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The Heavy Metal, Fatty Acid, and Proximate Composition of Wild

Brown Trout (Salmo trutta Sp.)

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ABSTRACT

The purpose of this study was to investigate the proximate composition, fatty acid profiles,

microbiology, and heavy metal concentrations of wild brown trout (Salmo trutta macrostigma)

that were obtained from the Munzur River in Tunceli, Turkey. According to the findings of the

study, wild brown trout had significantly higher concentrations of monounsaturated,

polyunsaturated, and saturated fatty acids, respectively. C18:1 ω-9, which is oleic acid,

followed by C16:0, which is palmitic acid, C18:3 π -3, which is linolenic acid, and C16:1 ω -7,

which is palmitoleic acid, were discovered to be the primary fatty acids, in that particular order.

The fatty acids that were found to be the most prevalent in polyunsaturated fatty acids (PUFAs)

were C22:6 ω-3 (docosahexaenoic acid, also known as DHA), C18:3 ω-3c (cis-linolenic acid),

C18:2 ω-6c (linoleic acid), and C20:5 ω-3 (eicosapentaenoic acid, also known as EPA). The

results of this investigation showed that all of the values obtained were significantly lower than

the values that are defined in the Turkish Food Codex for heavy metals. It is also possible to

consider Munzur trout to be safe from a microbiological standpoint. In conclusion, following

the examination of the nutritional quality results in terms of fatty acid composition, proximate,

and heavy metal data, it can be concluded that wild brown trout that inhabit the Munzur River

can be considered a reliable and essential source of protein and ω -3 fatty acids.

Keywords: Salmo trutta macrostigma, fatty acid, heavy metal, microbiology

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1. INTRODUCTION

Production and consumption levels of foods, whose basic nutritional properties are effective in improving human health or preventing the occurrence of diseases, are rapidly increasing all over the world. Fish plays a significant role in the dietary habits of individuals. In the course of industrial processing, a portion of the entire fish that is not suitable for direct consumption is classified as a by-product (Olsen et al., 2014). Fish meat, with its rich protein content and polyunsaturated fatty acids in its structure, is considered among the important nutrients in the prevention of diseases and maintaining a healthy life in terms of meeting the body's need for basic nutritional components and having a positive effect on human physiology and metabolic functions.

Fats are one of the most important organic substances necessary for human nutrition. These organic substances are not only a source of high energy, but also contain vitamins that are soluble in fat; lipoproteins by combining with proteins to form metabolic activities are also important (Mol, 2008). It is very important that the fats in the foods consumed are rich in unsaturated fats. The significance of omega-3 fatty acids in human nutrition is widely acknowledged (Simopoulos, 2004). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are the two primary linolenic omega-3 fatty acids, are found in every form of seafood, but they are not found in any other foods. It is believed that these two fatty acids trigger substantial physiological and biochemical shifts within the organism (Gordon & Ratliff 1992). EPA and DHA, which are found in abundance in fish tissues, have been shown to have beneficial effects on bone formation, metabolism, and against the development of cardiovascular disease, according to recent research (Arts et al., 2001; Lauritzen et al., 2000; Su et al., 2003; Watkins et al., 2003). The measurement of fatty acids and fat in seafood is essential. The variations in lipid and fatty acid compositions among and within fish species are

influenced by factors such as food availability, seasonal changes, habitat, sex, diet, and age, which can differ even among individuals of the same species (Akpınar et al., 2009). Since fish consumption plays an important role in human nutrition in terms of nutritional composition and essential fatty acids, it is important to determine the nutritional content of fish species in different regions, as well as to determine microbial quality and heavy metal levels for food safety.

There is a little information on chemical composition, of *Salmo trutta macrostigma* in Munzur River, Turkey while there is no information on microbiological quality of *S. trutta macrostigma*. Studies by Kayım et al. (2011) and Ateş et al. (2013) demonstrate that wild brown trout in the Munzur River serve as a substantial source of protein and ω-3 fatty acids, according to evaluations of their nutritional quality concerning proximate and fatty acid composition. Numerous research have examined the fatty acid composition of total lipids in S. trutta macrostigma (Aras et al., 2003; Akpınar et al., 2009; Kayım et al., 2011; Ateş et al., 2013). Furthermore, two published studies have specifically examined the fatty acid profile of phospholipids and triacylglycerols in this species located in Erzurum (Bayır et al., 2010) and the Munzur River (Kayhan et al., 2015). Therefore, the aim of this study was to investigate the fatty acid profile and proximate composition as well as microbiological quality and heavy metals of the *S. trutta macrostigma* caught in the Munzur River, Turkey.

