DNA damage parameters were observed in the present study. Additionally, we first reported the successful use of the electrochemical technique in the determination of trace metal concentrations in mullet. Moreover, L. aurata constitutes a key tool as a sentinel organism for biomonitoring of coastal ecosystems.

Keywords Metals · DNA damage · Comet assay · *Liza aurata*

Introduction

Environmental pollution by trace metals is a critical problem worldwide. Toxic heavy metals and non-biodegradable elements can cause detrimental effects to terrestrial and aquatic organisms. Both natural and anthropogenic events generate heavy metals that contaminate aquatic habitats through drainage channels, river inputs and atmospheric deposition. Extensive amounts of unprocessed or inadequately treated wastewater from intense industrial activities and domestic drainage discharge into rivers, which degrades water quality and damages the marine ecosystem. Human health is threatened by toxic levels of trace metals in the water.Therefore, the toxic pollutants in marine ecosystems with a wide variety of contaminants have become a major concern for the past decade (Anandkumar et al. 2019; Turan et al. 2020a).

The interaction of DNA-damaging agents, such as heavy metals, with genetic material in cells, in relation to the consequences on the health of aquatic organisms is within the scope of geno-ecotoxicology. DNA integrity can be damaged owing to environmental toxic substances, causing genotoxic disorders which lead to induction of mutations, chromosomal abnormalities, tumors, and cell death in the aquatic organism (Bogoni et al. 2014). Therefore, it is crucial to evaluate the effect of genotoxins and the amount of DNA chain breakage in aquatic ecosystems as both an indicator of genotoxicity and a biomarker in ecological monitoring (Turan et al. 2020b). Comet testing is widely applied to study the genotoxic effects of contaminants on fish and is a reliable, sensitive, and quick technique used for the detection of DNA strand breakage and alkali-labile regions in cells of the organism (Martins and Costa 2017; Turan et al. 2020a).

Fish are one of the most important aquatic organisms widely used as a model for evaluating the health of aquatic ecosystems, considered a bioindicator of environmental pollution due to their sensitive responses to biochemical and

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physiological changes in the ecosystem (De Lemos et al. 2007; Cazenave et al. 2009). Determination of reactions of aquatic organisms to various pollutants such as heavy metals can be performed by using a range of biomarkers as a significant instrument in the marine ecosystem (Dalzochio et al. 2017). Furthermore, fish as a valuable human food can also provide information on the bioavailability of pollutants, promoting the process of bio-magnification (heavy metals) and threats to human health. Bioassay research using fish has stated an important correlation with DNA damage in human cells exposed to mutagens (Marcon et al. 2010). The fish species selected for the present study is the golden grey mullet, *Liza aurata*, which is generally distributed in the Mediterranean and the Black Seas, as well as along the Atlantic coast from Scotland and the southern coast of Norway and Sweden south towards Morocco (Turan 2016). Together with other members of the Mugilidae family, it inhabits coastal lagoons, marine neritic and coastal/supratidal zones, and estuaries. The feeding behaviour of *L. aurata* is generally characterized by regular contact with the sediment. Therefore, it is a good candidate for use as a biomarker for monitoring xenobiotic water contamination over a wide range of lipophilicities (Pacheco et al. 2005; D'Costa et al. 2017). Additionally, these species are known to bioconcentrate toxic pollutants (Bouzenda, Khebbeb (2017)).

The Mediterranean is under a great toxicological threat due to its unique hydrographic features and high anthropological activity (Storelli et al. 2011; Ayas et al. 2018). The Payas-Dörtyol coast of Iskenderun Bay is located on the Mediterranean coast of Turkey where there are many important international industrial plants (iron-steel plants, cement plants, fertilizer plants, liquefied petroleum gas plants, oil transfer docks, and other industrial plants) (Yilmaz et al. (2010), Duysak 2019). In this region, the iron and steel industries are the most important (Yücel and Çam 2021). There have been several studies that report heavy metal accumulation in numerous fish species, seawater, sediment and seston in Iskenderun Bay (Türkmen et al. 2005; Turan et al. 2009; Yilmaz et al. (2010); Dural et al. 2011; Manasirli et al. (2015); Dural Eken and Akman 2018, Duysak 2019; Yücel and Çam 2021). Although relationships between the metal bioaccumulation and DNA damage in the marine ecosystem are very important for environmental safety, there is no research regarding the assessment of the genotoxic potential of the Iskenderun Bay.

Recent investigations have been focused on electrochemical approaches in heavy metal ion detection. Electrochemical procedures are an easy technique and are suitable for fabricating small circuits in the form of mobile devices for in-situ monitoring of contaminated samples (Bansod et al. 2017). It is important to have a simple, inexpensive technique in order to selectively and sensitively measure toxic chemical pollutants. However, there are few studies about these techniques monitoring environmental pollution (Pujol et al. 2014; Qi et al. 2017). With this background, the aim of this study is to report the accumulation of trace metals and associated genetic damage using *L. aurata* as a bioindicator species in the Payas coast of the Iskenderun Bay, North-Eastern Mediterranean by COMET assay and electrochemical technique.

Material and methods

Sampling area

The sampling site was the Payas coast of the Iskenderun Bay (Turkey), the North-Eastern Mediterranean. The Payas coast (36°45'13.9"N 36°11'31.6"E) is downstream of Payas Stream which flows into the Mediterranean Sea and is surrounded by intense industrial activities (such as iron-steel factories and iron-steel waste plant), chemical manufacturing, domestic drainage, and shipping (Duysak 2019) (Fig. 1). Golden grey mullet (*L. aurata*) specimens and coastal seawater samples were seasonally collected from the same location at the sampling site for one year (September 2019 to July 2020). Cultured golden grey mullet supplied by the Iskenderun Technical University Aquaculture Research Centre (Iskenderun, Turkey) and water samples from the culture tanks were used as references for genotoxicological analyses.

Sampling procedure

Sea water samples from the sampling sites were taken 2 m below the surface using sterile 500 mL glass bottles and acidified to pH 2 with ultrapure 6 M HNO_3. The samples taken in triplicate were brought to the laboratory as quickly as possible and cooled at 4 °C until analysis. Live mullet (10 individuals per sampling site for each seasonal sampling) were captured using a fyke net by local fishermen and brought to the laboratory as quickly as possible. In the laboratory, total body length and wet weight were measured (Table 1). Cultured *L. aurata* were also seasonally sampled from the culture tanks on the same day as field sampling. Temperature, dissolved oxygen and pH from the sampling site and culture tanks were taken in situ by a YSI type oxygen-meter pH meter. The salinity, electrical conductivity, and total dissolved solids of the samples were taken by the portable YSI type salinity/conductivity meter.

