



-RESEARCH ARTICLE-

The Chemical Composition of the Lionfish (*Pterois miles*, Bennett 1828), the New Invasive Species of the Mediterranean Sea

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Abstract

In this study, the proximate composition of muscle tissue was investigated in lion fish (*P. miles*), which has a high invasion potency in the Mediterranean. At the same time, the potentials of accumulation of essential and toxic metals in brain, gill, muscle and liver tissues have been examined. The study was carried out with 4 lion fish individuals (1st individual 278 g, 28 cm, 2nd individual 55 g, 19 cm, 3rd individual 68 g, 20 cm, 4th individual 92 g, 22 cm) caught from the Yeşilovacık Bay in 2016. Water, crude protein, total lipid, total mineral substance (TMS) percentages were found to be 75.68-77.62%, 20.05-21.08%, 1.11-1.84%, 1.22-1.54 in *P. miles*, respectively. The percentage of fatty acids in lionfish ranged from 34.26% to 37.57% saturated fatty acids (SFAs), from 24.99% to 27.99% monounsaturated fatty acids (MUFAs), and from 20.49% to 49.31% polyunsaturated fatty acids (PUFAs). Dominant fatty acids are palmitic acid and stearic acid from SFAs, palmitoleic acid, oleic acid, cetoleic acid from MUFAs, EPA and DHA from PUFAs. The accumulation levels of heavy metals in the tissues were found as Fe > Zn > As > Cu > Cr > Pb, respectively. The accumulation levels of each metal in the tissues were determined as follows: liver > gill > brain > muscle (29.19-384.43 $\mu\text{g g}^{-1}\text{dw}$) for Fe, gill > brain > liver > muscle (16.08-56.68 $\mu\text{g g}^{-1}\text{dw}$) for Zn, muscle > brain > gill > liver (2.69-7.88 $\mu\text{g g}^{-1}\text{dw}$) for As, liver > brain > gill > muscle (0.74-7.05 $\mu\text{g g}^{-1}\text{dw}$) for Cu, brain > gill > muscle > liver (0.35-2.67 $\mu\text{g g}^{-1}\text{dw}$) for Cr, brain > gill > muscle > liver (0.26-2.11 $\mu\text{g g}^{-1}\text{dw}$) for Pb. As a result; while lionfish muscle tissue contains high levels of protein, unsaturated fatty acids, minerals and trace elements, it has been determined that levels of heavy metals in this consumable tissue are not at levels that could threaten human health.

Keywords:

Mersin Bay, *Pterois miles*, Lionfish, Chemical Composition

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Introduction

After the opening of the Suez Canal, the Mediterranean Sea has been heavily exposed to Indo-Pacific species for a long time (Zenetos et al., 2012; Katsanevakis et al. 2014). With the impact of climate change, the ever-increasing sea water temperature in the Mediterranean Sea leads to the transition of tropical marine species to the Mediterranean Sea, the establishment of populations and the expansion of their distribution: Tropicalisation of the Mediterranean Sea (Kletou & Hall-Spencer 2012; Lejeusne et al. 2010; Montefalcone et al. 2015). One of the last species of this tropic process is the lion fish that recently passed to the Mediterranean Sea. *P. miles* is currently among the most successful marine invaders on the history of underwater invasions (Bariche et al., 2013). The entry of this species into the Mediterranean Sea has posed a potential threat to local species. *P. miles* has recently started to be seen in the Mediterranean Sea (Golani & Sonini, 1992; Bariche et al., 2013; Evripidou, 2013; Turan & Öztürk, 2015; Kletou et al., 2016), which increases the concern that this species will affect the structure and function of local ecosystems in a negative way.

Lion fish is hunter-predator species fed with small fishes, invertebrates and crustaceans. Lion fish is a venomous species that transmit their poison to a victim with a needle (Gallagher, 2001) and, they do not have poisonous in their tissues such as balloon fishes (Mosher & Fuhrmann, 1984). For this reason, its consumption is suitable for human nutrition. Johnston & Purkis (2014) stated that the occupation pressure from the Red Sea in the Mediterranean Sea was clearly high. While this species is a risk for other species in the Mediterranean Sea, it can be considered as an opportunity because of the consumption as human food. For this purpose, lion fish hunting is encouraged and its flesh is consumed in many countries to prevent damage created in the ecosystem (Gallagher, 2013; Lund, 2015; Morris, 2012).

Fish is a nutritional and very useful food because it is a source of high protein, essential elements and especially n-3 polyunsaturated fatty acids (n-3 PUFAs) (Daviglius et al., 2002; Michael & Butler, 2005). These nutrients, especially docosahexaenoic acid, are beneficial to the development of the brain and visual system in infants and reduce the incidence of paralysis and cholesterol levels in adults and reduce the risk of some heart diseases (Oken, 2012; Mahaffey, 2011; Bouzan et al., 2005). For this reason, the Nutrition Committee of the American Heart Association recommends eating fish of any kind two or three times a week (Kris-Etherton et al., 2003). In addition, fish consumption is an important way of exposing many environmental pollutants, including heavy metals. For this reason, consumers can accumulate environmental pollutants at considerable levels in their bodies. Since muscle tissue is the edible part of fish, the level of exposure to these toxic elements is important.

Mercury (Hg), cadmium (Cd) and lead (Pb), which are known to have no function in metabolic events, are also found in aquatic environments, while heavy metals such as copper (Cu), zinc (Zn) and iron (Fe) are used by organisms at certain levels for metabolic events. In this study, the chemical composition of muscle tissue was investigated in lion fish (*P. miles*), which has a high invasion potency in the Mediterranean Sea. At the same time, the potentials of accumulation of essential and toxic metals in brain, gill, muscle and liver tissues have been examined.

