

Comparison of micro and macro element contents of commercial fish species sold in the Erzurum province

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Abstract

The purpose of this study was to investigate changes in macro element (Sodium (Na⁺), Potassium (K⁺), Calcium (Ca⁺²), Magnesium (Mg⁺²) and Phosphorus (P)) and microelement (Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni), Zinc (Zn), and Cadmium (Cd)) levels in muscle tissues of commercial fish species sold in the Erzurum, Turkey. In the study, 6 individuals of each species were used. Tissue samples were analyzed for Na, K, Ca, Mg, P, Cu, Fe, Mn, Ni, Zn, and Cd levels, separately, by ICP-OES method. There were significant changes between the macro and microelement levels of freshwater and marine fish. The results of this study showed that environmental differences may be the reason for the changes in macro and microelement levels of freshwater and marine fish.

Keywords: Fish, muscle tissue, ICP-OES, microelement, macroelement.

1. Introduction

Today, industrial and urban wastes are dumped directly into the aquatic environment and lead to bioaccumulation and bio-adhesion of heavy metals in fish. Therefore, fish are known as the best indicators of metal pollution in water systems depending on size and age (Burger *et al.*, 2002; Karatas, 2014). Elements in fish are examined in two groups, macro and micro elements. Microelements required for plant, animal

and human life have beneficial or harmful effects depending on concentration and amount. Therefore, aquatic pollutants have significant toxic potential (Gagnaire *et al.*, 2004; Karatas, 2014). Inadequacy of macro element slows down the growth and nutrition due to low productivity in living things. There are a number of factors that affect mineral intake in fish (Altıntaş, 2013). These factors can be listed as production of the organism and the nature, age, relation with other foods, mineral intake, adaptation of the living organism to nutrition and growth (Tchounwou *et al.*, 2014). Na and K provide the sustainability of the osmotic pressure in blood and the acid-base balance (Karnaky 1998; Keleştemur 2012). Mg plays an important role in neurochemical impulse transmission and muscle stimulation. In addition, Mg has an important role in the absorption of calcium ions from the intestines (Karnaky, 1998; Railo *et al.*, 1985; Keleştemur 2012). As in all living beings, the main factors affecting acid-base balance in fish are nutrition and environment. In intensive farming, the immune system must be strengthened for the sustainability of the acid-base balance (Moeller and Robert, 2001; Moraes, 2004). This study was carried out to determine the macro (Na, K, Ca, Mg and P) micro (Cu, Fe, Mn, Ni, Zn, and Cd) element levels of freshwater fish (rainbow trout (*Oncorhynchus mykiss*)), and marine fish (sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*), and anchovy (*Engraulis encrasicolus*)) offered for human consumption.

2. Materials and methods

2.1 Experimental animals: Rainbow trout (*Oncorhynchus mykiss*), sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*), and Anchovy (*Engraulis encrasicolus*) were purchased from the

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supermarket in Erzurum, Turkey (2011). 6 fish were used for each group.

2.2 Determination of micro and macroelement levels in muscle tissue: The fish were made into a fillet after their internal organs were removed and a sample of 10 g muscle tissue was taken for each fish. Muscle samples were thoroughly disintegrated with the help of a bisturia, and the disintegrated muscle samples were allowed to dry at 105°C for one day. The dried samples were placed in airtight polyethylene bags after being powdered into the air. The mineral content of muscle tissues was determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). The 2 gr weighing dried samples were finely crushed by using mortar and pestle. The tissues were digested overnight followed by hot plate method (120 °C) after mixing with 20 ml concentrated nitric acid and perchloric acid (2:1 v/v) (Merck) (Mertens, 2005b). All samples were completed to 50 mL with 20% conc. nitric acid in distilled water and then filtered with Whatman filter paper (11 ml). ICP-OES with radial torch equipped with argon saturation assembly was used for the determination of Na, K, Ca, Mg, P, Cu, Fe, Mn, Ni, Zn, and Cd. High purity (99.99%) argon was used as plasma, auxiliary and nebulizer gas. Element concentrations were calculated in milligrams/kilogram. Detailed information on ICP-OES can be found elsewhere (Karatas, 2014).

