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First Report on the Elemental Composition of the Largest Bony Fishes in the World, the Ocean Sunfish (*Mola mola*) from the Mediterranean Sea

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Abstract

In this study, element levels (Cr, Mn, Fe, Co, Ni, Cu, Zn, Al, V, As, Ba, Sr, K, Pb) in the liver, gill, muscle, and stomach tissues of Ocean Sunfish (*Mola mola*) caught from Mersin Bay in 2018 were determined, and the first report on the elemental composition of M. mola from the Mediterranean sea presented. Spectrophotometric methods were used in the determination of tissue element levels. Metal analysis of the tissues was performed on the ICP-MS device. The reference material IAEA-436 was used to follow the quality of the analytical process. Metal accumulation in *M. mola* tissues was determined as K> Fe> Zn>Sr> Cu> As> Al> V>Mn> Ni> Ba> Cr>Pb> Co. A statistical difference was found between tissues in terms of metal levels. It was determined that Fe and Cu were higher in the liver, Mn, Zn, Sr in gill, Al, Pb, Cr in muscle tissue. There were no statistical differences between the levels of As, V, and Ba detected in gill and muscle tissues. The K levels were similar in all tissues except the liver.

Keywords:
Mola mola, Mersin Bay, heavy metal, level, tissues
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Introduction

Mola mola is a species belonging to the Molidae family of Tetraodontiformes. It is known that they are the most massive bony fish, and they give the most eggs. It reported that the maximum total length of this species is 333 cm (Claro, 1994), and the weight is 2.3 tons (Roach, 2003). They are spotted in the subtropical waters of the Pacific, the Atlantic Ocean, and the Mediterranean Sea, in addition to are pelagic fish, and lives at a depth of 30-480 m (Tortonese, 1990; Allen & Erdmann, 2012). They feed on fish, mollusks, zooplankton, jellyfish, shellfish, and starfish (Clemens & Wilby, 1961; Scott & Scott, 1988; Kuiter & Tonozuka, 2001). California sea lions consume M.

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mola cubs in Monterey Bay (Powell, 2001). In general, Tetradontiformes species are not preferred for consumption by humans because of their toxins.

Metals are the major inorganic pollutant for the aquatic environment for centuries. Some metals are abundant in the earth's crust, such as As, Al, Sr, etc., and take part in the aquatic environment through natural processes. However, the metals' ambient concentrations increase more under the influence of anthropogenic activities and can reach toxic levels for marine organisms (Duruibe et al., 2007; Sokolova & Lanning, 2008). Metal accumulation in bodies can cause metabolic and physiological damages. Metals are taken from the environment through the gills and food, accumulate in tissues, and are carried to the upper trophic levels through the food web (Jezierska & Witeska, 2006; Shah et al., 2009; Lucia et al., 2014).

The primary uptake route of metals is Ca channels, which are located in gills interacting directly with the contaminated seawater. The liver is a metabolically active tissue, which has a vital role in detoxifying and storing toxic substances such as metals (Vinodhini & Narayanan, 2008). The stomach is a part of the digesting system. The low pH secretions secreted by the cells and glands in the inner stomach wall provide the physical and chemical breakdown of nutrients. Muscle tissues do not play an active role in the binding of metals. However, if active metabolic tissues exceed the metal carrying capacity, they are delivered to the muscle tissue for storage (Honda et al., 1985). For this reason, the investigation of metal accumulation in tissues provides information about the environmental concentration of metal and its level in the food web.

Metals may be categorized according to their functions in biological systems. While the daily requirement of macro minerals such as Ca, Mg, Na, and K is more than 100 mg, the necessity of micro minerals such as Fe, Cu, Zn, Se is less than 100 mg per day. The toxic elements such as Hg, Cd, Pb, As are not required, even at low levels in biological systems. All three groups are known to cause toxic effects in animals on a specific level (Larsson et al., 1985). However, insufficient intake of macro and microelements provided by nutrients and water in animals also causes functional disorders.

In this study, it was aimed to determine the levels of oligo, trace, and toxic elements in the liver, gill, muscle, and stomach of *M. mola* and is also aimed to report the first elemental composition record of *M. mola* from the Mediterranean Sea.

