The First Assessment on Metal Contamination in the Critically Endangered Sawback Angelshark (*Squatina Aculeata*) From North-Eastern Mediterranean

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Abstract

A Sawback angelshark (*Squatina aculeata*) classified as “Critically Endangered” Global Red List by the IUCN (2017) and CITES (2017) is one of the threatened elasmobranch groups and the increased pollution in the marine environment negatively impact angelshark species. Despite this, limited information exists about the effects of toxic chemical contamination in angelsharks. Therefore, present study was conducted to elucidate heavy metal accumulation in *Squatina aculeata* captured from the Iskenderun Bay, North-Eastern Mediterranean by electrochemical technique. Arsenic (As), Mercury (Hg), Copper (Cu), Iron (Fe), Zinc (Zn) and Mangan (Mn) were found by electrochemical analyzer. These results showed that Fe and Zn were the highest concentrations in all tissues of *S. aculeata* and, the accumulations of Hg, Fe, Zn and Mn were significantly different in the gill, liver and muscle tissues ($P < 0.05$). As and Hg concentrations exceeded maximum limit legalized by FAO (2003) and UNEP (2008). This results confirmed that the metal contamination in North-Eastern Mediterranean area may pose a potential threat to the sustainability of *S. aculeata* in marine ecosystem

Introduction

The environment pollution by trace metals is an important global problem. Toxic metals reason damaging impacts to terrestrial and aquatic animals. Both natural and anthropogenic events generally generate heavy metals that contaminate the aquatic habitats through sewage canals, river loads and atmospheric depositions (Turan et al. 2020).

Sharks are susceptible to heavy metals due to the slow elimination and large capacity to incorporate metals and, accumulate trace metals in their tissues because of their diet as they feed on many trophic organisms (Lopez et al. 2013). Heavy metal accumulation in each aquatic organism show variation according to its diet and habit. Hereafter, sharks as a top predator feeding on a large number of organisms would be having in a higher level of heavy metal accumulation into their body. Furthermore, their widespread distribution and significance to the ecosystem makes them ideal sentinel organisms for biomonitoring the marine pollution (Adel et al. 2018; Lozano-Bilbao et al. 2018; Kim et al. 2019). Earlier researches have reported the capacity of elasmobranch species to accumulate chemical pollutants with dense concentration (Lopez et al. 2013, Adel et al. 2018; Kim et al. 2019) which is particularly important for biota inhabiting in contaminated marine areas.

A notable number of elasmobranch species (about 25%) are currently regarded as threatened on the authority of International Union for Conservation of Nature (IUCN). Angel sharks (Squatinidae) are among the critically endangered fish species in marine ecosystem and actions goaling to these species conservation has been recommended in lately times (Martins et al. 2020). Populations of the three Critically Endangered angel sharks have been severely depleted in the Mediterranean Sea. Sawback angelshark (*S. aculeata*) is classified as “Critically Endangered” Global Red List by IUCN (2017) and CITES (2017). Sawback angelshark is an Atlanto-Mediterranean coastal shark live in continental shelf and tropical eastern Atlantic, and is extremely rare in the east and west parts of the Mediterranean

(Ergenler et al. 2020). Although the occurrence of the species in the Turkish marine waters has been reported before (Basusta, 2002; Filiz et al. 2005; Ergüden and Bayhan 2015), the new information on the rare occurrence of a male specimen of Sawback angelshark *S. aculeata* from the northeastern Mediterranean coast of Turkey was made by our laboratory team on February 19, 2019 (Ergenler et al. 2020). Hereby, the preliminary data on metal contamination in threatened sawback angelsharks from the northeastern Mediterranean will be available with this research. This species inhabits coastal areas of the northeastern Mediterranean coast of Turkey regions under great anthropogenic impact. It has been often reported that the Mediterranean Sea is at high toxicological risk (Storelli et al. 2011), as a result of specific hydrographical features and actual density of the anthropological effect. Especially Iskenderun bay is highly exposed to heavy metal accumulation impact due to agricultural, industrial and urban wastes. This bay has a major input of contaminants from maritime, domestic and industrial sources (Polat et al. 2018). Nevertheless, trace metal concentrations in shark's species especially angelsharks are not well documented either in the Mediterranean area or in other marine regions, and the few studies concentrate on different angel shark species (Lozano-Bilbao et al. 2018; Martins et al. 2020). Ellis et al. (2020) reported that there is no indication as to whether contaminants have effected on angel sharks at their populations, and more research on the possible effects of environment degradation and water quality are needed for natural areas of angel shark that occur in dense industrial areas.

