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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Integrated assessment with biomarker responses and metal concentrations on some fish species from İzmir Bay: A preliminary investigation

İzmir Körfezi'ndeki bazı balık türlerinde biyoişaretçi yanıtlarının metal konsantrasyonları ile birlikte değerlendirilmesi: Bir ön araştırma

Mustafa Bilgin[1] ● Esin Uluturhan-Suzer[2]* ● Enis Darılmaz[3]

[1] Dokuz Eylül University, Institute of Natural and Applied Sciences, Tınaztepe Campus, 35390, İzmir, Türkiye https://orcid.org/0000-0003-4680-5931
[2] Dokuz Eylül University, Institute of Marine Sciences and Technology, İnciraltı, 35340, İzmir, Türkiye https://orcid.org/0000-0002-7886-922X
[3] Dokuz Eylül University, Institute of Marine Sciences and Technology, İnciraltı, 35340, İzmir, Türkiye https://orcid.org/0000-0002-9090-9319

*Corresponding author: esin.uluturhan@deu.edu.tr
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Abstract: Antioxidant related biomarkers (superoxide dismutase, catalase, malondialdehyde) were investigated to evaluate metal (Hg, Cd, Pb, Cr, Cu, Zn, and Mn) bioaccumulation in some organs of fish species (*Sparus aurata*, *Chelon labrosus*, *Diplodus vulgaris*) from the İzmir Bay. Samples were collected at November 2019 from Inner and Outer Bays. Metal and biomarker analyses were carried out by Atomic Absorption Spectrometer and microplate reader, respectively. For metal analyses in organs, higher metal bioaccumulations were found at liver tissues. Higher Hg and Zn concentrations were found in *S. aurata*, higher Cd, Cr, Cu and, Mn concentrations were detected in *C. labrosus* and higher Pb concentrations were determined in *D. vulgaris*. In biomarker results, superoxide dismutase (SOD) and catalase (CAT) activities were generally higher in gills, however, MDA contents were higher at liver. The highest biomarker results were detected at *C. labrosus.* Statistical analyses were demonstrated that especially MDA content were expressed strong responses for the metal bioaccumulations. Also, Mn levels were considerably correlated with all biomarkers as expected. This study revealed that combined utilization of biomarkers and metal concentrations could be a vital indicator to investigate health status of the marine ecosystems.

Keywords: Catalase, superoxide dismutase, malondialdehyde, trace metals, mullet, Sparidae sp.

Öz: İzmir Körfezi'ndeki balık türlerinin (*Sparus aurata*, *Chelon labrosus*, *Diplodus vulgaris*) organlarında metal (Hg, Cd, Pb, Cr, Cu, Zn ve Mn) biyobirikimini değerlendirmek için antioksidanla kökenli biyoişaretçiler (süperoksit dismutaz, katalaz, malondialdehit) incelenmiştir. Balıklar Kasım 2019'da İç ve Dış Körfez'den toplanmıştır. Metal ve biyoişaretçi analizleri sırasıyla Atomik Absorpsiyon Spektrometresi ve mikroplaka okuyucu ile yapılmıştır. Organlarda bulunan metal değerlerine göre karaciğer dokularında daha yüksek metal biyobirikimleri bulunmuştur. *S. aurata*'da daha yüksek Hg ve Zn konsantrasyonları, *C. labrosus*'ta daha yüksek Cd, Cr, Cu ve Mn konsantrasyonları ve *D. vulgaris*'te ise daha yüksek Pb konsantrasyonları tespit edilmiştir. Biyoişaretçi sonuçlarına göre, solungaçlarda SOD ve CAT aktiviteleri genel olarak daha yüksek, ancak MDA içerikleri karaciğerde daha yüksek bulunmuştur. En yüksek biyoişaretçi bulguları ise *C. labrosus*'ta tespit edilmiştir. İstatistiksel hesaplamalar, özellikle MDA içeriğinin, metal biyobirikimleri için kayda değer cevaplar verdiğini göstermiştir. Ayrıca Mn seviyeleri, beklendiği gibi tüm biyoişaretçiler ile anlamlı korelasyonlar göstermiştir. Bu çalışma, biyoişaretçilerin ve metal konsantrasyonlarının birlikte kullanımının deniz ekosistemlerinin sağlık durumunu araştırmak için önemli bir belirteç olabileceğini ortaya koymuştur.

Anahtar kelimeler: Katalaz, süperoksit dismutaz, malondialdehit, iz metaller, kefal, Sparidae sp.

INTRODUCTION

Due to natural components of Earth's crust, metals interfere with living resources all the time. Main routes of metals trough the aquatic environment can be defined as two separate ways: natural and anthropogenic activities. Natural phenomenon includes erosion, volcanic eruptions, evaporation, and corrosion. Besides, industrial, agricultural, urban run-offs can be defined as anthropogenic sources. Eventually, aquatic organisms are exposed to essential and toxic metals which they can be fatal when they reach to the food chain with biomagnification mechanisms. (Briffa et al., 2020).

As it is known, the concentrations of pollutants in the aquatic ecosystem increase as a result of domestic and industrial activities. For this reason, environmental monitoring studies are needed to identify, evaluate and manage risks of the negative effects of organic and inorganic pollutants, on the environment and natural resources (Cajaraville et al., 2000). These environmental monitoring programs generally focus on determining the levels of physical (such as salinity, temperature, pH) and chemical (nutrients and pollutants) parameters in the water column. Although particle/grain size analysis from sediment, determination of pollutants and physicochemical analyses provide the information about contamination status of the environment, they cannot provide sufficient information about the effects of pollutants on the marine biological systems (Lam, 2009).

Since the second half of the 20th century, researches on early detection of the pollution effects on aquatic life have become increasingly important. The indicators obtained as a result of these researches are called "biomarkers". Basically, biomarkers defined as "measurable biological responses in a biological system that occur as a result of exposure to pollutants".

Biomarker responses are measured in cells, body fluids, tissues or organs; although they are limited to chemical, molecular or physical changes and these are an indicator of the effects of pollutants (Lam and Gray, 2003).