2. MATERIALS AND METHODS

2.1 Fish sampling

The Munzur River originates from Munzur Mountain, situated north of Ovacık. The Pülümür stream converges near the Tunceli city center before flowing into Keban Dam Lake. The primary source of the river is located in Tunceli Province. The river harbors a significant population of red-spotted trout (S. trutta macrostigma Dumeril, 1858). Munzur Valley features

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abundant indigenous flora, waterfalls, steep slopes, remarkable rock formations and canyons, and has been designated as Munzur Natural Park for conservation efforts. Samples of wild brown trout (Salmo trutta macrostigma Dumeril, 1858) were collected from fishermen in the Munzur River, Turkey, on three occasions between January and March 2018. Following capture, the specimens were immediately placed on ice within an insulated container and subsequently sent to the National Food Reference Laboratory, Ministry of Food, Agriculture and Livestock, Mersin, Turkey.

2.2 Proximate analyses

Standard methodologies were employed to assess the chemical composition of fish samples in accordance with AOAC (1995). The sections of the fish samples that may be consumed were homogenized before they were examined. The moisture amount was evaluated using a 3 g sample, dehydrated in a thermoventilated oven at 105°C overnight (Ludorff & Meyer 1973). The protein content was determined via the nitrogen (N × 6.25) Kjeldahl method (James, 1995), while the ash content was assessed through incineration at 550°C in a muffle furnace (Mattissek et al., 1988). The samples underwent treatment with cyclohexane, 2-propanol, and water to extract the lipids (Smedes & Thomasen, 1996). A duplicate of every chemical analysis was conducted.

2.3 Lipid extraction and FAME analyses

Following the homogenization of the edible components of the fish specimens, 40 grams of samples were removed. Subsequently, 2-propanol and cyclohexane were introduced, homogenized using an ultra turrax, followed by the addition of distilled water; the mixture was homogenized again with the ultra turrax and subsequently centrifuged. The supernatant was transferred to a funnel containing anhydrous sodium sulfate, which had previously been tared to a flask. This method was reiterated two additional times. The solvent was subsequently evaporated using a rotary evaporator, and the flask was later placed in the oven overnight

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(Smedes & Thomasen, 1996). The extracted lipids were next examined for their fatty acid makeup. To create fatty acid methyl esters, 0.1 g of fish oil was introduced into a tube containing 10 ml of n-hexane. Subsequently, 0.5 mL of 2N KOH in methanol was added. The mixture was then homogenized using a vortex and allowed to settle for one hour. The supernatant was pipetted and then filtered through a 0.45 µm filter disk. Prior to injection into the Agilent GC-FID (TS EN ISO 12966-2, 2017), it was transferred to a 2 ml vial. A Hewlett Packard (HP) Agilent 6890N gas chromatograph (GC) with a flame ionization detector (FID) and a JW 1222362, DB-23 capillary column (60 m x 0.25 mm i.d. x 0.25 µm thickness) was utilized to evaluate fatty acids methyl esters (FAME). Nitrogen served as the carrier gas at a constant pressure of 40.02 psi, with an initial flow rate of 1.4 mL/min and an average velocity of 23 cm/sec. The injection port was set to 220°C, and the sample was introduced in split mode with a split ratio of 25:1. The temperature of the detector was 280°C. The column temperature commenced at 100°C for 5 minutes, subsequently increased at a rate of 5°C/min to 180°C, followed by a ramp to 200°C at 2°C/min, and was then maintained at 200°C for 30 minutes. Nitrogen served as the carrier gas at a constant flow rate of 30 mL/min, while hydrogen and dry air functioned as the detecting gases (TS EN ISO 12966-4, 2015). Fatty acid identification involved comparing the retention times of sample FAME peaks with those of Supelco standards, specifically the Supelco 37 Compounds FAME mix (10 mg/ml in CH2Cl2 – 47885 U) and the Supelco 1819-1 Ampule FAME mix (C4-C24). The results for each fatty acid were expressed as percentages of the FID response area in relation to the total measured fatty acids (TS EN ISO 12966-1, 2014).

