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Metal accumulation in economically important fish species from Gökova Bay, Türkiye: approaches of statistical modeling and risk assessment for consumer's health

by

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Abstract

The concentrations of eight metals (Cu, Fe, Mn, Zn, Cd, Pb, Se, and Hg) in the tissues (liver, gill, and muscle) of *Dicentrarchus labrax* and *Saurida undosquamis* living in Gökova Bay and their relationship with health risk indexes (target hazard quotient [THQ], total target hazard quotient [TTHQ], estimated daily intake [EDI], and carcinogenic risk [CR]) and condition factor (CF) were determined. The calculated EDI for metals shows a low value for adults in comparison with the recommended intake limit, but for children the values for Cd and Mn in both fish were above the recommended values. Based on the metal analyses, the THQ and TTHQ for fish were calculated to be <1 for both adults and children. CR for Cd and Pb were determined at negligible levels. In addition, metal accumulations were modeled by random forest regression using season, tissue type, THQ weight, and fish length as covariates. The models explained about 55%–93% of the variation in metal concentrations and had high correlations between the observed and predicted values (mostly above 0.80), so they can be used as a practical tool to predict tissue-specific metal levels in similar studies.

Key words: Metal pollution, *Dicentrarchus labrax*, *Saurida undosquamis*, Random Forest

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1. Introduction

Fish is almost the only source of dietary quality protein for humans, providing 16% of the world's animal protein consumption (Tidwell & Allan, 2002). They contain beneficial polyunsaturated fatty acids and are low in cholesterol (Ersoy & Çelik, 2010). A major concern, however, is the level of contaminants in fish. Heavy metal discharges into the marine environment are a major concern worldwide and are of great ecological worry due to their toxicity to marine species, especially fish, which are consumed in large quantities (Genç, 2021). It is also a major source of toxins that are harmful to human health, including infertility, liver disease, skin and bladder cancers, and fatalities (Wei et al., 2014). Due to their important role in biological systems, metals such as Fe, Cu, Mn, Se, and Zn are considered as essential metals. Essential metals can also have toxic effects when the metal intake is too high. Hg, Pb, and Cd are not considered as essential metals because they are toxic even in trace amounts. This has the potential to harm both marine biodiversity and the ecosystem (Ahmed et al., 2020; Kontaş & Bostancı, 2020). Therefore, the determination of metal concentrations in fish and the assessment of possible risks to human health after fish consumption are important. Unsurprisingly, many previous studies have focused on assessment of the potential health risks associated with metal accumulation in various fish species (Bošković et al., 2023; Zhou et al., 2024). Brush-tooth lizardfish (*S. undosquamis*) is a commercially interesting coastal demersal species in the Eastern Mediterranean. It is one of the Lessepsian species that invaded the Mediterranean via the Suez Canal, first reported in Turkish Seas by Kosswig (1951). In addition, studies on *S. undosquamis* have been reported more in the eastern Mediterranean region of Türkiye (Çiçek et al., 2008; Türkmen et al., 2005). *S. undosquamis* is an important species preferred by the local people for consumption due to its high catch and cheap price in recent years. European sea bass (*D. labrax*), which has a very tasty and high-quality meat, is among the most preferred fish by the public. Sea bass production in Muğla reached 65.750 tonnes in 2020 (Yıldırım & Çantaş, 2021). This represents approximately 54% of Türkiye's total sea bass production (TURKSTAT, 2013). In this study, European sea bass were caught from the sea by seine not aquaculture. The habit of eating European sea bass in the Gökova region is higher than in many other regions of Türkiye. European sea bass can always be found in the fish stalls of Muğla, a fishing city. This is the first time that metal accumulation is reported for *S. undosquamis* and *D. labrax* living in the Aegean Sea. From a public health perspective, this study could provide consumers with better information on metal contamination issues of these economic species. Furthermore, possible relationships between concentrations and seasons were also researched. Additionally, this paper considers the statistical modeling of the accumulations for each metal by random forest regression. The inclusion of modeling in this research offers several benefits. First, it enhances predictive accuracy by leveraging the complex interactions between multiple covariates such as season, tissue type, and fish characteristics. This improved accuracy may provide benchmark scores for similar studies in the future, aiding in the standardization and comparability of results across research efforts. Additionally, the model's ability to efficiently handle non-linear relationships may lessen the laboratory burden at least for similar research by streamlining data analysis processes, thereby allowing researchers to focus resources more effectively on experimental design and data collection. Therefore, the objectives of this study are to: (1) provide a better understanding of the cause–effect relationships regarding metal accumulation in the tissues (liver, gill, and muscle) of some target species inhabiting Gökova Bay: *D. labrax* and *Saurida undosquamis*; (2) quantify the relationship between metal bioaccumulation and well-being (condition) of the fish; (3) estimate health risk of metals via consumption of fish; (4) model the metal accumulations for each metal by random forest regression using variables such as season, tissue type, target hazard quotient (THQ), fish length and weight; and (5) evaluate the performance and variable importance results of these models to see which factors have more effect on metal accumulation and to support future studies.

2. Materials and methods

2.1. Study area

Gökova Bay (Fig. 1) is one of Türkiye's largest and most productive gulfs, located at the intersection of the Aegean and Mediterranean Seas (37° 01′ 25.92″ N 28° 15′ 04.58″ E). Gökova Gulf is in the Aegean Sea within the borders of Muğla province; its sea area is 1851 km^2 and its entire coastal length is approximately 500 km (Kiraç, 2010). Gökova Bay is rich in biodiversity and is one of the most important

