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METAL CAUSING DNA DAMAGE IN LION FISH *PTEROIS MILES* FROM THE NORTHEASTERN MEDITERRANEAN

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ABSTRACT: The influence of trace metal contamination on marine ecosystems and marine waters is a complex construct. Metal concentrations can cause carcinogenic and toxicogenic harm in marine environments. With a variety of sizes, ages, and trophic levels, fish are excellent markers of pollution from heavy metals for the identification of genotoxic factors in freshwater as well as marine systems. The current study used the damage frequency (%), arbitrary unit (%), and genetic damage index (%) in the gill and liver cells of *P. miles* to assess DNA damage brought along by toxic metals. Under the constant levels criteria used in this investigation, the levels of Cr, Hg, Fe, and Zn in the surrounding water are only slightly above the tolerance level. According to our findings, the frequency of damage in the lionfish's gill and liver cells was 53.66 to 73.512% and 39.33 to 45.033%, accordingly. The gill tissue had a higher frequency of injury than the liver tissue. Furthermore, Pb, Hg, Cr, Co, Fe, Ni, and Cu contents in seawater were shown to have a positive correlation with DNA damage levels in *P. miles*. As a result, our study revealed the first time genotoxic damage resulting from metal contamination in *P. miles.*

KEYWORDS: comet assay**,** genotoxic effect**,** lionfish**,** metal pollution

INTRODUCTION

Completely natural metals are those with atomic masses greater than 20 and elemental density greater than 5 g cm^3 (Ali and Khan, 2018; Ahmed *et al.*, 2022). The primary causes of aquatic water contamination are discharges of industry, agriculture, and municipal pollutants that are defined as consisting of multiple chemicals. High quantities of hazardous metals in the marine ecosystem may jeopardize the well-being of marine species. As a result of their bioaccumulation from main manufacturing to consumer consumption, metal accumulates over time and has negative impacts just on the food supply chain (Heng *et al.*, 2004). According to multiple research metals have accumulated in saltwater, soils, and marine animals during the past few decades, having negative consequences that have led to genotoxic effects (Kumar *et al.*, 2015; Kumar *et al.*, 2017; Arumugam *et al.*, 2018). Therefore, it is essential that we emphasize the impact of genotoxins released into the marine environment, the degree to which they cause DNA damage, and the surveillance of these factors (Faria *et al.*, 2010; Turan *et al.*, 2020a). The comet test, which detects DNA damage in cellular to determine the genetic risk involved with toxic compounds inputs, is frequently used to study the toxicants effects of pollutants on fish. A trusted, sensitive, and quick way to detect DNA damage is a comet test (Turan & Ergenler, 2019; Turan & Ergenler, 2021).

Because ofits peculiar hydrogeological characteristics and strong anthropological pressure, the Mediterranean is facing a serious toxicological danger (Storelli *et al.*, 2011; Ayas & Kosker, 2018). There are several significant international industrial units in Iskenderun Bay, which is located on Turkey's northeastern coastline ((LPG (liquefied petroleum gas) mills, oil drilling ports, cement factories, fertilizer industries, metal vegetation, etc.). Recent times have seen a significant rise in plastics, and heavy metals, including water contamination as a result of these urbanizations (Turan *et al.*, 2009; Duysak and Azdural, 2017; Gündodu & Cevik, 2017; Dural-Eken & Akman, 2018; Can *et al.*, 2021). According to Froese and Pauly (2023), the lionfish *Pterois miles* (Bennett 1828), a species that is typically found in the Indian Ocean and Red Sea, has invaded the Mediterranean Sea and is now a regular sight in the eastern and central Mediterranean coastal waters (Golani & Sonin, 1992; Turan *et al.*, 2014; Turan, 2020). Afterwards, *P. miles* became widespread along the coasts of northern Africa, including Egypt, Libya, and Tunisia, in addition to the massive Mediterranean island nations (Cyprus, Rhodes, Crete, and Sicily), the Aegean Sea, in the eastern and central Mediterranean (Di Martino & Stancanelli, 2021). And while the lionfish is incredibly risky for fishermen and divers because of its toxic chemical dorsal, anal, and pelvic fins, its yield is growing due to the quality of its seafood (Turan, 2018; Turan and Dogdu, 2022).

P. miles, which has been improved in information as an invasive species in the Mediterranean, was not the subject of any investigations on its genotoxicity. Consequently, the purpose of this investigation was the first time the DNA damage brought about by metal contamination in the lionfish, *Pterois miles*.