Metal analysis

Tissue sample

Trace metal determination in muscle and liver tissue was performed by acid digestion adapted from AOAC Official

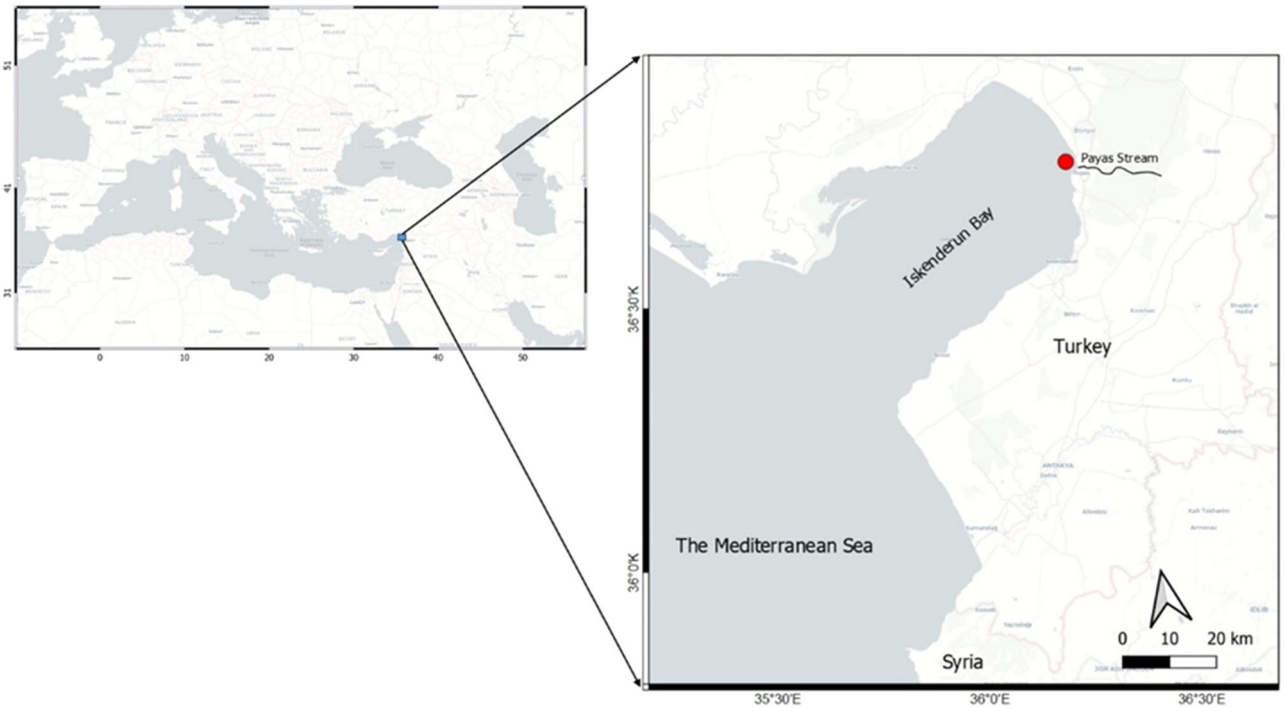

Fig. 1 Map of sampling site (•) in the Payas coast (Turkey) of the North-Eastern Mediterranean

Table 1 Mean length and weight of *L. aurata* from Payas coast of the North-Eastern Mediterranean and the reference (culture tanks) ($\bar{x} \pm SD$) ($n = 10$)

	Winter	Spring	Summer	Autumn
Sampling site				
Length (cm)	12.22 ± 1.02	15.26 ± 5.07	17.10 ± 10.83	18.68 ± 6.40
Weight (g)	16.21 ± 2.65	38.98 ± 12.78	43.80 ± 25.05	61.77 ± 40.46
Reference				
Length (cm)	19.85 ± 1.75	19.60 ± 1.05	21.18 ± 2.15	21.05 ± 1.75
Weight (g)	250.26 ± 42.60	275.65 ± 30.38	381.84 ± 42.02	360.45 ± 35.47

Method 999.10 (2002) on a wet weight basis. Firstly, 1 g of the sample (taken with a scalpel from the central part of the muscle and liver) was digested by a mixture of 10 mL of nitric acid (HNO_3), 0.25 mL of hydrogen peroxide (H_2O_2) and kept in a bath-water at 60 °C for one hour to perform acid digestion, after which the samples were allowed to cool at room temperature. Later, the solution was filtered and increased to 100 mL with distilled and deionized water. After the acid digestion, the metal concentration was determined by the electrochemical method in triplicate. The values of the heavy metal content of the samples were measured as μg g^{-1} wet weight (w.w.) respectively by mathematical methods. In addition, the analytical grade of chemicals and standard solutions (SIGMA) were used in this research.

Evaluation of trace metals by Electrochemical method

Cadmium (Cd), Lead (Pb), Mercury (Hg), Chromium (Cr), Cobalt (Co), Iron (Fe), Zinc (Zn) Copper (Cu), Nickel (Ni), and Mangan (Mn) were determined by electrochemical measurements with samples being previously acid digested. Electrochemical measurements were carried out by Gamry Reference 600 work-station (Gamry, USA) and BAS-100B electrochemical analyser. Triple electrode system comprising glassy carbon electrode as indicator electrode, Ag/AgCl/KCl (sat) as reference electrode, and platinum wire as auxiliary electrode were employed for all electrochemical measurements. Moreover, the cleaning protocol of glassy carbon electrodes were performed according to our previous paper (Yola et al. 2012). After the supporting electrolyte (pH 7.4, phosphate buffer, 3.0 mL) was put into the electrochemical cell, the standard solutions (Cr, Cu, Pb, Co, Cd, Fe, Ni, Zn and Mn) were added into phosphate buffer by micropipette. This process was separately carried out for each metal ion. Before the measurements, the sample solutions were passed through argon gas (99.999 %) during 15 min. Then, the electrochemical potential scan was applied to electrochemical cell including trace metal

Table 2 Comparison of some physicochemical parameters (mean ± standard deviation) of the reference (culture tanks) samples and Payas coastal seawater samples of the North-Eastern Mediterranean