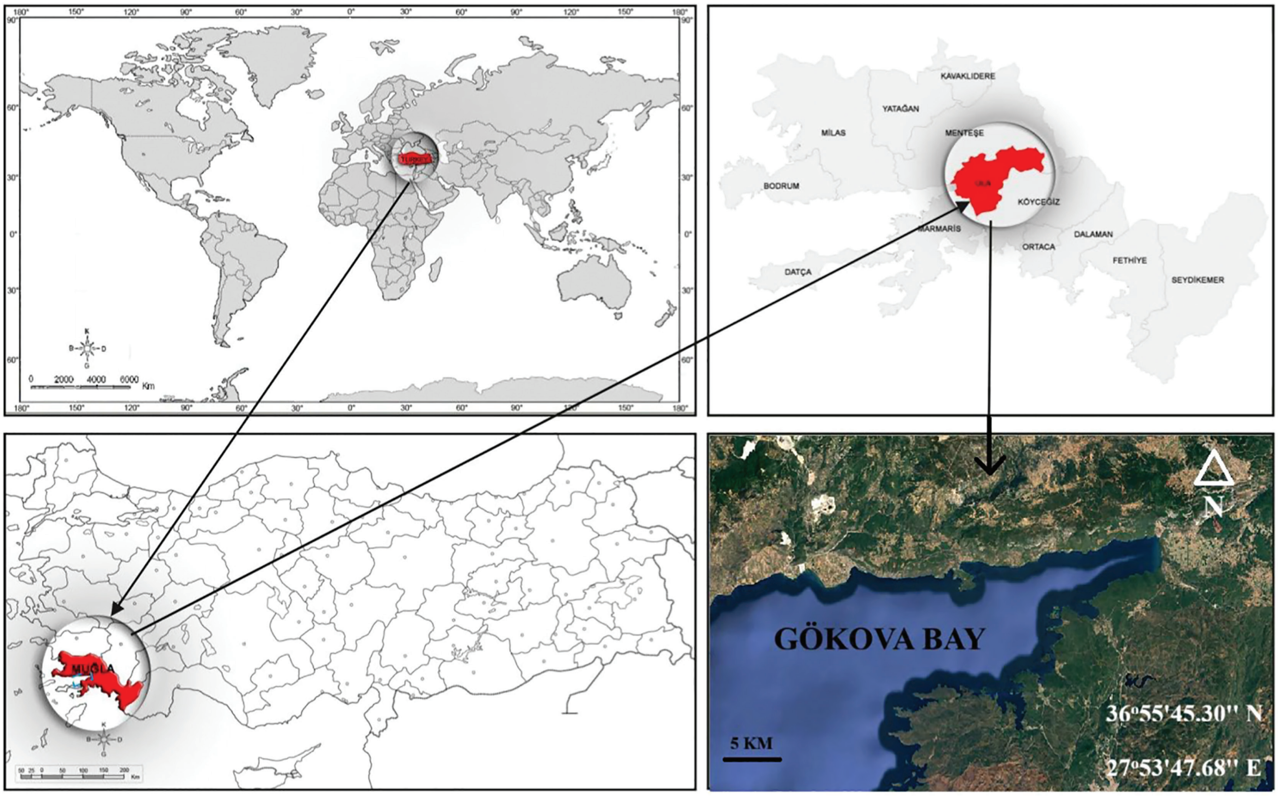

Figure 1
Gökova Bay.

marine protected areas in Türkiye (Cihangir et al., 1998). Small-scale fishing is common because trawling and purse seine fishing are prohibited in much of the Gulf (Ayaz et al., 2010). The Gulf was also affected by Indo-Pacific species that cross the Suez Canal into the Mediterranean. The Gulf has become an important center of Lesepsian migration in the past 20 years (Çoker & Akyol, 2014).

2.2. Sample collection

Two fish species (sea bass and brush-tooth lizardfish) were randomly collected by Akyaka Aquaculture Cooperative in the Gökova Bay. A total of 80 samples (40 of each species) were collected. Ten Sea bass samples were obtained in each season between May 2022 and February 2023, while 10 brush-tooth lizardfish samples were obtained in each season between September 2022 and August 2023. Fishermen working in the cooperative used seines to collect fish samples. The caught fish were washed in distilled water and then properly iced and packed in labeled plastic bags. Finally, the packed samples were taken into the Hydrobiology Laboratory of Muğla Sıtkı Koçman University.

2.3. Analysis of metals

2.3.1. Sample preparation

The length (standard length [SL] ± 1 mm) and weight (total weight [TW] ± 0.001 g) of the individual fish of both species were measured. Deionized water (resistivity: 18.0 MΩ cm) was used for all fish samples prior to dissection. Liver, gills, and muscles of the fish were dissected. Approximately 1.0 g wet of each tissue sample was placed in Teflon containers containing 7.0 mL HNO_3 (65% Suprapur, Merck, Darmstadt, Germany) and 3.0 mL H_2O_2 (30% Suprapur, Merck). The tissues were digested using a microwave system (Berghof Speedwave MWS-3, Germany) as described by Genç et al. (2015).

2.3.2. Reagents and detection system

All solutions utilized in the experiments were of analytical grade and were provided by Merck. Ultrapure water from the Milli-Q Plus water purification system (Millipore, Bedford, MA, USA, 18.2 MΩ cm) was used for the preparation and dilution of the solutions. The employed standard solutions of Cu(II), Fe(III), Mn(II), Cd(II), Zn(II), Pb(IV), Se(IV), and Hg(II) were prepared by diluting the relevant 1000 mg L^{-1} stock standard solutions. The determination of Fe concentration utilized a flame atomic absorption spectrometer (FAAS) (Agilent Technologies, 240FS AA) equipped with a döterium (D_2) background correction system. Detection of Cu, Mn, Cd, Zn, and Pb concentrations was conducted using an Agilent Technologies GTA 120 graphite furnace atomic absorption spectrometer (GFAAS), featuring a Zeeman background technique and PSD120 autosampler. Se and Hg concentrations were determined employing hydride generation atomic absorption spectrometry (HGAAS) and cold vapor absorption spectrometry (CVAAS) methods through a FAAS (Agilent Technologies, 240FS AA) equipped with a VGA 77 hydride generation accessory. Hollow cathode lamps were operated for Cu (327.4 nm and slit width 0.5 nm, lamp current 10.0 A), Fe (248.3 nm and slit width 0.2 nm, lamp current 10.0 A), Mn (279.5 nm and slit width 0.2 nm, lamp current 10.0 A), Cd (228.8 nm and slit width 0.5 nm, lamp current 4.0 A), Zn (213.9 nm and slit width 0.7 nm, lamp current 15.0 A), Pb (217.0 nm and slit width 1.0 nm, lamp current 10.0 A), Se (196.0 nm and slit width 1.0 nm, lamp current 10.0 A), and Hg (253.7 nm and slit width 0.5 nm, lamp current 4.0 A). In the FAAS method, an air–acetylene mixture was used as a flame source. In the GFAAS method, Argon gas was used to protect and purge the graphite tubes during the furnace program procedures and the absorbance measurements were based on the peak area. The peak height mode was selected for absorbance measurements of analyte signals in the FAAS and HGAAS methods.

2.3.3. Accuracy evaluation of the methods

The accuracy of the methods applied for the analysis of heavy metals in fish samples was evaluated by analyzing DOLT-5 (dogfish liver, Ottawa, ON, Canada) certified reference material (CRM). CRM was digested employing the same digestion procedure as for the fish samples. In the analysis of CRM, external calibration plots were generated for each metal and no standard addition procedure was needed. Three consecutive measurements were taken for each metal and the results are given as average values. The results obtained and the certified values demonstrated good agreement at the 95% confidence level. The recovery values of the metals analyzed were all quite well, ranging from 95.2% to 104.9%. The results are presented in Table 1.