MATERIALS AND METHODS

Sample collection and preparation: Specimens of lionfish *Pterois miles* were collected from Iskenderun Bay (36°24'28.9"N 35°50'45.0"E) in the northeastern Mediterranean Sea during the period of January-February 2022 by trawler net (Fig. 1).

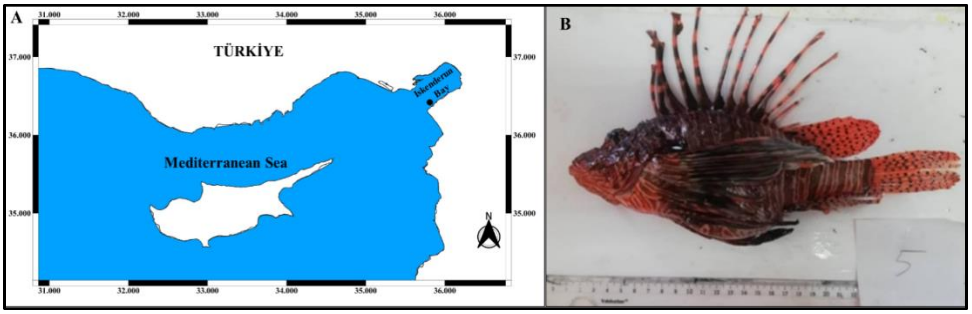

Fig. 1. A, Map of sampling site (•) in the Iskenderun Bay (Turkey) of the northeastern Mediterranean; B, Lionfish (*Pterois miles*) caught in the sampling site (Orginal).

In total, fifteen specimens of *P. miles* (average length 26.40±3.00 cm; average weight 150.05±22.05 g) ($\bar{x}$±SD) were sampledand immediately transported to the laboratory on ice in an insulated box. Species identification was carried out according to Turan, *et al.* (2014) and the length and weights of lionfish were measured to the nearest cm and nearest 0.01 g, respectively.The dissected gill and liver were carefully washed with phosphate buffer. Sea water samples from the lionfish captured area were taken from 30 cm below the seawater surface in two repetitions in 1000 ml dark-colored polyethylene (PE) bottles, which were previously cleaned with HNO_3 and deionized water, by first shaking them with some water samples and filling them from the flowing water (Boyd, 1990). It was brought to the laboratory after being kept in cooler containers (+4 °C).Water temperature (°C), pH and Dissolved O_2 (mg L^{-1}) amounts were determined in the sampling area with YSI brand Oxygen meters and pH meters.

Trace elements analysis: The water samples brought to the laboratory were pre-treated within 72 hours by filtering with blue band filter paper, or if the analysis would not be started, the pH value could be adjusted to 2-3 with a 1+1 HNO_3 solution and stored for a longer time. 100 ml of the water samples to be analyzed were taken and placed in 250 ml containers, and 5 ml of HNO_3 (55%) was added. Then, the acidified samples were placed on the heating plate and evaporated down to 20 ml. Then, 5 ml of HNO_3 (55%) and 10 ml of $HClO_4$ (70%) were added, and this process was continued until the brown smoke turned into white smoke. Water samples cooled to room temperature were made ready for reading by completing 100 ml with distilled water (Lopez *et al.*, 2002).Metal (Cadmium, Chromium, Cobalt, Copper, Iron, Mercury, Manganese, Nickel, Lead and Zinc) measurements in seawater were made using Analytik Jena/Plasma aquant ICP-MS Elite at Iskenderun Technical University, Science and Technology Research and Application (ISTE-BTM) according to APHA (2005) protocols. As a result of the measurements, the values of the heavy metal amounts contained in the samples were calculated as µg L^{-1} wet weight using mathematical methods. The following absorption lines were used; Pb 217.0 nm, Mn 279.5 nm, Cr 357.9 nm, Ni 232.0 nm, Fe 248.3 nm, Cu 324.8 nm, Co 240.7 nm, Cd 228.8 nm and Zn 213.9 nm. The calibration and blank standard solutions were inspected similarly to the samples. The standards for instrument calibrations were used with a diluting standard (1000×10^{-6}) delivered by Merck.

Comet assay: The Faculty of Marine Science and Technology Laboratories at Iskenderun Technological University conducted the Comet Assay analyses related to DNA damage detection in lionfish tissues. The liver tissue from lionfish and blood cells from the gill were separated using the cellular dissociation technique, which was adapted from Cavalcante, *et al.* (2008). Samples were made by combining 10 l of cell suspension with 100 l of 0.7% LMPAand then plating them on slides covered with regular agarose (NMPA). At 4 °C for 20 minutes, the preparations were first treated in lysis solution and subsequently in electrophoresis buffer. By using fluorescence microscopy and the scoring system, calculations for the Arbitrary Unit and other indices were established. DNA damage was measured using an image analysis system that visually assessed 100 photos of cells. The genetic damage index (GDI), the arbitrary unit values (AU), and the damage percentages (%DF) were computed to better understand the DNA damage caused by the comet test (Pitarque, *et al.*, 1999; Collins, 2004).