Biomarkers could be classified in three sub-groups for the effects in organisms; a) stress-sensitive biomarkers, b) genotoxicological biomarkers, and c) exposure biomarkers (Viarengo et al., 2007). While catalase (CAT) and superoxide dismutase (SOD) were associated into the stress-sensitive biomarkers class; malondialdehyde (MDA) is one of the exposure biomarkers (Viarengo et al., 2007).

Atmospheric O_2 has a distinctive feature because it has two unpaired electrons in its ground state. For this important property, O_2 is described as "paramagnetic" and therefore has limited interaction with organic molecules unless there is an activation. Univalent reduction of molecular oxygen results in mostly unstable intermediates such as superoxide radical (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^-), before producing water (H_2O) (Markovitch, 2005). These structures are defined as reactive oxygen species (ROS). Since ROS products are involved in many metabolic reactions, they can cause cellular damage and also have disease-causing effects such as DNA damage (Lee and Steinert, 2003; Lesser, 2006). In addition to metabolic reactions in living cells, organic and inorganic pollutants such as metals and pesticides also cause the formation of free radicals.

There are some antioxidant enzymes that help remove the affection of ROS in the cell. Superoxide dismutase (SOD), an antioxidant enzyme, is a metalloprotein that distributes in different organelles or cytoplasm inside the cell and contains different metals (such as Mn, Fe, and Zn) as cofactors. Main function of SOD catalyzes the conversion reaction of O_2^- radical ion to H_2O_2 and O_2 molecules. Catalase (CAT), another oxidative stress biomarker, is an enzyme containing a heme group; It enables the conversion of H_2O_2 to H_2O and O_2. In the use of hydroxyl (-OH) scavenging agents, SOD and CAT determine the role of oxygen radicals involved in biological processes. (Lesser, 2006; Di Giulio et al., 1989).

Lipid peroxidation (LPO) is a mechanism that occurs under the control of enzymes in biological systems without radical ions. One of the final products formed in this reaction is malondialdehyde (MDA). MDA molecule is formed by the breakdown of unsaturated polylipids by ROS. MDA can have a mutagenic effect by binding to DNA as a result of the reactions it enters in the cell. The presence of MDA or other LPO products is expressed as an important "fingerprint" about the entry of xenobiotics that cause oxidative stress in the cell (Pryor and Stanley, 1975; Romero et al., 1998).

Chelon labrosus (Risso, 1827) (thicklip grey mullet) in one of the common species of Mugilidae family. Its individuals generally distribute coasts and stream mouths, especially rocky and sandy bottoms. It also prefers living with larger groups (Parrino et al., 2021). *Chelon labrosus* is an omnivorous scavenger that feeds with small organisms and particles from water column and sediment (Abumourad et al., 2014).

Sparus aurata (Linneaus, 1758) (gilthead seabream) is one of the common fish species at the Eastern Atlantic Ocean and the Mediterranean Sea. It generally lives about 0 to 30 meters depth, but it can excess to 150 m (Bauchot and Hureau, 1990; Seginer, 2016). This species is carnivore and usually feeds on generally molluscs and arthropods (Hadj Taieb et al., 2013).

Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817) (common two-banded seabream) is a demersal marine fish that lives generally inshore waterbeds and posidonia covered areas with 0 – 90 m depth (Cengiz et al., 2019). Along with *S. aurata*, it is also member of Sparidae family (Da Palma, 2002). Main diet of this species contains crustacea such as amphipoda, isopoda, prawns, fish and polychaeta (Osman and Mahmoud, 2009).

These species have spawning period between late autumn – winter session (Abumourad et al., 2014; Hadj Taieb et al., 2013; Osman and Mahmoud, 2009).

İzmir Bay, chosen as the study area, is heavily under the influence anthropogenic and industrial pollution. Various industrial facilities (such as agricultural production, food processing, tanneries, paper production, textile, metal industries and beverage manufacturing) and urban wastes discharge to the Bay and these are prime reasons for the pollution (Kucuksezgin et al., 2006).

Many streams and domestic discharge channels carry the pollution load to the bay. Gediz River, one of the most important sources of pollution, is located in the northern part of the bay. Inner Bay has intensity of industrial and anthropogenic activity. In addition, İzmir Port, one of Türkiye's most important natural ports, operates actively in the Inner Bay. Thanks to the wastewater treatment plant, which was put into operation in 2000, the water quality and number of living organisms in the bay has increased. Accordingly, the people of İzmir can hunt fish with high economic value such as sea bream, sea bass and mullets, especially in the Inner Bay. According to many studies (Uluturhan Suzer et al., 2015; Kacar et al., 2016; Taş et al., 2018; Taş and Sunlu, 2019), the pollution in the sediment continues despite the improvement in water quality in the bay. For this reason, organisms like fish and mussels caught from

Izmir Bay, a potential risk to public health in seafood via consumption continues (Çakal Arslan et al., 2010; Kucuksezgin et al., 2011).

In this context, the aim of this study is integrated evaluation of the metal concentrations and the investigation of the metabolic responses by using biomarkers sensitive to reactive oxygen species distributed in fish species in the İzmir Bay

MATERIALS AND METHODS

Sampling: Fish samples were collected coastal sites of Foça (Outer Bay) and İnciraltı (Inner Bay) in November 2019 by fishing rods (Figure 1). While *Sparus aurata*, *Diplodus vulgaris* and *Chelon labrosus* were sampled in Foça, only *C. labrosus* individuals were sampled in İnciraltı (Table 1).

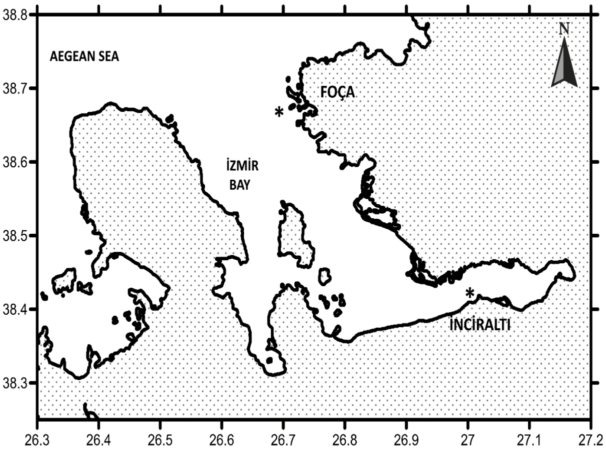

Figure.1. Sampling points (Foça and İnciraltı) at Izmir Bay (*)

The fish were sampled and quickly decapitated. Muscle, liver and gill tissues were separated from fish. Samples that used in biomarker analysis were placed in cryo-tubes and stored in a liquid nitrogen tank (-196 °C), while tissues for metal analysis were stored in deep freezers (-18 °C) in plastic bags (UNEP/FAO/IAEA/IOC 1984).