Recent investigations have been focused on electrochemical approaches in the field of heavy metal ion detection. Electrochemical method is simple procedure and suitable to manufacture on minor circuits in the form of portable instrument for monitoring of polluted examples (Bansod et al. 2017). It is central issue to have a facile and inexpensive technique in order to selectively and sensitively determine toxic chemical pollutants. However, there exist few studies about these techniques in the monitoring of environmental (Pujol et al. 2014; Qi et al. 2017).

With this background, this study aims to elucidate heavy metal accumulation by electrochemical techniques in critically endangered Sawback angelsharks (*Squatina aculeata*) from the Iskenderun Bay, North-Eastern Mediterranean Coast of Turkey.

Material And Methods

Sampling area and Procedure

A male specimen of the Sawback angelshark (*Squatina aculeata* Cuvier, 1829) commercially trawled from the Konacik coast (36°36'050"N, 35°80'599"E), Iskenderun Bay, North-Eastern Mediterranean Coast of Turkey at a depth of 47 m on 19 February 2019 were collected from fishermen (Figure. 1).

This species was transported to the laboratory. Morphometric measurements were carried out to the nearest 0.1 mm by a caliper. The specimen was identified as *S. aculeata* with the diagnostic characteristics described by Compagno (1984) and Serena (2005). Measurements such as total body length, standard length and wet body weight of sampling shark (mean $\pm$ SD) were found as 1170 mm,

1020 mm and 13500 g, respectively. The epaxial muscles were sampled at the level of the first dorsal fin. Liver and gill tissues were also dissected with the aid of a scalpel with stainless steel blade, and samples were stored in plastic bags at -18°C until analyses.

Trace Metal Analysis

Briefly, the trace metal determination in the muscle and liver tissue was performed on a wet weight basis through an acid digestion adapted from AOAC Official Method 999.10 (2002), where a mixture of 10 mL of nitric acid (HNO_3), 0.25 mL of hydrogen peroxide (H_2O_2) and 1 g of the sample (taken with a scalpel from the central part of the muscle) kept in a bath-water at 60°C for one hour to perform acid digestion, after which the samples were allowed to cool at room temperature. Later, the resulting solution was filtered and increased to 100 mL with distilled and deionized water. To avoid contamination, all glassware used was previously washed with 10% (v/v) HNO_3 solution according to the procedure recommended by AOAC Official Method 999.10 (2002). After the acid digestion, the metals concentration was determined by electrochemical method in triplicate. At the end of all the analyses, the values of heavy metal content of the samples were measured as $\mu\text{g g}^{-1}$ wet weight (ww) respectively by mathematical methods.

Evaluation of Trace Metals by Electrochemical Method

Arsenic (As), Mercury (Hg), Copper (Cu), Iron (Fe), Zinc (Zn) and Mangan (Mn) were determined by electrochemical measurements, with samples being previously acid digested. Electrochemical measurements were carried out by Gamry Reference 600 work-station (Gamry, USA) and BAS-100B electrochemical analyzer. Triple electrode system including glassy carbon electrode as indicator electrode, $\text{Ag}/\text{AgCl}/\text{KCl}_{(\text{sat})}$ as reference electrode and platinum wire as auxiliary electrode was utilized for all electrochemical measurements. The cleaning protocol of glassy carbon electrodes were performed according to our previous paper (Yola et al. 2012). After the supporting electrolyte (pH 7.4, phosphate buffer, 3.0 mL) put into the electrochemical cell, the standard solutions (As, Hg, Cu, Fe, Zn and Mn) were added into phosphate buffer by micropipette. This process was separately carried out for each metal ion. Before the measurements, the sample solutions were passed through argon gas (99.999 %) during 15 min. Then, the electrochemical potential scan was applied to electrochemical cell including trace metal solutions in range from -1.00 to 0.0 V. After the recording of electrochemical voltammograms based on at pulse height of 5 mV, square wave amplitude of 50 mV and frequency of 50 Hz, the peak signals (μA) attributing to trace metal concentrations were evaluated for trace metal detections.