Statistical analysis: Based on 2000 cells, the comet tail's intensity in the samples was determined. All data were given a mean and standard deviation (SD) of the mean.

According to Zheng, *et al.* (2016),the Pearson Correlation analysis was used to ascertain the connection between the parameters and DNA damage. R-Studio and IBM SPSS Statistics 21 were used to perform all statistical analyses.

RESULTS AND DISCUSSION

Trace Metal: Temperature, dissolved oxygen concentration, and pH values from the sample point were all determined to be 18.50 0.5 °C, 6.95 0.45 mg L-1, and 8.25 0.05, respectively. Table 1 lists the values for the concentrations of Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn in the water columns at the sample location. In the water columns of the sample location, Fe, Zn, Cr, and Cu were at greater concentrations, while Cd was at the lowest dose. The relative abundance of heavy metals at the sample location wasin the following order: Fe>Zn>Cr>Cu>Ni>Mn>Pb>Hg>Co>Cd (Table 1).

Table 1. The mean values of trace metal concentrations in the Iskenderun Bay with a comparison of the Turkish Environmental Guideline (TEG) and Environmental Protection Agency (EPA, USEPA).

Metals	Iskenderun Bay	TEG (2016)		USEPA (2004)		EPA (2007, 2016)	
		CMC	CCC	CMC	CCC	CMC	CCC
Cd	0.036±0.024	<0.45(Class1)	0.2	40	8.8	33	7.9
Co	0.067±0.020	2.6	0.3	-	-	-	-
Cr	**10.439±0.708**	88	4.2	-	-	1.1	50.0
Cu	3.631±0.538	5.7	1.3	4.8	3.1	4.8	3.1
Fe	**90.516±21.104**	101	36	-	-	-	-
Hg	**0.128±0.024**	0.07	-	-	-	1.8	0.94
Mn	1.391±0.039	-	-	-	-	50	100
Ni	2.697±0.301	34	8.6	74	8.2	74	8.2
Pb	0.629±0.124	14	1.3	210	8.1	-	5.6
Zn	**43.803±3.641**	76	5.33	90	81	90	81

Data are shown as mean ± standard deviation, TEG: Turkish Environmental Guideline; EPA: Environmental Protection Agency; CMC: Criterion Maximum Concentrations; CCC: Criterion Continuous Concentrations. . US EPA: the United States Environmental Protection Agency.

The amounts of Cd, Co, Cu, Mn, Ni, Mn, and Pb in the seawater columns just at the sample location were below EPA (Environmental Protection Agency 1999, 2016) and TEG standards (Turkish Environmental Guideline, 2016). Fe, Zn, Cr, and Hg contents were found to be 90.51621.104 g L^{-1}, 43.8033.641 g L^{-1}, 10.4390.708 g L^{-1}, and 0.1280.024 g L^{-1}, correspondingly, in the sample site. Nonetheless, following the criteria of continuous variables that have been sufficient to have adverse impacts on the marine ecosystem, Cr, Fe, Hg, and Zn concentrations at the water columns of the sample site exceeded the limitations permitted by TEG (2016).

Genotoxicity: The results of the comet assay of percent damage frequency (DF %), the arbitrary unit values (AU), and the genetic damage index (GDI %) of DNA damage in *P. miles* are given in Table 2. The level of DNA damage in gill cells was 53.667±3.512% DF, 117.00±5.201 AU and 1.170±0.052% GDI. DNA damage in *P. miles* was higher in gill cells compared to liver cells in all samples (Table 2).

Table 2. Means and standard deviations of DNA damage in the gill and liver cells of lionfish were obtained from Iskenderun Bay (n=15).

Fish/Tissues	Damage Frequency (%)	Arbitrary Unit (AU)	Genetic Damage Index (%)
***P. miles*/Gill**	53.667±3.512	117.00±5.201	1.170±0.052
***P. miles*/Liver**	39.334±5.033	101.667±11.015	1.016±0.110

The data are shown as arithmetic mean ± standard deviation.

