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First Report on the Elemental Composition of the Bigeye Thresher Shark *Alopias superciliosus* Lowe, 1841 from the Mediterranean Sea

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Abstract

Cartilaginous fish species have ecological importance. Besides, the ecotoxicological studies on these species are pretty insufficient. In this study, Al, Cr, Mn, Fe, Cu, Zn, As, Pb, Cd, and Sr levels were determined in muscle, liver, gill, kidney, spleen, stomach, and gonad tissues of *Alopias superciliosus* (Female, 240 cm TL) caught from Mersin Bay. Tissue metal analysis was determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). A statistical difference was found among the tissues in terms of the metals. Fe was determined to be the highest level in all tissues ($p<0.05$). The relation between tissues in terms of Fe level was determined as Liver>Gill>Spleen>Gonad>Kidney>Stomach>Muscle. Zn was detected at higher levels in the liver and stomach and As in other tissues after Fe. Al has the highest level after Zn and As and was mainly found in the gills. The tissue Cu and Zn levels were found in the same order from highest to lowest as Liver>Gonad>Kidney>Spleen≥ Stomach>Gill>Muscle. Sr was higher in the stomach, gonad, and kidney than in the other tissues. Cd levels were found in higher than Pb levels in the examined tissues. Liver Cd level was determined as 57.37 µg g^{-1} dw. Except for the liver, Mn levels were found low than Cr levels in the examined tissues. The distinction between the tissue levels of the investigated elements has changed depending on the functional differences between the tissues and metal metabolisms.

Keywords:

Alopias superciliosus, elemental composition, tissue level, Mersin Bay

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Introduction

Studies examining the accumulation and toxic effects of metals that participate in the marine ecosystem under the influence of natural and anthropogenic sources in marine organisms have

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proven that metals taken through water and food are easily transported in the food chain. Accumulation varies according to species, organization level, habitat, sex, texture, and metal is known (Jardine et al., 2013). The continuity of the participation of metals in nature and their long half-life of them cause vertical and horizontal transfer of them in aquatic ecosystems and progress from the shore to the open waters. In addition, the density of vegetation and the amount of dissolved organic matter in the environment can change the concentration of metals in the aquatic environment.

Cartilaginous fish with a long lifespan can accumulate metals at high levels because they are apex predators at the upper trophic level and make long migrations circumglobally (Delshad et al., 2012; Nicolaus et al., 2016; Adel et al., 2017; Lara et al., 2020). Furthermore, due to the widespread use in different industries such as fin, muscle, liver oil, cartilage, and squalene production, it is necessary to determine the metal levels in the tissues of cartilaginous fish.

Diet is the main route of metal uptake and accumulation in cartilaginous fish (Corsolini et al., 2014), and it has been reported that accumulation differs between tissues. The liver, which is a metabolically active tissue, can accumulate metals at higher concentrations than other tissue due to the leading synthesis site of metallothionein, a metal-binding protein, and glutathione, a tripeptide rich in low molecular weight cysteine (Mull et al., 2012).

The species and tissue examined in ecotoxicological studies with cartilaginous fish are minimal. The gills are target organs because they directly interact with the metal in the environment and are essential in terms of reflecting the metal environment concentration. The digestive organs should be examined in terms of reflecting dietary intake, and the kidneys as the most basic route of excretion. Gonads show a high affinity for accumulation during the long maturation phase in cartilaginous fish. This is important for reproductive success.

A study conducted with *Galeocerdo cuvier* determined that Cu, Zn, and Hg levels vary depending on the growth rate and tissue (Endo et al., 2008). In a study, Cu, Zn, Cd, Hg, and Pb levels were investigated in the fin and muscle tissues of four Elasmobranch species; and it was stated that heavy metal levels varied according to species, metal, and tissue (Ong & Gan, 2017).

A. superciliosus, known as the big-eyed thresher shark, is a pelagic oceanodromous species systematically belonging to the Alopiidae family of the Lamniformes order (Riede 2004). It shows a circumglobal distribution in tropical and temperate waters at a depth of 0-730 m (McMillan et al., 2011) and generally 0-100 m (Compagno, 2001). It is located in the pelagic and benthopelagic zones on the continental shelf. It feeds on lancetfish, herring, mackerel, small fish in the pelagic zone, and bottom fish such as European hake and squid in the benthic area.