Materials and Method

One female individual of *M. mola* was caught by a trammel net on 5 June 2018 in Mersin Bay (Bozyazı Coast). Its total size and its weight are measured 122 cm, 80 kg, respectively. The species sampling point on Turkey's Mediterranean Sea coast is presented on the map (Figure 1). Coordinates: 36°05'15.2"N-32°58'28.7"E. The photograph of the specimen is shown in Figure 2.

Figure 1. The black point indicates the location where the specimen caught

Figure 2. The specimen of *M. mola* caught from Mersin Bay (Bozyazı coast)

Metal analysis

The samples (0.1 g dry weight) used for metal analysis were dried at 105°C to reach constant masses. Then concentrated nitric acid (4 mL, Merck, Darmstadt, Germany) and perchloric acid (2 mL, Merck, Darmstadt, Germany) were added to the samples, and they were put on a hot plate set to 150°C until all tissues were dissolved (Canli & Atli 2003).

An inductively coupled plasma mass spectrometer (ICP-MS, Agilent, 7500ce Model, Japan) was used to determine metals. 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; nebuliser pump (rps), 0.1; extract lens (V), 1.5. The levels of Cr, Mn, Fe, Co, Ni, Cu, Zn, Al, V, As, Ba, Sr, K, Pb in samples detected as µg metal g-1 dry weight. High Purity Multi-Standard (Charleston, SC 29423) was used for the determination of the metal analyses. Standard solutions for calibration curves prepared by dilutions of the trace elements and potentially toxic metals. Solutions have prepared for the toxic metals that had a content of lead, cadmium, arsenic, and chromium in the range of 1-50 ppb (0.001 to 0.050 mg/L), for the trace elements had a content of copper, iron, and zinc in the range of 1-50 ppm (1 to 50 mg/L). IAEA-436 reference material was used to follow the quality of the analytical process. IAEA-436 was analyzed for all elements except for V, Ba, K, Pb. 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 ($\mu g\ g^{-1}$)	**Certified value $\bar{X} \pm S_x$**	**95% Confidence interval**	**Observed value $\bar{X} \pm S_x$**
Al	3.06±0.42	2.68 – 3.44	3.385±0.325
As	1.98±0.17	1.91 – 2.06	1.997±0.013
Co	0.042±0.006	0.039 – 0.045	0.043±0.001
Cr	0.194±0.058	0.168 – 0.219	0.199±0.005
Cu	1.73±0.19	1.66 – 1.79	1.768±0.038
Fe	89.3±4.2	87.8 – 90.9	90.17±5.867
Mn	0.238±0.042	0.218 – 0.257	0.241±0.003
Ni	0.069±0.041	0.040 – 0.099	0.062±0.008
Sr	0.564±0.062	0.523 – 0.606	0.528±0.037
Zn	19.0±1.3	18.6 – 19.4	18.657±0.343

Statistical analyses

Before the analyses, all data were checked for outliers, and Levene's homogeneity of variance also applied for variance homogeneity. Statistical analysis of data was carried out with the IBM SPSS STATISTICS 22.0 statistical program. ANOVA (Analysis of Variance) was used to evaluate the differences in metal levels of the species.

Results

In this study, Cr, Mn, Fe, Co, Ni, Cu, Zn, Al, V, As, Ba, Sr, K, Pb levels in liver, gill, muscle, and stomach tissues of *M. mola* was determined (Table 2). Metal accumulation in tissues of *M. mola* was determined as K> Fe> Zn>Sr> Cu> As> Al> V>Mn> Ni> Ba> Cr>Pb> Co. It was found that Fe and Cu were higher in the liver, Mn, Zn, Sr in gill, Al, Pb, Cr in muscle tissue. The As, V, and Ba levels detected in gill and muscle and K levels in all tissues except the liver were similar ($p>0.05$). Co and Ni showed no statistical difference in the examined tissues ($p>0.05$).

Our findings indicate that toxic elements were high in gill and muscle tissues, trace elements in liver tissue except for Zn and Mn. K, an oligo-element, was more accumulate in gill, muscle, and stomach than liver tissue ($p<0.05$). The level of toxic metals in different tissues of *M. mola* was found as Sr, As, Al, Ba, and Pb from the highest to the lowest level, respectively (Table 2).