Materials and Methods

Fish collection, identification and measurements

Four specimens of the common lionfish *P. miles* were caught alive by a commercial trawl fishing boat in the North-Eastern Mediterranean (Yeşilovacık Bay) ($36^{\circ}06'37''\text{N}$ $33^{\circ}40'04''\text{E}$ to $36^{\circ}06'27''\text{N}$ $33^{\circ}40'41''\text{E}$) in 2016 (Fig. 1). Taxonomic identification was based on diagnostic characters provided by Turan et al. (2017). Their size (cm) and weight (g) were measured (Table 1).

Table 1. The weight and length of the specimens

Specimen	Weight (g)	Length (cm)
1.	278	28
2.	55	19
3.	68	20
4.	92	22

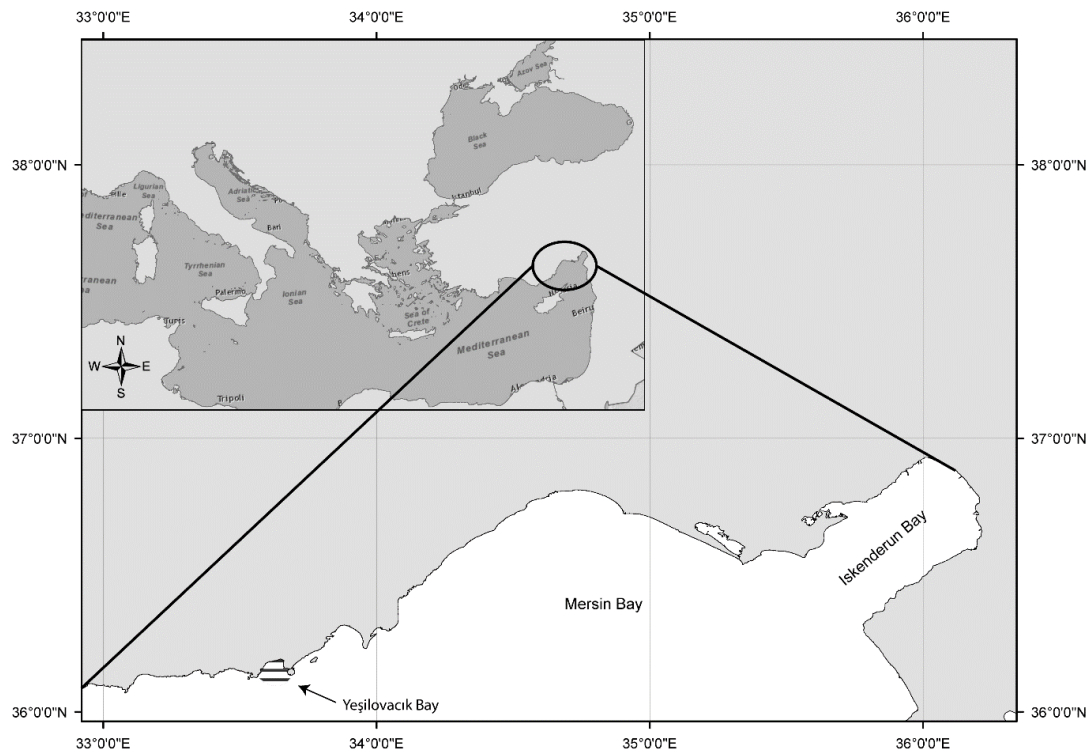


Figure 1: Sampling location map. (The shaded region (Yeşilovacık Bay) is the sampling area)

Proximate Analyses

AOAC (935.47, 1998) method was used for the TMS (total mineral substance) measurements, AOAC (1990) method was used for water measurements. Total protein measurement was taken using Kjeldahl method (AOAC 981.10, 1998), and lipid analysis was performed using Bligh & Dyer (1959) method.

Fatty Acids Analyses

Total lipids extracted by Bligh & Dyer (1959) method and fatty acid methyl esters were obtained using the Ichibara *et al.* (1996) method. Fatty acid composition was analyzed using a Gas Chromatography (GC) Clarus 500 device (Perkin–Elmer, USA), one flame ionization detector (FID) and SGE (60 m x 0.32 mm ID BPX70 x 0.25 μ m, USA or Australia) column. Injector and detector temperatures were set as 260 °C and 230 °C respectively. During this time, the furnace temperature was kept at 140 °C for 8 minutes. After that, it was increased by 4 °C per minute until 220 °C, and from 220 °C to 230 °C by increasing the temperature 1°C per minute. It was kept at 230 °C for 15 minutes to complete analysis. Sample scale was 1 μ l and carrier gas was controlled at 16 ps. For split flow 40, 0 mL/minute (1:40) level was used. Fatty acids were determined using a comparison to the exit times of the FAME mix that contains 37 standard components (Fatty acid methyl ester, Supelco, PA, USA).

Atherogenicity index (AI) and thrombogenicity index (TI)

The AI and TI linked to the fatty acid composition were calculated according to Ulbricht and Southgate (1991).

$$AI = [(a*12:0)+(b*14:0)+(c*16:0)] / [d*(PUFA\ n-6+n-3)+e*(MUFA)+f*(MUFA-18:1)]$$

$$TI = [g*(14:0+16:0+(18:0))] / [(h *MUFA)+i*(MUFA-18:1)+(m*n-6)+(n*n-3)+(n-3/n-6)]$$

a, c, d, e, f=1, b=4, g=1, h, i, m=0.5 n=3

Analyses of Elements

The samples (0.1 g dry weight) used for element analysis were dried at 105°C to reach constant weights, and then concentrated with nitric acid (4 mL, Merck, Darmstadt, Germany) and percholoric acid (2 mL, Merck, Darmstadt, Germany) were added to the samples, and they were heat up to 150°C on a hot plate heater. The process was run until all tissues were dissolved in acid solution (Canli & Atli, 2003).