Table 1. Biometric data of sampled fish species in November 2019

Locations	Species	n	Weight (g)	Fork Length (cm)
Foça	*S. aurata*	3	254-271	24.6-25.7
Foça	*C. labrosus*	3	361-455	29.5-33.0
Foça	*D. vulgaris*	3	209-237	18.8-23.0
İnciraltı	*C. labrosus*	8	252-272	26.5-37.0

Metal analysis: Firstly, tissue samples were first be subjected to drying using a lyophilizer and then, the dried samples were weighed approximately 0.50 g in at least triplicate and digested in a Milestone microwave digestion system in an acid mixture of 5 mL HNO_3 and 1 mL $HClO_4$. The mixture with fragmented samples were completed to 25mL and stored in HDPE bottles. Within the scope of metal analysis, mercury (Hg), cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu), zinc (Zn) and manganese (Mn) analyzes were carried out. Varian Atomic Absorption Spectrometer (AAS) was performed on 280FS and 280Z instruments using flame technique (Cu, Zn and Mn), graphite furnace (Pb, Cd and Cr) and cold vapor (Hg) (UNEP/FAO/IAEA/IOC 1984; UNEP 1990). Recovery rates were set with standard solutions and the percentages of ratios were set 100%±10 to verify the accuracy of analyses. All metal analyses were expressed as “mg/kg dry weight” unit.

Biomarker analyzes: SOD, CAT and MDA analyzes were performed with Biotek Synergy HTX model microplate reader. Homogenization processes were carried out with ISOLAB homogenizer.

Determination of SOD activity: This method was described by Sun et al. (1988) and adapted to the microplate reader. The supernatants from the homogenates prepared with Tris-HCl (pH 7.5) containing EDTA were measured at 560 nm wavelength against blank reading in the presence of xanthine/xanthine oxidase reaction and nitroblue tetrazolium. Depending on the inhibition rate obtained, the SOD activity was determined as mU/mL.

CAT activity determination: CAT activity method was adapted from the methods of (Aebi, 1984) for the microplate reader. The activity was determined at 230 nm as mU/mL by kinetic reading against H_2O_2.

Determination of Total Protein: Total protein analysis was performed to calculate SOD and CAT activities as specific activities. Protein determination was measured at a wavelength of 562 nm by using the “Pierce™ BCA Protein Assay Kit” from ThermoFisher Scientific. As a result of protein determination, CAT and SOD results were calculated as mU/mg protein.

Determination of MDA content: Concentration of MDA content was determined by adapting the method of Ohkawa et al. (1979) to the microplate. It was determined by spectrophotometric measurement of the tissues against 1,1,3,3-tetramethylpropane (TMP) at a wavelength of 532 nm after the reaction with a mixture of thiobarbituric acid and acetic acid and incubation at 95 °C for 1 hour. The level of MDA contents is expressed in pmol MDA/mg tissue.

Statistical analysis and evaluation of data: Statistical calculations were carried out JASP statistical software (version 0.16.3.0). Evaluation of metal and biomarker analyses; by using parametric (ANOVA) and non-parametric (Kruskal-Wallis) statistical tests, the interactions of the analysis results with each other were examined by considering the changes depending on the region, species and tissues ($p < 0.05$). Data were log-transformed where necessary to meet normal distribution and Levene’s homogeneity tests. In addition, the interactions of metals and biomarkers were investigated by Pearson Product-Moment Correlation tests ($p < 0.05$).˝R≥ |0.40|” values were evaluated and interpretation of absolute magnitudes on correlation coefficients were described as

Schober and Schwarte (2018). Also, Network Analysis were carried out to exhibit interferences of sub-groups (metals and biomarkers) with each other.

RESULTS AND DISCUSSION

Metal analyses

Metal concentrations at organs of fish species were given at Table 2. According to the tissue-based investigation, the highest Hg concentrations were detected at muscle tissues for *S. aurata* (0.68 mg/kg). In addition, highest levels of Cd (1.41 mg/kg), Pb (3.98 mg/kg), Cu (79.6 mg/kg), and Zn (368 mg/kg) were found at livers, while the highest levels of Cr (1.30 mg/kg) and Mn (26.7 mg/kg) were obtained at gills (Table 2).

For *C. labrosus*, maximum Hg (0.51 mg/kg), Cd (4.51 mg/kg), Cr (1.50 mg/kg), Cu (775 mg/kg), and Zn (180 mg/kg) levels found at liver tissues. Also, maximum Pb (4.28 mg/kg) and Mn (33.2 mg/kg) concentrations were determined at gills (Table 2).

For, *D. vulgaris* the highest concentrations of Hg (0.60 mg/kg) were detected at muscle tissues. The highest Cu (41.3 mg/kg), Cd (3.82 mg/kg) and Zn (166 mg/kg) levels were obtained at liver, while the highest Pb (16.5 mg/kg), Cr (0.75 mg/kg) and Mn (25.3 mg/kg) levels were found at gills.

To evaluate minimum concentrations for all species; only Hg levels of *S. aurata* and *D. vulgaris* were detected at gills, other minimum levels of metals were all found at muscle tissues (Table 2).