0.5 grams' samples were taken from the muscle tissue of each fish and they were placed in a plastic balcony tube and they were allowed to stand for 10 minutes immediately after the addition of 2 ml of NO₃ (nitrite oxide), the mixture. Then the mouths of the tubes were closed and subjected to a burning process in the microwave for half an hour. The burning process is carried out in 3 different steps (step 1: 5 minutes at 75% microwave power at 145°C, step 2: 10 minutes at 90% microwave power at 180°C and 10 minutes at 40% microwave power at 100°C) in a pressurized microwave wet combustion unit (SpeedWave MWS-2 Berghof product + Instruments Harresstr.1. 72800 Enien Germany) resistant to 40 bar (Mertens, 2005a). After the burning process, the lids of the tubes are slightly loosened to allow gas to escape from the tube. After the gas outlet is completed, the tubes are opened and the dissolved samples are passed through the filter paper and they

are made ready to be read by adding distilled water over the total volume of 25 ml (Karatas, 2014).

2.3 Statistical analysis: All data obtained from this study were analyzed by Duncan-test. SPSS 15.0 statistical package program was used for this purpose. Duncan-test was used to determine the differences between micro, and macro element levels of fish. P<0.05 was statistically considered significant.

3. Results and Discussion: The micro and macro element content in muscle tissues of freshwater and marine fish are summarized in Table 1 and Table 2. Potassium (K), the main cation of intracellular fluid is the main ion of sodium (Na) and Na and K are important ions that ensure the acid-base balance, permeability of cell membranes, functioning of nerves and muscles as well as the osmotic pressure of the body fluids (Lilly *et al.*, 2017). Sodium (Na) and potassium (K) levels in the water are important to meet the basic needs of fish, but it should be supplemented with nutritionally best Na⁺ and K⁺ ratios in fish growth (Kalantarian *et al.*, 2013). K deficiency in fish has been reported to cause loss of appetite (Kopp *et al.*, 2013). Respiration in hunger and toughness is necessary for maintaining mineral balance and maintaining vital functions (Salem *et al.*, 2007). There were significantly different in the Na (290.0-1162.6 mg/kg) and K (3335.2-4576.0 mg/kg) levels in muscle tissues of freshwater and marine fish (p<0.05) (Table 1). The Na values were observed as 464.0-1247.0 mg/kg in rainbow trout, 289-1350 mg/kg in *Sparus aurata*, 773-1089 mg/kg in *Dicentrarchus labrax* (Erkan and Özden, 2007; Rubio *et al.*, 2011; Karatas, 2014), 911-1181 mg/kg in *Engraulis encrasicolus* (Erdemet *et al.*, 2021), 2300 mg/100g in *Eriphia verrucosa* and 1662 mg/100g in *Crangon crangon* (Erdemet *et al.*, 2015). K values were determined as 4261-4615 mg / kg in *Oncorhynchus mykiss*, 3035 mg/100g in *Eriphia verrucosa* and 2883mg/100g in *Crangon crangon* (Erdemet *et al.*, 2015), 2890 mg/kg in *Carangoides chrysophrys* (Lourenco *et al.*, 2009), 4140 mg/kg in *Carangoides chrysophrys* (Lilly *et al.*, 2017), 996.0 mg/kg in *Parapenaeus longirostris* and 644.9 mg/kg in *Parapenaeus martia* (Oksuzet *et al.*, 2009), 4140 mg/kg in *Hemiramphus far* (Lilly *et al.*, 2017), 4597mg/kg in *Dicentrarchus labrax* and 3938 mg/kg in *Sparus aurata* (Erkan and Ozden, 2007). 4604 mg/kg in *Sparus aurata*, 3510 mg/kg in *Dicentrarchus labrax*

(Rubio *et al.*, 2011) and 2655-4274 mg/kg in *Engraulis encrasicolus* (Erdemet *et al.*, 2021).