Correlation between metals and genetic damage: Significantly positive correlations ($P<0.001$) were detected between Pb, Hg, Cr, Co, Fe, Ni, and Cu in water columnsand DNA damage parameters in gill and liver cells. Moreover, a significantly negative ($P<0.001$) correlation was detected between Zn in water columns of the sampling site and DNA damage parameters in both gill and liver cells (Fig. 2).

Discussion: Owing to their toxic and bioaccumulation characteristics, most heavy metals pose a major concern to human health, aquatic life, and the marine ecosystem. Several of these substances have even been identified as fatal or cancer-causing to people (Nasser, 2013). As a result, it is becoming more important to monitor the genotoxicity of damaged ecosystems due to these harmful contaminants. New information on the genotoxic reactions of lessepsian *P. miles* in the Iskenderun Bay, the northern Mediterranean, is provided by the current study. The quantities of Cr, Hg, Fe, and Zn in the seawater column at the sample site in the current research were higher than those permitted by the TEG (2016), which is sufficient to negatively impact the marine environment for the Northeastern Mediterranean. Our findings generally agreed with the information in the literature. As an instance, Agca & Ozdel (2014) observed that high Cr, Fe, Mn, Pb, and Zn concentrations in soils of a comparable location of Iskenderun Gulf were found in industrial lands, especially near the steel, nail, plastic, steel pipe, carton, and motor industries. According to Yücel & Cam (2021), Iskenderun Bay coastal saltwater samples included significant quantities of several elements, including iron, aluminium, chromium, and zinc. Additionally, past studies (Türkmen, *et al.*, 2005; Manasırlı *et al.*, 2015; Duysak & Azdural, 2017; Gündogdu *et al.*, 2018; Dural & Akman, 2018; Can *et al.*, 2021) have reported the development of heavy metals in a variety of marine fish species, seawater, as well as soil inside the Iskenderun Bay, in the North-Eastern Mediterranean. The following metals, because of their consistency and durability. Because of their long-term stability and durability, the abovementioned metals present in the water represent a major threat to the well-being of affected species.