Bioaccumulation order for the tissues in all fish species can be described as liver > gills > muscle. Kruskal – Wallis test was performed to the whole data to observe differences among locations, species and tissues ($p < 0.05$). All metals showed significant differences between tissues. Besides, Hg concentrations were showed significances between locations and species. On the other hand, Pb was showed statistical differences only for species ($p < 0.05$). Fish physiologies and behaviours, life cycle, and feeding habits affect variations on metal bioaccumulations such as Hg and Pb (Vetsis et al., 2021; Kontas et al., 2022). Fish individuals tend to store and magnify metals in the fatty organs such as liver (Omar et al., 2014). Previous studies also suggest that main source for the increased concentrations of heavy metals such as Hg and Cd is through the feeding (benthic organisms) for fish species (Cretì et al., 2010; Pan et al., 2022). Liver is the most efficient organ for detoxification and liver include many enzymes related to metals like metallothioneins. Therefore, liver contains high levels of metals (Cretì et al., 2010; Pan et al., 2022). In addition, metal ions were firstly deposited at gills through the passive diffusion, therefore higher concentrations of gills can be related with this natural phenomenon (da Silva et al., 2021). Increased levels of metal bioaccumulation at livers, gills, and muscle tissues of fish species are under the influenced of feeding habits, lipid contents, swimming activity, and, physiology (Vetsis et al., 2021). Similar profiles of minimum concentrations in *S. aurata* and *D. vulgaris* could be related with they are both member of Sparidae family (Da Palma, 2002).

To compare between species in Foça, the maximum concentrations of Hg and Zn were found at *S. aurata*; maximum concentrations of Cr, Cu, and Mn were detected at *C. labrossus* and the maximum Cd and Pb concentrations were determined at *D. vulgaris* (Table 2). Two-way ANOVA tests were performed to distinguish significances between tissues and species for metal levels and all variables were showed significant differences ($p < 0.05$). Previous studies claim that variations of metal bioaccumulation in different fish species may be related both exposure levels of metals, biochemical activities, reproduction and also ingestion, filtration and detoxification capabilities (Islam et al., 2015; Luoma and Rainbow, 2008; Wang and Rainbow, 2008).

Data of *C. labrosus* were evaluated from two locations. The maximum levels of Pb, Zn and Mn were found at Foça, however, maximum levels of Hg, Cd, Cr, and Cu were detected at İnciraltı (Table 2). Kruskal – Wallis tests were performed to detect significances between locations, but no statistical differences were observed ($p < 0.05$). It is well known that, inorganic (metal) pollutants that originated from natural and anthropogenic resources through the inputs such as atmospheric depositions, sewage, industrial and urban discharges (Zhang et al., 2015). İzmir Bay is heavily affected from municipal, agricultural, mining, and industrial sources where transport along with Gediz River that cause elevated concentrations of Hg, Pb and Cr (Uluturhan Suzer et al., 2015).

Biomarker analyses

Results of SOD, CAT, and MDA were given at Table 3. According to the biomarker results, *S. aurata* and *C. labrosus* generally showed similar profile. The maximum SOD and CAT activities were determined at gills, while maximum MDA contents were found at liver tissues. On the other hand, minimum levels of all biomarkers were detected at muscle tissues. For *D. vulgaris*, the highest levels of biomarker were obtained at livers, however, the lowest SOD and CAT activities were found at gills and the lowest MDA contents were found at muscle tissues (Table 3). The reason of higher concentrations on livers and gills than the muscle; liver tissues responded more for the antioxidant enzymes (SOD and CAT) and gills responded to biomarker of effects (MDA) (Gusso-Choueri et al., 2015) In addition, gills have thinnest epithelium and pollutants could be exposed through the respiration. Also, liver can concentrate metals and other pollutants to the considerable degree (Farombi et al., 2007). Kruskal – Wallis tests showed that SOD activities were reflected the significant differences for locations, besides, CAT activities and MDA contents were exhibited significant differences for tissues ($p < 0.05$). Higher activities of biomarkers were having connections with the exposure of elevated concentrations of metals and/or other pollutants (Beg et al., 2015; He et al., 2012). In our results, CAT and MDA levels were generally followed close patterns.

Table 2. Maximum and minimum metal concentrations in organs of selected fish species from inner and outer bays of Izmir Bay (mg/kg dry weight)

	Hg	Cd	Pb	Cr	Cu	Zn	Mn
S. aurata							
Foça (Outer Bay)							
Muscle	0.67-0.68	0.0008-0.0010	0.28-0.37	0.16-0.21	0.69-0.91	16.6-17.2	0.50-0.81
Liver	0.437-0.438	1.34-1.41	3.61-3.98	0.49-0.58	77.5-79.6	367-368	12.9-13.3
Gills	0.147-0.149	0.0036-0.0039	2.18-.2.21	1.18-1.30	2.83-2.99	86.4-91.8	26.1-26.7
C. labrosus							
Foça (Outer Bay)							
Muscle	0.25-0.27	0.001-0.002	0.24-0.33	0.12-0.69	0.55-0.66	12.4-14.7	0.18-0.20
Liver	0.056-0.058	3.19-3.66	2.17-2.30	1.15-1.44	567-568	162-165	3.20-3.24
Gills	0.056-0.064	0.003-0.004	3.55-4.28	0.50-0.53	2.62-2.84	56.3-61.9	31.0-33.2
İnciraltı (Inner Bay)							
Muscle	0.01-0.03	0.001-0.002	0.20-0.34	0.23-0.35	0.95-1.72	11.5-19.0	0.48-0.88
Liver	0.22-0.51	2.40-4.51	1.59-1.89	0.52-1.50	56.1-775	100-180	3.78-4.36
Gills	0.02-0.03	0.0038-0.0040	2.56-2.71	0.49-0.52	2.54-6.79	54.4-59.8	23.4-30.0
D. vulgaris							
Foça (Outer Bay)							
Muscle	0.57-0.60	0.0021-0.0023	1.00-1.16	0.11-0.12	1.34-1.63	18.4-18.7	1.09-1.25
Liver	0.20-0.22	3.30-3.82	9.78-10.3	0.43-0.58	40.9-41.3	165.5-165.9	9.05-9.36
Gills	0.11-0.12	0.0040-0.0042	16.2-16.5	0.70-0.75	2.44-3.13	84.5-91.7	24.2-25.3

This situation suggests that production of extensive amounts of H_2O_2 that cause oxidative stress. In that case, CAT activities could not able to sufficiently eliminate hydrogen peroxides, which provides lipid peroxidation products such as MDA (Vlahogianni et al., 2007).