Magnesium (Mg) is the most abundant mineral after potassium in the cell, and it carries out many metabolic events including maintaining the correct distribution of Na, K, and Ca in the cell (Terech-Majewska *et al.*, 2015). Mg is involved in the formation of bones and teeth, in metabolism, in the synthesis of nucleic acids and proteins, and in thermoregulation (Lilly *et al.*, 2017). In this study, the Mg values were in the range of 238.3-345.4 mg/kg in muscle tissues of freshwater and marine fish ($p < 0.05$) (Table 1). The Mg values were in range of 335.7-336.6 mg/kg in *Oncorhynchus mykiss* (Karatas, 2014; Siemianowska *et al.*, 2016), 101.4 mg/100g in *Eriphia verrucosa* and 323.4mg/100g in *Crangon crangon* (Erdemet *et al.*, 2015), 382.0 mg/kg in *Parapenaeus longirostris* and 579.0 mg/kg in *Parapenaeus martia* (Oksuzet *et al.*, 2009), 403.8-509.4 mg/kg in *Engraulis encrasicolus* (Erdemet *et al.*, 2021), 300.5 mg/kg in *Dicentrarchus labrax* and 384.4 mg/kg in *Sparus aurata* (Rubio *et al.*, 2011), 222 mg/kg in *Sparus aurata* and 326 mg/kg in *Dicentrarchus labrax* (Erkan and Ozden, 2007).

Calcium (Ca) has an important role in the regulation of muscle, nerve and heart functions as well as the development of bones and teeth (Lilly *et al.*, 2017). Ca, one of the most abundant cations in the body of a fish, plays an important role not only in skeletal development but also in the realization of many physiological processes (Terech-Majewska *et al.*, 2015). In this study, the Ca levels and were in the range of 248.2-939.4 mg/kg in muscle tissues of freshwater and marine fish ($p < 0.05$) (Table 1). Ca levels may vary according to fish species such as 61.20 mg/100g in *Dussumieria acuta*, 28.30 mg/100g in *Hemiramphus far*, 188.4-517.4 mg/kg in *Oncorhynchus mykiss* (Siemianowska *et al.*, 2016), 1.51 mg/100g in Indian mackerel 3.03 mg/100g in *sixbar grouper*, 1.04 mg/100g in *Japanese threadfin bream* and 1.02 mg/100g in *Spanish mackerel* (Lilly *et al.*, 2017), 978.2-1265.5 mg/kg in *Engraulis encrasicolus* (Erdemet *et al.*, 2021), 192- 3475 mg/kg in *Sparus aurata* and 636-3391mg/kg in *Dicentrarchus labrax* (Erkan and Ozden, 2007; Rubio *et al.*, 2011), 896.8 mg/kg in *Eriphia verrucosa*, 387.1 mg/kg in *Crangon crangon* (Erdemet *et al.*, 2015), 495 mg/kg in

Parapenaeus longirostris and 322.5 mg/kg in *Parapenaeus martia* (Oksuzet *et al.*, 2009).

Phosphorus (P) is a component of bones, teeth, high energetic compounds, nucleic acids, caffeine, lecithin, cell membranes and blood (Lilly *et al.*, 2017). Phosphorus is also a mineral that is responsible for balancing the normal pH value in intracellular and extracellular fluids. Contrary to calcium, phosphorus is the main source of nutrients (Terech-Majewska *et al.*, 2015). In this study, P levels were in the range of 2287.8-3592.0 mg/kg in muscle tissues of freshwater and marine fish ($p < 0.05$) (Table 1). P values in studies conducted on different species of fish were determined as 2485.7-2738.1 mg/kg in *Oncorhynchus mykiss* (Siemianowska *et al.*, 2016), 3119.0 mg/100g in *Eriphia verrucosa* and 2118.0mg/100g in *Crangon crangon* (Erdemet *et al.*, 2015), 933.0 mg/kg in *Parapenaeus longirostris* and 1344.6 mg/kg in *Parapenaeus martia* (Oksuzet *et al.*, 2009), 2282.1 mg/kg in *Engraulis encrasicolus* (Kocet *et al.*, 2016), 3560 mg/kg in *Sparus aurata* and 3736 mg/kg in *Dicentrarchus labrax* (Erkan and Ozden, 2007).