Table 2. Metal levels of some tissues of *M. mola.*

Analyte ($\mu g\ g^{-1}$)	**Liver** $\bar{X} \pm S_x$ *	**Gill** $\bar{X} \pm S_x$ *	**Muscle** $\bar{X} \pm S_x$ *	**Stomach** $\bar{X} \pm S_x$ *
Cr	0.137±0.010^{a}	0.263±0.023^{b}	0.349±0.041^{c}	0.123±0.009^{a}
Mn	0.274±0.017^{a}	2.611±0.100^{c}	0.549±0.055^{b}	0.255±0.018^{a}
Fe	342.51±19.957^{c}	231.1±13.096^{b}	192.05±24.222^{b}	77.892±7.098^{a}
Co	0.072±0.004^{a}	0.059±0.002^{a}	0.028±0.002^{a}	0.131±0.114^{a}
Ni	0.048±0.004^{a}	0.127±0.016^{a}	0.172±0.022^{a}	1.098±1.049^{a}
Cu	52.295±3.44^{b}	3.617±0.200^{a}	5.548±1.300^{a}	2.411±0.305^{a}
Zn	43.314±1.958^{a}	136.61±4.440^{b}	51.869±5.591^{a}	48.394±1.456^{a}
Al	1.424±0.139^{a}	4.269±0.570^{c}	6.364±0.524^{d}	2.964±0.324^{b}
V	0.855±0.104^{a}	2.795±0.241^{b}	3.519±0.517^{b}	0.970±0.107^{a}
As	6.479±0.628^{b}	11.120±0.551^{c}	10.473±1.325^{c}	3.910±0.113^{a}
Ba	0.207±0.036^{a}	0.753±0.579^{b}	0.635±0.076^{b}	0.286±0.052^{a}
Sr	0.766±0.057^{a}	61.856±3.282^{b}	3.586±0.420^{a}	1.734±0.224^{a}
K	651.49±36.255^{a}	1789.3±99.418^{b}	1790.06±221.868^{b}	1746.3±187.674^{b}
Pb	0.027±0.003^{a}	0.093±0.195^{b}	0.141±0.010^{c}	0.063±0.020^{ab}

* Duncan; The letters a, b, and c indicate the statistical differences of tissues. The statistical difference was $p<0.05$.

$\bar{X} \pm S_x$: mean ± Standard error

Discussion

M. mola is the biggest teleost fishes migrated long distance from Pacific, Atlantic to Mediterranean waters. The metal level in tissues of *M. mola* can be affected by the environmental concentrations and can also be carried by their prey. In the present study, metal levels in tissues of *M. mola* were determined as K> Fe> Zn>Sr> Cu> As> Al> V>Mn> Ni> Ba> Cr>Pb> Co. The highest concentrations of Zn and Fe in teleost fish sampled from the Atlantic and the Mediterranean Sea have been reported to be determined (Romeo et al., 1999; Storelli et al., 2006; Abdallah, 2008; Türkmen et al. 2008; Uysal et al. 2008; Yipel & Yorsan 2014). The Zn level in fish species sampled from Mauritania Coast was 507 µg g-1dw (Romeo et al., 1999), from Tunisia was 293 µg g-1dw (Zohra & Habib 2016), from Egypt, was 31.5 µg g-1dw (Shreadah et al., 2015) and 57 µg g-1dw (Abdallah, 2008), from Italy was 38.52 µg g-1 ww (Perugini et al., 2014), from Antalya bay 339.76 µg g-1 ww (Uysal et al., 2008), from Turkish Seas was 11.2 µg g-1 ww (Türkmen et al., 2008). Iron level in fish species sampled from Tunus was found 157.83 µg g-1dw (Zohra & Habib 2016), from Antalya bay, was 117.73 µg g-1 ww (Uysal et al., 2008), from Turkish seas was 40.1 µg g-1 dw (Türkmen et al., 2008). The distinction among metal concentrations in fish species sampled in the Atlantic and the Mediterranean Sea may vary depending on the ambient concentration, sampling time, or species. The metal levels in the tissues of M. mola may indicate the transport of a significant part of these metals by the food web. Another reason why these metals have higher accumulation than other metals is that they have a biological role in animals. Iron participates in hemoglobin and myoglobin structure in animals and has a crucial role in oxygen transport (Hevesy et al., 1964). Zinc is found in the formation of more than 200 enzymes and has an active role in protein synthesis, digestion, nucleic acid synthesis, carbohydrate metabolism, oxygen transport (Hodson, 1988). In the previous study, Fe and Zn levels were found in liver tissues of *M. mola* sampled from the Florida coast were 2311 µg g-1ww and 47 µg g-1(ww), respectively (Perrault et al., 2014). The liver Fe, Zn levels were more than our results (342.51 µg g-1dw, 43.314 µg g-1 dw). Fe levels found in tissues as liver>gill>muscle>stomach while Zn as gill>muscle>stomach>liver. The distinction between Fe and Zn levels in tissues can be explained by more than one result. Fe is the most abundant essential element in vertebrates besides is used relatively few; therefore, over amount is stored in the liver. Zn is the second most plentiful after Fe, though it is used in the body more diversely (Hogstrand, 2012). The excess amount of zinc can be excreted by gill, which is being the major excretory route in fish (Hardy et al., 1987)