Foça results showed that the highest biomarker levels were recorded at *C. labrosus*. The lowest activities of SOD and CAT were found at *S. aurata*, while the lowest MDA contents were detected at *D. vulgaris* (Table 3). Moreover, ANOVA tests were performed to test statistical variations between species significant differences were detected for all variables ($p < 0.05$).

Higher levels of biomarkers on *C. labrosus* could be linked their bioaccumulation of pollutants by their feeding habit. It is widely known that these sampled species can feed from bottom such as benthic organisms and sedimented detritus, also, this species has ability of feeding small organisms and suspended materials (Osman and Mahmoud, 2009; Hadj Taieb et al., 2013; Abumourad et al., 2014).The individuals of *C. labrosus* could be confronted floating or suspended pollutants such as oil spill and garbage, microplastics etc. (Bilbao et al., 2010; Chacón Aranda et al., 2021). To assess *C. labrosus* results between locations, SOD and CAT activities in Foça were higher than İnciraltı. MDA results were not able to distinguish locations. ANOVA tests also confirm these situations; SOD and CAT activities had significant differences; however, no significant differences were observed for MDA. Higher antioxidant enzyme activities could be related greater accumulations of metals. In conclusion, our results could confirm that, variations of MDA and metal levels were occurred parallel trends with each other. Marine organisms like fish species elevated enzyme activity levels may be triggered due to SOD's scavenging capacity and CAT removes H_2O_2 (Kumar et al., 2021).

Inter-relationship between metal concentrations and biomarker levels

According to the Pearson Product-Moment Correlation tests, especially MDA levels were showed significant correlations between all metals (Figure 2a). MDA contents were provided positive moderate correlations with Cd (0.509), Pb (0.476), Cr (0.589), Zn (0.627), and Mn (0.589). Also, MDA-Cu relationship were calculated as 0.394 ($p < 0.05$). Besides these, MDA levels were showed negative moderate correlations with Hg (-0.493) ($p < 0.05$). Also, Network Analysis confirmed that MDA contents have strong interaction between metals (Figure 2b).

Table 3. Maximum, minimum and (mean ± SD) levels biomarker levels in organs of selected fish species from inner and outer bays of İzmir Bay.

	CAT (mU/mg protein)	SOD (mU/mg protein)	MDA (pmol MDA/ mg tissue)
S. aurata			
Foça (Outer Bay)			
Muscle	1.89-2.88 (2.24±0.56)	36.8-37.7 (37.2±0.51)	9.11-11.0 (10.3±1.05)
Liver	1.53-2.89 (2.08±0.72)	22.3-31.3 (28.2±5.07)	26.9-31.0 (29.6±2.39)
Gills	3.80-7.60 (5.77±1.90)	69.4-90.7 (77.5±11.6)	21.7-23.2 (22.7±0.85)
C. labrosus			
Foça (Outer Bay)			
Muscle	2.47-17.3 (7.84±8.18)	23.2-27.0 (25.2±1.91)	9.97-11.3 (10.4±0.74)
Liver	4.74-7.51 (6.39±1.46)	29.5-36.9 (32.5±3.85)	31.9-33.1 (32.3±0.69)
Gills	14.9-22.4 (17.6±4.19)	111-131 (124±10.9)	28.7-30.1 (29.3±0.75)
İnciraltı (Inner Bay)			
Muscle	1.90-2.70 (2.36±0.41)	17.8-22.7 (20.5±2.47)	9.44-11.6 (10.5±1.07)
Liver	1.76-4.53 (3.01±1.41)	36.6-40.3 (38.6±1.83)	20.9-28.2 (25.0±3.76)
Gills	2.90-4.78 (3.79±0.94)	21.0-24.2 (21.3±2.73)	25.4-33.2 (30.4±4.33)
D. vulgaris			
Foça (Outer Bay)			
Muscle	2.56-3.12 (2.85±0.28)	28.1-30.6 (29.5±1.25)	8.32-8.49 (8.43±0.10)
Liver	4.70-6.27 (5.69±0.86)	48.7-51.0 49.6±1.19)	30.2-30.6 (30.4±0.22)
Gills	2.23-3.97 (3.26±0.91)	26.9-29.0 (28.1±1.08)	26.3-27.6 (26.9±0.66)

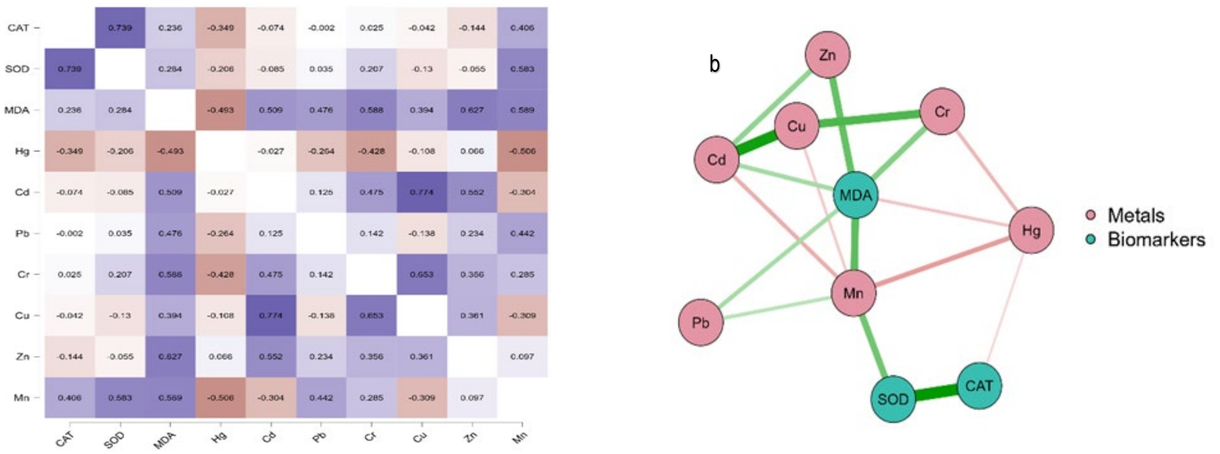

Figure 2. **a)** Heat map **b)** Network Analysis of combined correlations between biomarkers and metals

Previous studies also describe those levels of MDA contents had a strong link to essential and non-essential metals (Beg et al., 2015; Nesto et al., 2007). Lipid degradation by ROS starts a chain reaction which results MDA as products. Therefore, MDA is a vital compound to diagnose oxidative stress (Mishra, 2022). In addition, Mn concentrations were exhibited positive moderate correlations with SOD (0.583) and CAT (0.406) (p < 0.05). Hence one of the main components (as co-factor) of

SOD and having directly involved to ROS production mechanism, (Roche and Bogé, 1993) Mn and antioxidant biomarkers relationships were obviously revealed in this study.