2.4 Microbiological analysis

Fish samples brought to Mersin Food Control Laboratory were kept at room temperature until dissolution and then samples were taken from muscle tissue and microbiological analyzes were performed. Salmonella spp., Coagulase positive Staphylococus aureus, Escherichia coli,

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Escherichia coli 0157, Listeria monocytogenes, Vibrio paraheamolyticus, Vibrio cholera, total mesophilic aerobic bacteria (TMAB) and Coliform group bacteria were investigated. The detection of Salmonella spp. was carried out using the microbiological protocol ISO 6579: 2002, the detection of Staphylococcus aureus was performed using the microbiological protocol FDA / BAM. E. coli detection was performed with National Standard Method (F 20, UK). L. monocytogenes detection was performed using microbiological protocol ISO 11290-1:199. (ISO, 2004), V. paraheamolyticus and V. cholera were performed using FDA/BAM 2004. In the detection of coliform group bacteria, Halkman's method (Halkman, 2005) was used.

2.5 Heavy metal analysis

Microwave extraction was performed in an oven at the Mersin Food Control Laboratory. To minimize contamination, all materials used in the experiments were rinsed with ultra-pure water before application, and a stainless steel knife was utilized for tissue cutting. Before analysis, the tissues were thawed, and a 0.5 g sample was obtained from the muscle tissue. The modified microwave method was employed for the digestion procedure of samples. Three 0.5 g thawed homogenates from each tissue were put in a Teflon digestion tube with two milliliters of hydrogen peroxide (H2O2 30%) and eight milliliters of concentrated nitric acid (HNO3 65%). The samples in the vessels were subjected to digestion using a microwave digestion program (Table 1). They were then permitted to cool to room temperature. The residues were dissolved and diluted to a final volume of 25 ml (Anonymous, 1998). The diluted digests were measured directly using ICP-MS, with parameters outlined in Table 2 (NMKL-186, 2007).

ISSN: 0369-8963

Table1: Microwave digestion programme

Step	Time	T1	T2	p	Power
1	00:10:00	180°C	100°C	45 bar	Max power*
2	00:15:00	180°C	100°C	45 bar	Max power*

^{*}Max power: 1500 W for Ethos and 1200 W for Start units.

Table 2. ICP-MS Parameters

Instrument model	Agilent 7500 cx
RF power	1550 W
Carrier gas flow rate	0,9 L/min
Make-up gas flow rate	0,15 L/min
Plasma gas flow rate	15 L/min
Auxiliary gas flow rate	1 L/min
Nebulizer	Concentric
Spray chamber	Water-cooled double-pass (2°C)
Interface cones	Nickel
Mass resolution	0,8 u
Integration time	0,1 sec

3. RESULTS AND DISCUSSION

3.1 Fatty acid results

The consumption of essential fatty acids through daily dietary intake is crucial for health maintenance. Essential fatty acids should be consumed daily with food to promote healthy eating and longevity. All analyzed samples exhibited high levels of fatty acids with significant nutritional value (Table 3). The significant fatty acids relevant to nutrition include Σ SFA 30%, C18:1 ω -9-Oleic acid 25%, C18:3 ω -3-Linolenic acid 13%, C20:5 ω -3-Eicosapentaenoic acid (EPA) 6%, C22:6 ω -3-Docosahexaenoic acid (DHA) 3%, and a combined total of EPA+DHA 7%. Wild brown trout exhibited elevated concentrations of saturated, polyunsaturated, and monounsaturated fatty acids, respectively. The primary fatty acids identified were C18:1 ω -9 (oleic acid), C16:0 (palmitic acid), C18:3 ω -3 (linolenic acid), and C16:1 ω -7 (palmitoleic acid).