The distribution of *A. superciliosus* in the Mediterranean is quite limited. Although there are restricted ecotoxicological studies with *A. vulpinus* and *A. pelagicus*, there are no ecotoxicological studies related to *A. superciliosus*. It is important to be toxicological reports in species with ecological and economic importance. Therefore, it was aimed to determine the Al, Cr, Mn, Fe, Cu, Zn, As, Pb, Cd, and Sr levels in the liver, gill, muscle, kidney, spleen, stomach and gonad of a female *A. superciliosus* specimen caught from Mersin Bay in the present study.

Materials and Methods

Alopias superciliosus, which was used as a material in the research, was caught incidentally from the Mersin Bay Taşucu coast from 25 m with a trammel net in January 2020. It was determined that the individual was a young female with a total length of 240 cm. The individual was brought to the Museum of Marine Life, Mersin University, and recorded with the catalog number (MEUFC-20-11-127). The liver, gill, muscle, kidney, spleen, gonad, and stomach tissue were dissected, and three samples were taken from each tissue. Al, Cr, Mn, Fe, Cu, Zn, As, Pb, Cd, and Sr levels were determined in tissues. The coordinate (Coordinate: 36°18'17.6"N, 33°51'41.0"E) and map of the area where the sample was caught are presented in Figure 1.

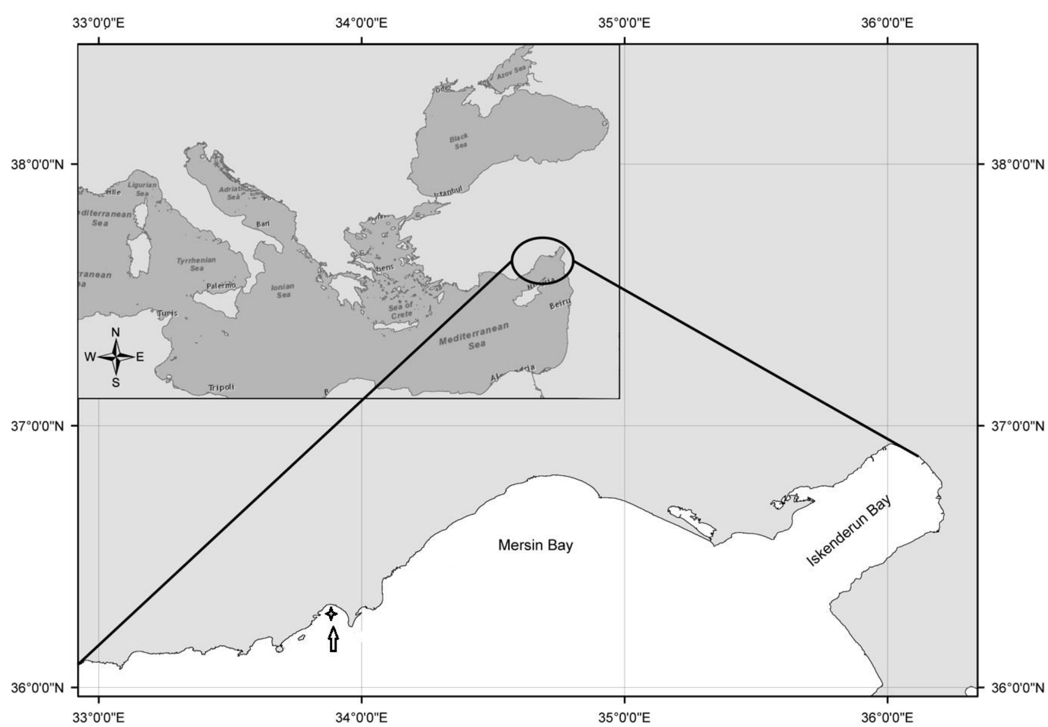

Figure 1. Incidentally captured locality for *Alopias superciliosus* from Taşucu Bay.