CONCLUSION

Integrated approach was processed to evaluate metal bioaccumulation and antioxidant enzymes-oriented biomarkers on fish species from İzmir Bay where heavily affected from natural and anthropogenic pollutions. Samplings were carried out at Inner (İnciraltı) and Outer (Foça) regions of İzmir Bay. Results of metal analyses showed that both sides of İzmir Bay should be monitored regularly. According to the statistics, significant differences were mainly detected at tissues. Simultaneous investigations between metals and biomarkers were demonstrated that especially MDA contents are directly linked to metal bioaccumulation. In addition, Mn concentration were showed significant correlations for all biomarkers as expected. This study represented that pollutant dependent biomonitoring studies, it is important to investigate combined utilization of biomarkers and metal concentrations in order to determine the effects on organisms.

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AUTHORSHIP CONTRIBUTIONS

Mustafa Bilgin: Literature, conceptualization, manuscript writing, field research, laboratory experiments, data analysis. Esin Uluturhan-Suzer: Supervision, conceptualization, laboratory experiments, reviewing and editing. Enis darilmaz: Field research, laboratory experiments, reviewing and editing.

CONFLICT OF INTEREST

Authors would like to declare that no financial or personal conflict of interests in this study entitled “Preliminary studies of integration between metal bioaccumulation and antioxidant enzymes related biomarkers on fish species from İzmir Bay”.

ETHICS APPROVAL

In order for the experimental design to be carried out on a legal basis, the approval dated 03.05.2019 and numbered 13/2019 was obtained from the Multidiscipline Laboratory Animal Experiments Local Ethics Committee of Dokuz Eylül University.

DATA AVAILABILITY

Further questions about datasets, the corresponding author should be contacted.

REFERENCES

Abumourad, I., Bizarro, C., Aragón, P., Maquieira, Á., Vallejo, A., Zuloaga, O., de Alda, M.L., Josep, B., Barceló, D., Cagaraville, M., & Ortiz-Zarragoitia, M. (2014). Hazards of the endocrine disruptors on mullets (*Chelon labrosus*) from the basque coast (Bay of Biscay) applying a biomarker based approach. *Cytologia*, *79*(4), 517–533. DOI: 10.1508/cytologia.79.517

Aebi, H. (1984). [13] Catalase in Vitro. *Methods in Enzymology*, *105*(C), 121–126. DOI: 10.1016/S0076-6879(84)05016-3

Bauchot, M.L., & Hureau, J.C. (1990). Sparidae. 790-812pp. *Check-List of the Fishes of the Eastern Tropical Atlantic (CLOFETA)*, *2*.

Beg, M.U., Al-Jandal, N., Al-Subiai, S., Karam, Q., Husain, S., Butt, S.A., Ali, A., Al-Hasan, E., Al-Dufaileej, S., & Al-Husaini, M. (2015). Metallothionein, oxidative stress and trace metals in gills and liver of demersal and pelagic fish species from Kuwaits' marine area. *Marine Pollution Bulletin*, *100*(2), 662–672. DOI: 10.1016/j.marpolbul.2015.07.058

Bilbao, E., Raingeard, D., de Cerio, O. D., Ortiz-Zarragoitia, M., Ruiz, P., Izagirre, U., Orbea, A., Marigómez, I., Cajaraville, M. P., & Cancio, I. (2010). Effects of exposure to Prestige-like heavy fuel oil and to perfluorooctane sulfonate on conventional biomarkers and target gene transcription in the thicklip grey mullet *Chelon labrosus*. *Aquatic Toxicology*, *98*(3), 282–296. DOI: 10.1016/j.aquatox.2010.02.018

Briffa, J., Sinagra, E., & Blundell, R. (2020). Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon*, *6*(9), e04691.

Cajaraville, M. P., Bebianno, M. J., Blasco, J., Porte, C., Sarasquete, C., & Viarengo, A. (2000). The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: A practical approach. *Science of the Total Environment*, *247*(2–3), 295–311. DOI: 10.1016/S0048-9697(99)00499-4

Çakal Arslan, Ö., Parlak, H., Katalay, S., Boyacioglu, M., Karaaslan, M.A., & Guner, H. (2010). Detecting micronuclei frequency in some aquatic organisms for monitoring pollution of Izmir Bay (Western Turkey). *Environmental Monitoring and Assessment*, *165*(1–4), 55–66. DOI: 10.1007/s10661-009-0926-5

Cengiz, Ö., Paruğ, Ş.Ş., & Kızılkaya, B. (2019). Maximum length record of common two-banded seabream (*Diplodus vulgaris* Geoffroy Saint-Hilaire, 1817) for Aegean Sea with Turkish Waters. *Alınteri Zirai Bilimler Dergisi*, *34*, 160–163. DOI: 10.28955/alinterizbd.638974

Chacón Aranda, M.F., Fanarraga Tasayco, G.B., Castañeda Olivera, C.A., Benites Alfaro, E.G., & Acosta Suasnabar, E.H. (2021). Microplastics in water and their influence in the species *Chelon labrosus*. *Proceedings of the LACCEI International Multi-Conference for Engineering, Education and Technology*, *2021-July*, 19–24. DOI: 10.18687/LACCEI2021.1.1.233

Cretì, P., Trinchella, F., & Scudiero, R. (2010). Heavy metal bioaccumulation and metallothionein content in tissues of the sea bream *Sparus aurata* from three different fish farming systems. *Environmental Monitoring and Assessment*, *165*(1–4), 321–329. DOI: 10.1007/s10661-009-0948-z

Da Palma, J. A. M. (2002). Morphological variation in eight *Sparidae* species of the Eastern Atlantic and Mediterranean Sea. *Academy of Management Journal*. 226 p. Portugal.

da Silva, C.A., Garcia, C.A.B., de Santana, H.L.P., de Pontes, G.C., Wasserman, J.C., & da Costa, S.S.L. (2021). Metal and metalloid concentrations in marine fish marketed in Salvador, BA, northeastern Brazil, and associated human health risks. *Regional Studies in Marine Science*, *43*, 101716. DOI: 10.1016/j.rsma.2021.101716

Di Giulio, R.T., Washburn, P.C., Wenning, R.J., Winston, G.W., & Jewell, C.S. (1989). Biochemical responses in aquatic animals: a review of determinants of oxidative stress. *Environmental Toxicology and Chemistry*, *8*, 1103–1123.