ISSN: 0369-8963

The primary fatty acids identified in polyunsaturated fatty acids (PUFAs) include C22:6 ω-3 (docosahexaenoic acid, DHA), C18:3 ω-3c (cis-linolenic acid), C18:2 ω-6c (linoleic acid), and C20:5 ω-3 (eicosapentaenoic acid, EPA). The findings are consistent with the research by Ates et al. (2013) and Kayım et al. (2011). The primary constituents of Σ SFA were 16:0 fatty acids, whereas 18:1 ω-9 and 16:1 ω-7 fatty acids represented the largest proportions among the MUFAs, consistent with the results of Kaçar et al. (2021). January is the spawning period; therefore, the significantly lower levels of DHA may be associated with this occurrence. Kris-Etherton et al. (2002) indicate that heart disease patients should target approximately one gram of EPA and DHA daily, in line with the dietary guidelines set by the American Heart Association. Sidhu (2003) indicates that a 227 g serving of wild brown trout from the Munzur River provides an adequate amount of EPA and DHA, with values between 0.27 and 0.69 g per meal. Authorities typically recommend a daily intake of 0.2–0.5 g (Innes & Calder, 2020). The values were lower than those documented by Ateş et al. (2013) and Kayım et al. (2011). Nutritional information variations are influenced by biological, physical, and chemical environmental properties, alongside factors including food habits, temperature, salinity, and age, time of year, genders, sexual maturity, and breeding period (Ackman, 1989; Saito et al., 1999; Özyurt and Polat, 2006; Özogul et al., 2009). Fisheries, aquaculture, and consumer health all benefit tremendously from the updating of nutritional information regarding economically significant fish species. The observed higher concentration of EPA relative to DHA is consistent with the findings of Innes and Calder (2020). The intake of EPA and DHA from dietary sources is largely influenced by fish consumption, particularly from fatty fish, which are the primary source of these fatty acids.

ISSN: 0369-8963

Table 3. Fatty acid composition of *Salmo trutta macrostigma* in Munzur River (% of total fatty acids)

Fish Samples	Fish									
_	1	2	3	4	5	6	7	8	9	10
FAME	%	%	%	%	%	%	%	%	%	%
C12:0-Lauric acid	0.40	2.80	1.95	3.45	0.05	2.08	1.80	0.44	0.53	1.18
C14:0-Myristic acid	3.29	3.73	2.86	3.73	3.97	4.75	3.68	3.61	3.84	3.78
C16:0-Palmitic acid	20.87	16.82	19.12	19.79	14.54	18.03	19.13	17.31	18.75	19.05
C17:0-Heptadecanoic acid										
(Margaric acid)	0.14	0.12	0.51	0	0.21	0.17	0.12	0.23	0.26	0.18
C18:0-Stearic acid	4.54	3.50	3.58	3.72	3.41	4.66	3.96	5.11	3.86	4.21
C20:0-Arachidic acid	0	0.44	0	0	0.52	0.58	0.35	0.59	0.46	0.38
C22:0-Behenic acid	0	0	2.22	0	1.42	1.95	1.89	1.81	1.96	1.78
ΣSFA	29.23	27.41	30.24	30.68	24.12	32.22	30.94	29.10	28.42	29.15
C16:1 ω-7-Palmitoleic acid	12.43	13.21	10.16	10.01	12.34	10.59	12.53	11.53	10.75	11.68
C17:1 ω-7-Heptadecenoic acid	1.67	1.46	1.34	1.38	2.17	1.14	1.39	1.59	1.42	1.49
C18:1 ω-9-Oleic acid	24.08	24.30	26.30	26.67	26.84	19.82	21.29	20.98	25.18	23.42
C20:1 ω-9-Eicosenoic acid										
(Gadoleic acid)	3.01	2.57	2.57	2.74	5.73	6.93	4.54	5.57	4.97	3.81
ΣMUFA	41.20	41.54	40.38	40.79	47.08	38.49	39.74	39.67	40.65	39.95
C18:3 ω-3-Linolenic acid	10.77	13.17	12.85	11.90	8.84	14.13	14.25	13.23	12.56	13.65
C20:3 ω-3-Eicosatrienoic acid										
(11c-14c-17c)	3.11	1.63	2.05	1.63	2.91	3.84	2.30	3.14	2.85	3.68
C20:5 ω-3-Eicosapentaenoic										
acid (EPA)	6.06	6.93	1.77	2.59	8.05	5.74	6.24	7.27	6.21	5.25
C22:6 ω-3-Docosahexaenoic										
acid (DHA)	1.55	2.25	2.51	2.29	3.06	1.75	2.27	2.16	1.65	1.70
Σω-3PUFA	21.49	23.99	19.17	18.41	22.86	25.46	25.06	25.80	18.55	21.23
C18:2 ω-6-Linoleic acid	6.09	5.68	5.30	5.34	5.94	3.83	4.27	5.42	5.48	4.16
C20:4 ω-6-Arachidonic acid	1.99	1.39	4.91	4.78	0	0	0	0	1.21	0
Σω-6PUFA	8.08	7.07	10.21	10.11	5.94	3.83	4.27	5.42	5.65	4.85
ΣΡυγΑ	29.57	31.06	29.39	28.53	28.81	29.29	29.32	31.23	29.62	28.53
ω3/ω6 ratio	2.66	3.39	1.88	1.82	3.85	6.64	5.87	4.76	3.25	5.34
EPA+DHA	7.61	9.18	4.27	4.88	11.11	7.49	8.51	9.43	8.52	14.23