Iron (Fe) and copper (Cu), which have an important role in biological systems of organisms and fish, are basic elements (Karatas, 2014). In this study, Fe, Cu and Mn levels were found in the ranges of 3.10-15.8, 0.40-0.86, and 0.39-1.27 mg/kg in muscle tissues of freshwater and marine fish, respectively ($p < 0.05$) (Table 2). Fe, Cu and Mn levels were determined as 3.20, 1.42 and 0.42 mg/kg in *D. labrax* and 3.09, 2.52 and 0.36 mg/kg in *S. aurata* (Rubio *et al.*, 2011) and 3.52-0.42-0.82 mg/kg in *Oncorhynchus mykiss* (Varol and Sünbül, 2017), and 17.93-52.9, 1.33-2.21, 0.57-1.55 mg/kg in *Engraulis encrasicolus* (Akaydin 2004; Erdemet *et al.*, 2021). Fe contents were determined as 225 mg/kg in *Sparus aurata*, 24.7 mg/kg in *Dicentrarchus labrax* (Erkan and Ozden, 2007) and 10.2-38.9 mg/kg in *Engraulis encrasicolus* (Turhanet *et al.*, 2004). According to the Turkish Food Codex, the maximum level of Cu is 20 mg/kg.

Nickel (Ni) is involved in the maintenance and production of cells and is an activator of certain enzyme systems such as oxidoreductases and hydrolysis. It is found in the structure of RNA and DNA (Blandzicet *et al.*, 2018). In this study, Ni levels of

fish were in the range of 0.03-11.4 mg/kg in muscle tissue of freshwater and marine fish ($p < 0.05$) (Table 2). Ni levels were determined as 9.63-12 mg/kg in *D. labrax* and 13-27.6 mg/kg in *S. aurata* (Rubio *et al.*, 2011; Bilandžić *et al.*, 2018) and 0.085-0.890 mg/kg in *Oncorhynchus mykiss* (Karatas, 2014; Varol and Sünbül, 2017), and 17.93-52.9, 1.33-2.21, 0.57-1.55 mg/kg in *Engraulis encrasicolus* (Akaydin 2004; Erdem *et al.*, 2021).

Zinc (Zn) is present in most of the metabolic pathways in humans and zinc deficiency can cause loss of appetite, loss of growth, skin alterations and immunological abnormalities (Karatas, 2014). In this study, Zn levels in muscle tissues of freshwater and marine fish were in the range of 4.7-15.4 mg/kg ($p < 0.05$) (Table 2). Zn levels of fish living in the Swan and Boeuf lakes have been reported as 45-60.9 and 4.62-14.6 mg/kg (Park and Presley, 1997; Aucoinet *et al.*, 1999). Varol and Sünbül (2017) reported that Zn content of *Oncorhynchus mykiss* was 4.42 and 4.41 mg/kg. The zinc levels in muscle tissue of *Engraulis encrasicolus* reported as 7.2 and 30.72 mg/kg, respectively (Akaydin 2004; Bat *et al.*, 2014; Erdem *et al.*, 2021), 4.9-8.11 mg/kg in *Sparus aurata* and 5.4-10.11 mg/kg in *Dicentrarchus labrax* (Rubio *et al.*, 2011; Bilandžić *et al.*, 2018). According to the Turkish Food Codex, the maximum level of Zn is 50 mg / kg.