Inductively coupled plasma mass spectrometer (ICP-MS) (Agilent, 7500ce Model, Japan) was used to determine elements in samples. 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 (mention the detection limits for the ICP/MS). The levels of trace element (Fe, Cu, Zn) and potential toxic metal (Cr, As, Pb) in samples were detected as μ g metal g⁻¹ dry weight. High Purity Multi Standard (Charleston, SC 29423) was used for determination of the metal analyses. Standard solutions for calibration curves were prepared by dilutions of the trace elements and

potential heavy metals. Solutions have prepared for the toxic metals had a content of lead, 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).

Metal analysis were conducted with stable samples with fixed weighing (dw). Mathematical transformation of wet weight (in muscle tissue) was carried out by using the percent dry matter value in order to compare the levels of potential toxic metals with the toxic limits of food codex.

Daily and weekly metal intake are calculated using the following formulas:

EWI (mg/week/70 kg body weight): $c \times FIR \times 7$

EDI (mg/day/70 kg body weight): $c \times FIR \times 1$

Concentration (c): mean metal level in the tissue ($mg\ kg^{-1}$).

Consumption Rate (FIR): Daily amount of fish consumed in Turkey is 0.017 kg/person/day according to Turkish Statistical Institute (TURKSTAT, 2016). Yearly amount of fish consumed in Turkey is 6.2 kg/person/year in 2015 according to Turkish Statistical Institute (TURKSTAT, 2016).

Statistical analysis

Prior to the analyses, all data were checked for outliers and Levene's homogeneity of variance was also applied for variance homogeneity. Statistical analysis of data was carried out with the IBM SPSS STATISTICS 22 statistical program. ANOVA (Analysis of Variance) was used to evaluate the differences of metals levels of the tissues (Gündoğdu, 2014).

Results

Water, crude protein, total lipid, total mineral substance (TMS) levels were found to be 75.68-77.62%, 20.05-21.08%, 1.11-1.84%, 1.22-1.54 in *P. miles*, respectively (Table 2). The fatty acids of lionfish ranged from 34.26% to 37.57% saturated fatty acids (SFAs), from 24.99% to 27.99 % monounsaturated fatty acids (MUFAs) and from 20.49% to 49.31% PUFAs (Table 3).

Table 2. The proximate composition of the *P. miles* (%)

Component	Specimen (1) $\bar{X} \pm S_x$	Specimen (2) $\bar{X} \pm S_x$	Specimen (3) $\bar{X} \pm S_x$	Specimen (4) $\bar{X} \pm S_x$
Water	77.62±0.10 ^b	77.11±0.24 ^b	77.06±0.08 ^b	75.68±0.60 ^a
Protein	20.07±0.68 ^a	20.16±0.21 ^a	20.05±0.06 ^a	21.08±0.46 ^a
Lipid	1.11±0.13 ^a	1.21±0.19 ^a	1.84±0.02 ^b	1.54±0.05 ^b
TMS	1.22±0.04 ^a	1.54±0.04 ^b	1.30±0.01 ^a	1.50±0.12 ^b

Uppercases in same lines indicates difference ($p < 0.05$). $\bar{X} \pm S_x$: Average±Standard deviation

It was determined that the fatty acid profile was composed of 25 fatty acids. These fatty acids are lauric acid (C12: 0), myristic acid (C14: 0), pentadecanoic acid (C15: 0), palmitic acid (C16: 0), heptadecanoic acid (C17: 0), stearic acid (C18: 0), arachidic acid (C20: 0), behenic acid (C22: 0), myristoleic acid (C14: 1), pentadecenoic acid (C15:1), palmitoleic acid (C16: 1), heptadecenoic acid (C17: 1), oleic acid (C18:1n9), vaccenic acid (C18:1n7), gadoleic acid

(C20:1n9), cetoleic acid (C22:1n11), nervonic acid (C24:1n9), linoleic acid (C18:2n6), alfa linolenic (C18:3n3), eicosadienoic acid (C20:2cis), eicosatrienoic acid (C20:3n6), arachidonic acid (C20:4n6), eikosapentaenoic acid (C20:5n3), docosahexaenoic acid (C22:6n3), docosadienoic acid (C22:2cis) (Table 3).

Dominant fatty acids are palmitic acid and stearic acid in SFAs, palmitoleic acid, oleic acid, cetoleic acid in MUFAs, EPA and DHA in PUFAs. Palmitic acid and stearic acid levels ranged from 20.60% to 23.50% and from 9.32% to 12.32%, respectively. Palmitoleic acid, oleic acid and cetoleic acid levels from the MUFAs were found to be between 3.05% and 6.21%, 10.83% and 12.23%, and 2.85% and 4.46%, respectively. EPA levels were found to be between 3.21% and 5.07% and DHA levels between 18.77% and 20.74% (Table 3).