	Sampling Site				
	Reference	Winter	Spring	Summer	Autumn
Temperature (°C)	25.30 ± 1.50	18.05 ± 0.50	22.60 ± 0.56	30.40 ± 0.65	29.50 ± 1.05
D.O (mg L^{-1})	6.55 ± 0.06	7.50 ± 0.16	8.55 ± 0.06	8.12 ± 0.12	7.35 ± 0.07
pH	8.20 ± 0.05	8.45 ± 0.04	8.25 ± 0.14	8.52 ± 0.17	8.10 ± 0.08
Salinity (‰)	36.12 ± 0.52	37.12 ± 0.55	37.12 ± 1.45	38.12 ± 0.75	38.85 ± 1.15
Electrical Conductivity (µS cm^{-1})	35200 ± 126	45500 ± 150	47750 ± 120	48100 ± 135	49300 ± 196
Total Dissolved solid (g L^{-1})	45.00 ± 0.15	48.50 ± 0.10	47.30 ± 0.15	48.10 ± 0.05	46.85 ± 0.10

solutions in range from −1.00 to 0.0 V. After the recording of electrochemical voltammograms based on at pulse height of 5 mV, square wave amplitude of 50 mV and frequency of 50 Hz, the peak signals (µA) attributing to trace metal concentrations were evaluated for trace metal detections.

Comet assay

Comet assay was done according to cellular dissociation technique improved from Cavalcante et al. (2008). Firstly, liver and gill tissues of *L. aurata* were homogenized and centrifuged at 3000 rpm at 4 °C for 5 min for the cell suspension, and then the cell pellet was retained. Singh et al. (1988) were followed for performing the single-cell gel electrophoresis. The slides were neutralized with ice-cold 0.4 M Tris buffer (pH 7.5), stained with 80 ml ethidium bromide (20 mg mL^{-1}). The slides were then examined at X400 magnification using a fluorescence microscope (Carl Zeiss Aksiostar Plus). Images of 100 cells from each sample (gill and liver) were monitored and scored as proposed by Collins (2004) by classifying the nucleoids, which were assigned to one of five classes (0–4; with 0 signifying no visible tail and 4 almost all DNA in the tail) according to intensity of the comet tail. For comparison of the data from the comet assay, the damage percentage (%DF), the arbitrary unit values (AU), and genetic damage ındex (GDI) were calculated as defined by Pitarque et al. (1999) and Collins (2004).

Statistical analysis

Before statistical treatment, all collected data were tested for the normality (Shapiro–Wilk test) and homogeneity (Levene analyse test). Furthermore, a one-way analysis of variance (ANOVA) was applied for significance assessments ($P < 0.05$) (Zar 1996). Principal component analysis (PCA) was applied to define the most important parameters involved in DNA damage. Additionally, Pearson's chi-squared test was also used to determine the relationship between trace metal and DNA damage (Zheng et al. 2016). The statistical analysis was made using IBM SPSS Statisticsv21 and R-Studio.

Results

Physicochemical parameters

The seasonally analysed physicochemical parameters in the sampling and reference sites during one year are presented in Table 2. At the reference site (cultured tanks), average temperature 25.30 °C, dissolved oxygen 6.55 mg L^{-1}, pH 8.20, salinity 36.12‰, electrical conductivity 35,200 µS cm^{-1} and total dissolved solids 45.00 g L^{-1} were determined for one year.

The average temperature values of the sampling site were between 18.05 °C and 30.40 °C for one year. It is known that the temperature of the seawater varies depending on the season and flows. The dissolved oxygen and pH and salinity values of the sampling sites were between 7.35–8.55 mg L^{-1}, 8.10–8.52, and 37.12–38.85 ‰, respectively. The measurements of electrical conductivity and total dissolved solids were obtained between 45500–49300 µS cm^{-1} and 46.85–48.50 g L^{-1}, respectively. The coastal seawater samples of sampling site were suitable in the quality range with respect to the temperature, pH and salinity parameters as described by Coastal Waters Quality Criteria of Turkish Environmental Guidelines 2015.

Bioaccumulation

Mean values of Cd, Pb, Hg, Cr, Co, Fe, Zn, Cu, Ni, and Mn concentrations in different tissues (muscle and liver) of *L. aurata* collected from the Payas coast and references for one year are given in Tables 3, 4 and 5.

The concentrations of all the trace metals, except Co were significantly different in the liver and muscle tissues of L. aurata collected from the Payas coast and the culture tanks for all seasons ($P < 0.01$, $P < 0.001$) (Tables 3, 4). Cd and Pb concentrations at the Payas coast highly exceeded the maximum limits allowed by the TFC Turkish Food Codex (2011), EU European Union (2005) in summer and autumn and were sufficient to have negative effects on coastal ecosystems.

During the study period, the highest Cd accumulation in liver tissues was determined in autumn at Payas coast

Table 3 Trace metal concentrations in the liver of *Liza aurata* in Payas coast of the North-Eastern Mediterranean and the reference (culture tanks) (concentration unit as μg g^{-1} w.w.)

