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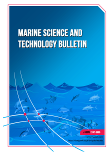

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RESEARCH ARTICLE

Macroelement and microelement compositions in the liver of smooth-hound *Mustelus mustelus* in fall and spring from Iskenderun Bay, Northeastern Mediterranean Sea

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ABSTRACT

The shark is an important cartilaginous fish species in the Northeastern Mediterranean and has occupied a wide range of areas. They are landed for different kinds of reasons but mostly as by catch. Sharks have a great proportion of livers that are generally not used. The livers may have potential usage for different materials e.g., fish meal, food ingredients, and fish oil materials. Therefore, they should be used, not wasted. Determining its macro element and microelement composition could be beneficial for any raw material. According to this current study's data, Cd and Pb levels were detected only in Fall. The Cr levels in the livers were not detected all year long. The amounts of the Cu, Mn, and Zn in the livers of common sharks all year long did not exceed 1 mg/kg, 1 mg/kg, and 2.4 mg/kg respectively. The livers contained high levels of Fe which differed from season to season. The difference in Fe amounts was found to be statistically significant between seasons ($P>0.05$). Even though changes were observed in the levels of microelements in the livers of common sharks in this study, there were no statistically significant changes except for Zn and Fe. There should be more testing to support the result suggested. Results of this study showed that the predominant macro minerals were Na and P for sharks in fall and spring (3968 and 3710 mg/kg, respectively throughout the year).

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Introduction

Mustelus mustelus (Linnaeus, 1758) is one of the members of the class Chondrichthyes, which includes fish that have skeletons made of cartilage. Sharks are one of the two subclasses of Elasmobranchii e.g., skates and rays. Elasmobranch fish are generally bycatch fish and have larger livers (Lamas & Massa, 2017, 2019; Yığın et al., 2019; Ayas et al., 2019; Gupta et al., 2020; Pagliarini et al., 2020; Kumar et al., 2021; Özyılmaz & Öksüz, 2021; Spada, 2021; Fordham et al., 2022; Yogi et al., 2023; Alvarez et al., 2023). Studies showed that livers have a great amount of crude lipid capacity (Özyılmaz & Öksüz, 2015; Quero-Jiménez et al., 2020; Pagliarini et al., 2020)

Some researchers pointed out in previous reporting that livers of cartilaginous and bycatch fish can be considered as raw materials which can turn into beneficial materials (Kebir et al., 2007; Néchet et al., 2007; Özyılmaz & Öksüz, 2015; Özyılmaz, 2016a; Ayas et al., 2019; Yığın et al., 2019). The materials should be checked for different aspects of their biochemical features. Especially, the lipid they have considered as beneficial for health owing to their fatty acids. Health-promoted fatty acids have many different positive health effects (Šimat et al., 2015; Bagge et al., 2017). However, the raw materials should be safe regarding their nutritional aspects such as heavy metals, microelements, and macro elements.

Even though there are few studies conducted on these fish species (Gökçe et al., 2004; Özyilmaz, 2016b; Küçükgülmez et al., 2018; Šimat et al., 2020), there should be more studies needed in this field. The aim of the study is to search the macro elements and microelements content of the shark to see if it is safe to use them as possible raw materials for people or animals. From this point of view, knowing the macro and micro elements of the liver of the shark biannually may give an idea of whether it is suitable to use as raw materials.

Material and Methods

Study Area and Sample Collection

Sharks (*Mustelus mustelus*, Linnaeus 1758) (n=18 for each season) were caught in a trawling operation in fall and spring in Iskenderun Bay (Northeastern Mediterranean Sea, Türkiye, fall 2010 and spring 2011). Iskenderun Bay is a main sea area in the Northeast Mediterranean, where port management, maritime traffic, industrial facilities, and fishery activities are intense (Demirhan et al., 2020; Yılmaz et al., 2022). A map of the study area is shown in Figure 1. The livers of the fish were taken out and stored at -20°C for the element analysis.

Figure 1. Map of the study area

Extraction and Determination of Trace Elements

In this study, the liver samples were subjected to the wet digestion method. The procedure was performed according to AOAC Method 975.03 with a few minor modifications. The samples (approximately 1 g) were weighed and a total of 3 mL of 60% perchloric acid (Merck, Darmstadt, Germany) was added to the samples in a fume hood. The liver samples were incubated until the dense gas output decreased. This took a while because liver samples contained a high amount of oil. After the gas dense was gone, a total of 7 mL of 65% HNO_3 (Merck, Darmstadt, Germany) was added samples to complete digestion. The next step was to heat the samples on a hot plate for at least 8 hours. The samples were allowed to cool down. The digested materials were filtered through a 15 mL volumetric flask, using ash-free filter paper, and made up to 15 mL with ultra-pure water.