The dissected tissues were dried at 95 °C for 72 hours. After the dry weight of the tissues was determined, 2 ml of nitric acid was added (HNO3, 65%, Merck) and digested at 120 °C for 4 hours. The acid-digested samples were then transferred to falcon tubes and filled with 10 ml of bidistilled water (Çiftçi et al., 2021). Finally, samples were analyzed with an Inductively-Coupled

Plasma Mass Spectrometer (ICP-MS, Agilent, 7500ce Model, Japan). ICP-MS operating conditions were the following: radio frequency (RF) (W), 1500; plasma gas flow rate (L min^{-1}),15; auxiliary gas flow rate (L min^{-1}), 1; carrier gas flow rate (L min^{-1}), 1.1; spray chamber T (°C), 2; sample depth (mm), 8.6; sample introduction flow rate (ml min^{-1}), 1; nebulizer pump (rps), 0.1; extract lens (V), 1.5. Metals in samples were detected as µg metal g−1 dry weight. High Purity Multi-Standard (Charleston, SC 29423) was used to determine the metal analyses. Standard solutions for calibration curves were prepared by dilutions of the trace elements and potentially toxic metals. International Atomic Energy Agency (IAEA-436) reference material was used to follow the quality of the analytical process. IAEA-436 was analyzed for all elements. The certified value and observed value of the IAEA-436 reference material were compared. Replicate analysis of this reference material showed good accuracy (Table 1).

Table 1. The certificated value and the observed value of reference material IAEA-436.

Analyte	Certified value	95% Confidence interval	Observed value
Al	3.06 ± 0.42	2.68–3.44	3.419 ± 0.11
Cr	0.194 ± 0.058	0.168–0.219	0.179 ± 0.01
Mn	0.238 ± 0.042	0.218–0.257	0.238 ± 0.005
Fe	89.3 ± 4.2	87.8–90.9	99.04 ± 0.826
Cu	1.73 ± 0.19	1.66–1.79	1.827 ± 0.092
Zn	19.0 ± 1.3	18.6–19.4	19.876 ± 0.072
As	1.98 ± 0.17	1.91–2.06	2.19 ± 0.035
Cd	0.052 ± 0.007	0.050-0.054	0.049 ± 0.021
Sr	0.564 ± 0.062	0.523–0.606	0.598 ± 0.026

The statistical analyses of data were performed using IBM 22 SPSS package program. The metal levels in tissues of *A. superciliosus* were carried out using variance analysis (ANOVA), and Duncan's Multiple Range tests were used to compare the distinction between each group.

Results

Al, Cr, Mn, Fe, Cu, Zn, As, Pb, Cd, and Sr levels in the liver, gill, muscle, kidney, spleen, stomach, and gonad tissues of *A. superciliosus* are shown in Table 2. There were statistically significant differences between metals in terms of tissue levels ($p<0.05$).

Fe was found at the highest and Cr at the lowest level in all examined tissues. The tissue Fe level was determined as Liver>Gill>Spleen>Gonad>Kidney>Stomach>Muscle, respectively. Zn in the liver and stomach, As in other tissues, was found to be second place as a higher element after Fe. The tissues were listed as liver>gonad>spleen>gill> kidney>stomach>muscle in order to As level. Al was followed by Zn and As, and the highest level was found in the gill. The tissue Cu and Zn levels were found in the same order from highest to lowest as Liver>Gonad>Kidney> Spleen≥Stomach>Gill>Muscle. Sr was the highest in the stomach, followed by the gonad and kidney. Cd was found at higher levels than Pb in the examined tissues. Liver Cd level was determined as 57.37 µg g^{-1} d.w. Mn was found in low levels after Cr in tissues except for the liver.