2.3.4. Statistical analysis and modeling

The outcomes obtained from modeling the accumulation of specific metal quantities in tissues utilizing random forest regression, incorporating THQ, weight, tissue type, and seasonal variables, are presented below for Cu, Fe, Mn, Cd, Zn, Pb, Se, and Hg. Here, season and tissue variables are weighted before the modeling according to their quantities at each level. In ordinary linear case, the mathematical expression of the model under the linearity assumption, for example, Cu accumulation can be formulated as follows:

$$\begin{aligned} Cu_i &= \beta_0 + \beta_1 \text{weight}_i + \beta_2 \text{length}_i + \beta_3 \text{CuTHQ}_i \\ &+ \beta_4 \text{season}_i + \beta_5 \text{tissue}_i + \varepsilon_i, 1 \le i \le n \end{aligned} \quad (3.1)$$

Here, the Cu_i values are referred to as the response variable (dependent variable), while the variables on the right-hand side of the equation are termed as explanatory variables. Additionally, $\beta = (\beta_1, \ldots, \beta_5)^T$ denotes the regression coefficients, representing the effects of the explanatory variables on Cu_i in terms of both direction and magnitude. Finally, $\varepsilon_i \sim N(0, \sigma_\varepsilon^2)$

Table 1

Analysis results of the metals in the CRM.

Analyte	Certified value (mg · kg^{-1})	Found value (mg · kg^{-1})	Recovery (%)
Cu	35.0 ± 2.4	35.8 ± 1.3	102.3
Fe	1070 ± 80	1075 ± 28	100.5
Mn	8.91 ± 0.70	9.23 ± 0.54	100.6
Cd	14.5 ± 0.6	14.7 ± 0.4	103.6
Zn	105.3 ± 5.4	100.2 ± 3.6	95.2
Pb	0.162 ± 0.032	0.170 ± 0.018	104.9
Se	8.3 ± 1.8	8.6 ± 0.8	104.8
Hg	0.44 ± 0.18	0.42 ± 0.11	95.5

*Results are presented as the average value with the associated SD (n = 3).
CRM, certified reference material; SD, standard deviation.

signifies the random error terms of the model. Note that model (3.1) can be adapted similarly for other analytes in terms of both species *S. undosquamis* and *D. labrax*. The fundamental issue here lies in estimating the regression coefficients, for which conventional regression solutions exist. However, due to the non-linearity of the relationship between the Cu variable and the explanatory variables, the results obtained would be biased and unreliable. In this context, the random forest regression method, capable of describing the relationship between variables in a non-linear manner, has been employed. Also, to evaluate the performance of the models, some common metrics for random forest method are used that are root mean squared error (RMSE), % variance explanation, optimal number of trees, mean squared residuals, and correlation between the predicted and actual values of the analytes.

2.3.5. Pollution and health risk assessment

Several criteria/indices were used to calculate the risks to human health from the metals identified in fish species through their consumption: THQ, total target hazard quotient (TTHQ), estimated daily intake (EDI) of metals, and carcinogenic risk (CR). Table 2 provides summary information on these hazard parameters.

Based on data from the United States Environmental Protection Agency (USEPA), body weight and life expectancy for children were assumed to be 32 kg and 7 years, respectively (USEPA, 2008). According to the data of the Statistical Institute of the Republic of Türkiye, the average life expectancy is 77 years (TSI, 2013). RfD (mg · person^{-1} · day^{-1}) of metals, namely, Cu, Fe, Mn, Zn, Cd, Pb, Se, and Hg is 0.04, 0.7, 0.14, 0.3, 0.001, 0.004, 0.005, and 0.0003, respectively (USEPA, 2009).

3. Results and discussion

3.1. Bioaccumulation of metals in tissue of fish and condition factor

The weight/length ratio may be used to estimate the state of health of the fish, with the assumption that the larger the fish, the better the state of health. The

Table 2

Description of some of the indices that were used in the study of the present fish samples.

Indexes	Aim	Methods	Reference
Condition coefficients (CF)	Estimation of the condition of the fish by looking at the length–weight relationship	CF = $(W/TL^3) \times 100$ where *W* is the weight (g), TL is the total length (mm)	Bolger and Connolly (1989)
EDI	The EDI was evaluated based on the metal concentrations in the fish studied and their consumption characteristics	EDI (mg · day^{-1} · person^{-1}) = $C_{metal} \times W_{food}/Bw$ The variable C_{metal} (μg · g^{-1}, on fresh weight basis) is the concentration of heavy metals in contaminated fish; W_{food} represents the daily average consumption of fish in this region; and BW is the body weight	Griboff et al. (2017)
THQ	Assessment of the risk to human health from the consumption of fish contaminated with heavy metals	$THQ = \frac{EFr \times EDtot \times FIR \times C}{RfDo \times BWa \times ATn} \times 10^{-3}$; EFr is exposure frequency (365 days · year^{-1}); EDtot is the exposure duration (77 years for adults and 7 years for child, average lifetime); FIR is the food ingestion rate (18.6 g · day^{-1}); *C* is the heavy metal concentration in fish (mg · g^{-1}); RfDo is the oral reference dose (mg · kg^{-1} · day^{-1}), BWa is the average adult BW (70 kg for adults and 32 kg for child); and ATn is the averaging exposure time for non-carcinogens (365 days · year^{-1} number of exposure years, assuming 77 years). RfD (mg · person^{-1} · day^{-1}) of metals	TSI (2010, 2013); USEPA (2011)
TTHQ	To assess the overall potential health risk of multiple metals, each metal's THQ is summed and called the TTHQ	Total THQ (TTHQ) = THQ (toxicant 1) + THQ (toxicant 2) + THQ (toxicant 3) + ... + THQ (toxicant *n*)	Chien et al. (2002)
CR	CR is an individual's increased lifetime probability of developing cancer from exposure to a potential carcinogen	CR = CSF × EDI where, CSF is the carcinogenic slope factor of 0.0085 (mg · kg^{-1} · day^{-1}) for Pb and 1.7 (mg · kg^{-1} · day^{-1}) for Ni. Since Fe and Cu don't cause any carcinogenic effects as their CSF have yet not been established (USEPA, 2012)	USEPA (2000)

CF, condition factor; CR, carcinogenic risk, EDI, estimated daily intake, THQ, target hazard quotient, TTHQ, total target hazard quotient.

mean values of length, weight, and condition factor (CF) are shown in Table 3.