Table 3. The fatty acid profile of the *P. miles* (%)

Fatty acids	Specimen(1) $\bar{X} \pm S_x$	Specimen (2) $\bar{X} \pm S_x$	Specimen (3) $\bar{X} \pm S_x$	Specimen (4) $\bar{X} \pm S_x$
Lauric acid (C12:0)	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Myristic acid (C14: 0)	2.47±0.71 ^b	2.26±0.23 ^{ab}	1.66±0.44 ^a	2.25±0.05 ^{ab}
Pentadecanoic acid (C15: 0)	0.52±0.16 ^{ab}	0.60±0.07 ^b	0.45±0.08 ^a	0.48±0.02 ^{ab}
Palmitic acid (C16: 0)	20.60±1.15 ^a	22.55±0.31 ^b	20.80±1.29 ^a	23.50±0.36 ^b
Heptadecanoic acid (C17: 0)	0.69±0.12 ^a	0.97±0.10 ^b	0.71±0.06 ^a	0.62±0.03 ^a
Stearic acid (C18: 0)	9.54±1.14 ^a	9.32±0.15 ^a	12.32±1.21 ^b	9.83±0.80 ^a
Arachidic acid (C20: 0)	0.31±0.01 ^a	0.37±0.03 ^b	0.47±0.05 ^c	0.40±0.02 ^b
Behenic acid (C22: 0)	0.13±0.02 ^{bc}	0.09±0.01 ^{ab}	0.16±0.06 ^c	0.04±0.05 ^a
ΣSFA	34.26	36.16	37.57	37.12
Myristoleic acid (C14: 1)	0.07±0.08 ^a	0.12±0.02 ^a	0.03±0.06 ^a	0.08±0.10 ^a
Pentadecenoic acid (C15:1)	0.05±0.01 ^b	0.06±0.01 ^b	0.00±0.00 ^a	0.00±0.00 ^a
Palmitoleic acid (C16: 1)	3.49±0.77 ^a	3.21±0.04 ^a	3.05±0.31 ^a	6.21±0.10 ^b
Heptadecenoic acid (C17: 1)	0.28±0.11 ^a	0.23±0.02 ^a	0.20±0.02 ^a	0.33±0.17 ^a
Oleic acid (C18:1n9)	12.08±1.07 ^b	10.83±0.18 ^a	12.23±0.95 ^b	10.90±0.23 ^a
Vaccenic acid (C18:1n7)	1.76±0.37 ^a	1.77±0.06 ^a	1.63±0.12 ^a	1.50±0.09 ^a
Gadoleic acid (C20:1n9)	0.67±0.13 ^a	0.62±0.04 ^a	0.61±0.06 ^a	0.81±0.04 ^b
Cetoleic acid (C22:1n11)	4.40±0.58 ^b	3.21±0.24 ^a	4.46±0.05 ^b	2.85±0.09 ^a
Nervonic acid (C24:1n9)	0.32±0.30 ^a	0.43±0.05 ^a	0.39±0.08 ^a	0.48±0.12 ^a
ΣMUFA	23.12	20.48	22.60	23.16
Linoleic acid (C18:2n6)	1.15±0.07 ^b	1.39±0.08 ^c	1.04±0.07 ^a	1.08±0.05 ^{ab}
Alfa linolenic (C18:3n3)	0.23±0.06 ^{bc}	0.25±0.03 ^c	0.15±0.03 ^{ab}	0.08±0.09 ^a
Eicosadienoic acid (C20:2cis)	0.20±0.12 ^b	0.09±0.01 ^a	0.23±0.01 ^{bc}	0.33±0.02 ^c
Eicosatrienoic acid (C20:3n6)	0.19±0.09 ^a	0.09±0.00 ^a	0.16±0.05 ^a	0.19±0.07 ^a
Arachidonic acid (C20:4n6)	0.26±0.02 ^b	0.28±0.03 ^b	0.22±0.01 ^a	0.26±0.03 ^b
Eikosapentaenoic acid (C20:5n3)	3.21±0.10 ^a	5.07±0.10 ^c	4.38±0.17 ^b	4.23±0.12 ^b
Docosahexaenoic acid (C22:6n3)	20.27±1.69 ^{ab}	20.74±0.16 ^b	18.77±0.67 ^a	19.47±1.05 ^{ab}
Docosadienoic acid (C22:2cis)	0.15±0.02 ^b	0.08±0.00 ^a	0.04±0.05 ^a	0.04±0.04 ^a
ΣPUFA	25.66	27.99	24.99	25.68
SFA/PUFA	1.34	1.29	1.50	1.45

Σn7	1.76	1.77	1.63	1.50
Σn6	1.60	1.76	1.42	1.53
Σn3	23.71	26.06	23.30	23.78
Σn9	13.07	11.88	13.23	12.19
Σn11	4.40	3.21	4.46	2.85
n6/n3	0.07	0.07	0.06	0.06
n3/ n6	14.82	14.81	16.41	15.54
DHA/EPA	6.31	4.09	4.29	4,60
AI	0.63	0.65	0.58	0.67
TI	0.33	0.33	0.35	0.36
Unidentified	16.96	15.37	14.84	14.04

Uppercases in same lines indicates difference (p<0.05). $\bar{X} \pm S_x$: Average±Standard deviation

The accumulation amounts of heavy metals in the tissues were found as Fe> Zn> As> Cu> Cr> Pb, respectively. The accumulation levels of each metal in the tissues were determined as follows: liver>gill>brain>muscle (29.19-384.43 $\mu\text{g g}^{-1}\text{dw}$) for Fe, gill>brain>liver>muscle (16.08-56.68 $\mu\text{g g}^{-1}\text{dw}$) for Zn, muscle>brain>gill>liver (2.69-7.88 $\mu\text{g g}^{-1}\text{dw}$) for As, liver>brain>gill>muscle (0.74-7.05 $\mu\text{g g}^{-1}\text{dw}$) for Cu, brain>gill>muscle>liver (0.35-2.67 $\mu\text{g g}^{-1}\text{dw}$) for Cr, brain>gill>muscle>liver (0.26-2.11 $\mu\text{g g}^{-1}\text{dw}$) for Pb (Table 4).