Seasons/Metals	STATIONS					
WINTER	Payas Site	Reference	EU European Union (2005)	EPA (1989)	WHO (1989)	TFC Turkish Food Codex (2011)
Cd	0.0219 ± 0.008	0.019 ± 0.005	0.05	1.4	1.0	0.05
Pb**	1.647 ± 0.338	0.135 ± 0.010	0.2	1.0	2.0	0.3
Hg***	0.089 ± 0.007	0.0091 ± 0.006	0.02	0.1	0.4	0.50
Cr**	1.460 ± 0.253	0.406 ± 0.097	–	4.1	–	–
Co*	0.013 ± 0.012	0.053 ± 0.005	–	–	–	–
Fe	69.646 ± 4.090	54.904 ± 9.125	–	410	100	50
Zn***	28.825 ± 1.216	8.090 ± 0.895	50	410	100	50
Cu***	5.745 ± 0.505	1.230 ± 0.211	10	54	30	20
Ni	0.847 ± 0.112	0.878 ± 0.122	–	4.6	–	–
Mn**	0.510 ± 0.129	2.615 ± 0.440	–	100	1.0	20
SPRING						
Cd*	0.255 ± 0.119	0.017 ± 0.008	0.05	1.4	1.0	0.05
Pb**	1.607 ± 0.138	0.110 ± 0.032	0.2	1.0	2.0	0.3
Hg*	0.143 ± 0.057	0.011 ± 0.006	0.02	0.1	0.4	0.50
Cr*	1.793 ± 0.772	0.306 ± 0.106	–	4.1	–	–
Co*	0.677 ± 0.315	0.117 ± 0.055	–	–	–	–
Fe*	106.979 ± 17.361	63.237 ± 16.05	–	410	100	50
Zn**	45.491 ± 10.829	7.757 ± 0.259	50	410	100	50
Cu***	8.745 ± 0.505	1.059 ± 0.126	10	54	30	20
Ni	1.180 ± 0.550	0.798 ± 0.153	–	4.6	–	–
Mn	2.844 ± 1.282	1.948 ± 1.111	–	100	1.0	20
SUMMER						
Cd**	0.768 ± 0.192	0.020 ± 0.013	0.05	1.4	1.0	0.05
Pb***	5.058 ± 0.428	0.151 ± 0.045	0.2	1.0	2.0	0.3
Hg**	0.307 ± 0.063	0.009 ± 0.002	0.02	0.1	0.4	0.50
Cr***	2.019 ± 0.108	0.406 ± 0.097	–	4.1	–	–
Co	1.088 ± 0.720	0.084 ± 0.056	–	–	–	–
Fe***	174.549 ± 6.988	56.237 ± 14.365	–	410	100	50
Zn***	85.220 ± 8.755	7.090 ± 1.299	50	410	100	50
Cu***	12.347 ± 0.368	1.230 ± 0.360	10	54	30	20
Ni*	1.381 ± 0.247	0.831 ± 0.155	–	4.6	–	–
Mn	2.523 ± 1.191	2.282 ± 1.090	–	100	1.0	20
AUTUMN						
Cd***	1.610 ± 0.110	0.023 ± 0.011	0.05	1.4	1.0	0.05
Pb**	2.627 ± 0.601	0.159 ± 0.035	0.2	1.0	2.0	0.3
Hg**	0.189 ± 0.061	0.008 ± 0.001	0.02	0.1	0.4	0.50
Cr*	1.879 ± 0.746	0.473 ± 0.052	–	4.1	–	–
Co*	0.115 ± 0.047	0.050 ± 0.001	–	–	–	–
Fe***	249.495 ± 74.827	55.904 ± 5.718	–	410	100	50
Zn**	24.822 ± 3.730	9.090 ± 0.506	50	410	100	50
Cu***	57.623 ± 3.606	1.563 ± 0.280	10	54	30	20
Ni	1.253 ± 0.489	0.865 ± 0.101	–	4.6	–	–
Mn**	1.284 ± 0.274	2.948 ± 0.334	–	100	1.0	20

The data are shown as arithmetic mean ± standard deviation. Indicate significance level between different tissues of *L. aurata* collected from the sampling site and reference station (*$P < 0.05$; **$P < 0.01$, ***$P < 0.001$)

Table 4 Trace metal concentrations in the muscle of *L. aurata* in Payas coast of the North-Eastern Mediterranean and the reference (culture tanks) (concentration unit as μg g^{-1} w.w.)

Seasons/Metals	STATIONS					
WINTER	Payas Site	Reference	EU European Union (2005)	EPA (1989)	WHO (1989)	TFC Turkish Food Codex (2011)
Cd***	0.058 ± 0.005	0.001 ± 0.000	0.05	1.4	1.0	0.05
Pb	0.237 ± 0.152	0.032 ± 0.005	0.2	1.0	2.0	0.3
Hg***	0.076 ± 0.010	0.001 ± 0.000	0.02	0.1	0.4	0.50
Cr***	0.872 ± 0.121	0.087 ± 0.027	–	4.1	–	–
Co	0.012 ± 0.003	0.017 ± 0.004	–	–	–	–
Fe**	19.177 ± 1.852	9.879 ± 0.759	–	410	100	50
Zn**	13.996 ± 1.517	3.353 ± 1.203	50	410	100	50
Cu**	1.763 ± 0.188	0.483 ± 0.253	10	54	30	20
Ni	0.316 ± 0.015	0.166 ± 0.151	–	4.6	–	–
Mn	0.434 ± 0.080	0.199 ± 0.178	–	100	1.0	20
SPRING						
Cd	0.103 ± 0.071	0.000 ± 0.000	0.05	1.4	1.0	0.05
Pb**	0.709 ± 0.139	0.041 ± 0.021	0.2	1.0	2.0	0.3
Hg*	0.130 ± 0.051	0.000 ± 0.000	0.02	0.1	0.4	0.50
Cr**	1.340 ± 0.276	0.078 ± 0.019	–	4.1	–	–
Co	0.029 ± 0.011	0.010 ± 0.004	–	–	–	–
Fe*	25.012 ± 4.360	9.212 ± 1.135	–	410	100	50
Zn***	19.108 ± 2.014	3.687 ± 1.305	50	410	100	50
Cu**	1.366 ± 0.344	0.251 ± 0.134	10	54	30	20
Ni**	0.621 ± 0.066	0.066 ± 0.052	–	4.6	–	–
Mn**	0.787 ± 0.216	0.134 ± 0.009	–	100	1.0	20
SUMMER						
Cd*	0.120 ± 0.057	0.001 ± 0.000	0.05	1.4	1.0	0.05
Pb**	0.809 ± 0.181	0.028 ± 0.009	0.2	1.0	2.0	0.3
Hg*	0.154 ± 0.063	0.001 ± 0.000	0.02	0.1	0.4	0.50
Cr***	1.131 ± 0.102	0.091 ± 0.012	–	4.1	–	–
Co	0.023 ± 0.011	0.013 ± 0.009	–	–	–	–
Fe***	28.679 ± 2.333	10.212 ± 0.815	–	410	100	50
Zn**	29.775 ± 7.309	4.687 ± 2.015	50	410	100	50
Cu**	1.700 ± 0.267	0.317 ± 0.074	10	54	30	20
Ni***	0.688 ± 0.049	0.100 ± 0.070	–	4.6	–	–
Mn**	0.877 ± 0.067	0.167 ± 0.126	–	100	1.0	20
AUTUMN						
Cd*	0.085 ± 0.050	0.001 ± 0.000	0.05	1.4	1.0	0.05
Pb***	0.561 ± 0.051	0.035 ± 0.007	0.2	1.0	2.0	0.3
Hg*	0.153 ± 0.001	0.001 ± 0.000	0.02	0.1	0.4	0.50
Cr***	1.016 ± 0.026	0.094 ± 0.016	–	4.1	–	–
Co	0.024 ± 0.010	0.023 ± 0.026	–	–	–	–
Fe**	21.980 ± 2.591	10.545 ± 0.401	–	410	100	50
Zn***	17.856 ± 1.593	3.687 ± 1.788	50	410	100	50
Cu**	1.383 ± 0.920	0.350 ± 0.126	10	54	30	20
Ni**	0.602 ± 0.055	0.133 ± 0.103	–	4.6	–	–
Mn**	0.778 ± 0.110	0.165 ± 0.129	–	100	1.0	20