The maximum length of *D. labrax* was 380.07 mg in winter while the minimum weight was 230.12 mg in autumn. The maximum weight of *S. undosquamis* was 174.18 mg in spring, while the minimum weight was 39.79 mg in autumn. The average CF for *D. labrax* was approximately twice as high as for *S. undosquamis*. Mean concentrations and standard deviations of Cu, Fe, Mn, Zn, Cd, Pb, Se, and Hg from three tissues (muscle, gill, and liver) of two fish species during winter, spring, summer, and autumn are presented in Table 4.

Fe had the highest concentrations in all tissues of the investigated species. The second most common metal after Fe was Zn. In general, metal accumulation in the liver was found to be considerably higher than in the muscle and gill. All metals accumulated in *D. labrax* (except Cu accumulation in the gill in the autumn and summer) were found to be higher in the liver, while all metals were analyzed higher in the liver of *S. undosquamis*. Fe, Cd, and Hg accumulation in the liver tissue of *D. labrax* was found to be high in the summer, while Cu, Mn, and Pb accumulation was high in the autumn. Metal accumulation in gill (except Cd and Hg) and muscle (except Cd and Pb) of *D. labrax* was analyzed high in autumn. The average levels of metals in the gill of *S. undosquamis* (only Se was high in autumn) were found to be high during the summer. In line with expectations, the levels of essential metals were higher than the levels of toxic metals. The metal accumulation values obtained in this study were compared with the same species in nearby regions. Dural et al. (2006) determined seasonal accumulation of Fe, Zn, and Cd in tissues of *D. labrax* caught from Iskenderun Bay in 2001. They found that Cd accumulation in the liver was much higher than our mean Cd value, but in this study, the mean Cd value was 2 times higher in muscle. Although Zn accumulation was found to be very high in the muscle and gill in their studies, it was found to be higher in the liver in this study. The Fe accumulation analyzed in this study was significantly higher in all three tissues compared with their study. Duran et al. (2014) reported the accumulation of Cu, Pb, Co, Ni, Cr, Mn, Cd, and Fe in the muscle tissues of *D. labrax* that were supplied by retailers in Kayseri. Comparing the metals accumulated in the muscle of *D. labrax*, Cu, Fe, and Mn, known as essential elements, are higher in this study, while the toxic elements Pb and Cd are at very high levels in their study. Türkmen et al. (2011) examined the metal (Fe, Zn, Cu, Mn, Cr, Ni, Pb; Cd, and Co) concentrations in the muscle, liver, gonad, and gill of *D. labrax* from Paradeniz Lagoon, in the Mediterranean coastal area. Similar to other compared studies, metals known as essential (Fe, Zn, Cu and Mn) were found at higher levels in all three tissues in this study, while toxic metals (Pb and Cd) were analyzed at higher levels in their study. In their study conducted at Lake Bardawil (Egypt), Zaghloul et al. (2024) analyzed the levels of Cd, Pb, Cu, and Zn in the muscle and liver tissue of *D. labrax*. In comparison to the results of this study, they analyzed approximately 30 times more Pb accumulation in the liver and around 10 times more in muscle tissue. Cd showed similar accumulation in muscle the *D. labrax* analyzed in this study, while twice as much accumulation was detected in the liver tissue of the fish samples analyzed in this study during summer and autumn. The Cu accumulation analyzed in this study was found to be 10 times higher in the muscle tissue of *D. labrax* living in Lake Bardawil in all

Table 3

Length, weight, and CF in fish.

Species	Season	Average length (cm)	Min-Max length (cm)	Average weight (g)	Min-Max weight (g)	CF
D. labrax	Winter	32.18 ± 1.31	30.8–34.5	345.19 ± 20.16	319.05–380.07	1.04 ± 0.12
	Spring	31.25 ± 2.04	27.50–33.50	337.30 ± 36.01	284.33–377.11	1.13 ± 0.27
	Summer	28.04 ± 3.28	22.5–32	295.57 ± 26.51	252.89–325.41	1.40 ± 0.4
	Autumn	27.59 ± 2.29	23.70–31.5	275.84 ± 30.54	230.12–324.85	1.35 ± 0.31
	Mean	29.77 ± 3.02	22.50–34.50	313.48 ± 40.24	230.1–380.07	1.23 ± 0.32
S. undosquamis	Winter	26 ± 2.69	21–29	122.18 ± 36.81	49.59–165.69	0.67 ± 0.11
	Spring	25.25 ± 1.96	22.5–29	119.53 ± 32.9	78.25–174.18	0.73 ± 0.10
	Summer	24.11 ± 3.34	19.8–29.5	97.55 ± 38.64	46.35–162.39	0.66 ± 0.07
	Autumn	24.25 ± 2.95	19.5–29	87.11 ± 37.55	39.79–140.5	0.57 ± 0.08
	Mean	24.903 ± 2.8	19.50–29.50	106.6 ± 38.16	39.79–174.18	0.66 ± 0.11

CF, condition factor.

Table 4

Mean metal concentrations (mg · g^{-1} wet weight ± SD) in fish tissues from Gökova Bay and species comparison