Farombi, E.O., Adelowo, O.A., & Ajimoko, Y.R. (2007). Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogun River. *International Journal of Environmental Research and Public Health*, *4*(2), 158–165. DOI: 10.3390/ijerph2007040011

Gusso-Choueri, P.K., Choueri, R.B., de Araújo, G.S., Cruz, A.C.F., Stremel, T., Campos, S., Abessa, D.m.S., & Ribeiro, C.A.O. (2015). Assessing pollution in marine protected areas: the role of a multi-biomarker and multi-organ approach. *Environmental Science and Pollution Research*, *22*(22), 18047–18065. DOI: 10.1007/s11356-015-4911-y

Hadj Taieb, A., Sley, A., Ghorbel, M., & Jarboui, O. (2013). Feeding habits of *Sparus aurata* (Sparidae) from the Gulf of Gabes (central Mediterranean). *Cahiers de Biologie Marine*, *54*(2), 263–270.

He, X., Nie, X., Yang, Y., Liu, X., Pan, D., Cheng, Z., & Liang, X. (2012). Multi-biomarker responses in fishes from two typical marine aquaculture regions of South China. *Marine Pollution Bulletin*, *64*(11), 2317–2324. DOI: 10.1016/j.marpolbul.2012.09.002

Islam, M.S., Ahmed, M. K., Raknuzzaman, M., Habibullah-Al-Mamun, M., & Masunaga, S. (2015). Metal Speciation in Sediment and Their Bioaccumulation in Fish Species of Three Urban Rivers in Bangladesh. *Archives of Environmental Contamination and Toxicology*, *68*(1), 92–106. DOI: 10.1007/s00244-014-0079-6

Kacar, A., Kocyigit, A. & Uluturhan, E. (2016) Concentration Of Heavy Metals and Investigation of Bacterial Toxic Metal Resistance in Costal City Sediments (Eastern Aegean Sea) *Fresenius Environmental Bulletin, 25*(1), 55-66.

Kontas, A., Alyuruk, H., Bilgin, M., Uluturhan, E., Ünlüoğlu, A., Darilmaz, E., & Altay, O. (2022). Metal Bioaccumulation and Potential Health Risk Assessment in Different Tissues of Three Commercial Fish Species (*Merluccius merluccius*, *Mullus barbatus*, and *Pagellus erythrinus*) from Edremit Bay (Aegean Sea), Turkey. *Biological Trace Element Research*, *200*(2), 868-880.

Kucuksezgin, F., Kontas, A., Altay, O., Uluturhan, E., & Darılmaz, E. (2006). Assessment of marine pollution in Izmir Bay: Nutrient, heavy metal and total hydrocarbon concentrations. *Environment international*, *32*(1), 41-51.

Kucuksezgin,F., Kontas, A., & Uluturhan, E. (2011) Evaluations of heavy metal pollution in sediment and *Mullus barbatus* from the Izmir Bay (Eastern Aegean) during 1997–2009. *Marine Pollution Bulletin*, *62*, (7), 1562-2011. DOI: 10.1016/j.marpolbul.2011.05.012

Kumar, N., Bhushan, S., Gupta, S.K., Kumar, P., Chandan, N.K., Singh, D.K., & Kumar, P. (2021). Metal determination and biochemical status of marine fishes facilitate the biomonitoring of marine pollution. *Marine Pollution Bulletin*, *170*(June), 112682. DOI: 10.1016/j.marpolbul.2021.112682

Lam, P.K.S. (2009). Use of biomarkers in environmental monitoring. *Ocean and Coastal Management*, *52*(7), 348–354. DOI: 10.1016/j.ocecoaman.2009.04.010

Lam, P.K.S., & Gray, J.S. (2003). The use of biomarkers in environmental monitoring programmes. *Marine Pollution Bulletin*, *46*(2), 182–186. DOI: 10.1016/S0025-326X(02)00449-6

Lee, R.F., & Steinert, S. (2003). Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. *Mutation Research - Reviews in Mutation Research*, *544*(1), 43–64. DOI: 10.1016/S1383-5742(03)00017-6

Lesser, M.P. (2006). Oxidative stress in marine environments: Biochemistry and physiological ecology. *Annual Review of Physiology*, *68*(3), 253–278. DOI: 10.1146/annurev.physiol.68.040104.110001

Luoma, S.N., Rainbow, P.S., (2008). *Metal contamination in aquatic environments: science and lateral management.* Cambridge university press.

Markovitch, D. (2005). *Oxidative Stress-Induced Expression of Haem Oxygenase-1 (HO-1) in Human Mononuclear Cells in vivo and ex vivo and the Impact of Lycopene Supplementation*. Doctoral dissertation, University of Bath (United Kingdom).

Mishra, D.B. (2022). Environmental pollution biomarker: Fish antioxidant défense system alterations. *Eurasian Journal of Research, Development and Innovation*, *7*, 38-46.