The data are shown as arithmetic mean ± standard deviation. Indicate significance level between different tissues of *L. aurata* collected from the sampling site and reference station (*$P < 0.05$; **$P < 0.01$, ***$P < 0.001$)

Table 5 Trace metal concentrations in the liver and muscle tissues of *Liza aurata* in Payas coast of the North-Eastern Mediterranean and Reference (culture tanks) (concentration unit as μg g^{-1} w.w.) and guidelines

Annual/Metals	STATIONS					
LIVER	Payas Site	Reference	EU European Union (2005)	EPA (1989)	WHO (1989)	TFC Turkish Food Codex (2011)
Cd**	0.664 ± 0.145	0.020 ± 0.009	0.05	1.4	1.0	0.05
Pb***	2.745 ± 1.503	0.139 ± 0.034	0.2	1.0	2.0	0.3
Hg***	0.182 ± 0.095	0.009 ± 0.002	0.02	0.1	0.4	0.50
Cr***	1.788 ± 0.519	0.398 ± 0.099	–	4.1	–	–
Co*	0.473 ± 0.565	0.076 ± 0.034	–	–	–	–
Fe**	150.167 ± 78.832	57.570 ± 10.838	–	410	100	50
Zn***	46.090 ± 25.698	8.007 ± 1.039	50	410	100	50
Cu*	21.115 ± 12.205	1.270 ± 0.329	10	54	30	20
Ni*	1.165 ± 0.393	0.843 ± 0.119	–	4.6	–	–
Mn	1.790 ± 0.358	2.448 ± 0.804	–	100	1.0	20
MUSCLE						
Cd***	0.091 ± 0.050	0.001 ± 0.000	0.05	1.4	1.0	0.05
Pb***	0.579 ± 0.255	0.034 ± 0.012	0.2	1.0	2.0	0.3
Hg***	0.128 ± 0.057	0.001 ± 0.000	0.02	0.1	0.4	0.50
Cr***	1.090 ± 0.224	0.087 ± 0.017	–	4.1	–	–
Co	0.022 ± 0.010	0.016 ± 0.014	–	–	–	–
Fe***	23.712 ± 4.463	9.962 ± 0.868	–	410	100	50
Zn***	20.184 ± 6.975	3.853 ± 1.668	50	410	100	50
Cu***	1.553 ± 0.315	0.350 ± 0.194	10	54	30	20
Ni***	0.557 ± 0.154	0.116 ± 0.098	–	4.6	–	–
Mn***	0.719 ± 0.209	0.166 ± 0.117	–	100	1.0	20

The data are shown as arithmetic mean ± standard deviation. Indicate significance level between different tissues of *L. aurata* collected from the sampling site and reference station (*$P < 0.05$; **$P < 0.01$, ***$P < 0.001$). *TFC* Turkish Food Codex, Communiqué on Maximum Limits of Contaminants in Foodstuffs in Turkey

EPA Environmental Protection Agency, *EU* European Union, *WHO* World Health Organization

(1.610 ± 0.110 μg g^{-1}), and the lowest was determined in spring in the reference (0.017 ± 0.008 μg g^{-1}). Except winter season, Cd showed significant differences between studied sites for all seasons ($P < 0.01$) (Tables 3, 4). The highest Pb accumulation was found in summer at Payas coast (5.058 ± 0.428 μg g^{-1}), and the lowest was in summer in muscle tissues of *L. aurata* from the culture tanks reference site (0.028 ± 0.009 μg g^{-1}). For all seasons, Pb accumulation was higher than the maximum limits of TFC Turkish Food Codex (2011) and EU European Union (2005) in liver and muscle tissues from the Payas coast. Significant differences in the concentration of Fe in liver and muscle tissues were detected in samples from the Payas coast, relative to the reference site ($P < 0.05$) (Tables 3 and 4). The highest Fe concentration in liver tissues ranged from 249.495 ± 74.827 to 69.646 ± 4.090 μg g^{-1}, much higher than the values described by the TFC Turkish Food Codex (2011), WHO (1989) for all seasons. The annual values were sufficient to have negative effects on coastal ecosystems (Table 5). In the liver tissue, the highest content of Cu (57.623 ± 3.606 μg g^{-1}) was detected in Payas coast at autumn. Cu concentration in the Payas coast was above the EU European Union (2005), EPA (1989), WHO (1989) and TFC Turkish Food Codex (2011) limits at summer season.

Trace metal concentrations in the liver tissues of *L. aurata* can be ranged as follows: Fe > Zn> Cu > Pb> Cr > Cd> Hg> Co> Mn > Ni for studied site and Reference site; in the muscle tissues of *L. aurata* can be ranged as follows Fe > Zn> Cu > Cr> Pb> Cd> Hg> Co> Mn> Ni for Payas coast and Reference site (Tables 3 and 4). Accumulations of Hg, Cr, Co, Zn, Ni and Mn in liver and muscle tissues of *L. aurata* collected from the Payas coast and the references weren't higher than the maximum limits (EU European Union (2005); EPA 1989; WHO 1989 and TFC Turkish Food Codex (2011)) in both all seasons and annual (Tables 3, 4 and 5). Hence, the assessment of human health risk wasn't conducted to estimate the risk posed by these metals.

DNA damage

Percent damage frequency (DF %), the arbitrary units values (AU) and genetic damage index (GDI %) of DNA damage in *L. aurata* sampled from Payas coastal site and the reference was evaluated through comet assay and results are given in Table 6.

In the COMET analysis, the DNA damage levels of both gill and liver tissues showed significant seasonal differences. Gill tissue in Payas coast site revealed the highest level of DNA damage (94.00 ± 4.35% DF) in winter. However, the lowest level of DNA damage (35.33 ± 0.57% DF) was observed in liver tissue at the reference in the

Table 6 DNA damage in the gill and liver cells of *L. aurata* from Payas coast of the North-Eastern Mediterranean and reference (culture tanks) analysed by Comet Assay

