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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Assessment of element concentrations in widely consumed cultured fish in Turkey

Türkiye'de yaygın olarak tüketilen kültür balıklarında element konsantrasyonlarının değerlendirilmesi

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Özet: Balık çiftlikleri, insanların tüketmesi için yüksek miktarlarda besin üretmektedir. Fakat sucul ekosistemlerde ağır metal kirliliği, ciddi bir problem olabilir. Bu çalışmada, Aralık 2011 ve Ocak 2013 tarihleri arasında Kocaeli ilinde bulunan balık hallerinden alınan üç kültür balığı türünün (*Oncoryhynicus mykiss* (gökkuşağı alabalığı), *Dicentrarchus labrax* (levrek) ve *Sparus aurata* (çipura)), yenilebilir dokularında bulunan mikronütrient ve esansiyel olmayan element konsantrasyonları izlenmiştir. Ca, K, Mg, Na, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb, Se, ve Zn konsantrasyonları ICP-MS kullanılarak analiz edilmiş ve bölge halkı için bu balıkların tüketimi sonucu bu elementlerin yıllık alım miktarları hesaplanmıştır. Balık kas dokusunda bulunan element konsantrasyonlarının hiç biri, Birleşmiş Milletler Gıda ve Tarım Örgütü ve Türk Gıda Kodeksi tarafından belirlenen limitleri aşmamıştır. Hesaplanan element alım miktarları, bu çiftlik balıklarının insanlar tarafından tüketilmesinin risk oluşturmadığını göstermiştir. Temel bileşenler analizi, deniz çiftlik balıklarının kas dokusundaki elementlerin üç bileşen altında gruplanabileceğini göstermiştir.

Anahtar kelimeler: Çiftlik balığı, çipura, element, gökkuşağı alabalığı, levrek.

Abstract: Fish farms are able to produce high amount of quality food for human consumption. On the other hand, heavy metal pollution in aquatic ecosystems can be a serious problem. In this study, some micronutrients and non-essential element concentrations in edible tissue of three cultured fish species, (*Oncoryhynicus mykiss* (rainbow trout), *Dicentrarchus labrax* (seabass) and *Sparus aurata* (seabream)) which were taken from primary fish markets of Kocaeli province between December 2011 and January 2013 were monitored. Concentrations of Ca, K, Mg, Na, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb, Se, and Zn were analyzed using ICP-MS and annual intakes of these elements via consumption of fish were calculated for inhabitants. None of the element concentrations in the fish muscle tissue exceeded the threshold levels which were suggested by Food and Agriculture Organization and by the Turkish Food Codex and based on calculated element intake rates, there were no risk for humans to consume these farm fishes. Principal Component Analysis reveals that elements can be grouped under three components in cultured marine fish muscle tissue.

Keywords: Farm fish, element, rainbow trout, seabass, seabream.

INTRODUCTION

Fish is on the top of aquatic food pyramid and is an important protein source for human diet. With progressing technology in fish culture, this important protein source is becoming available for many people. Heavy metal pollution of marine environment has long been recognized as a serious environmental concern. In aquatic environments, pollutants are potentially accumulated in aquatic organisms and sediment. Therefore, heavy metals in aquatic environments are transferred through food chain into humans. It is well known that edible parts of fish (muscle and skin) are not active tissues in accumulating heavy metals. (Kalay et al. 1999) But it was informed that heavy metal levels of edible portions of some fish in polluted regions exceeded acceptable levels (Tüzen, 2003; Uysal et al. 2008). It has been recognized for many years that concentrations of metals found in coastal areas, whether they are in the dissolved or the particulate phase may be derived from a variety of anthropogenic and natural sources. Through atmospheric deposition, sewage outfalls, urban storm water, and agricultural and industrial runoff, heavy metals may enter marine and pond fish culture areas and subsequently pose a human health risk. On the other hand, the residual content of contaminants (metals and organics) in cultured fish diet constituents (fish), and of Cu, which is commonly used in antifouling chemicals for the treatment of net-pen cages, has drawn little attention regarding impacts to the aquatic environment (Burridge et al. 1999; Cheung et al. 2008).

Aquaculture currently provides a considerable proportion of edible fish which is expected to increase in future decades in order to meet the needs of the growing human population (Duarte et al. 2009).

Heavy metal load from fish is a serious problem especially for people living in settlements which are densely populated.

Kocaeli, which is located at the Marmara region, is a good example for its dense population. Also the city has undergone heavy industrialization since the 1960s, which was followed by a rapid increase in population and an irregular urbanization (Karademir 2004).

In this study the concentrations of metals in edible tissue of culture breed *Oncoryhynicus mykiss, Sparus aurata* and *Dicentrarchus labrax* that are commercially available in the Kocaeli fish markets were determined using Inductive Coupled Plasma-Mass Spectrometry (ICP-MS). The aims of this study are to determine changes in concentrations of some non-essential elements and some micronutrients in the muscle tissue of culture breed fish over a year, and to calculate metal loads for individuals in Kocaeli exposed via consumption of these fish.