Nesto, N., Romano, S., Moschino, V., Mauri, M., & Da Ros, L. (2007). Bioaccumulation and biomarker responses of trace metals and micro-organic pollutants in mussels and fish from the Lagoon of Venice, Italy. *Marine Pollution Bulletin*, *55*(10–12), 469–484. DOI: 10.1016/j.marpolbul.2007.09.009

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*(2), 351–358. DOI: 10.1016/0003-2697(79)90738-3

Omar, W.A., Saleh, Y.S., & Marie, M.A.S. (2014). Integrating multiple fish biomarkers and risk assessment as indicators of metal pollution along the Red Sea coast of Hodeida, Yemen Republic. *Ecotoxicology and Environmental Safety*, *110*, 221–231. DOI: 10.1016/j.ecoenv.2014.09.004

Osman, A.M., & Mahmoud, H.H. (2009). Feeding Biology of *Diplodus sargus* and *Diplodus vulgaris* (Teleostei, Sparidae) in Egyptian Mediterranean Waters. *World Journal of Fish and Marine Sciences*, *1*(4), 290–296.

Pan, B., Wang, Y., Li, D., Wang, T., & Du, L. (2022). Tissue-specific distribution and bioaccumulation pattern of trace metals in fish species from the heavily sediment-laden Yellow River, China. *Journal of Hazardous Materials*, *425*(August 2021), 128050. DOI: 10.1016/j.jhazmat.2021.128050

Parrino, V., de Marco, G., Minutoli, R., Lo Paro, G., Giannetto, A., Cappello, T., De Plano, L. M., Cecchini, S., & Fazio, F. (2021). Effects of pesticides on *Chelon labrosus* (Risso, 1827) evaluated by enzymatic activities along the north eastern Sicilian coastlines (Italy). *European Zoological Journal*, *88*(1), 540–548. DOI: 10.1080/24750263.2021.1905090

Pryor, W.A., & Stanley, J.P. (1975). A Suggested Mechanism for the Production of Malonaldehyde during the Autoxidation of Polyunsaturated Fatty Acids Nonenzymic Production of Prostaglandin Endoperoxides during Autoxidation. *Journal of Organic Chemistry*, *40*(24), 3615–3617. DOI: 10.1021/jo00912a038

Roche, H., & Bogé, G. (1993). Effects of Cu, Zn and Cr salts on antioxidant enzyme activities in vitro of red blood cells. *Toxic. in Vitro*, *7*(5), 623–629.

Romero, F.J., Bosch-Morell, F., Romero, M.J., Jareño, E.J., Romero, B., Marín, N., & Romá, J. (1998). Lipid peroxidation products and antioxidants in human disease. *Environmental Health Perspectives*, *106*(SUPPL. 5), 1229–1234. DOI: 10.1289/ehp.98106s51229

Schober, P., & Schwarte, L.A. (2018). Correlation coefficients: Appropriate use and interpretation. *Anesthesia and Analgesia*, *126*(5), 1763–1768. DOI: 10.1213/ANE.0000000000002864

Seginer, I. (2016). Growth models of gilthead sea bream (*Sparus aurata* L.) for aquaculture: A review. *Aquacultural Engineering*, *70*, 15–32. DOI: 10.1016/j.aquaeng.2015.12.001

Sun, Y.I., Oberley, L.W., & Li, Y. (1988). A simple method for clinical assay of superoxide dismutase. *Clinical chemistry*, *34*(3), 497-500.

Taş, E.Ç., Ergen, Z. & Sunlu, U. (2018). An Investigation on Cd, Cu, Zn, Pb, Cr, Fe Levels in *Diopatra neapolitana* (Delle Chiaje, 1841) and Sediments that Inhabit (in Turkish with Engish abstract). *Türk Tarım – Gıda Bilim ve Teknoloji Dergisi*, *6*(10): 1493-1500. DOI: 10.24925/turjaf.v6i10.1493-1500.2140

Taş, E.Ç., & Sunlu, U. (2019). Heavy Metal Concentrations in Razor Clam (*Solen marginatus*, Pulteney, 1799) and Sediments from Izmir Bay, Aegean Sea, Turkey. *Turkish Journal of Agriculture - Food Science and Technology*, *7*(2): 306-313.

Uluturhan Suzer, E., Kontaş, A., & Yılmaz, E. C. (2015). Assessment of heavy metal pollution of surface sediments from lagoon areas of Gediz Delta (Izmir Bay). *Ege Journal of Fisheries and Aquatic Sciences*, *32*(2), 79–87. DOI: 10.12714/egejfas.2015.32.2.04

UNEP/FAO/IAEA/IOC. (1984). Determination of total mercury in selected marine organisms by cold vapour atomic absorption spectrophotometry. *Reference Methods for the Marine Pollution Studies, No. 8, Rev. 1.*

UNEP. (1990). Contaminant monitoring programmes using marine organisms: Quality assurance and good laboratory practice. *Reference Methods For Marine Pollution Studies*, *57*(4), 467–475.

Vetsis, E., Kalantzi, I., Pergantis, S.A., Kokokiris, L., & Karakassis, I. (2021). Metals in tissues of marine fish from the Thermaikos Gulf, Eastern Mediterranean Sea: Detection of changes with trophic level. *Marine Pollution Bulletin*, *173*(PA), 113024. DOI: 10.1016/j.marpolbul.2021.113024

Viarengo, A., Lowe, D., Bolognesi, C., Fabbri, E., & Koehler, A. (2007). The use of biomarkers in biomonitoring: A 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, *146*(3), 281–300. DOI: 10.1016/j.cbpc.2007.04.011

Vlahogianni, T., Dassenakis, M., Scoullos, M.J., & Valavanidis, A. (2007). Integrated use of biomarkers (superoxide dismutase, catalase and lipid peroxidation) in mussels *Mytilus galloprovincialis* for assessing heavy metals' pollution in coastal areas from the Saronikos Gulf of Greece. *Marine Pollution Bulletin*, *54*(9), 1361–1371. DOI: 10.1016/j.marpolbul.2007.05.018

Wang, W.X., & Rainbow, P.S. (2008). Comparative approaches to understand metal bioaccumulation in aquatic animals. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, *148*(4), 315–323. DOI: 10.1016/j.cbpc.2008.04.003

Zhang, L., Shi, Z., Jiang, Z., Zhang, J., Wang, F., & Huang, X. (2015). Distribution and bioaccumulation of heavy metals in marine organisms in east and west Guangdong coastal regions, South China. *Marine Pollution Bulletin*, *101*(2), 930–937. DOI: 10.1016/j.marpolbul.2015.10.041