Table 4. The metal levels of some tissues of the lionfish ($\mu\text{g g}^{-1}$)

Metal	Brain $\bar{X} \pm S_x$	Gill $\bar{X} \pm S_x$	Muscle $\bar{X} \pm S_x$	Liver $\bar{X} \pm S_x$
Cr	2.67±0.71 ^b	1.01±0.04 ^a	0.51±0.02 ^a	0.35±0.04 ^a
Fe	166.32±20.36 ^b	216.20±32.87 ^b	29.19±7.69 ^a	384.43±57.96 ^c
Cu	4.79±1.29 ^{bc}	2.22±0.15 ^{ab}	0.74±0.12 ^a	7.05±1.50 ^c
Zn	36.78±0.63 ^c	56.68±1.62 ^d	16.08±0.78 ^a	23.73±3.19 ^b
As	5.85±1.77 ^{ab}	3.74±0.88 ^{ab}	7.88±1.67 ^b	2.69±0.56 ^a
Pb	2.11±0.40 ^b	0.57±0.04 ^a	0.26±0.07 ^a	0.26±0.09 ^a

Uppercases in same lines indicates difference (p<0.05). $\bar{X} \pm S_x$: Average±Standard error

Discussion

Nutritional benefits of fish consumption relate to the use of certain biologically valued proteins as well as certain minerals and vitamins provided by fish. It was determined that lion fish had high protein level (20.05-21.08%) in the study.

Lambertsen (1978) distinguishes 4 groups according to the amount of fat they have, and they are classified as lean fish (<2%), low-fat fish (2-4%), medium-fat fish (4-8%), fatty fish (> 8%). In another similar study (Polish Standard PN-A-86770, 1999), it was defined as lean fish (<2%), low-fat fish (2-7%), fatty fish (7-15%), very fatty fish (> 15%). In our study, since the fat level of the lion fish varied between 1.11-1.84%, it was defined as a lean fish.

Long chain omega-3 polyunsaturated fatty acids (LC n3 PUFAs) including eicosapentaenoic acid (EPA, 20: 5 ω -3), docosapentaenoic acid (DPA, 22: 5 ω -3) and docosahexaenoic acid (DHA, 22: 6 ω) are the main nutrients responsible for the potential cardiovascular effects of fish-based consumption (Schmidt, 1997; Sidhu, 2003). The biochemical, cellular and physiological functions of these three PUFAs in other vertebrates are the same in fish, and are divided into two categories: (a) a generalized role in protecting the structural and functional integrity of cell membranes; (b) being the precursor of biologically highly active paracrine hormones, collectively known as eicosanoids (Sargent et al., 1999). The ideal n-6/n-3 ratio of 4.0 at a maximum is recommended by the UK Department of Health (HMSO UK, 1994). Values higher than 4.0 have negative effects on health and may promote cardiovascular diseases (Moreira et al., 2001). In this study, the ratio of n-6/n-3 was in the range of 0.06-0.07 for lionfish. The index of AI and TI varied between 0.58 and 0.67, 0.33 and 0.36, respectively.

DHA is taken by the brain according to other fatty acids, it is necessary for the growth and functional development of the brain in babies. In adults, DHA is also needed to maintain normal brain function. While the abundance of DHA in the diet improves learning ability, DHA deficiencies are associated with learning gaps (Horrock & Yeo, 1999). Chuang et al. (2012) reported that the level of DHA in *Mullus barbatus* was 9.97%. In a similar study, DHA levels of *Boops boops*, *Mugil cephalus*, *Saurida aurita* and *Solea solea* were found to be 18.7%, 7.69%, 13.3%, 18.7%, respectively by Ozoğul & Ozogul (2007). It has been found that *P. miles* has a higher DHA value (%18.77-20.74) than the commercially consumed species in the Mediterranean Sea. This result is important to ensure that this species is consumed by humans.

Arsenic (As), Mercury (Hg), cadmium (Cd) and lead (Pb), which are known to have no function in metabolic events, are also found in aquatic environments, while heavy metals such as copper (Cu), zinc (Zn) and iron (Fe) are used by organisms at certain levels for metabolic events. In fish, heavy metal accumulation changes depending on the tissues and organs. Studies by various researchers have shown that significant differences were obtained among the tissues in various fish species and that accumulation was highest in the liver and lowest in muscle (Kalay et al., 1999; Karayakar et al., 2010). A similar relationship was observed in this study in terms of iron and copper levels. The highest zinc level in this study was accumulated in the gills. In fish, gill is a respiratory organ and is directly related to the environment, so it is selected as the target organ in toxicology studies (Pelgrom et al., 1995). Cu in *Oncorhynchus kisutch* (Buckley et al., 1982), Pb in *Tilapia zilli* (Karatas & Kalay, 2002) and Zn in *Cyprinus carpio* (Cicik, 2003) have been found to be the highest accumulation in the gill tissue according to other tissues and organs. In this study, zinc level was found to be the highest accumulation in the gill from the other investigated metals, while the lowest accumulation was in the muscle.