Statistical Analysis

Normality (Shapiro–Wilk test) and homogeneity (Levene analyze test) tests were performed on the data before the statistical analyses. One-way analysis of variance (ANOVA) was used for statistical evaluations and, Duncan test was used to compare the means of the trace metal concentrations in different tissues with P-values below 0.05 as significant (Zar 1996).

Results

Mean values for trace metals measured in the different tissues of Sawback angelshark are illustrated in Table 1. The mean concentrations of Fe and Zn were the highest concentrations in gill, liver and muscle tissues of *S. aculeata* (Fig. 2). The mean concentrations of Cu and Mn were the lowest concentrations in all tissues of *S. aculeata*.

The concentrations of all the heavy metals were significantly different in the gill, liver and tissues ($P < 0.005$) except As and Cu in *S. aculeata* (Table 1). Hg concentrations in muscle of *S. aculeata* was actual high ($19.9942 \pm 1.6116 \mu\text{g g}^{-1}$) from other tissues and, statistically significant between tissues of the Sawback angelshark (Table 1)

Table 1
Average concentrations ($\mu\text{g g}^{-1}$ wet weight) of trace metals in gill, liver and muscle tissues of *Squatina aculeata*.

<i>TISSUES</i>			
	<i>GILL</i>	<i>LIVER</i>	<i>MUSCLE</i>
As	10.1595 ± 0.4240^a	8.8507 ± 1.9755^a	7.5416 ± 0.6548^a
Hg	0.1546 ± 0.0384^a	0.4022 ± 0.0424^a	19.9942 ± 1.6116^b
Cu	0.0242 ± 0.0049^a	0.0275 ± 0.0034^a	0.0101 ± 0.0086^a
Fe	82.3220 ± 9.1082^c	50.5782 ± 9.0230^b	26.0000 ± 8.2491^a
Zn	23.1071 ± 3.0512^c	17.8102 ± 1.8952^b	10.8575 ± 1.7281^a
Mn	0.0762 ± 0.0098^b	0.0452 ± 0.0065^a	0.0351 ± 0.0102^a

*Results are expressed as mean $\pm$ S.D. (Standard deviation). Indicate significance level between the trace metal concentrations in different tissues of *S. aculeata* with different superscripts in each line indicate significant differences. ($P < 0.05$ for Fe, Zn and Mn and $P < 0.001$ for Hg).

In general, the concentration orders of heavy metals in the tissues were found to be gill > liver > muscle for the studied sawback angelshark, except for Hg (Table 3). The concentrations order of Hg was found to be muscle > liver > gill. Among the gill and liver tissue samples of *S. aculeata*, Fe and Zn were detected as highest concentrations followed by As, Hg, Mn and Cu; Among the muscle tissue of *S. aculeata*, Fe and Zn were detected as highest followed by Hg, As, Mn and Cu (Table 1; Fig. 2).

Discussion

The present study provides firstly, the preliminary data on metal contamination in the Critically endangered Sawback angelshark from the northeastern Mediterranean by electrochemical technique. Angel sharks often occupy coastal waters and thus exposed to different contaminants, the high trophic

level of this environment could increase the accumulation of such pollutants (Ellis et al., 2020). Therefore, the use of shark species as bio indicators has been underlined by numerous investigators for environmental monitoring studies (Adel et al. 2018; Lozano-Bilbao et al. 2018; Kim et al. 2019). The present result of trace metal concentrations is compared with those of other researchers who investigated the angelsharks from the Mediterranean Sea and other part of the world as shown at Table 2.