MATERIALS AND METHODS

Oncoryhynicus mykiss, Sparus aurata and *Dicentrarchus labrax* which are produced in fish farms were purchased from the primary fish markets of Kocaeli province in December 2011 and January 2013. Fish samples (i.e. *O. mykiss*, which are fed in Akyazı district of Sakarya province, and *S. aurata* and *D. labrax* which are fed in Aegean Sea on offshore of Aydın province) were put into polyethylene containers and kept cool for immediate transportation to the laboratory. Before dissection length and weight measurements of all samples were done (Table 1). Ten individuals per fish species were filleted using porcelain knives to avoid any metal contamination. Dissected muscle tissues were homogenized and fifty grams freeze dried until constant dry weight. Dried samples were gently powdered and homogenized by porcelain mortar and pestle. Muscle dry/wet weight ratios were calculated gravimetrically (Table 1).

0.5 g dried samples were digested for 30 minutes with mixture of i.e. 7 mL HNO_3, 1 mL HCl and 1 mL H_2O_2 in a microwave digestion system at 200 °C (1000 W). Suprapure Merck chemicals were used upon digestion. After digestion samples were diluted up to 50 mL with ultra-pure water. All measurements were done with at least 3 replicates. Digested samples and analytical blanks that were prepared in the same acid matrix were analyzed with Perkin Elmer Elan DRC-e model ICP-MS which equipped with Cetax ADX–500 auto sampler and diluter. Internal standards were used for calibration. The relative standard deviations were determined as <5 %. Interferences due to Cl matrix were removed in Dynamic Reaction Cell mode using CH_4 gas. In order to validate the method for accuracy and precision, standard reference material (SRM-NIST 2977 Mussel Tissue) was analyzed for the corresponding elements. Analyze results for SRM were given in Table 2.

To determine the annual exposures of metals to adults a modified version of equation developed by the United States Environmental Protection Agency (EPA 2000) was used:

$$E_m = (C_m\ DC)\ 365.25 \qquad (1)$$

Where; E_m is the annual individual exposure to chemical contaminant m from ingesting fish (mg year^{-1}), C_m is the concentration of chemical contaminant m in the edible portion of the fish (mg kg^{-1}), DC is the mean daily consumption rate of the fish in Kocaeli Province (0.039 kg day^{-1}) considering the study of Karademir (2004) conducted in the city.

Table 1. Sampling dates, locations, muscle tissue dry weight ratios, total lengths, standard lengths and fork lengths of fish that purchased from the primary fish markets in Kocaeli Province

Species	Common Name	Sampling Location	Sampling Date	Total Length (cm)	Standard Length (cm)	Fork Length (cm)	Muscle Dry Weight Ratio (%)
Oncorchynchus mykiss	Rainbow trout	Akyazı	Dec-11	28.6 ± 3.8	26.3 ± 3.1	-	25.2
			Jan-13	29.0 ± 1.0	26.6 ± 0.9	-	23.5
Dicentrarchus labrax	Seabass	Aydın offshore	Dec-11	32.3 ± 0.3	26.8 ± 1.1	30.1 ± 0.6	26.5
			Jan-13	31.0 ± 0.5	25.7 ± 1.0	29.8 ± 0.4	26.6
Sparus aurata	Seabream	Aydın offshore	Dec-11	29.2 ± 0.3	23.4 ± 1.6	26.8 ± 0.2	28.7
			Jan-13	25.9 ± 1.8	22.0 ± 1.1	24.9 ± 2.1	28.4

Table 2. Certified and observed (mean ± standard deviation) values of elemental concentrations in NIST 2977 Mussel tissue standard reference material.

Element	Replicates	Certified	Observed Value	Recovery (%)
Ca (g kg^{-1})	5	8.30	8.40±0.07	101
K (g kg^{-1})	5	12.0	12.4±0.04	103
Mg (g kg^{-1})	5	39.0	40.1±0.47	103
Na (g kg^{-1})	5	24.0	20.4±0.23	85
As (mg kg^{-1})	5	8.83	8.16±0.82	92
Cd (mg kg^{-1})	5	0.18	0.16±0.02	91
Co (mg kg^{-1})	5	0.48	0.44±0.05	93
Cr (mg kg^{-1})	5	3.91	3.34±0.27	86
Cu (mg kg^{-1})	5	9.42	8.64±0.99	92
Fe (mg kg^{-1})	5	274	285±11.9	104
Hg (mg kg^{-1})	5	0.10	0.11±0.01	107
Mn (mg kg^{-1})	5	23.9	22.9±0.86	94
Se (mg kg^{-1})	5	1.78	1.58±0.11	89
Pb (mg kg^{-1})	5	2.27	2.21±0.18	98
Zn (mg kg^{-1})	5	135	128±14.6	90

RESULTS AND DISCUSSION

Concentrations of Ca, K, Mg, Na, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb, Se, and Zn in the muscle tissue of *O. mykiss*, *D. labrax* and *S. aurata* which were harvested in December 2011 and January 2013 are given in Table 3. Average annual element intakes per adult in Kocaeli province via consuming these fishes between December 2011 and January 2013 were calculated according to formula (1) and given in Table 4. Measured element concentrations in all samples were below recommended threshold levels for human consumption which were indicated by FAO (1983) and Turkish Food Codex (TFC, 2002; TFC 2011) (i.e. 1 mg kg^{-1} for As, 0.5 mg kg^{-1} for Cd, 20 mg kg^{-1} for Cu, 1 mg kg^{-1} for Hg, 0.3 mg kg^{-1} for Pb, 50 mg kg^{-1} for Zn).