		D. labrax			*S. undosquamis*		
		Muscle (*n* = 40)	**Gill (*n* = 40)**	**Liver (*n* = 40)**	**Muscle (*n* = 40)**	**Gill (*n* = 40)**	**Liver (*n* = 40)**
Cu	**1 (*n* = 10)**	2.712 ± 0.341	15.541 ± 1.791	66.53 ± 7.481	1.053 ± 0.175	3.520 ± 0.175	12.219 ± 0.896
	2 (*n* = 10)	2.452 ± 0.189	17.685 ± 1.322	73.736 ± 4.059	1.551 ± 0.188	5.385 ± 0.581	22.270 ± 2.627
	3 (*n* = 10)	2.144 ± 0.325	62.714 ± 9.295	11.285 ± 1.715	3.827 ± 0.285	12.066 ± 0.964	38.98 ± 2.693
	4 (*n* = 10)	2.550 ± 0.215	87.783 ± 4.170	14.876 ± 1.025	1.749 ± 0.215	6.951 ± 0.785	20.895 ± 2.136
Fe	**1 (*n* = 10)**	60.348 ± 6.196	376.47 ± 34. 064	633.675 ± 40.696	15.305 ± 1.273	86.202 ± 11.073	125.754 ± 9.536
	2 (*n* = 10)	60.802 ± 7.25	371.517 ± 41.689	605.858 ± 50.505	27.858 ± 3.438	170.896 ± 20.424	225.57 ± 28.44
	3 (*n* = 10)	83.29 ± 4.919	468.623 ± 31.02	697.461 ± 35.796	59.568 ± 6.785	353.878 ± 38.523	474.38 ± 54.56
	4 (*n* = 10)	86.215 ± 5.648	474. 849 ± 33.317	683.419 ± 22.297	20.512 ± 1.905	136.027 ± 12.614	174.715 ± 18.09
Mn	**1 (*n* = 10)**	3.136 ± 0.695	11.898 ± 2.584	17.572 ± 3.06	1.892 ± 0.116	10.802 ± 0.319	18.837 ± 0.486
	2 (*n* = 10)	3.552 ± 0.502	13.11 ± 1.719	25.019 ± 2.452	3.305 ± 0.187	13.711 ± 0.737	23.363 ± 0.952
	3 (*n* = 10)	5.68 ± 0.205	23.106 ± 0.523	44.639 ± 1.744	4.718 ± 0.299	19.541 ± 1.219	30.596 ± 1.735
	4 (*n* = 10)	6.50 ± 0.072	26.975 ± 0.628	51.156 ± 1.832	2.703 ± 0.229	12.571 ± 0.527	22.057 ± 0.989
Zn	**1 (*n* = 10)**	15.454 ± 2.042	29.678 ± 3.308	65.701 ± 5.815	3.781 ± 0.273	8.926 ± 0.559	20.169 ± 1.222
	2 (*n* = 10)	25.289 ± 3.618	50.069 ± 6.264	78.796 ± 7.839	5.468 ± 0.696	16.918 ± 1.963	59.274 ± 6.378
	3 (*n* = 10)	16.569 ± 1.439	51.814 ± 3.151	108.958 ± 6.671	9.396 ± 1.168	41.961 ± 4.380	75.406 ± 6.367
	4 (*n* = 10)	19.667 ± 1.374	52.973 ± 4.137	92.881 ± 3.598	5.669 ± 0.734	21.172 ± 3.374	44.546 ± 6.194
Cd	**1 (*n* = 10)**	0.117 ± 0.010	0.193 ± 0.013	0.252 ± 0.015	0.104 ± 0.013	0.236 ± 0.032	0.456 ± 0.043
	2 (*n* = 10)	0.141 ± 0.017	0.232 ± 0.015	0.296 ± 0.018	0.177 ± 0.015	0.354 ± 0.033	0.575 ± 0.0387
	3 (*n* = 10)	0.275 ± 0.020	0.446 ± 0.018	0.672 ± 0.014	0.241 ± 0.021	0.483 ± 0.045	0.708 ± 0.060
	4 (*n* = 10)	0.258 ± 0.022	0.408 ± 0.029	0.630 ± 0.034	0.141 ± 0.014	0.288 ± 0.027	0.484 ± 0.039
Pb	**1 (*n* = 10)**	0.004 ± 0.001	0.020 ± 0.002	0.028 ± 0.002	0.003 ± 0.0003	0.006 ± 0.0006	0.011 ± 0.001
	2 (*n* = 10)	0.005 ± 0.001	0.023 ± 0.003	0.038 ± 0.007	0.006 ± 0.0007	0.011 ± 0.002	0.015 ± 0.002
	3 (*n* = 10)	0.104 ± 0.001	0.022 ± 0.002	0.032 ± 0.003	0.011 ± 0.001	0.024 ± 0.002	0.03 ± 0.002
	4 (*n* = 10)	0.011 ± 0.001	0.024 ± 0.003	0.036 ± 0.004	0.006 ± 0.001	0.012 ± 0.002	0.019 ± 0.002
Se	**1 (*n* = 10)**	0.005 ± 0.0003	0.006 ± 0.0003	0.015 ± 0.008	0.005 ± 0.0003	0.007 ± 0.0003	0.009 ± 0.0002
	2 (*n* = 10)	0.005 ± 0.0002	0.007 ± 0.0002	0.008 ± 0.0003	0.006 ± 0.0003	0.007 ± 0.0003	0.009 ± 0.0003
	3 (*n* = 10)	0.004 ± 0.0003	0.006 ± 0.0005	0.007 ± 0.0005	0.004 ± 0.0003	0.005 ± 0.0003	0.008 ± 0.0004
	4 (*n* = 10)	0.004 ± 0.0002	0.006 ± 0.0003	0.007 ± 0.0003	0.004 ± 0.0003	0.006 ± 0.0003	0.008 ± 0.0003
Hg	**1 (*n* = 10)**	0.0115 ± 0.0005	0.0262 ± 0.001	0.0471 ± 0.002	0.012 ± 0.0004	0.023 ± 0.001	0.043 ± 0.001
	2 (*n* = 10)	0.0114 ± 0.0005	0.028 ± 0.001	0.0469 ± 0.002	0.016 ± 0.0005	0.028 ± 0.0008	0.043 ± 0.001
	3 (*n* = 10)	0.010 ± 0.0005	0.023 ± 0.001	0.041 ± 0.002	0.024 ± 0.0006	0.042 ± 0.0008	0.058 ± 0.001
	4 (*n* = 10)	0.010 ± 0.0003	0.020 ± 0.0007	0.037 ± 0.001	0.014 ± 0.0005	0.026 ± 0.001	0.043 ± 0.002

1 = winter, 2 = spring, 3 = summer, 4 = autumn.
SD, standard deviation.

seasons and 5 times higher in the liver in winter and spring. Al-Halani (2021) investigated the accumulation of Cu, Zn, Mn, Ni, Cd, Pb, Cr, and Fe in the liver and muscle tissues of European sea bass (*D. labrax*) collected from the Damietta fishing harbor in winter and spring. Compared with the liver tissue of *D. labrax* caught at the Damietta fishing harbor, the liver tissue of *D. labrax* in this study showed 20 times more Fe, 10 times more Cu, 8 times more Zn, and 5 times more Mn accumulation. Again, in this study, 5 times more Fe and 10 times more Cu were analyzed in muscle tissue. Unlike other metals, Al-Halani (2021) also found that Pb accumulated in the liver and muscle at levels 25 and 100 times higher, respectively. Žvab Rožič et al. (2014) found higher concentrations of trace elements (i.e., As, Cu, Hg, Se) in wild fish tissues compared with cultured fish in their study on metal accumulation in *D. labrax* tissues. They stated that this was due to the presence of additional metal sources, such as fish feed and natural and/or anthropogenic sources. Ersoy and Çelik (2010) found that essential and toxic elements (Pb and Cd) were determined seasonally in the muscle and liver of the brush-tooth lizardfish from the Iskenderun Bay, in the Eastern Mediterranean Sea. All metals except Pb showed excessive accumulation in the muscle and liver of *S. undosquamis* caught in Gökova Bay when compared with their studies. The concentrations of Cd, Fe, Pb, Zn, Cu, Mn, Ni, Cr, Co, and Al were determined in the *S. undosquamis* collected from the Bay of Iskenderun in August 2003 by Türkmen et al. (2005). In comparison to our study, they found 10 times higher levels of cadmium and 100 times higher levels of lead in the muscle. Trace elements (Zn, Fe, Mn, and Cu) were found to accumulate more in muscle in our study. Abdallah and Abdallah (2008) investigated the concentrations of Cd, Cu, Co, Zn, Mn, and Fe in *S. undosquamis* collected from the Eastern Harbour and El-Mex Bay in the Mediterranean Sea, Egypt. In their study, they found that all metals except Fe and Mn were high in *S. undosquamis* muscle. The accumulation of Fe, Cu, and Zn in the muscle tissues of *S. undosquamis* from İskenderun Bay (Türkiye) was investigated by Manasirli et al. (2015). Compared with this study, Fe and Cu accumulation showed similarities, while they found Zn accumulation to be quite high. A study was performed by Li et al. (2023) to analyze the concentrations of seven heavy metals (including Cu, Cr, Cd, Pb, Zn, As, and Hg) in the muscles of *S. undosquamis* in the Beibu Gulf. When the samples from Beibu Gulf were compared with the samples in this study, Cu and Zn, which showed accumulation in muscle tissue, were detected at approximately 5 times lower levels, while Hg was analyzed at 4 times higher levels. Compared with other studies, it can be said that in general, essential metals were found to be high in the tissues of both fish in this study. Factors such as feeding habits, size, length, or age of the fish may cause differences in metal accumulation (Genç & Yılmaz, 2018).