Table 2. Comparison of trace metal concentrations ($\mu\text{g g}^{-1}$ ww) in angelsharks from the North-Eastern Mediterranean Coast of Turkey with the other locations worldwide.

Species	Tissues	Cd	As	Hg	Cu	Fe	Zn	Mn	Location	Referans
<i>S. aculeate</i> (n=1)	Gill	-	10.15±0.42	0.15±0.03	0.02±0.00	82.32±9.10	23.10±3.05	0.07±0.00	North-Eastern Mediterranean	This research
<i>S. aculeate</i> (n=1)	Liver	-	8.85±1.97	0.40±0.04	0.02±0.00	50.57±9.02	17.81±1.89	0.04±0.00	North-Eastern Mediterranean	This research
<i>S. aculeate</i> (n=1)	Muscle	-	7.54±0.65	19.99±1.61	0.01±0.00	26.00±8.24	10.85±1.72	0.03±0.01	North-Eastern Mediterranean	This research
<i>S. squatina</i> (n = 1)	Liver	-	-	0.07	-	-	-	-	Cardigan Bay (UK)	Morris <i>et al.</i> 1989
<i>S. dumeril</i> (n = 1)	Muscle	-	-	0.08	-	-	-	-	North-western Atlantic	Greig <i>et al.</i> 1975
<i>S. argentina</i> (n = 2)	Muscle	-	-	0.304	-	-	-	-	Southern Brazilian	Kütter <i>et al.</i> 2009
<i>S. californica</i> (n=18)	Muscle	-	-	0.47±0.15	-	-	-	-	Tomales Bay	Gassel <i>et al.</i> 2004
<i>S.guggenheim</i> (n=9)	Liver	0.22 ±0.21	-	0.02 ± 0.01	2.79 ± 2.08	61.11 ± 23.29	-	-	Southeastern Brazil	Martins <i>et al.</i> 2020
<i>S.guggenheim</i> (n=9)	Muscle	0.16 ±0.04	-	0.02 ± 0.05	1.02 ± 0.66	42.20 ± 11.98	-	-	Southeastern Brazil	Martins <i>et al.</i> 2020

As seen in Table 2, the restricted data is available on the levels of metal contamination in angel sharks. Especially, no study on metal accumulations of Critically endangered Sawback angelshark was found in the literature searches. Ellis et al. (2020) reported that few published studies on organic and inorganic (metals) contaminants in different *Squatina* species have been available. The high concentrations of mercury (Hg, $0.24 \pm 0.27 \mu\text{g g}^{-1}$) in the muscle of *S. californica* from the Gulf of Mexico was reported by Escobar-Sánchez et al. (2016). Morris et al. (1989) reported on the accumulation of Hg in the liver of *S. squatina* (n = 1) from Cardigan Bay (UK), which was $0.07 \mu\text{g g}^{-1}$. The concentrations of Hg are not found at levels that would exceed most current guidelines for seafood products in the former studies on the other angelshark species, *S. californica* and *S. squatina* (Morris et al. 1989; Escobar-Sánchez et al. 2016). Ellis et al. (2020) reported that there is no report on the contaminants that have impacted on angel sharks, and further studies are required on the potential impacts of habitat degradation and water quality for inhabiting areas of angel shark, especially industrialized areas. Therefore, the present finding with exceeded legalized limit of Hg concentrations are very valuable for the conservation and sustainability of *S. aculeata* as management consideration.

Human Health Risk Assessment

The regulatory limits of As, Hg, Cu, Fe, Zn and Mn concentrations are directly compared in Table 5. Concentrations of Cu, Fe, Zn and Mn as found in this study did not constitute a risk factor for human health being below the permissible limits for human consumption reported by FAO (2003) and UNEP (2008).

Table 3
Regulatory maximum limits of metals in sawback angel shark muscle.