3.2 Microbiological analysis

The Microbiological Analysis of wild Munzur trout is shown in Table 4. analysis results for microorganisms showed that *E. coli*, *E. coli* O157, *Listeria monocytogenes, Salmonella spp., Vibrio parahaemolyticus* and *Vibrio cholera* microorganisms were not observed in any sample. These results showed that Munzur trout in the natural environment is sterile in terms of these organisms. Specifically, a typical colony APİ 20E test was performed at the end of *Vibrio* analysis. As a result of this test, the colony was found to be *Vibrio fluvialis*. However, coliform microorganism was observed in six samples. These are 2.55x10³ in fish 1; 7.05x10³ fish 2; 1x10² fish 3; 1x10² fish 7; 1x10² in fish 8 and 1,5x10² in fish 9, respectively. In addition, Coagulase (+) *Staphylococcus aureus* was found in only 5x10 (in Fish 8) in only one sample.

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In addition, Total Mesophilic Aerobic Bacteria (TMAB) analysis was found in all samples taken. Values were close to each other and generally between $4x10^2$ and $1.45x10^3$. The values identified in our study align with the findings from research conducted on trout samples sourced from their natural habitat (Öksüztepe et al., 2011). As a result, we can say that there are no harmful or dangerous microorganisms in the Munzur Trout, which are grown in natural environment, and the ones found are not at the limit values that would create a danger to human health. Total Mesophilic Aerobic Bacteria (TMAB) results were lower than the values described in ICMSF (1986).

Table 4. Microorganism analysis results

Microorganisms	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10
E. coli	-	-	-	-	-	-	-	-	-	-
Coliform	$2,55x10^3$	$7,05x10^3$	$1x10^{2}$	-	-	-	$1x10^{2}$	$1x10^{2}$	$1,5x10^2$	-
E. coli O157	-	-	-	-	-	-	-	-	-	-
Coagulase (+)	-	-	-	-	-	-	-	5x10 ¹	-	-
Staphlococcus										
aureus										
Listeria	-	-	-	-		=.	-	-	-	-
monocytogenes										
Salmonella spp.	-	-	-	-	-	-	-	-	-	-
Vibrio	-	-	-	-	-	-	-	-	-	-
parahaemolyticus										
Vibrio cholerae	-	-	-	-	-	-	-	-	-	-
Total Mesophilic	$1,03x10^4$	1,44x10 ⁴	$1,45 \times 10^3$	$4x10^{2}$	1,77x10 ⁴	$1,45 \times 10^3$	$2,5x10^3$	$2,37x10^4$	$2,15x10^3$	1.85×10^{3}
Aerobic Bacteria										
(TMAB)										

3.3 Proximate analysis

The results of proximate analysis of Munzur Trout show that protein, moisture, lipid and ash analysis results were close to each other. For example, protein analysis values of all fish samples were close to each other. Similarly, moisture, lipid and ash analyzes were found to be proportionally similar. Totally 10 fish samples used in the analysis, protein results are generally 17.96 to 20.15; moisture results from 75.47 to 77.31; lipid values were between 2.42 and 2.97 and ash values were between 1.05 and 1.40, respectively (Table 5). The results align with the studies carried out by Ateş et al. (2013) and Kayım et al. (2011). The chemical composition of fish is influenced by various variables, including dietary habits, species,

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variations in the seasons, geographical location, biological conditions, age, gender maturity, reproductive status, and temperature (Taşbozan & Gökçe, 2017).