Table 5 shows the correlation coefficient between the metals accumulated in the commercial fish in Gökova Bay and the CF. Between all metals in two

Table 5

Correlation coefficients of metals and CF in fish.

	Cu	Fe	Mn	Zn	Cd	Pb	Se	Hg	CF
S. undosquamis									
Cu	1.000								
Fe	0.837**	1000							
Mn	0.933**	0.851**	1000						
Zn	0.892**	0.834**	0.914**	1000					
Cd	0.831**	0.740**	0.878**	0.844**	1000				
Pb	0.789**	0.784**	0.797**	0.820**	0.726**	1000			
Se	0.613**	0.500**	0.686**	0.572**	0.626**	0.404**	1000		
Hg	0.902**	0.840**	0.927**	0.897**	0.882**	0.776**	0.651**	1000	
CF	-0.061	0.001	0.018	-0.008	0.098	0.043	0.123	0.033	1000
D. labrax									
Cu	1000								
Fe	0.657**	1000							
Mn	0.597**	0.820**	1000						
Zn	0.555**	0.768**	0.788**	1000					
Cd	0.377**	0.601**	0.767**	0.715**	1000				
Pb	0.632**	0.745**	0.755**	0.790**	0.701**	1000			
Se	0.621**	0.673**	0.598**	0.638**	0.363**	0.677**	1000		
Hg	0.647**	0.802**	0.682**	0.765**	0.429**	0.762**	0.903**	1000	
CF	1000	0.200*	0.161	0.002	0.285**	0.018	-0.014	-0.076	1000

**Correlation is significant at the 0.01 level (two-tailed).
CF, condition factor.

fish species, positive correlation coefficients were found. No significant correlation was detected between all metals and CF in *S. undosquamis*, whereas a positive correlation was detected between Fe (r = 0.200) and Cd (r = 0.285) and CF in *D. labrax*. While the highest positive correlation coefficient was found between Mn and Cu (r = 0.933) in *S. undosquamis*, it was found between Hg and Se (r = 0.903) in *D. labrax*. Positive relationships between Cr, Cu, Fe, Mn, Zn, and length and weight of *Scomber japonicus* were calculated by Tekin-Özan and Aktan (2012). In contrast to the other example, Yi and Zhang (2012) found that there was a negative correlation between the size of fish in *Silurus asotus* and *Pelteobagrus fulvidraco*. The mean accumulation values of both essential (Cu, Fe; Mn and Zn) and non-essential (Hg, Se, Cd, and Pb) metals in three tissues (liver, gill, and muscle) of two fish species varied greatly. This variation may be due to differences in ecological needs and metabolic activities (Canli & Atli, 2003; Öğlü et al., 2015).

3.2. Human health risk assessment

THQ, TTHQ, EDI, carcinogenic risk (CR) values of metals via consumption of *D. labrax* and *S. undosquamis* are given in Table 6.

EDI for various metals were calculated from consumption of fish species sold commercially in different markets nationwide, and EDI were compared for adults and children. Fe had the highest EDI for both fish species (15.642 mg · day^{-1} · person^{-1} for an adult and 34.217 mg · day^{-1} · person^{-1} for children in *D. labrax* and 6.63 mg · day^{-1} · person^{-1} for an adult and 14.509 mg · day^{-1} · person^{-1} for children in *S. undosquamis*). Pb with 0.0017 mg · day^{-1} · person^{-1} for adults and Se with 0.002 mg · day^{-1} · person^{-1} for children were the lowest intakes in *D. labrax*. Similarly, the minimum intake in *S. undosquamis* is Pb for adults and Se for children are 0.0013 mg · day^{-1} · person^{-1} and 0.0022 mg · day^{-1} · person^{-1}, respectively. The calculated EDI for metals shows a low value for adults in comparison with the recommended intake limit, but for children the values for Cd and Mn in both fish were above the recommended values. It was reported that EDI values did not exceed the tolerable intake for Cd, Pb, Fe, Cu, and Zn in a study of metal accumulation in four different fish species in Malaysia (Salam et al., 2019). Risk assessment results for some metals (Pb, Cd, Zn, Cu, Cr, As, Mn, and Ni) for fresh and cultured fish in a study conducted in Northern China showed that EDI values did not exceed the tolerable daily intake (Zhong et al., 2018). However, the health of the local population may be adversely affected by an increase in the frequency of fish consumption or by metal contamination in fish. In general, the CR <10^{-6} are considered negligible. The CR above 10^{-4} are considered unacceptable and CR between 10^{-6} and 10^{-4} are considered acceptable (Wang et al., 2005). CR for Cd and Pb were determined at negligible levels. Therefore, it is not possible to explain a situation that poses a potential health risk to the inhabitants. Töre et al. (2021) determined the accumulation of some metals (Mn, Cd, Fe, Cu, Ni, Zn, Co, Pb, Cr, As) in six fish species in the Tigris River (Türkiye) and possible risks to public health. In their study, while the CR values calculated for Pb in all species did not pose a risk, for Cr, Ni, and As, they determined above 10^{-4} value in some fish species. Demirak et al. (2022) determined some metal (Mn, Cd, Zn, Pb, and Cu) levels in eels living in Köyceğiz (Türkiye) and Võrtsjärv (Estonia) lakes. In their study, they found that the CR values for Pb and Cd in Lake Köyceğiz were negligible, while the CR value for Pb in Lake Võrtsjärv was very close to the hazard limits. The allowable THQ guideline is 1 (USEPA, 2011). The THQ values were <1 for all individual heavy metals in two fish species. This indicates that there is no non-cancer health risk associated with the intake of any individual heavy metal through the consumption of these fish. Based on the metals analyzed, the THQ value has been calculated to be <1 for all the fish. THQ is only interested in a single heavy metal, but often foods contain >1 heavy metal, as seen in the analyzed samples. Therefore, the calculation of the TTHQ is mandatory. The average TTHQ values were calculated as <1 for both adults and children. Results for THQ and TTHQ are <1, indicating that there are no potential adverse health effects. Percentage contribution of metals to TTHQ in *D. labrax* were Cd 42.30%, Fe 22.22%, Zn 13.73%, Cu 13.18%, Mn 7. 21%, Hg 0.75%, Pb 0.41%, and Se 0.17% for adults while the contribution rates for children are Cd 44.81%, Fe 23.57%, Zn 14.54%, Cu 14.04%, Mn 1.5%, Hg 0.83%, Pb 0.50%, and Se 0.16. TTQH percentage contribution rates for *S. undosquamis* in adults: Cd 45.91%, Hg 15.06%, Cu 14.21%, Fe 12.24%, Mn 6.27%, Zn 5.65%, Pb 0.44%, and Se 0.26%, while in children: Cd 45.91%, Hg 14.89%, Cu 14.08%, Fe 12.24%, Mn 6.32%, Zn 5.51%, Pb 0.40%, and Se 0.20%. TTHQ values in both fish indicate that Cd is the major risk contributor in adults and children. Fe in *D. labrax* but Hg in *S. undosquamis* is another high-risk element in adults and children. The THQ results in this study are similar to the THQ findings of Zhong et al. (2018); Yin et al. (2020); Bat et al. (2020); Genç (2021). On the other hand, Javed and Usmani (2016) found THQ levels >1 for Ni and Co in their study conducted in the Kasimpur channel of India in 2016.