Table 2. Metal levels (µg g^{-1} dw) in tissues of *A. superciliosus*

	Spleen	Stomach	Gonad	Kidney	Gill	Liver	Muscle
	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
Al	6.26±1.72^{a}	6.96±1.05^{a}	5.84±0.68^{a}	6.95±1.65^{a}	56.65±9.08^{b}	4.80±0.27^{a}	5.67±1.49^{a}
Cr	0.39±0.06^{ab}	0.15±0.05^{a}	0.40±0.04^{ab}	0.26±0.02^{a}	0.85±0.15^{c}	0.54±0.06^{b}	0.31±0.10^{ab}
Mn	0.57±0.05^{a}	1.79±0.22^{a}	1.61±0.30^{a}	1.87±0.51^{a}	1.52±0.22^{a}	5.00±0.83^{b}	1.00±0.15^{a}
Fe	676.94±110.71^{c}	74.23±4.93^{a}	478.31±88.82^{bc}	316.55±96.26^{b}	1300.01±96.14^{d}	1831.3±48.62^{e}	48.27±7.03^{a}
Cu	3.59±0.51^{a}	5.81±0.62^{a}	20.66±6.78^{b}	10.37±1.02^{ab}	2.71±0.41^{a}	142.41±9.37^{b}	2.32±0.57^{a}
Zn	50.51±6.52^{ab}	49.05±0.58^{ab}	92.53±14.20 ^{bc}	75.14±16.47^{bc}	42.50±3.78^{ab}	309.66±18.87^{c}	25.70±2.99^{a}
As	141.58±16.37^{b}	47.10±5.60^{a}	213.74±19.29^{c}	104.26±8.12^{b}	124.95±12.43^{b}	243.85±22.32^{c}	42.81±2.47^{a}
Pb	2.98±0.63^{a}	3.02±0.61^{a}	3.46±0.21^{a}	3.68±0.21^{a}	3.85±0.52^{a}	2.84±0.04^{a}	3.56±0.86^{a}
Cd	2.93±0.37^{ab}	2.89±0.18^{ab}	9.13±2.21^{b}	5.98±0.92^{ab}	1.96±0.21^{ab}	57.37±5.24^{c}	0.97±0.17^{a}
Sr	5.82±0.50^{a}	19.11±3.21^{b}	15.71±1.22^{b}	15.63±4.2^{b}	8.85±1.11^{a}	5.95±0.37^{a}	4.01±0.42^{a}

*The different letters (a, b, c, d) in each row indicate the statistical differences ($p<0.05$) between tissues
$\bar{x} \pm s_x$: Mean±standard error

Discussion

Increasing concentrations of essential elements such as Fe, Cu, and Zn, which are vital for animals at a certain concentration, and deficient concentrations of non-essential elements such as Cd and Pb, which are not biologically required, cause accumulation and toxicity in tissues. In the study, the gill, liver, spleen, muscle, kidney, and gonad tissue levels of essential (Fe, Cu, Zn, Mn, Cr, Sr) and non-essential (Al, As, Pb, and Cd) elements of *A. superciliosus* were determined. Among the essential elements, Fe was found at the highest level. Tissue Fe levels of the species were determined as Liver>Gill>Spleen>Gonad>Kidney> Stomach>Muscle, respectively ($p<0.05$).

Fe is responsible for many biochemical reactions, takes part in oxygen transport by participating in the structure of hemoglobin and myoglobin, and takes part in the electron transport system by participating in the structure of cytochromes in animals. In animals, 60% of the total Fe is found in blood hemoglobin, 3-7% in myoglobin in muscle tissue, while the rest is in the liver, spleen, kidney, bone marrow, and muscles. The excretion of Fe, whose absorption in the body is very low, is controlled by Fe homeostasis. The Fe level in animals varies depending on the species, developmental stage, sex, feeding preference, and disease. Muscle Fe levels in *Prionace glauca*, *Carcharhinus falciformis*, and *A. pelagicus* sampled from the Mexico coast were determined as 445.3±673.63, 420.0±393.22, 396.3±306.50 mg kg^{-1} ww, respectively, and muscle Fe levels were found to be higher (41-61%) in females compared to males (Álvaro-Berlanga et al., 2021). In the present study, the muscle Fe level of *A. superciliosus* was determined as 48.27±7.03 µg g^{-1} dw. Fe was found at the highest level in the liver of *A. superciliosus* among the other tissues. The liver is the most important iron reserve area. The gills have the highest Fe levels after the liver (Doğdu et al., 2021). The wide capillary network that provides gas exchange during respiration explains the high level of Fe in the gills. Spleen is a hematopoietic tissue and Fe level was found to be high in the third place. Fe, together with Cu, is an essential element responsible for reproduction. "Sertoli and Leydig cells" in the male reproductive system are important sources of ferritin and serve as a ready source of Fe for developing spermatozoa as well as protecting testicular tissue (Toebosch et al., 1987; Wise et al., 2003). Fe level in *A. superciliosus* gonad tissue may indicate that the individual is either in the reproductive period to reach reproductive or maturity. It is known that the known reproductive maturity range of the species is 154-341 cm, with an average of 253 cm (Compagno 1984). The kidneys are responsible for homeostasis and excretion in fish (Goldstein & Schnellmann 1996; Wendelaar Bonga & Lock 2008). Since cartilaginous fish are hyperosmotic, freshwater and marine bony fish show kidney functions together. In this study, kidney tissue Fe level can be explained by homeostatic control. Heme iron is the most important source of Fe in top predator species with long lifespans, such as sharks. The low pH of the stomach allows Fe to be converted to the ionic form during digestion, and cellular absorption of iron in the ionic form is faster. This may explain why the Fe level in *A. superciliosus* stomach tissue is higher than the muscle tissue level.