Table 5. Proximate analysis (%) of Munzur trout

Samples name	Protein (%)	Moisture (%)	Lipid (%)	Ash (%)
Fish 1	19.87	76.39	2.65	1.08
Fish 2	20.15	75.47	2.97	1.40
Fish 3	19.33	76.56	2.75	1.35
Fish 4	19.98	76.37	2.56	1.09
Fish 5	19.48	76.13	2.76	1.31
Fish 6	19.27	76.43	2.42	1.18
Fish 7	19.14	77.05	2.74	1.05
Fish 8	19.65	76.54	2.84	1.12
Fish 9	18.94	77.25	2.64	1.17
Fish 10	18.65	77.31	2.86	1.17

3.4 Heavy metal analysis

The findings of the heavy metal analysis show that in most of the samples a small amount of all heavy metals was found (Table 6). However, although some metals (such as copper) are in the heavy metal class, their trace amounts are nutritious for aquatic organisms. According to the results of the analysis, Pb heavy metal was found to be 35 µg/kg in 4 samples but not in the other 4 samples. Cd and As heavy metals were found in similar proportions in all samples, even in trace amounts. However, there is not a proportional balance between the groups of fish in terms of Cd and As results, and the values found in the fish vary greatly. These two heavy metals were found in some fish but not in other fish samples. Hg heavy metal values were above 50 µg/kg in each fish sample analyzed. Finally, Cu analysis showed a high rate in all fish. Generally Cu is not as toxic as other heavy metals, but is usually between 355 and 2119 µg/kg in all fish. All the values determined in this study were so lower than the values described in the Turkish Food Codex Regulation on contaminants in foodstuffs (Turkish Legislation, 2011). There are no values described for As and Cu in the Turkish legislation, therefore it was not possible to evaluate these values determined in the study. Cd and Pb results were found lower than the values found by Kayım et al. (2011), whereas the Cu values were found almost same in both studies. Although there are lower and higher values in terms of As, Cd and Cu in

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this study, in general all the values were found as comparable with the results of Can et al. (2012). Although all of the values in this investigation were lower than those reported in Turkish Legislation (2011), the Hg levels were much higher than those observed by Can et al. (2012). The concentration and toxicity of heavy metals in aquatic organisms vary based on the specific metal and the species involved. Even within the same species, the accumulation levels and toxicity of the same heavy metal might vary. Once accumulated by an aquatic organism, heavy metals can be passed on to higher classes of the food chain. For the health of humans and ecological equilibrium, it is crucial to ascertain the levels of heavy metal accumulation in fish, which make up a large portion of the food chain's protein sources (Gündoğdu & Erdem, 2008).

Fish tissues and organs are immediately impacted by the buildup of heavy metals from industrial waste that is dumped into the water, which causes environmental disruption and loss (Pasha, 2016).

Table 6. Heavy metal concentrations of *Salmo trutta macrostigma* in Munzur River (μg/kg fresh weight)

Heavy metals (μg/kg)	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10
Pb	35.400	2.483	34.730	35.350	n.d.*	n.d.	n.d.	n.d.	n.d	34.525
Cd	10.435	1.220	11.760	0.850	0.518	1.294	0.924	0.592	1.083	0.896
As	59.920	39.660	127.700	18.340	14.930	n.d.	7.848	11.410	16.42	43.65
Hg	92.469	75.885	56.950	102.300	95.160	50.640	65.510	63.530	60.215	72.456
Cu	609.500	489.100	709.500	550.600	541.200	2119.000	394.800	355.100	518.600	378.956

^{*}not detected

4. CONCLUSION

In summary, wild brown trout from the Munzur River may be considered a major and safe source of protein and ω -3 fatty acids based on the nutritional profile evaluated in terms of microbiological and heavy metal results as well as proximate and fatty acid content. A high percentage of ω -3 PUFA and a significant amount of DHA, EPA, LNA, and LA—all of which are regarded as crucial nutrients for fish to eat—were characteristics of the lipid structure.

Conflict of interest

The authors have declared no conflicts of interest for this article.

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Author statement

Gülderen Kurt Kaya: Investigation; Methodology; Software; Writing –original draft.

Halil Yalçın: Investigation; Methodology; Software; Writing –original draft. Gül Çelik

Çakıroğulları: Methodology; Software; Writing –original draft.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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