Table 6

THQ, TTHQ, EDI, CR values of metals via consumption of *D. labrax* and *S. undosquamis*

			EDI (mg · day^{-1} · person^{-1})	Recommended limits (mg · day^{-1} · person^{-1})	Ref.	THQ	TTHQ Adults	TTHQ Children	CR
D. labrax	**Cu**	Adults	0.5305	30	FAO/WHO (2002)	0.0382	0.2898	0.0598	-
		Children	1.1605			0.0084			-
	Fe	Adults	15.6424	100	WHO (2003)	0.0644			-
		Children	34.2178			0.0141			-
	Mn	Adults	1.0154	1	FAO/WHO (2002)	0.0209			-
		Children	2.2211			0.0009			-
	Zn	Adults	4.1428	30	FAO/WHO (2011)	0.0398			-
		Children	9.0625			0.0087			-
	Pb	Adults	0.0017	0.3	FAO/WHO (2022)	0.0012			4.11E–08
		Children	0.0037			0.0003			8.99E–09
	Cd	Adults	0.0426	0.06	FAO/WHO (2009)	0.1226			0.0008
		Children	0.0931			0.0268			0.0002
	Se	Adults	0.0009	1	FAO/WHO (1983)	0.0005			-
		Children	0.002			0.0001			-
	Hg	Adults	0.0023	0.03	FAO/WHO (2009)	0.0022			-
		Children	0.005			0.0005			-
S. undosquamis	**Cu**	Adults	0.4402	30	FAO/WHO (2002)	0.0317	0.223	0.049	-
		Children	0.963			0.0069			-
	Fe	Adults	6.6327	100	WHO (2003)	0.0273			-
		Children	14.509			0.0060			-
	Mn	Adults	0.6079	1	FAO/WHO (2002)	0.0140			-
		Children	1.4855			0.0031			-
	Zn	Adults	1.3085	30	FAO/WHO (2011)	0.0126			-
		Children	2.8624			0.0027			-
	Pb	Adults	0.0013	0.3	FAO/WHO (2022)	0.0010			3.28E–08
		Children	0.0029			0.0002			7.17E–09
	Cd	Adults	0.0357	0.06	FAO/WHO (2009)	0.1024			0.0006
		Children	0.0780			0.0225			0.0001
	Se	Adults	0.0010	1	FAO/WHO (1983)	0.0006			-
		Children	0.0022			0.0001			-
	Hg	Adults	0.0035	0.03	FAO/WHO (2009)	0.0336			-
		Children	0.0077			0.0073			-

CR, carcinogenic risk; EDI, estimated daily intake; THQ, target hazard quotient; TTHQ, total Target hazard quotient.

3.3. Results of estimated statistical models

In this section, outcomes obtained from estimated models with random forest method are presented. Table 7 and the following figures can be examined concerning the prediction results and performance of estimated random forest models for all analytes. Table 7 presents performance metrics for the model. The RMSE values in the model represent the square root of the mean squared errors of the prediction model. Here, the error is expressed as $e_i = (\text{Actual}_i - \text{Predicted}_i)$, where e_i represents the predictions of ε_i. Notice that it is expected that the RMSE values can be as small as possible, and upon examining the models, it can be seen that the accumulations of analytes are satisfactorily predicted. In terms of comparison of the predicted models, Hg and Mn models have smaller RMSE values and Cu has the largest RMSE value for *S. undosquamis*. For *D. labrax,* the model obtained for Cu has the smallest RMSE and Pb model has the largest one.

Additionally, "% Var Explained" denotes the percentage of variance in metal accumulation explained by the explanatory variables, and high values support the generalizability of the model. In this context, regarding *S. undosquamis*, the variation in analytes is explained by a minimum of 72.77% and a maximum of 92.45% by the five variables considered in the model: Weight, Height, THQ, Season, and Tissue. This interval indicates a reasonable model performance for all analytes. On the other hand, interval of% Var explained for *D. labrax*, is between 54.82% and 93.32%, which is sourced from the estimated model for Pb analyte.

The optimal number of trees presented in the table ensures the random forest regression model's prediction with the least error. "MS residuals" represent the mean squared residuals of the selected model. Additionally, correlations between the predicted and actual value of analytes are provided, and these values are obtained satisfactorily, being >0.80 for all models except for estimated model of Pb whose correlation is 0.764. These results can be observed from Table 7.

In Figs 2 and 3 predicted values obtained for certain analytes in the model are compared with the plot of actual values of the analytes. In the figures, only two analytes are considered that are Fe and Mn for both species to save space. The figures confirm the reasonable correlations given in Table 7 and as can be seen, random forest models show quiet close behaviors to the actual data spreading regarding different levels of either season and tissue variables. Upon examination of Table 7 and the mentioned figures, it can be observed that the random forest models achieved good performances in predicting metal accumulations that might provide a sustainable support on the cost of laboratory burden under similar cases for both species (Li et al., 2021; Petrea et al., 2020).