	Payas coast	Reference
GILL		
Damage Frequency (%)		
Winter**	94.00 ± 4.35	42.33 ± 12.71
Spring***	93.67 ± 1.52	41.00 ± 3.60
Summer***	79.67 ± 4.04	40.67 ± 5.13
Autumn**	93.00 ± 7.55	40.33 ± 7.57
ANNUAL***	90.08 ± 7.53	41.08 ± 6.89
Arbitrary Unit (AU)		
Winter**	275.00 ± 20.95	94.67 ± 33.38
Spring*	152.00 ± 37.80	81.33 ± 6.02
Summer***	213.00 ± 7.21	83.67 ± 21.03
Autumn	127.33 ± 22.89	83.66 ± 21.03
ANNUAL***	191.83 ± 63.41	85.33 ± 19.18
Damage Index (DI) (%)		
Winter**	2.75 ± 0.21	0.94 ± 0.33
Spring*	1.52 ± 0.37	0.81 ± 0.06
Summer***	2.13 ± 0.07	0.83 ± 0.21
Autumn	1.27 ± 0.22	0.83 ± 0.21
ANNUAL***	1.91 ± 0.63	0.85 ± 0.19
LIVER		
Damage Frequency (%)		
Winter***	82.33 ± 3.78	38.33 ± 3.51
Spring**	75.00 ± 7.93	39.33 ± 6.42
Summer***	76.67 ± 2.08	35.33 ± 0.57
Autumn***	79.00 ± 1.01	36.33 ± 0.57
ANNUAL***	78.25 ± 4.82	37.33 ± 3.55
Arbitrary Unit (AU)		
Winter*	159.33 ± 36.91	79.33 ± 20.79
Spring***	228.33 ± 17.67	74.00 ± 18.52
Summer***	197.00 ± 21.28	67.33 ± 6.65
Autumn**	237.33 ± 42.44	70.33 ± 7.51
ANNUAL***	205.50 ± 41.64	72.75 ± 13.45
Damage Index (DI) (%)		
Winter*	1.59 ± 0.36	0.79 ± 0.21
Spring***	2.28 ± 0.17	0.74 ± 0.18
Summer***	1.97 ± 0.21	0.67 ± 0.06
Autumn**	2.37 ± 0.42	0.70 ± 0.07
ANNUAL***	2.05 ± 0.41	0.72 ± 0.13

The data are shown as arithmetic mean ± standard deviation. Indicate significance level between different tissues of *L. aurata* collected from the sampling and reference site (*$P < 0.05$; **$P < 0.01$, ***$P < 0.001$)

summer (Table 6). Relatively a higher level of DNA damage in gill than that the liver was detected for all seasons and all sites ($p < 0.05$). Annual analysis of DNA damage DF%, AU and GDI%) in *L. aurata* both from sampling and the reference were significantly different

Table 7 Eigenvalue, proportion and cumulative contribution of heavy metal variables to DNA damage of *L. aurata* on first two Principal Components

Component	Eigenvalues	% of Variance	Cumulative %
1	17.526	67.408	67.408
2	2.557	9.836	77.243
3	1.808	6.955	84.198
4	1.060	4.077	88.275

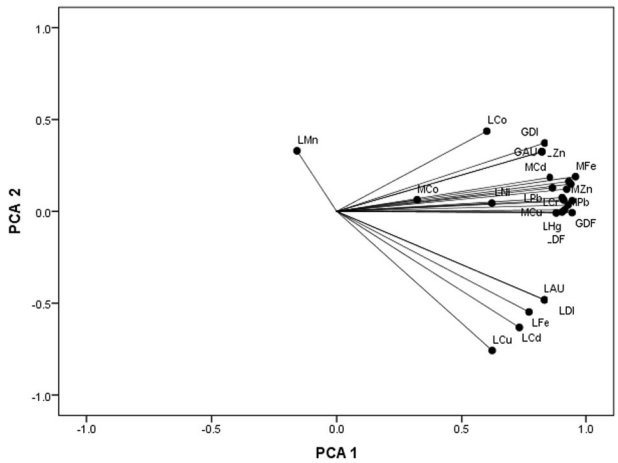

Fig. 2 Contribution and relation of analyzed parameters on first two Principal Components

($p < 0.001$) from each other. Besides, the highest levels of DNA damage were detected at Payas coastal site with 94.00 ± 4.35% DF, 275.00 ± 20.95 AU and 2.75 ± 0.20% GDI in the gill tissue (Table 6). Moreover, the highest levels of DNA damage were detected as 82.33 ± 3.78% DF, 237.33 ± 42.44 AU and 2.37 ± 0.42% GDI in the liver tissue at Payas coastal site (Table 6).

In the PCA, the first two principal components' loadings showed 67.408% and 9.836% of total variations, respectively (Table 7). Plotting the first principal components showed that the heavy metal variations could be related to DNA damage, which is scattered along with the principal components with high contributions (Fig. 2). Plotting the first two principal components showed that the heavy metals Hg, Pb, Fe, Zn, Cr, and Cu (in the liver tissue) revealed a strong contribution to the observed DNA damage (DF%) that were vectored in the same direction along with the principal component space (Fig. 2). Furthermore, Cd, Cu, and Fe metals (in liver tissue) were detected to be the most important parameters involved in DNA damage for AU and GDI parameters in the liver tissues. In addition, Zn and Cd (in liver and muscle) were also important parameters taking part in DNA damage for AU and GDI parameters in gill tissues (Fig. 2).