Four macro elements and seven microelements were determined for this study. Calibration curves for each of the individual elements were prepared from ICP Multi-element stocks. The determinations of the micro and macro elements were carried out by using ICP-AES (Inductively Coupled Plasma- Atomic Emission Spectrometry, Varian Model-Liberty series II) in Mustafa Kemal University, Natural Sciences Research and Application Center. ICP multi-element stocks were used for the measurement of elements. Wavelengths used for calcium (Ca, 396.847, λ (nanometer)), magnesium (Mg, 285.213), sodium (Na, 588.995), phosphorus (P, 213.618), cadmium (Cd, 228.802), lead (Pb, 220.353 λ), chromium (Cr, 267.716 λ), copper (Cu, 324.754 λ), iron (Fe, 259.940 λ), manganese (Mn, 257.610 λ), and Zn (zinc, 213.856 λ).

Statistical Analysis

Statistical analysis was performed with SPSS (22.0). Significance was established at P<0.05. The data of this study regarding seasons were subjected to a one-way analysis of variance (ANOVA), and a mean comparison was carried out by using the Tukey test. Normality and homogeneity were tested by using Kolmogorov–Smirnov and Levene's tests, respectively.

Results

A total of three heavy metals [cadmium (Cd), chromium (Cr), and lead (Pb)], and eight minerals [four macros; Mg (magnesium), P (phosphorus), Ca (calcium), K (potassium), and four micros; Mn (manganese), Cu (copper), Fe (iron), and Zn (zinc)] were seasonally investigated in shark livers. The levels of heavy metals in the livers of the sharks are shown in Table 1. The levels of the Cd, Pb, and Cr were detected under detection limit or no heavy metals were detected in spring in shark livers. However, the Cd and Pb were detected in fall.

Additionally, the levels of macro minerals in the livers of the sharks are shown in Table 2. Results of this study showed that the predominant macro elements were Na and P all year. The levels of Na and P for sharks throughout the year in the fall and spring were calculated to be 3968 and 3710 mg/kg, respectively. As can be seen from the table, the levels of P and Na, the major macro minerals, changed throughout the year, and the changes were statistically significant (P<0.05). The changes in the levels of Mg were also found to be statistically significant (P<0.05). The levels of Mg, Na, and P were higher in the spring than they were in the fall, except for the levels of Ca, which were higher in the fall.

The levels of micro minerals in the livers of the sharks are presented in Table 3. The highest macro mineral was measured to be Fe in the livers of the sharks in spring and fall. There were statistically significant changes between seasons regarding the amounts of the Fe (P<0.05). The levels of the Zn in livers of the sharks in the fall were found to be higher than those of Zn in the spring (P<0.05).

Table 1. The levels of heavy metals in livers of the sharks (mg/kg, wet weight)

Micro Elements	**Seasons**		
	Spring	***Fall***	***In a year***
Cd	0.000±0.000[a]	0.158±0.235[a]	0.079±0.172
Pb	0.000±0.000[a]	0.016±0.028[a]	0.008±0.020
Cr	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000

Note: Values were presented as mean SD (n=3). [a,b] Values within the same row with different superscripts diverge significantly at P<0.05.

Table 2. The levels of macro minerals in livers of the sharks (mg/kg, wet weight)

Seasons	**Macro Elements**			
	Ca	***Mg***	***Na***	***P***
Fall	321.73±34.32[a]	345.61±20.82[a]	3533.95±106.49[a]	3051.04±155.66[a]
Spring	155.47±61.65[a]	471.07±171.71[b]	403.88±1427.34[b]	4370.52±1949.02[b]
in a year	238.60±101.41	408.34±129.18	3968.92±1022.98	3710.78±1432.30

Note: Values were presented as mean SD (n=3). [a,b] Values within the same column with different superscripts diverge significantly at P<0.05.

Table 3. The levels of micro minerals in livers of the sharks (mg/kg, wet weight)

Micro Elements	**Seasons**		
	Spring	***Fall***	***In a year***
Fe	126.629±99.770[a]	459.399±43.144[b]	293.014±194.799
Zn	1.830±0.161[a]	2.642±0.261[b]	2.236±0.485
Mn	0.149±0.081[a]	0.122±0.062[a]	0.135±0.066
Cu	0.731±0.063[a]	0.626±0.050[a]	0.679±0.076

Note: n=3 for the chemical analysis (Spring n=3; Fall n=3). [a,b] Values within the same row with different superscripts diverge significantly at P<0.05.