K was found at a higher level in the gill, muscle, and stomach than the liver tissue of *M. mola* in the present study. The liver K level was found 651.49 µg g-1dw in the study. Perrault et al. (2014) reported that the liver K level of M. mola was 1350 µg g-1ww. The reason indicates that an excess amount of K in the liver may be sent to the gill tissue for excretion. Mitochondria-rich cells in gill epithelium in teleost fish have a homeostatic mechanism that provides ion regulation and acid-base balance (Furukawa et al., 2012). The muscle K level was found at 1790.06 µg g-1dw in the present study. The muscle K level in *Serranus cabrilla* sampled from Yeşilovacık, Mersin Bay, was 19042.19 µg g-1dw (Ayas et al., 2018). The stomach and muscle tissues higher K level may be due to the biological functions of potassium.

Sr is one of the most abundant metals in nature, and its compounds are soluble in water as a result of chemical reactions. Water-soluble forms are more toxic to organisms. Due to its similarity to Ca, it can be instantly accumulated by aquatic organisms (Pasqualetti et al., 2013). In this study, the gill tissue level of Sr was found to be relatively high. Since the number of studies on Sr accumulation in biota and ambient concentration is deficient, it could not be compared with the literature.

The Cu level in liver tissue (52.29 µg g-1d.w) of *M. mola* was found high than previous research (2.8 µg Cu g-1 ww) by Perrault et al. (2014) in liver tissue of M. mola. The muscle Cu levels determined in teleost fish species sampled from Iskenderun Gulf (5.36 µg g-1dw) by Yılmaz (2003), from Antalya Gulf (5.15 µg g-1dw) by Yipel & Yorsan (2014), and from Turkish Seas (6.48 µg g-1dw) by Türkmen et al. (2008) were similar with each other. Storelli et al. (2006) reported that the Cu liver level in *Mugil cephalus, Mugil capito,* and *Mugil labrosus* sampled from the Mediterranean Sea was 177.78 µg g-1ww. Cu acts as a cofactor by participating in more than 30 enzymes synthesized by the liver (Kamunde et al., 2002), which may cause a high level in the liver detected in this study.

Aquatic environments are rich in arsenic, which is both abundant in the ground and soluble in water easily (Sharma & Sohn 2009). Arsenic accumulated in the gill and muscle tissue of *M. mola* more than the liver and stomach in this study. Arsenic level in muscle tissue of *Engraulis encrasicholus*, *Scomber japonicus*, *Mullus barbatus*, and *Spicara smaris* sampled from the Adriatic Sea were 54.8, 70.90, 36.4 and 54.6 µg g-1 (Bilandzˇic' et al., 2011), in *M. barbatus* sampled from Italy was 59.9 µg g-1 ww (Perugini et al., 2014). However, Storelli et al. (2005) notified that the As level in muscle tissues of *Xiphias gladius* (7.44 µg g-1ww) and *Thunnus thynnus* (5.01 µg g-1ww) were low. In this study, the gill tissue level of arsenic can be explained by direct interaction with the environment, and muscle tissue level can be explained by arsenic storage in the form of organoarsenical compound.

The Al level was found in tissues of *M. mola* as muscle>gill>stomach>liver in the present study. Türkmen et al. (2005) reported that the Al level in different fish species sampled from Iskenderun Bay was 0.02-5.41 µg g-1dw. The Al level in *Spondylus spinosus* sampled from the uncontaminated and contaminated area of Iskenderun Bay was 78.8-66 and 113 µg g-1dw, respectively (Türkmen & Türkmen 2005). Al concentration was reported higher in invertebrates than fish tissues by Türkmen & Türkmen (2005). Al is abundant in earth crust (Brown et al., 2010), and no biological function in animals is known. The high level of aluminum in animals' tissues, which increases in the environment due to natural or anthropogenic sources, can be explained by chronic accumulation.