Cu and Zn are trace elements that function as cofactors by participating in the structure of many enzymes in animal organisms, and they cause disturbances in metabolic and physiological events over a certain concentration range (Michalska-Mosiej et al., 2016). Zn participates in the structure of more enzymes than Cu, so it is generally found at higher levels than Cu in Teleost and Chondrichthyes fish species (Mendil et al., 2010; Olgunoğlu et al., 2015; Raimundo et al., 2015; Álvaro-Berlanga et al., 2021). Adel et al. (2017) reported that the muscle Cu level *in Carcharhinus dussumieri* was higher than the Zn, which could be explained by the antagonistic effect between metals. Cu and Zn levels in tissues of *A. superciliosus* were determined as Liver>Gonad>Kidney >Spleen>Stomach>Gill>Muscle, respectively. The proportional similarity in the tissue levels of both elements can be explained by the functional role of the tissues and metal metabolism.

The arsenic originating from the earth's crust is highly involved in the aquatic ecosystem with anthropogenic sources (Kumari et al., 2017). Arsenic is a nonmetallic element found in different forms in biological systems. The presence of organic arsenic compounds in fish and other aquatic fauna and flora has been reported in many studies (Francesconi et al., 1994; Schmeisser et al., 2004; Soeroes et al., 2005; Grotti et al., 2008; Rahman & Hasegawa 2012). It is known that arsenic, which is in the inorganic form in water, turns into a harmless form as a result of methylation with aquatic flora and is stored in the muscle and liver as organic compounds such as arsenobetaine and arsenolipid in biota (Duker et al., 2005; Bears et al., 2006). The liver has an important role in the biotransformation of inorganic arsenic and is stored in the liver in various bony fish species (Cockell et al., 1991; Ohki et al., 2002). However, it has been reported that arsenic is found at a higher level in muscle tissue than liver in some teleost fish (Cˇelechovska´ et al., 2011; Tyokumbur et al., 2014; Çiftçi et al., 2021). Arsenic was found at the highest level in the liver and gonad tissues of *A. superciliosus* and lowest in muscle tissue in the present study. The accumulation of high biotransformation ability elements, such as arsenic, in tissues may vary depending on the species, life span, organization level, nutritional preference, and habitat. In this study, the gill and kidney tissue As levels were found to be higher than the muscle tissue, which may indicate that excretion is high.

The gills are one of the main uptake routes of metals in the aquatic environment by fish, and especially the uptake and transport of +2 valence elements in the body occur at a higher level. Aluminum is added to aquatic ecosystems mostly with the effect of anthropogenic factors. Its main uptake by fish takes place through gills, which are in direct interaction with the environment. Aluminum has a valence of +3. This situation weakens the competition of aluminum with the +2 valence elements that can be easily taken from the Ca channels in the gills (Rosseland et al., 1990; Exley et al., 1991; Monette et al., 2008). Its low water solubility is another factor limiting its accumulation. Acidic environments increase Al solubility and cause toxic effects in aquatic organisms. Wauer and Teien (2010) stated that gill tissue is a bioindicator for Al accumulation in fish. Al was found in the highest concentration in the gill tissue of *A. superciliosus* ($p<0.05$), and there was no statistical difference in other tissues ($p>0.05$). The most important reason for this may be the limited transport of aluminum between tissues as a result of binding to functional groups located both apically and within the lamellar epithelial cells on the gill surface (Exley et al., 1991). Muscle tissue Al level was reported as 0.83 µg g^{-1} dw (Marques et al., 2021) in *Scylorhinus canicula* sampled from the Atlantic Ocean and 1.34 mg kg-1 ww in *Mustelus mustelus* sampled from Langebaan Lagoon, South Africa (Bosch et al., 2016). Muscle Al level in *A. superciliosus* was found to be 5.67 µg g^{-1} dw in the present study. Our findings were higher than in previous studies.