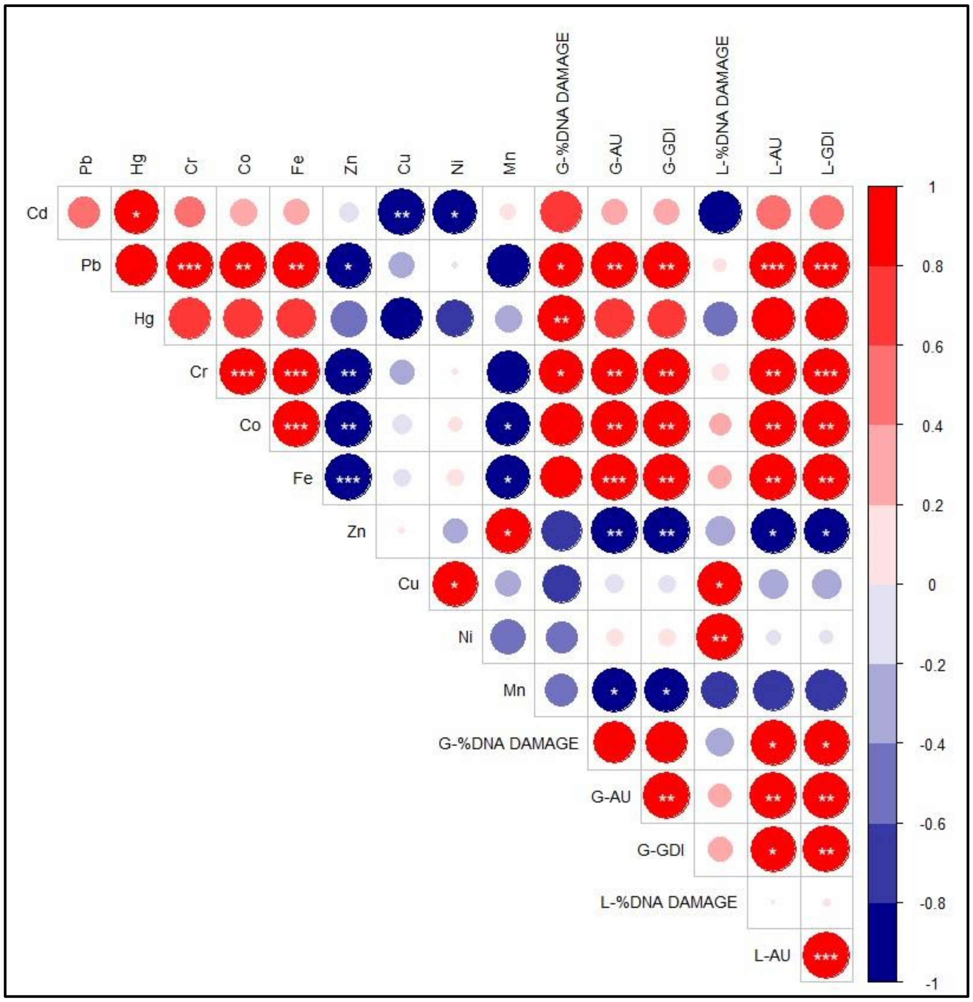

Fig. 2. Heat map of Pearson correlations between parameters with significance levels. The coloured correlation levels on the scale colour bar indicate a correlation between -1 and +1. The specified significance levels are given in the circled cells: *, $P<0.05$; **, $P<0.01$; $P<0.001$.

Heavy metals are hazardous substances that have been linked to cancer and Genetic damage (Barbaso, *et al.*, 2010). Because of the presence of certain contaminants in specific amounts in the aquatic environment, a series of genotoxicity analyses may be necessary. Vulnerable species, such as those used in situ testing and short-term research on plants and aquatic species, stand out as acceptable options among these assessments. One of the methods often used frequently these days in eco-genotoxicology to find DNA damage is the comet test. When used with aquatic biological indicators species, this test has extensive applicability and offers a quick, accurate, and useful approach for the investigation of environmental genotoxicity. Fish are also top-notch test subjects for studies on genotoxicity. For the reason that they may absorb and digest contaminants. Also, there are various sizes, ages, and trophic levels, as well as inhabiting (Scalon *et al.*, 2010).

The current work used the Comet test to assess DNA damage brought on by metal contamination in *P. miles*. According to our findings, the frequency of damage in the lionfish's gill and liver cells was 53.6673.512% and 39.3345.033%, significantly. The gill tissue had a higher frequency of injury than the liver tissue. With their high respiratory blood flow and ongoing interaction with saltwater, gills may be more vulnerable to pollutants than some other organ tissues, according to this report. The gill, with its practical structure for detecting environmental pollutants, is a critical tissue in other studies that have also made inferences (Omar *et al.* 2012; Turan, *et al.* 2020a, 2020b).Furthermore, because there was more damage seen in the gill than in the liver, oxidative stress was linked to DNA damage in mullet species' gill and liver cells (De Andrade *et al.*, 2004). Moreover, Pb, Hg, Cr, Co, Fe, Ni, and Cu in water columns were shown to strongly positively correlate ($p< 0.001$) with DNA damage indices in gill and liver cells. According to Matsumoto *et al.* (2006), *O. niloticus* exposed to water samples containing Cr had a considerable rise in the number of DNA breaks. Moreover, according to several studies, Cr, Pb, and Hg are all toxic elements of DNA in distinct ways. According to such authors (De León *et al.*, 2021).That DNA can provide Cr with a large number of binding sites, leading to breaking and other associated DNA damage. In addition to oxidative stress, Hg ions may bind to DNA and result in DNA-DNA crosslinks, strand breakage, and suppression of DNA repair (De León *et al.*, 2021). Fish have a considerably poorer ability for DNA repair than other creatures, therefore repeated exposure to contaminants can cause DNA strand breaks in these animals. This was discovered by D'Costa *et al.* (2017).

CONCLUSION

The outcomes of this research offer new information on the impact of heavy metals on toxicogenic harm in invasive alien fish like *P. miles* found in the Mediterranean. The sample region studied in Iskenderun Bay was discovered to have trace element pollution, and DNA damage to some extent was detected within the lionfish's gill and liver cells. Consequently, it is probable that chemicals that cause genetic toxicity inside the research region include the two metals discovered in this research and additional groups of environmental toxins. This work also demonstrates the significance of various physiological and biochemical concerns for the biomonitoring of coastal zones, such as genotoxicity analyses as well as pollutant indications.

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REFERENCES

Ağca, N. and E. Özdel, 2014. Assessment of spatial distribution and possible sources of heavy metals in the soils of Sariseki-Dörtyol District in Hatay Province (Turkey). *Environ. Earth Sci.* 71(3): 1033-1047. (doi:10.1007/s12665-013-2507-8).

Ahmed, M.S., T.T. Aurpa and M.A.K. Azad, 2022. Fish disease detection using image based machine learning technique in aquaculture. *J. K. S. Univ.-Comput. Inf. Sci.* 34(8): 5170518. (doi: 10.1016/j.jksuci.2021.05.03).