V accumulated a low level in the gill and muscle tissue of *M. mola* in this study. Specific information about the biological function of vanadium in animals is limited. It is known to be toxic to animals. Nevertheless, it has been reported to have a decisive role in controlling the development of diseases such as cancer and diabetes (Mukherjee et al., 2004).

Manganese level in gill tissue (2.611µg g-1 dw) was found higher than other tissues of *M. mola* in the present study. The liver Mn level in *M. mola* (0.274 µg g-1dw) was found lower than the previous research (0.85 µg g-1ww) (Perrault et al., 2014). Mn is the most critical antioxidant for animals and plays an essential role in protein synthesis, digestion, and energy production (Aliko et al., 2018).

The Cr level in the liver tissue of *M. mola* was determined as 0.137µg g-1dw that was similar to those obtained from the previous study (<0.2 µg g-1) by Perrault et al. (2014). The muscle Cr levels in different fish species were reported as 1.40 µg g-1 ww in Antalya bay (Uysal et al. 2008), 1.75 µg g-1dw in Turkish Seas (Türkmen et al. 2008), 3.22 µg g-1ww in Iskenderun bay (Yılmaz, 2003), 0.72 µg g-1dw in Sicilian (Copat et al. 2012). Cr level was found lower in cephalopods (0.40 µg g-1dw) sampled from the Mediterranean Sea (Storelli 2009) and in *Patella caerulea* (0.47-0.97 µg g-1ww) sampled from the Ionian Sea, Italy (Storelli & Marcotrigiano 2005). The maximum Cr level was reported as 20.1 µg g-1dw in *Boops boops* sampled from Egypt (Abdallah, 2008).

Co and Ni showed no statistical differences among the tissues of *M. mola* in this study. The liver Co level of *M. mola* was lower than (0.07 µg g-1dw) those obtained from the previous study (2.6 µg g-1 ww) by Perrault et al. (2014). Co level in tissues of Sard*inella aurita*, *Pagellus erthrynus*, *Balistes capriscus*, *Trachurus trachurus*, *Synodus saurus*, and *Dactylopterus volitans* sampled from Libya was informed to be higher than the levels (5.187 µg g-1) determined by World Health Organization (Khalifa et al., 2010). The Co levels detected in different fish species sampled from Turkish Seas and Antalya Gulf were 0.41 µg g-1 and 3.29 µg g-1 ww, respectively (Türkmen et al., 2008). Ni level in cephalopods sampled from the Mediterranean Sea was detected 1.13 µg g-1 ww by Storelli (2009). Co and Ni have a similar role in animals. They support the formation of vitamin B12 (Blust, 2012).

The lowest Ba and Pb levels are toxic elements for animals (Martinez et al., 2004; Hoskote et al., 2008). Both of them were found low levels in the tissues of *M. mola* in the present study. Numerous studies noticed that Pb level in teleost fish sampled from Turkish Seas was 0.86 µg g-1 (Turkmen et al., 2008), from Antalya Gulf was 0.22-0.29 µg g-1dw (Yipel & Yorsan 2014), from Sicilia was 0.32 µg g-1 (Copat et al., 2012), from the Adriatic Sea was 0.24-0.46 µg g-1 (Bilandzˇic' et al., 2011) and from the Mediterranean Sea was 0.39 µg g-1ww (Storelli et al., 2005). However, high Pb levels have been reported in fish species sampled from Libya (Khalifa et al., 2010) and Egypt (Abdallah, 2008) as 1.210 µg g-1 and 4.7 µg g-1dw, respectively.

In conclusion, *M. mola* is the largest bony fish, stands into the food chain's upper trophic level, and can be migrated over a long distance. The determination of metal levels in different tissues of *M. mola* obtain some information about the metal concentrations in seawater and the food web. The feeding preference of M. mola is quite extensive. Although *M. mola* is a predator of many species, it is not consumed by humans. Most importantly, this species is found relatively rare in the seas. Therefore, toxicological research in this species is quite limited. A study determined the metal level in the liver tissue of *M. mola*, captured from Florida. Our results will provide ecotoxicological data to the literature related to M. mola from the Mediterranean Sea.

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Author Contributions

All author contributions are equal for the preparation research in the manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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