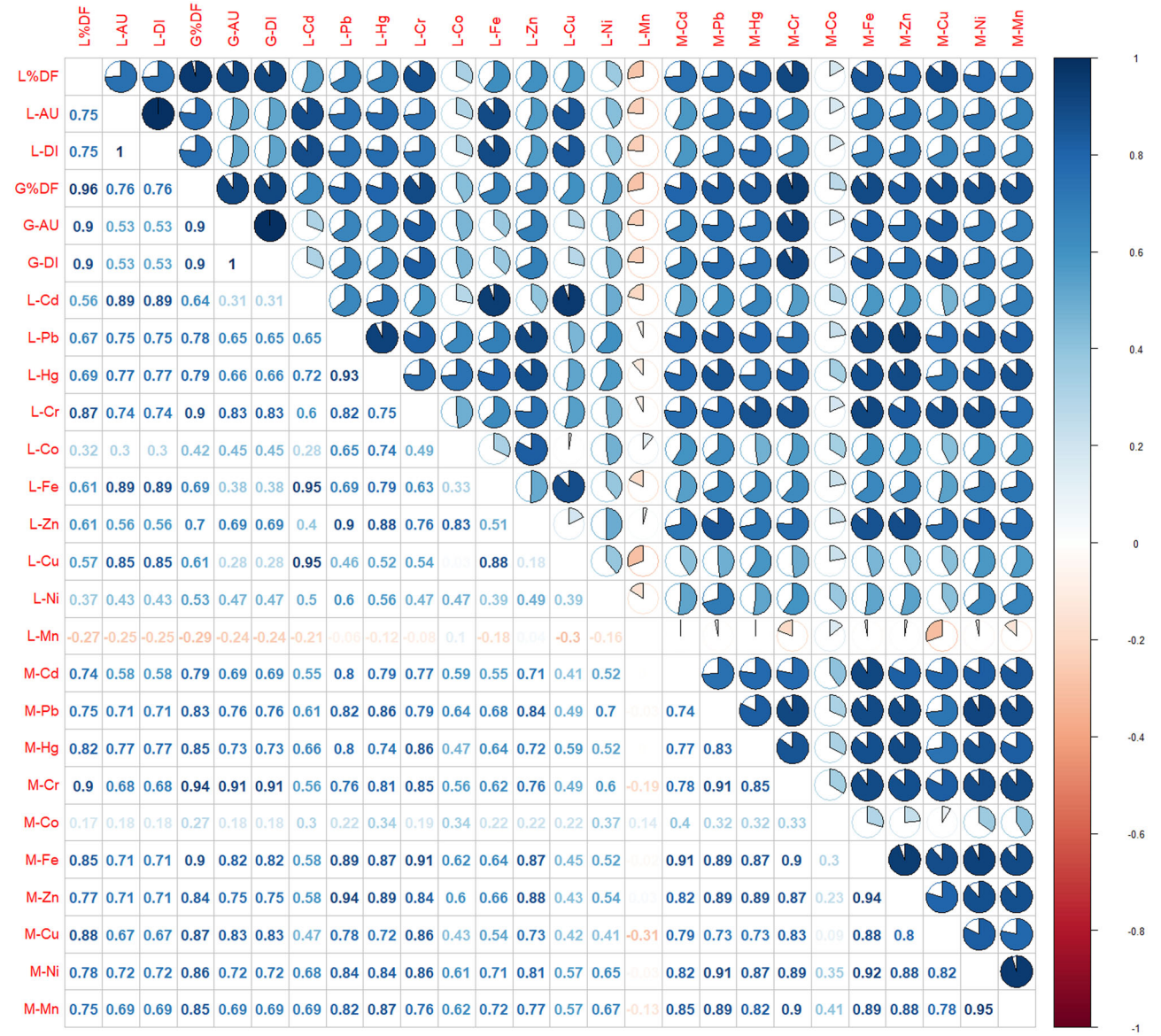

Fig. 3 Heat map of correlations between parameters with pie graph indicating the correlation level (above diagonal), and coloured correlation levels on the bases of the scale colour bar, indicating correlation between −1 and +1. The specified significance level is $P < 0.05$

Correlation between trace metals and genetic damage

Significant positive ($P < 0.001$) correlations were detected between Cd, Pb, Hg, Cr, Fe, Zn and Cu in liver and DNA damage parameters in gill and liver cells (Fig. 3). No significant correlations were observed between genotoxicity and Co, Ni and Mn accumulations in the liver of mullet. In addition, Pearson correlation analysis revealed a significant positive ($P < 0.001$) relationship between Cd, Pb, Hg, Cr, Fe, Zn, Cu, Ni and Mn accumulations in the muscle and DNA damage levels in the gill and liver (Fig. 3).

Discussion

Heavy metals in coastal and marine ecosystems are considered significant anthropogenic pollutants, which pose a serious risk to human health, aquatic species, and the ecosystem due to their toxicity and bioaccumulation features. Many heavy metals are recognized to be lethal or oncogenic to humans. (Naser 2013). These toxic pollutants are of increasing concern regarding genotoxicity and necessitate the use of sensible bioassays to monitor contaminated ecosystems. This study supplies novel information on genotoxic responses of Liza aurata as a bioindicator

species in the Payas coast of Iskenderun Bay, the North-Eastern Mediterranean.

Physicochemical parameters

According to physicochemical results, the seawater samples from the Payas coast of Iskenderun Bay were within the acceptable range with respect to the temperature, pH, and salinity parameters, as described by Coastal Waters Quality Criteria of Turkish Environmental Guidelines, 2015. When physicochemical parameters of coastal seawater in the literature are compared with the results of our study, it is clear that the values we obtained are in the similar range (Tekin Özcan 2015; Dural Eken and Akman 2017, 2018). The levels of dissolved oxygen (7.35–8.55 mg L^{-1}), pH (8.10–8.52), electrical conductivity (45500–49300 µS cm^{-1}), and total dissolved solids (46.85–48.50 g L^{-1}) for all seasons in our findings were similar to the known stated pH, electrical conductivity, dissolved oxygen and total dissolved solids detected from Iskenderun Bay coastal seawater by Yücel and Çam 2021.

Bioaccumulation

The present study provides the first data set on the accumulation of trace elements in golden grey mullet (*L. aurata*) from the Payas coast of Iskenderun Bay, North-Eastern Mediterranean using electrochemical techniques. Recently, much attention has been given to electrochemical detection methods which are inexpensive, highly sensitive, and easily adaptable for in situ assessment with short analytical periods in the field of detection of heavy metal ions (Pujol et al. 2014; Qi et al. 2017). Electrochemical techniques are more economic, user-friendly, reliable, and suitable for in-field applications. These electrochemical techniques are simple processes and are well suited to small circuits in the form of portable devices for in-situ observation of contaminated samples. The electrochemical techniques are also quick in terms of short analytical time in comparison to the other spectroscopic methods, authorizing online monitoring of the environment (Bansod et al. 2017). Nevertheless, there are few studies about its use in the analysis of environmental contamination of fish species. In this study, we reported that electrochemical techniques can be used successfully in the determination of trace metal concentrations in golden grey mullet.