	Metal concentration comparison with regulatory maximum limits			
	As	Hg	Cu	Zn
Regulatory maximum limits (As, Hg: mg/kg) (Cu and Zn: mg/day)	3 ^a	0.4-1 ^{bc}	5 ^d	25 ^d
<i>Squatina aculeata</i>	7.5416 ± 0.6548	19.9942 ± 1.6116	0.0101 ± 0.0086	0.1195 ± 0.0216

The regulatory maximum limits and mean heavy metal concentrations of As, Hg, Fe, Cu, Zn and Mn are shown in mg/kg. For Cu and Zn, the regulatory maximum limits and mean daily heavy metal intake amounts from shark meat are shown in mg/day. The regulatory limit of Fe and Mn has not been specified. Red box: mean value exceeding the regulatory maximum limit. Green box: every data is under the regulatory maximum limit. a: DOH (2004); b: FAO (2003); c: UNEP (2008) d: SCF (2006).

Average total arsenic contents in *S. aculeata* was 7.5416 ± 0.6548 µg g⁻¹ wet weight, which exceeded maximum limit legalized for any kind of food. Similarly, Total mercury average content in *S. aculeata* was 19.9942 ± 1.6116 µg g⁻¹ (wet weight), which exceeded the proposed limit value which is 0.2-2 µg g⁻¹, confirming that the consumption of *S. aculeata* is a high risk threatening the health of consumer (Table 3). On the other hand, several researchers reported that the concentrations of Hg in *S. californica*, *S. squatina*, *S. dumeril* and *S. guggenheim* did not constitute a risk factor for human health (Greig et al. 1975; Morris et al. 1989; Gassel et al. 2004; Martins et al. 2020). Nonetheless, the differences in the habitat, size, age, feeding nature, physiological tolerance and metabolic reactions of shark species complicate the comparison and interpretation of the metal concentrations in the same types of tissues. The detected absorptions of trace metals in different tissues of shark species can be attributed to the exposure time and the elemental concentration in their present environment (Arulkumar et al. 2017). The longevity and slow growth rates of shark species as biological traits in conjunction with their high trophic status, agreed upon to explain the high levels of metals encountered (Lozano-Bilbao et al. 2018; Kim et al. 2019).

Recently, much attention has been given on electrochemical detection methods which are inexpensive, highly sensitive and easily adaptable for in situ assessment with short analytical periods in the field of detection of heavy metal ions (Pujol et al. 2014; Qi et al. 2017). Electrochemical techniques are relatively

more economic, user friendly, reliable and suitable for in-field applications. The electrochemical methods allow the simple processes and well suited to fabricate on small circuits in the form of portable devices for in-situ observing of contaminated samples. The electrochemical methods are also quick in terms of short analytical time in comparison to the other spectroscopic methods, authorizing on-line monitoring of environment (Bansod et al. 2017). Nevertheless, there are few studies about its use in the analysis of environmental and fish species. In this study, we reported that it can be used successfully the electrochemical technique in determination of trace metal concentrations in angelshark species.

Conclusion

The present study revealed first data on metal contamination in the critically endangered Sawback angelshark from the northeastern Mediterranean by electrochemical technique. As and Hg concentrations exceeded maximum limit legalized by FAO (2003) and UNEP (2008). This results confirmed that the metal contamination in North-Eastern Mediterranean area may pose a potential threat to the sustainability of *S. aculeata* in marine ecosystem and the concentrations detected is the above the safety limits for angelshark that should be taken into consideration regarding human consumption. Our data also reveal that the electrochemical technique can be used successfully in determination of trace metal concentrations in sawback angel shark.

Declarations

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Conflict of Interest: The authors decelerate that they have no conflict of interests.

Ethical Approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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Figures

Figure 1

Map showing capturing site (black dot) of *Squatina aculeata* in the Iskenderun Bay. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Mean concentrations of metals in different tissues of *Squatina aculeata* from the North-Eastern Mediterranean Coast of Turkey.