Ali, H. and E. Khan, 2018. Bioaccumulation of non-essential hazardous heavy metals and metalloids in freshwater fish. Risk to human health. *Environ. Chem. Lett.* 16(3): 903-917. (doi: 10.1007/s10311-018-0734-7).

APHA, 2005. Standard methods for the examination of water and wastewater analysis 21st edition. American Public Health Association WEF, Washington.

Arumugam, G.K.S., D. Sharma, R.M. Balakrishnan and J.B.P. Ettiyappan, 2018. Extraction, optimization and characterization of collagen from sole fish skin. *Sustain. Chem. Pharma.* 9: 19-26. (doi:10.1016/j.scp.2018.04.003).

Ayas, D. and A.R. Köşker, 2018. The effects of age and individual size on metal accumulation of *Lagocephalus sceleratus* (Gmelin, 1789) from Mersin Bay, Turkey. *Nat. Engineer. Sci.* 3(1): 45-53. (doi:10.28978/nesciences.379321).

Barbosa, L.F. and F.M. Cerqueira, A.F. Macedo, C.C. Garcia, J.P.F. Angeli, R.I. Schumacher, M.C. Sogayar, O. Augusto, M.T. Carri, P. Di Mascio and M.H.G. Medeiros, 2010. Increased SOD1 association with chromatin, DNA damage, p53 activation, and apoptosis in a cellular model of SOD1-linked ALS. *Biochim. et Biophys. Acta (BBA)-Molecul. Bas. Diseas.* 1802(5): 462-471. (doi:10.1016/j.bbadis. 2010.01.011).

Boyd, C.E., 1990. Water quality in ponds for aquaculture. Agriculture Experiment Station, Auburn University, Alabama. 482 pp.

Can, M.F., Y. Mazlum, A.B. Yılmaz and A. Öksüz, 2021. A meta analysis on the heavy metal content of two shrimp species, *Penaeus semisulcatus* (de Hann, 1844) and *Metapenaeus monoceros* from İskenderun Bay. *J. agricult. Nat.* 24(4): 834-849. (doi:10.18016/ksutarimdoga.vi.811212).

Cavalcante, D.G.S.M., C.B.R. Martinez and S.H. Sofia, 2008. Genotoxic effects of Roundup fish *Prochilodus lineatus*. *Mutat. Res./Genet. Toxicol. Environ. Mutagen.* 655(1-2): 41-46. (doi:10.1016/j.mrgentox.2008.06.010).

Collins, A.R., 2004. The comet assay for DNA damage and repair. *Molecul. Biotechnol.* 26(3): 249-261. (doi:10.1385/mb:26:3:249).

D Costa, A., S.K. Shyama and M.P. Kumar, 2017. Bioaccumulation of trace metals and total petroleum and genotoxicity responses in an edible fish population as indicators of marine pollution. *Ecotoxicol. Environ. Safe.* 142: 22-28. (doi:10.1016/j.ecoenv. 2017.03.049).

De Andrade, V.M., J. Da Silva, F.R. Da Silva, V.D. Heuser, J.F. Dias, M.L. Yoneama and T.R. De Freitas, 2004. Fish as bioindicators to assess the effects of pollution in two southern Brazilian rivers using the Comet assay and micronucleus test. *Environ. Molecul. Mutagen.* 44(5): 459-468. (doi:10.1002/em.20070).

De León, J.S.D.D., G.E. Santos-Medrano, R. Rico-Martínez, I. Rubio-Franchini and D. Robles-Vargas, 2021. Preliminary Genotoxicity Assessment of Cadmium, Lead, and Mercury Planktonic Organisms. *Pol. J. Environ. Stud*. 30(6): 5519-5525. (doi:10.15244/pjoes/136481).

Di Martino, V. and B. Stancanelli, 2021. The alien lionfish, *Pterois miles* (Bennett, 1828), enters the Adriatic Sea, Central Mediterranean Sea. *J. Bl. Sea/Mediterran. Environ.* 27(1): 104-108.

Dural Eken, M. and B. Akman, 2018. Assessment of heavy metal pollution of seston from freshwater resources poured into the Northeast Mediterranean region. *Environ. Monitor. Assess.* 190(5): 1-7. (doi:10.1007/s10661-018-6642-2).

Duysak, Ö. and K. Azdural, 2017. Evaluation of heavy metal and aluminium accumulation in a gastropod, *Patella caerulea* L., 1758 in Iskenderun Bay, Turkey. *Pak. J. Zool.* 49(2): 629-637. (doi:10.17582/journal.pjz/2017.49.2.629.637).

EPA, 2007. Environmental Protection Agency, National Recommended Water Quality Criteria-Aquatic Life Criteria.