In the research area, Cd and Pb accumulation in liver and muscle tissues of golden grey mullet exceeded the maximum limits allowed by the TFC Turkish Food Codex (2011), EU European Union (2005) in the summer and autumn enough to have negative effects on coastal ecosystems.. In addition, Fe concentrations in liver tissues from the sample site highly exceed the values allowed by the TFC Turkish Food Codex (2011), WHO (1989) for all seasons and the overall annual value. In addition, the highest content of Cu (57.623 ± 3.606 µg g−1) was detected at the sampling site in autumn and was above the EU European Union (2005), EPA (1989), WHO (1989) and TFC Turkish Food Codex (2011) limits.In addition, the highest content of Cu (57.623 ± 3.606 µg g^{-1}) detected in the sampling site in autumn was above the EU European Union (2005), EPA (1989), WHO (1989), and TFC Turkish Food Codex (2011) limits. Agca and Özdel (2014) reported that the highest Cd, Cu, Fe, Mn, Pb, and Zn accumulations in soils of the same area were observed in industrial lands, particularly around the Iskenderun OIZ, having filter, nail, plastic, steel pipe, carton, engine, and steel factories. Yücel and Çam (2021) also reported similar heavy metal pollution from industrial and non-industrial coastal waters of the Iskenderun Bay. When our measurements of metals in seawater were compared with international regulations, average heavy metal levels for Cd, Pb, Cu and Ni exceeded the limit values.Yücel and Çam (2021) showed that coastal seawater samples in the Iskenderun Bay may have been affected by environmental contamination. In our study, all-metal accumulations were higher in the liver than in muscle with varying concentrations. Generally, muscle is a less active tissue than the liver which is the most metabolic tissue concerning xenobiotic metabolism. Thus, the differences among tissues may reveal differential physiological and metabolic capacities (Storelli et al. 2011). Hence, the results of the previous studies (Türkmen et al. 2005; Yilmaz et al. (2010); Manasirli et al. (2015); Dural et al. 2011; Turan et al. 2020b) and the present study prove that heavy metal concentrations in marine organisms are higher in liver tissues.

DNA damage and correlation between trace metals and genotoxicity

The DNA damage analyzed by the comet assay was related to a wide range of genotoxic and cytotoxic compounds, such as trace metals (Lee and Steinert 2003). Damage frequency (DF%) and arbitrary unit (AU) is commonly used for quantifying DNA strand breakage and represent the reliable parameter depending on the intensity of the comet tail (Collins 2004; De Andrade et al. 2004; Hosseinabadi et al. 2020). In the present study, the effects of the environmental pollutants were assessed using *L. aurata* as bioindicator species. The widespread distribution and resistance to different environmental conditions make this species a good bio-indicator (Pacheco et al. 2005). The present study showed that the DNA damage level in the gill was significantly higher than in the liver for all seasons ($p < 0.05$), indicating that gills may be more susceptible to contaminants than other tissues due to high respiratory blood flow and

continuous contact with the water. Similar types of research state that gill is the sensible target tissue for monitoring environmental pollution (Omar et al. 2012; Turan et al. 2020a, 2020b). De Andrade et al. (2004) reported genotoxic levels of heavy metals in mullet species (Mugil sp.) and that the DNA damage in gill and liver cells was linked to the oxidative stress since gill revealed higher damage than that liver. In this study, *L. aurata* collected from the sampling site showed significant DNA damage as compared to those obtained from the reference site. The high damage levels were detected at Payas coastal site with 94.00 ± 4.35% DF, 275.00 ± 20.95 AU and 2.75 ± 0.20% GDI in gill tissues and 82.33 ± 3.78% DF, 237.33 ± 42.44 AU and 2.37 ± 0.42% GDI liver tissue of *L. aurata*. Chronic contact with pollutants may cause an accumulation of DNA strand breaks in aquatic organisms such as fish since their DNA-repair capacity is much lower compared to that of other species (D'Costa et al. 2017). Also, D'Costa et al. (2017) reported that high levels of DNA damage are due to the accumulation of pollutants from the environment by conducting multiple regression analyses.

The PCA showed the relationship of heavy metals in tissues and DNA damage in golden grey mullet. It revealed a strong contribution by the heavy metals Hg, Pb, Fe, Zn, Cr and Cu (in the liver tissue) to the observed DNA damage (DF%). Moreover, Cd, Cu, and Fe metals (in liver tissue) were observed to be the most important parameters taking a role in the DNA damage for AU and GDI parameters. Furthermore, the detected correlations between parameters showed a positive relationship between Cd, Pb, Hg, Cr, Fe, Zn, and Cu accumulations in the liver and DNA damage parameters in gill and liver cells in the present study. Catalyze roles of heavy metals generate reactive oxygen species, which may cause oxidative stress and damage to tissues and macromolecules such as DNA, proteins, and lipids. Some studies also reported the higher DNA damage due to the exposure to heavy metals in mullets (Pacheco et al. 2005; Bouzenda, Khebbeb (2017)) and other different fish species such as *Arius arius, Leuciscus cephalus, Clarias gariepinus, Anguilla anguilla* (De Andrade et al. 2004; Abdel-Khalek 2015; D'Costa et al. 2017; Turan et al. 2020a, 2020b).

According to the present results, Hg, Pb, Cd, Cr, Cu, Fe and Zn accumulated in the tissues of the golden grey mullet from the Payas coast of Iskenderun Bay, and could be the cause of genotoxicity in *L. aurata*. These findings are in agreement with the results of D'Costa et al. (2017) who reported genotoxic effects of trace metals, such as Fe, Cu, Cd, and Pb in the and other marine environments. Similarly, Omar et al. (2012) also reported the genotoxic effects of trace metals Cu, Zn, Fe, Mn, and Pb in marine and estuarine environments. The high concentrations of these metals can lead to DNA damage. Besides, the genotoxicity of mercury (Hg) has been demonstrated in various research on different aquatic species. The mutagenic effect of Hg accumulation in fish tissues was also confirmed by the increase of MN frequency in peripheral erythrocytes (Bolognesi et al. 1999).

Conclusion

The present study reveals novel data on the effect of heavy metals on toxiogenetic damage in *L. aurata* from Payas coast of the Iskenderun Bay, north-Eastern Mediterranean. Our study strongly supports that contaminated heavy metals caused DNA damage in the gill and liver of *L. aurata*. The high levels of DNA damage observed in L. aurata from the Payas coast, compared to the reference site, is due to pollution in the sampling site that exceeds the maximum levels allowed by national and international limits for Cd, Pb, Fe and Cu. A significant positive correlation between Cd, Pb, Hg, Cr, Fe, Zn, and Cu accumulations and DNA damage parameters was reported in the present study. The results of this study indicated that the studied sampling area in the Iskenderun Bay may have been affected by trace metal contamination. Pollution indicators and genotoxicity tests combined with other physiological or biochemical parameters represent a vital instrument for biomonitoring coastal ecosystems.

Data availability

Data is available upon request from the corresponding author.

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Author contributions Funda TURAN: Conceptualization, Project administration, Methodology, Formal analysis, Writing- Original Draft-Reviewing and Editing; M.Bertan YILMAZ: Project administration Methodology; Mehmet Lütfi YOLA: Project administration Methodology; Aysegül ERGENLER: Resources, Investigation; **N**. Seda ILGAZ: Investigation**;** Hale OKSUZ: Investigation.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Consent to participate All authors consent to participate.

Consent for publication All authors consent to publication.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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