Table 7

Outcomes of estimated random forest models for accumulations.

Species	Analyte	RMSE	% Var exp.	Opt. no. trees	MS residuals	Correlation
S. undosquamis	Cu	0.515	72.77	733	0.266	0.881
	Fe	0.351	86.85	158	0.123	0.961
	Mn	0.292	91.00	406	0.085	0.978
	Cd	0.359	86.57	522	0.129	0.963
	Zn	0.324	87.66	77	0.105	0.966
	Pb	0.337	87.19	45	0.113	0.958
	Se	0.360	86.36	268	0.131	0.954
	Hg	0.259	92.45	182	0.067	0.978
D. labrax	Cu	0.232	88.08	1	0.054	0.972
	Fe	0.243	93.23	37	0.059	0.980
	Mn	0.251	92.90	186	0.063	0.980
	Zn	0.328	88.74	991	0.108	0.964
	Cd	0.393	84.33	258	0.155	0.941
	Pb	0.660	54.82	37	0.436	0.764
	Se	0.491	75.20	441	0.241	0.951
	Hg	0.250	93.32	806	0.062	0.983

RMSE, root mean squared error.

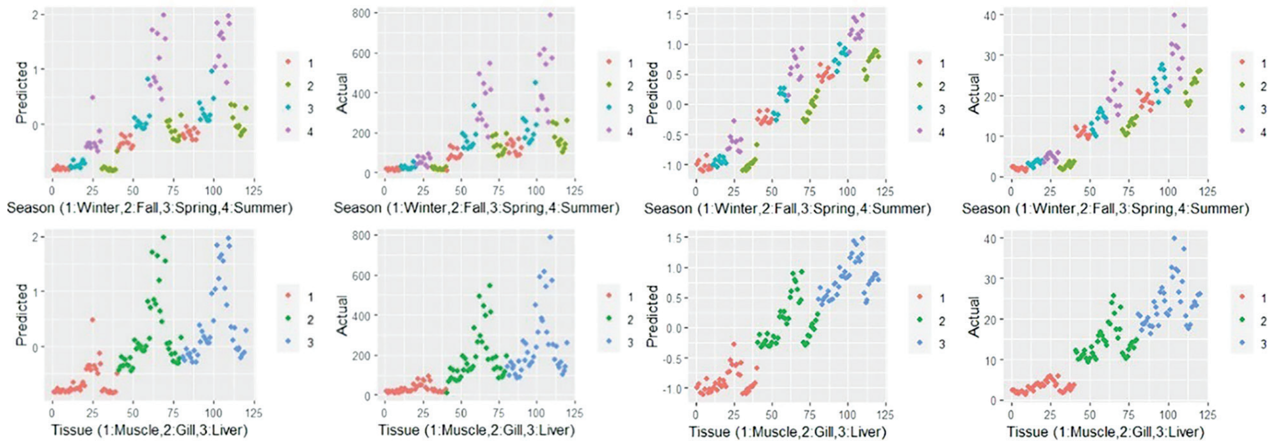

Figure 2

Scatter plots of predicted and actual values of analytes Fe and Mn for *S. undosquamis*. **(A)** Estimated Model of Fe. **(B)** Estimated Model of Mn.

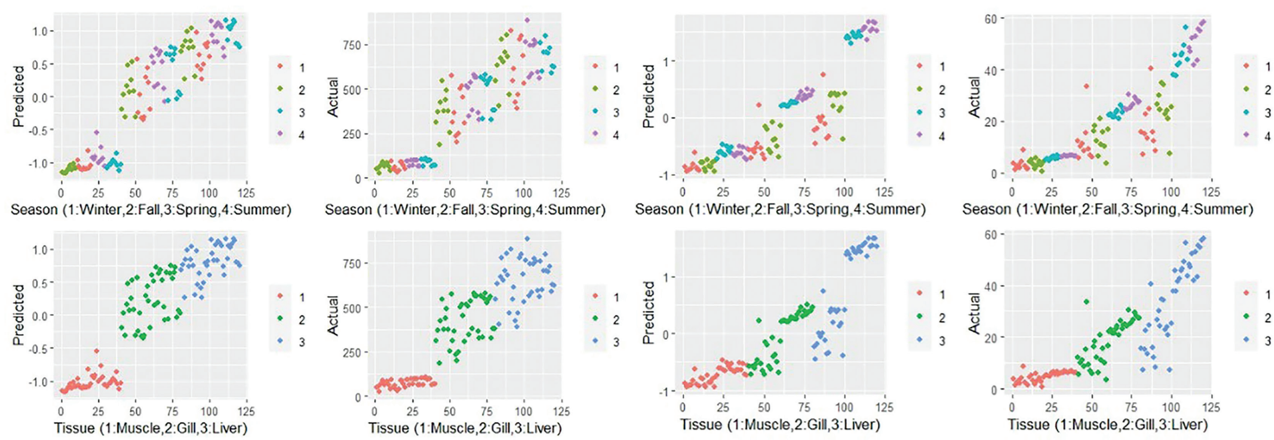

Figure 3

Similar to Fig. 1 but for analytes Fe and Mn for *D. Labrax*.

4. Conclusion

Gökova Bay is an important ecosystem in terms of fish fauna. This ecosystem has been the focus of studies on toxic metal levels in marine fish, whose pollution potential has been investigated in recent years, particularly due to the development of tourism in the region. In this study, it was determined that metal accumulation in the liver, gill, and muscle tissues varied according to metal types and seasons in two fish species (*S. undosquamis* and *D. labrax*). The average metal concentrations found in the muscle tissue of the two fish species were not high enough to pose a threat to human health and were within acceptable levels according to the World Health Organization and Food and Agriculture Organization. In addition, CR metal intake levels were found to be below tolerable levels. The reported EDI for metals for adults was low compared with the recommended intake limit, but for children the values for Cd and Mn in both fishes exceeded the recommendations. THQs and TTHQs were below 1, but Cd emerged as the major risk factor in both adults and children. It was also shown that random forest models, applied for the first time in this study, achieved good performances in predicting metal accumulations in similar situations for both species.

Declarations

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Ethics approval

This study was approved by Muğla Sıtkı Koçman University Local Ethics Committee for Aquatic Animal Experimentation (REC. id: E-38607093-604.01-742275 and E-38607093-604.01-742277)

Competing interests

The authors declare no competing interests.

Data availability

The datasets used and analyzed during the current study available from the corresponding author on reasonable request.

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