EPA, 2016. Environmental Protection Agency, National Recommended Water Quality Criteria-Aquatic Life Criteria.

Faria, J.J., J.R. Dyer, R.O. Clément, I.D. Couzin, N.C. Holt, A.J.W. Ward, D. Waters and J. Krause, 2010. A novel method for investigating the collective behaviour of fish: introducing ‘Robofish’. *Behavior. Ecol. Sociobiol.* 64(8): 1211-1218. (doi:10.1007/ s00265-010-0988-y).

Froese, R. and D. Pauly (Ed.), 2023. FishBase.World Wide Web electronic publication.www.fishbase.org, version (02/2023). Golani D. & Sonin O. 1992. New records of the Red Sea fishes, *Pterois miles* (Scorpaenidae) and *Pteragogus pelycus* (Labridae) from the eastern Mediterranean Sea. *Japan. J. Ichthyol.* 39(2): 167-169. (doi:10.1007/bf02906001).

Golani, D. and O. Sonin, 1992. New records of the Red Sea fishes, *Pterois miles* (Scorpaenidae) and *Pteragogus pelycus* (Labridae) from the eastern Mediterranean Sea. *Jpn. J. Ichthyol.* 39(2): 167-169. (doi.org/10.1007/bf02906001).

Gündoğdu, S., C. Çevik, B. Ayat, B. Aydoğan and S. Karaca S, 2018. How microplastics quantities increase with flood events? An example from Mersin Bay NE Levantine coast of Turkey. *Environ. Pollut.* 239: 342-350.

Heng, H.H.Q., S. Goetze, C.J. Ye, G. Liu, J.B. Stevens, S.W. Bremer, S.M. Wykes, J. Bode and S.A. Krawetz, 2004. Chromatin loops are selectively anchored using scaffold/matrix-attachment regions. *J. cell sci.* 117(Pt 7): 999-1008.

Kumar, G., S. Menanteau-Ledouble, M. Saleh and M. El-Matbouli, 2015. Yersinia ruckeri, the causative agent of enteric redmouth disease in fish. *Vet. Res.* 46(1): 1-10. (doi:10.1186/s13567-015-0238-4).

Kumar, S., Z. Sándor, N. Zoltan, G. Fazekas, H. Mate, A.K. Sinha, G. De Boeck and D. Gál, 2017. Potential of processed animal protein versus soybean meal to replace fish meal in practical diets European catfish (*Silurus glanis*): growth response and liver gene expression. *Aquacult. Nutrit.* 23(5): 1179-1189. (doi:10.1111/anu.12487).

López, F.F., C. Cabrera, M.L. Lorenzo and M.C. López, 2002. Aluminium content of drinking waters, fruit juices and soft drinks: contribution to dietary intake. *Sci. total environ.* 292(3): 205-213. (doi:10.1016/s0048-9697(01)01122-6).

Manaşırlı, M., 2015. Some properties reproductive of the speckled shrimp (*Metapenaeus monoceros* Fabricius, 1798) in the North-eastern Mediterranean. *Iran. J. Fisher. Sci.* 14(2): 485-493. (doi:10.1186/s41200-016-0072-z).

Matsumoto, S.T., M.S. Mantovani, M.I.A. Malaguttii, A.L. Dias, I.C. Fonseca and M.A. Marin-Morales, 2006. Genotoxicity and mutagenicity of water contaminated with tannery effluents, as evaluated by the micronucleus test and comet assay using the fish *Oreochromis niloticus* and chromosome aberrations in onion root-tips. *Genet.Molecul. Biol.* 29:148-158. (doi:10.1590/s1415-47572006000100028).

Nasser, N., P.D. Boyle and R.J. Puddephatt, 2013. Organoplatinum Chemistry with a Dicarboxamide–Diphosphine Ligand: Hydrogen Bonding, Cyclometalation, and a Complex with Two Metal–Metal Donor–Acceptor Bonds. *Organometal.* 32(19): 5504-5513. (doi:10.1021/om400780q).

Omar, W.A., K.H. Zaghloul, A.A. Abdel-Khalek and S. Abo-Hegab, 2012. Genotoxic effects of metal pollution in two fish species, *Oreochromis niloticus* and *Mugil cephalus*, highly degraded aquatic habitats. *Mutat. Res./Genet. Toxicol. Environ. Mutagene.* 746(1): 7-14. (doi:10.1016/j.mrgentox.2012.01.013).