Cadmium (Cd) is known as one of the environmental pollutants and is among the most dangerous substances (ATSDR, 2012). While acute effects of cadmium lead to intoxication, pulmonary edema, hemorrhage, testicular damage and mortality, long-term exposure to Cd is observed nephrotoxicity, osteotoxicity and immunotoxicity (ATSDR, 2012). In this study, Cd levels could not be determined in trout specie. But, Cd levels were observed in the range of 0.02-0.09 mg/kg in muscle tissues of different marine fish ($p < 0.05$) (Table 2). Varol and Sünbül (2017) reported that Cd content of *Oncorhynchus mykiss* was 0.00038 mg/kg. Zinc levels in muscle tissue of *Engraulis encrasicolus* were in the range of 0.0013-30.72 mg/kg, respectively (Akaydin 2004; Aygun and Abanoz, 2011; Bat *et al.*, 2014; Erdem *et al.*, 2021), 2.9 $\mu\text{g kg}^{-1}$ -1.36 mg/kg in *Sparus aurata* and 4.9 $\mu\text{g kg}^{-1}$ -3.33 mg/kg in *Dicentrarchus labrax* (Rubio *et al.*, 2011; Bilandžić *et al.*, 2018).

Table 1. Macroelement levels in muscle tissues of the Freshwater and Marine fish

Muscle Values (mg/kg)	Ca	P	Mg	Na	K
Freshwater fish					
Rainbow trout	248.2±5.53 ^d	2807.0±10.3 ^b	345.4±2.5 ^{7a}	1162.6±1.6 ^{9a}	4465.3±3.5 ^{0b}
Marine fish					
Sea bass	593.1±5.47 ^b	3592.0±19.2 ^a	333.0±10.3 ^{ab}	775.0±11.1 ^c	4576.0±4.1 ^{8a}
Sea bream	194.2±11.4 ^c	3574.0±43.9 ^a	238.3±10.1 ^c	290.0±7.90 ^d	4028.0±11.5 ^c
Anchovy	939.4±8.09 ^a	2287.8±12.4 ^c	343.0±19.8 ^a	1037.6±5.5 ^{9b}	3335.2±8.9 ^{2d}

The results were given as mean and standard deviation. Different letters indicate differences between groups

Table 2. Microelement levels in muscle tissues of the Freshwater and Marine fish

Muscle Values (mg/kg)	Cu	Fe	Mn	Ni	Zn	Cd
Rainbow Trout	0.4±0.0 ^{3d}	3.7±0.0 ^{.2b}	6±0.0 ^{.02b}	0.0±3±0 ^{.13d}	4.7±0.07 ^c	Not determined
Sea bass	0.5±4±0.0 ^{3b}	3.2±0.0 ^{.10c}	0.4±4±0.0 ^{.01c}	10.5±0.59 ^b	5.3±0.11 ^b	0.07±0.0 ^{1b}
Sea bream	0.4±8±0.0 ^{2c}	3.1±0.0 ^{.07c}	0.3±9±0.0 ^{.03c}	11.4±4±0.63 ^a	4.7±5±0.21 ^c	0.09±0.0 ^{2a}
Anchovy	0.8±6±0.0 ^{4a}	15.7±0.0 ^{.83a}	1.2±7±0.0 ^{.a}	0.5±4±0.0 ^{.04c}	15.4±4±0.29 ^a	0.02±0.0 ^{1c}

The results were given as mean and standard deviation. Different letters indicate differences between groups

4. Conclusion

It was determined that there were significant differences between Ca, P, Mg, Na, K, Cu, Fe, Mn, Ni, Zn and Cd levels in muscle tissues of marine and freshwater fish. However, Cu, Fe, Mn, Ni, Zn and Cd levels were below the upper limits allowed for trace elements in various standards such as health criteria (mg/kg), MAFF (1995) (mg/kg), Turkish standards (mg/kg) and other standards (mg/kg). Moreover, there was no negative situation in terms of heavy metal levels in muscle tissues of fish offered for human consumption. This study may provide useful information in terms of shedding light on future studies.

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