The aquatic organisms show a high accumulation capacity of arsenic (As) (1-1000 $\mu\text{g g}^{-1}$) (Maher & Batley, 1990; Francesconi & Edmonds, 1998). When they are fed and are exposed to arsenic in other ways / sources such as water, soil, particles, they accumulate As and turn them into their bodies. (Edmonds et al., 1997; Hasegawa et al., 2001; Suhendrayatna & Maeda, 2001). In this study, it was determined that arsenic accumulates at the highest levels in muscle tissue and at the lowest levels in liver tissue.

The amount of chromium in the food is important because Cr is an important micronutrient for homeostasis and is involved in lipid metabolism (Bratakos et al., 2002).

Bioaccumulation and physiological and toxicological results for chromium may be due to a number of reasons such as exposure concentration, exposure route, and Cr (VI) sensitive susceptibility of specific species (Chen et al., 2016; Woltering, 1984). In this study, total chromium was detected at the highest levels in brain tissue and at the lowest levels in liver tissue.

Lead was detected at the highest levels in brain tissue and at the lowest levels in liver and muscle tissues. The accumulation of lead in the brain most often can be attributed to the fact that the lead enters the neuronal intracellular environment by acting as calcium (Van Oasten, 1957), primarily due to chemical similarity, preventing the opening of voltage-sensitive calcium channels (Bressler & Goldstein, 1991; Sen & Karaytug, 2017).

Table 5. Estimated Weekly Intakes (EWI) and Estimated Daily Intakes (EDI) of the metals in tissue of the red mullet from the Black Sea coast of Turkey.

Metals	PTWI* (mg/week/70 kg body wt.)	PTDI* (mg/day/70 kg body wt.)	EWI (mean)	EDI (mean)
Fe	392 (FAO/WHO, 2011)	56	0,7774	0,1111
Cu	245 (EC, 2006; WHO, 1996)	35	0,0197	0,0028
Zn	490 (EC, 2006; WHO, 1996)	70	0,4282	0,0612
Cr	-	-	0,0136	0,0019
Pb	1.75 (FAO/WHO, 2010; WHO, 2000)	0.25	0,0072	0,0010
As	-	-	0,2096	0,0299

PTWI: Provisional Tolerable Weekly Intake, PTDI: Provisional Tolerable Daily Intake, EWI: Estimated Weekly Intake, EDI: Estimated Daily Intake * Internationally accepted safe levels for the studied metals.

Muscle tissue in fish is not an effective tissue for binding metals in general (Blevins & Pancorbo, 1986; Kargin, 1998). However, it is important for metals to be transported to people through food chain. Because of this, it is important for fish to have less metal accumulation in the muscles used as fish especially for human food. The lowest accumulation of all heavy metals except arsenic and chromium in the lion fish was detected in muscle tissue. However, all metal levels are within the safe limits recommended by FAO / WHO (JECFA, 2003) (Table 5).

Conclusion

Aquatic species are seen as a major source of protein by humans and their consumption is increasing day to day due to the nutrient molecules they contain. In addition, they contain the omega-3 fatty acids which reduce cholesterol levels and thus reduce the risk of diseases such as heart attack, stroke and premature birth. For this reason, knowing the quantities of the toxic substances contained in the tissues and organs of aquatic organisms is extremely important both in terms of "food safety" and in the health of other organisms.

Therefore, in this study, it is aimed to determine the chemical composition of *P. miles* that started to increase in invasive pressure in the Mediterranean Sea and to determine the levels of heavy metals such as Fe, Cu, Zn, Cr, As and Pb in consumable muscle tissues and to determine

the accumulation mechanism of these metals in tissues. As a result; while lionfish muscle tissue contains high levels of protein, unsaturated fatty acids, minerals and trace elements, it has been determined that levels of heavy metals in this consumable tissue are not at levels that could threaten human health.

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References

- Bariche, M. Torres, M. Azurro, E. (2013). The presence of the invasive lionfish *Pterois miles* in the Mediterranean Sea. *Mediterranean Marine Science*, 14(2), 292-294.
- Blevins, R.D. & Pancorbo, O.C. (1986). Metal Concentrations in Muscle of Fish from Aquatic Systems in East Tennessee. *U.S.A. Water, Air and Soil Pollution*, 29, 361-371.
- Bouzan, C., Cohen, J.T., Connor, W.E., Kris-Etherton, P.M., Gray, G.M., Konis, A., Lawrence, R.S., Savitz, D.A., Teutsch, S.M. (2005). A quantitative analysis of fish consumption and stroke risk. *American Journal of Preventive Medicine*, 29, 347-352.
- Bratakos, M.S., Lazos, E.S., Bratakos, S.M. (2002). Chromium content of selected Greek foods. *28 Science of the Total Environment*, 290, 47-58.
- Bressler, J.P. & Goldstein, G.W. (1991). Mechanisms of lead neurotoxicity. *Biochemical Pharmacology*, 41(4), 479-484.
- Buckley, J.T., Roch, M., McCarter, J.A., Rendell, C.A., Matheson, A.T. (1982). Chronic Exposure of Coho Salmon to Sublethal Concentrations of Copper-I. Effect on Growth, on Accumulation and Distribution of Copper and on Copper Tolerance. *Comparative Biochemistry & Physiology*, 72, 15-19.
- Chen, H., Mu, L., Cao, J., Mu, J., Klerks, P.L., Luo, Y., Guo, Z., Xie, L. (2016). Accumulation and effects of Cr(VI) in Japanese medaka (*Oryzias latipes*) during chronic dissolved and dietary exposures. *Aquatic Toxicology*, 176, 208-216.
- Chuang, L.T., Bülbül, U., Wen, P.C., Glew, R.H., Ayaz, F.A. (2012). Fatty Acid composition of 12 fish species from the Black Sea. *Journal of Food Science*, 77(5), 512-518.
- Cicik, B. (2003). Bakır-Çinko Etkileşiminin Sazan (*Cyprinus carpio* L.)'nin Karaciğer, Solungaç ve Kas Dokularındaki Metal Birikimi Üzerine Etkileri. *Ekoloji*, 12, 32-36.
- Daviglus, M., Sheeshka, J., Murkin, E. (2002). Health benefits from eating fish. *Comments Toxicology*, 8, 345-374.
- Edmonds, J., Shibata, Y., Francesconi, K., Rippingale, R., Morita, M. (1997). Arsenic transformations in short marine food chains studied by HPLC-ICP-MS. *Appl. Organometallic Chemistry*, 11, 281-287.
- Francesconi, K.A. & Edmonds, J.S. (1998). Arsenic species in marine samples Croat. *Chemica Acta*, 71, 343-359.
- Gallagher, S.A. (2001). Lionfish and stonefish. *Emedicine J.*, 2,7.
- Gallagher, S.E. (2013). Establishing a Culinary Market for Lionfish Species through a Market-based Organization to Mitigate the Environmental Impacts of the Invasive Species, MSc theses. College of Charleston, Charleston, South Carolina, p. 37
- Gündoğdu, S. (2014). The usage of common multiple comparison tests (post-hoc) in fisheries sciences. *Journal of Fisheries Sciences.com*, 8, 310-316.