Pitarque, M., A. Vaglenov, M. Nosko, A. Hirvonen, H. Norppa, A. Creus and R. Marcos, 1999. Evaluation of DNA damage by the Comet assay in shoe workers exposed to toluene and other organic solvents. *Mutat. Res./Genet. Toxicol. Environ. Mutagene.* 441(1): 115-127. (doi:10.1016/s1383-5718(99)00042-x).

Scalon, M.C.S., C. Rechenmacher, A.M. Siebel, M.L. Kayser, M.T. Rodrigues, S.W. Maluf, M.A. Rodrigues and L.B.D. Silva, 2010. Evaluation of Sinos River water genotoxicity using the comet assay in fish. *Brazil. J. Biol.* 70(4 Suppl.): 1217-1222. (doi: 10.1590/s1519-69842010000600011.).

Storelli M.M., G. Cuttone and G.O. Marcotrigiano, 2011. Distribution of trace elements in the tissues of smooth hound *Mustelus mustelus* (Linnaeus, 1758) from the southern–eastern waters of Mediterranean Sea (Italy). *Environ. Monitor. Assess.* 174(1): 271-281. (doi:10.1007/s10661-010-1456-x).

TEG, 2016. Turkish Environmental Guidelines 2015 Publications of Turkish Foundation of Environment.

Turan, C. and S.A. Doğdu, 2022. Preliminary Assessment of Invasive Lionfish *Pterois miles* Using Underwater Visual Census Method in the Northeastern Mediterranean. *Croat. J. Fisher.: Ribarst.* 80(1): 38-46.

Turan, C., 2018. The invasion and establishment of lionfish species (*Pterois* spp.) as a major threat to marine biodiversity of Turkey. *In*: Lionfish Invasion and Its Management in the Mediterranean Sea. pp.10-16.

Turan, C., 2020. Species distribution modelling of invasive alien species; *Pterois miles* for current distribution and future suitable habitats. *Glob. J. Environ. Sci. Manage.* 6(4): 429-440.

Turan, C., D. Erguden, M. Gurlek, D. Yaglioglu, A. Uyan and N. Uygur, 2014. First record of the Indo-Pacific lionfish *Pterois miles* (Bennett, 1828) (Osteichthyes:

Scorpaenidae) for the Turkish marine waters. *J. Bl. Sea/Mediterran. Environ.* 20(2): 158-163.

Turan, C., M. Dural, A. Oksuz and B. Öztürk, 2009. Levels of heavy metals in some commercial fish species captured from the Black Sea and Mediterranean coast of Turkey. *Bull. Environ. Contaminat. Toxicol.* 82(5): 601-604. (doi:10.1007/s00128-008-9624-1).

Turan, F. and A. Ergenler, 2019. Morphological Comparison of Thinlip mullet *Liza ramada* (Risso, 1827) Populations using the BioMorph Software. *Nat. Engineer. Sci.* 4(3): 18-24.

Turan, F. and A. Ergenler, 2021. DNA Damage in Hybrid Tilapia (*Oreochromis niloticus* x *O. aureus*) Exposed to Short-Transport Process. *NESciences*. 6(3): 190-196.

Turan, F., M. Eken, G. Ozyilmaz, S. Karan and H. Uluca, 2020b. Heavy metal bioaccumulation, oxidative stress and genotoxicity in African catfish *Clarias gariepinus* from Orontes river. *Ecotoxicol.* 29(9): 1522-1537. (doi:10.1007/s10646-020-02253-w).

Turan, F., S. Karan and A. Ergenler, 2020a . Effect of heavy metals on toxicogenetic damage of European eels *Anguilla anguilla*. *Environ. Sci. Pollut. Res.* 27(30): 38047-38055. (doi:10.1007/s11356-020-09749-2).

Türkmen, A., M. Türkmen, Y. Tepe and İ. Akyurt, 2005. Heavy metals in three commercially valuable fish species from Iskenderun Bay, Turkey. *Food Chem.* 91(1): 167-172. (doi:10.1016/j.foodchem.2004.08.008).

US-EPA, 2004. Environmental Protection Agency-US, Integrated risk information system.

Yücel, Y. and A.R. Çam, 2021. Assessment of industrial pollution effects in coastal seawater with chemometric approach. *Int. J. Environ. Analyt. Chem.* 101(1): 95-112. (doi:10.1080/03067319.2019.1660877).

Zheng, Q., C. Bao, W. Guo, S. Li, J. Chen, B. Chen, Y. Luo, D. Lyu, Y. Li, G. Shi, L. Liang, J. Gu, X. He and S. Huang, 2016. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. *Nat. communicat.* 7(1): 1-13.