In cartilaginous fish, tissue Pb level varies depending on the species. The muscle tissue Pb level was reported as 2.89 in *P. galuca,* 4.08 in *C. falciformis,* and 2.61 mg kg^{-1} ww in *A. pelagicus* (Álvaro-Berlanga et al., 2021). The muscle tissue Pb level of *A. superciliosus* was determined as 3.56 µg g^{-1} dw, and no statistical difference was found between the other tissues in terms of Pb level ($p>0.05$).

It has been determined that liver Cd levels are high in studies conducted with various shark species worldwide. In *P. glauca* sampled from Baja California Sur, Mexico, liver Cd level was

34.66 mg kg^{-1} ww (Barrera-Garcia et al., 2013), in *C. calciformis* 284.55 mg kg-1 ww (Terrazas-Lopez et al., 2016), 86.53 mg kg^{-1} ww in *A. pelagicus* (Lara et al., 2020) and 19.77 mg kg^{-1} ww in *Sphyrna zygaena* sampled from the Mediterranean (Storelli et al., 2003). In this study, the liver Cd level of *A. supercilosus* sampled from the Northeast Mediterranean was found to be 57.37 µg g^{-1} dw. High liver Cd levels in sharks may be related to their longevity, being top predators, and feeding preferences. One of the possible reasons for the high level of Cd in the liver is the synthesis of metallothionein and glutathione, which are low molecular weight, rich in cysteine, and function in metal binding, mainly in the liver.

Due to the functional properties of the liver, they can accumulate biologically required metals at high concentrations. Especially at the beginning of the developmental stage, Cu and Zn levels in the liver are quite high (Mull et al., 2012; Corsolini et al., 2014). Among these essential elements, Zn, besides its basic functions, has an important role in reducing Cd toxicity with its capacity to activate metal transcription factor (MTF-1), which stimulates metallothionein synthesis (Di Giulio & Meyer, 2008; Hahn & Hestermann, 2008). Barrera-García et al. (2013) emphasized that liver Zn level may be related to Cd level in *P. glauca*, for the reasons specified. The high level of *A. superciliosus* liver Zn and Cd in this study may also be due to the same mechanism. In this study, Cd was found the second-highest level in the gonad after the liver. Metals are actively transported to the gonad tissue during the reproductive period. In this study, the high level of Cd accumulation in the gonad tissue by *A. superciliosus* can be explained by the tendency to accumulate Cd at high levels in the gonad tissue due to its similarity to Ca to reach the individual's reproductive maturity.

Strontium was found at higher levels in the stomach, gonads, and kidneys of *A. superciliosus* than in other tissues in the present study. It is possible that this element, which is responsible for the mineralization of bone and cartilage tissue and invertebrate shells, may have been taken by food.

Cr and Mn were found at the lowest level among the metals examined in this study. *Rhincodon typus* has been reported as Cr 5.21 µg g^{-1} ww and Mn 4.45 µg g^{-1} ww in muscle tissue (Pancaldi et al., 2021). Muscle Cr and Mn levels in *A. superciliosus* were found to be lower than in previous studies. This may be related to the concentration of these metals in the environment, their ranks in the food chain, or their metabolism in the organism.

In conclusion, sharks are predators at the upper trophic level of the food chain and have a very long lifespan. The long periods of growth and reproductive maturity cause chronic accumulation of metals added to the environment through different sources in these species. The level of accumulation of metals taken into the body, mainly through food and water, cannot be adequately explained by regional pollution for species that make long migrations between oceans and seas, such as sharks. While migratory species may accumulate in certain concentrations in polluted areas, they may decrease tissue metal levels as a result of natural detoxification and depuration in areas far from metal effects. Therefore, tissue metal levels can be interpreted with nutritional preferences. In sharks that feed on benthic, benthopelagic fish, and invertebrate species, it is possible that the accumulation may be acquired mostly through contaminated prey. It is very important to monitor tissue metal concentrations in order to protect human health in these species, which are widely used in different industrial areas. *A. superciliosus* is a species with a limited number of individuals in the Mediterranean. The data obtained in this study is important in forming the species' first ecotoxicological report.

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Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

N.Ç. wrote the main manuscript, DA: Sampled the species and designed the research, BC: prepared table and figure.

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