- Hasegawa, H., Sohrin, Y., Seki, K., Sato, M., Norisuye, K., Naito, K., Matsui, M. (2001). Biosynthesis and release of methylarsenic compounds during the growth of freshwater algae. *Chemosphere*, 43, 265-272.
- HMSO UK. (1994). Nutritional aspects of cardiovascular disease. *Report on health and social subjects* no.46. London: HMSO.
- Horrocks, L.A. & Yeo, Y.K. (1999). Health benefits of docosahexaenoic acid (DHA). *Pharmacological Research.*, 40(3), 211-225.
- JECFA. (2010). Evaluation of certain food additives and contaminants. 72th Report of the Joint FAO/WHO Expert Committee on Food Additive. *WHO Technical Report Series*, 959.
- JECFA. (2010). Evaluation of certain food additives and contaminants. 73th Report of the Joint FAO/WHO Expert Committee on Food Additive. *WHO Technical Report Series*, 960.
- Johnston, M.W. & Purkis, S.J. (2014). Lionfish in the eastern Pacific: a cellular automaton approach to assessing invasion risk. *Biol Invasions*. 16(12), 2681–2695.
- Kalay, M., Ay, Ö., Canlı, M. (1999). Heavy Metal Concentrations in Fish Tissues from the Northeast Mediterranean Sea. *Bulletin of Environmental Contamination and Toxicology*, 63, 673-681.
- Karatas, S. & Kalay, M. (2002). Tilapia zilli'nin Solungaç, Karciger, Böbrek ve Beyin Dokularında Kursun Birikimi. *Turk J. Vet. Anim. Sci.*, 26, 471-477.
- Karayakar, F., Karaytuğ, S., Cıçık, B., Erdem, C., Ay, Ö., Çiftçi, N. (2010). Heavy Metal Levels in Five Species of Fish Caught from Mersin Gulf. *Fresenius Environmental Bulletin*, 19(10), 2222-2226.
- Kargın, F. (1998). Metal Concentrations in Tissues of the Freshwater fish *Capoeta barroisi* from the Seyhan River (Turkey). *Water, Air and Soil Pollution*, 60 (5), 822-828.
- Katsanevakis, S., Coll, M., Piroddi, C., Steenbeek, J., Ben Rais, L.F., Zenetos, A., Cardoso, A.C. (2014). Invading the Mediterranean Sea: biodiversity patterns shaped by human activities. *Front Mar Sci.*, 1-32.
- Kletou, D. & Hall-Spencer, J.M. (2012). Threats to ultraoligotrophic marine ecosystems. In: Dr. Cruzado A, editor. *Marine Ecosystems*. p. 1–34.
- Kletou, D., Hall-Spencer, J.M., Kleitou, P. (2016). A lionfish (*Pterois miles*) invasion has begun in the Mediterranean Sea. *Marine Biodiversity Records*. 9, 46.
- Kris-Etherton, P.M., Harris, W.S., Appel, L.J. (2003). Fish consumption, fish oil, omega_3 fatty acids and cardiovascular disease. *Circulation*, 106, 2747-2757.
- Lambertsen, H. (1978). *Fresh Fish Quality and Quality Changes*, H. Huss (ed.), FAO 17-19, Rome, 1998.
- Lejeusne, C., Chevaldonné, P., Pergent-Martini, C., Boudouresque, C.F., Pérez, T. (2010). Climate change effects on a miniature ocean: The highly diverse, highly impacted Mediterranean Sea. *Trends Ecol. Evol.* 25(4), 250–260
- Lund, T. (2015). Lionfish: You Have to Eat Them to Beat Them Florida Today. <http://www.usatoday.com/story/money/business/2015/02/16/fight-against-lionfish/23504661/>
- Mahaffey, K.R., Sunderland, E.M., Chan, H.M., Choi, A.L., Grandjean, P., Marien, K., Oken, E., Sakamoto, M., Schoeny, R., Weihe, P., Yan, C.H., Yasutake, A. (2011). Balancing the benefits of n-3 polyunsaturated fatty acids and the risks of methylmercury exposure from fish consumption. *Nutrition Reviews*, 69 (9), 493-508.
- Maher, W. & Batley, G. (1990). Organometallics in the near shore marine environment of Australia. *Applied Organometallic Chemistry*, 4, 419-437.