Discussion

The Cd and Pb in shark livers did not exist or existed under detection limits in the spring. This is a good sign that the fish livers are supposed to be safe for possible usage as raw materials based on the measured heavy metals amounts. They have a certain amount of Cd and Pb in shark livers in the fall. They should be checked before any possible usage. Because there are certain limits for specific heavy metals in foods to decide whether they are safe for consumption.

Özyılmaz & Öksüz (2015) investigated the liver oil of six different cartilaginous fish species including two different shark species and found out the levels of Cd and Pb in the range of 0.02-0.07 mg/kg and 0.15-0.90 mg/kg, respectively. Özyılmaz (2016a) investigated livers of thornback ray in two different regions for males and females and measured their Cd and Pb levels to be in the range of 0 to 0.5 mg/kg in wet weight. The levels of Cd and Pb in shark livers in the fall and spring in this current study are comparable to previous studies.

Cr in the livers of the sharks did not appear throughout the year in this study. Ayas et al. (2019) searched four ray species (*Dasyatis pastinaca, Raja radula, Raja clavata* and *Torpedo marmorata*) caught from Mersin Bay in the Northeastern Mediterranean Sea and found out the Cr levels in muscle and liver in higher than detection limits, which is also higher than those in this study. The levels of Cd and Pb in shark livers in the fall in this current study is similar with previously reported study of Ayas et al. (2019). Having no or very low amounts of these heavy metals can be considered a good sign for a clean environment and safe raw materials.

The levels of Ca, Mg, Na, and P in the livers of the sharks in this study showed that the livers were rich in macro minerals. Ayas et al. (2019) released the data showing that ray species (*D. pastinaca, R. radula, R. clavata* and *T. marmorata*) which are cartilaginous fish species also rich in macro minerals. On the other hand, the amounts of the macro minerals in the liver of cartilaginous fish species have higher amounts of macro minerals than those of many bony fish e.g., bogue, European hake, live sharksucker, wild trout (Öksüz et al., 2010, 2011; Kayım et al., 2011; Ozyılmaz et al., 2017a, 2017b; Ozyılmaz & Miçooğulları, 2020).

The livers contained high levels of Fe, which differed from season to season. The difference in Fe amounts was found to be statistically significant between seasons ($P>0.05$). Iron is known as an important essential micro element which has very important tasks in the human system. One of them is to serve as a carrier of oxygen to tissues from the lungs by red blood cells. Taking enough Fe in diet is recommended to stay away from some health problems (Belitz & Grosch, 2001; Camara et al., 2005). according to the Turkish Standards Institution (TSI, 2000), adequate Fe amount should be less than 10 mg in a kg and exceeding that level of iron is not permitted.

On the other hand, marine systems have the potential possibilities to receive environmental nutrients, residues, pollutants, etc. all territorial activities find a way to reach water (Yilmaz et al., 2018). The lives in this water are quite affected by their environment (Yılmaz et al., 2017). The lives get their bodies needs in the environment mostly through their food chains. The cartilaginous fish used in this current study was a part of this environment. There were a lot of companies that ran their business (e.g., iron and steel facilities, oil pipeline facilities) and a very busy harbor around the environment where cartilaginous fish live. These factors may be part of the reason that the level of Fe is higher in our study.

The amounts of Cu, Mn, and Zn in the livers of common sharks changed throughout the year in this study. According to Ayas et al. (2019) four different ray species have levels of the micro minerals in the following order: Mn<Cu<Zn. This previously reported data regarding the amounts of the Mn, Zn, Cu is like this current study relating their existence in the liver of the shark investigated seasonally.

Conclusion

As a result, this study contains seasonal changes in shark livers heavy metals, macro and micro minerals that could be beneficial, especially for producers who seek good raw materials for their products (such as, fish oil supplements for humans and pets, fish feed, cosmetics, greases, caulking compounds, buffing agents, paints, industrial coatings, pharmaceutical purposes, lubricants, textile auxiliaries, water repellents, soaps, and candles), consumers who care about what they consume, and scholars who intend to study in this very particular area. Because these livers may have potential usage as raw materials if they are safe for any possible usage.

Compliance With Ethical Standards

Authors' Contributions

AO: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing - original draft, Writing-review and editing

EŞ: Investigation, Writing-review and editing

SD: Conceptualization, Investigation, Writing-review and editing

AD: Investigation, Writing-review and editing

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Funding

Not applicable.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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