- McMichael, A.J. & Butler, C.D. (2005). Fish, health, and sustainability. *Am. J. Prev. Med.*, 29, 322–323.
- MHPRC. (1994). (Ministry of Health of the People's Republic of China). Maximum levels of arsenic in foods (GB4810-1994). Beijing, China: MHPRC (in Chinese).
- MHPRC. (2012). (Ministry of Health of the People's Republic of China). Maximum levels of contaminants in foods (GB2762-2012). Beijing, China: MHPRC (in Chinese).
- Montefalcone, M., Morri, C., Parravicini, V., Bianchi, C.N. (2015). A tale of two invaders: divergent spreading kinetics of the alien green algae *Caulerpa taxifolia* and *Caulerpa cylindracea*. *Biol Invasions*. 17, 2717–28.
- Moreira, A.B., Visentainer, J.V., de Souza, N.E., Matsushita, M. (2001). Fatty acids profile and cholesterol contents of three Brazilian Brycon freshwater fishes. *J Food Comp Anal.*, 4, 565-574.
- Morris, J.A. Jr. (Ed.) (2012). Invasive Lionfish: A Guide to Control and Management. *Marathon*, Florida, USA.
- Mosher, M. & Fuhrmann, F.A. (1984). *Occurrence and origin of tetrodotoxin*. In: *Seafood Toxins* (Ragelis EP, ed). Washington, DC: American Chemical Society, 333–344.
- Oken, E., Choi, A.L., Karagas, M.R., Marien, K., Rheinberger, C.M., Schoeny, R., Sunderland, E. & Korrick, S. (2012). Which fish should i eat? Perspectives influencing fish consumption choices. *Environmental Health Perspectives*, 120 (6), 790-798.
- Ozogul, Y. & Ozogul, F. (2007). Fatty acid profiles of commercially important fish species from the Mediterranean, Aegean and Black Seas. *Food Chemistry*, 100(4), 1634-1638.
- Polish Standard PN-A-86770. (1999). *Fish and fishery*. Takeuchi, T., Watanabe, T., Ogino, C., Saito, M., products – terminology (in Polish).
- Sargent, J., Bell, G., McEvoy, L., Tocher, D., Estevez, A. (1999). Recent developments in the essential fatty acid nutrition of fish, *Aquaculture*, 177 (1–4), 191-199.
- Schmidt, E.B. & Dyerberg, J. (1994). Omega-3 fatty acids. Current status in cardiovascular medicine. *Drugs*, 47, 405-424.
- Sen, G. & Karaytug, S. (2017). Effects of Lead and Selenium Interaction on Acetylcholinesterase Activity in Brain and Accumulation of Metal in Tissues of *Oreochromis niloticus* (L., 1758). *NESciences*, 2, 21-32.
- Sidhu, K.S. (2003). Health benefits and potential risks related to consumption of fish or fish oil. *Regul Toxicol Pharmacol.* 38(3), 336-344.
- Suhendrayatna, A.O. & Maeda, S. (2001). Biotransformation of arsenite in freshwater food chain models. *Appl. Organomet. Chem.*, 15, 277-284.
- Turan, C. & Öztürk, B. (2015). First record of the lionfish *Pterois miles* from the Aegean Sea. *Journal of Black Sea/Mediterranean Environment*, 21, 334-338.
- Turan, C., Uygur, N., İğde, M. (2017). Lionfishes *Pterois miles* and *Pterois volitans* in the North-eastern Mediterranean Sea: Distribution, Habitation, Predation and Predators. *Natural and Engineering Sciences*, 2(1), 35-43.
- TURKSTAT. (2016). Turkey Statistical Institute, Fisheries Statistics.
- Ulbricht, T. L. V. & Southgate, D. A. T. (1991). Coronary heart disease: seven dietary factors. *Lancet*, 338, 985-992.
- Van Oosten, J. (1957). *The skin and scales*. The Physiology of Fishes, Academic Press, New York, 1, 207.

- Varanasi, L. & Markey, D. (1978). Uptake and Release of Lead and Cadmium in Skin and Mukus of Coho Salmon (*Oncorhynchus kisutch*). *Comparative Biochemistry & Physiology*, 60, 187-191.
- Wang, W.-X., Griscom, S.B., Fisher, N.S. (1997). Bioavailability of Cr(III) and Cr(VI) to marine mussels from solute and particulate pathways. *Environmental Science and Technology*, 31, 603-611.
- Weihrauch, J. L., Posati, L. P., Anderson, B. A. ve Exler, J. (1975). Lipid conversion factors for calculating fatty acid contents of foods. *Journal of the American Chemists' Society*, 54, 36-40.
- WHO. (1995). *Reliable Evaluation of Low-Level Contaminants of Food*. Report on a Workshop in the Frame of GEMS/Food-EURO.
- WHO. (2015). Evaluation of Certain Food Additives: Seventy-ninth Report of the Joint FAO/WHO Expert Committee on Food Additives.
- Woltering, D.M. (1984). The growth response in fish chronic and early life stage toxicity tests: a critical review. *Aquatic Toxicology*, 5, 1-21.
- Zenetos, A., Ballesteros, E., Verlaque, M. (2012). Alien species in the Mediterranean Sea by 2012. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part 2. Introduction trends and pathways. *Mediterranean Marine Science*, 13, 328-52.