Presence of heavy metals i.e. As, Cd, Hg and Pb were determined in muscle tissue of cultured fish species in 2011 and 2013. Although below recommended values (i.e. 1 mg kg^{-1}, 0.05 mg kg^{-1}, 1 mg kg^{-1}, and 0.3 mg kg^{-1} wet weight for As, Cd, Hg, and Pb respectively) by FAO (1983) and Turkish Food Codex (TFC 2002, TFC 2011), it is noteworthy that these fish species include non-essential elements. While the highest As concentration was determined in *D. labrax* (i.e. 2.53 mg kg^{-1}), the lowest in *O. mykiss* (i.e. 0.66 mg kg^{-1}) in December 2011 in the present study. In a previous study As was found in lower concentration (i.e. average 0.37 mg kg^{-1} - wet weight) in cultured *D. labrax* (Ersoy et al. 2006) from Mediterranean Sea. On the other hand, higher As concentrations (i.e. average 4.9 mg kg^{-1}) was reported in cultured *S. aurata* (Minganti et al. 2010) from Mediterranean Sea. Fish, those analyzed in the present study, contains an average of 0.07 mg kg^{-1} inorganic As and could be consumed without restriction according to EPA (2000). Because approximately 80% of As in fish muscle is estimated to be in the form of arsenobetaine (Larsen and Francesconi 2003), while inorganic As remain below the recommended level by Turkish Food Codex (2011) and FAO (1983). Cadmium concentrations in the present study varied between 0.001 and 0.004 mg kg^{-1} for all species. In a previous study higher Cd concentration was determined (i.e. 0.012 mg kg^{-1}) in *S. aurata* in a fish farm from Adriatic Sea (Cretì et al. 2010). Both the lowest (i.e. 0.01 mg kg^{-1}) and the highest (i.e 0.25 mg kg^{-1}) Hg levels were determined in *O. mykiss* in 2011 and 2013 respectively in the present study. In a previous study, while average Hg content was reported 0.02 mg kg^{-1} in *O. mykiss* it was reported as 0.04 mg kg^{-1} in *D. labrax* in a fish farm from Canary Island (Hardisson et al. 2012). In another study 0.12 mg kg^{-1} average total Hg concentration was reported in *S. aurata* in a fish farm from Mediterranean Sea (Minganti et al. 2010). All Hg concentrations reported from other studies were lower than the results in the present study (Table 3). Pb values varied between 0.01 and 0.03 mg kg^{-1} in the present study. In previous studies higher Pb concentrations were determined (i.e. 0.16 mg kg^{-1}) in *S. aurata* in a fish farm from Adriatic Sea (Cretì et al. 2010) and in cultured *D. labrax* (i.e. 0.28 mg kg^{-1} - wet weight) (Ersoy et al. 2006) from Mediterranean Sea. Food pellets, precipitated and/or resuspended sediment, and cage materials and equipment including sea vessels can be sources of these non-essential elements in the muscle tissue of fish samples. On the other hand, because of lack of studies on metal contents of artificial food from fish farms, it is difficult to estimate sources of non-essential elements. Though there are some surface sediment studies below the fish farms in the region (Kalantzi et al. 2013; Basaran et al. 2010) it is also difficult to say presence of non-essential elements in the muscle tissue of farm fishes, originated from sediments.

Table 3. Element concentrations (dry weight ± standart deviation) in cultured fish species (i.e. *O. mykiss, D. labrax* and *S.aurata*) harvested from Akyazı and Aegean Sea, on December 2011 and January 2013.

Element	*O. mykiss* Akyazı		*D. labrax* Aydın offshore		*S. aurata* Aydın offshore	
	Dec - 2011	Jan - 2013	Dec - 2011	Jan - 2013	Dec - 2011	Jan - 2013
Ca (g kg^{-1})	1.00±0.20	0.80±0.30	4.90±0.50	2.80±3.30	1.60±0.10	1.20±0.80
K (g kg^{-1})	11.3±6.40	20.9±0.40	0.20±0.10	16.9±0.50	7.50±10.4	19.0±0.30
Mg (g kg^{-1})	1.10±0.10	1.40±0.10	1.60±0.10	1.30±0.10	1.60±0.10	1.30±0.10
Na (g kg^{-1})	4.30±1.50	2.90±0.10	0.01±0.01	6.00±0.10	2.50±3.50	2.60±0.10
As (mg kg^{-1})	0.66±0.01	1.56±0.08	2.53±0.08	0.89±0.06	1.67±0.07	1.17±0.18
Cd (mg kg^{-1})	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01
Co (mg kg^{-1})	0.03±0.01	0.04±0.01	0.09±0.01	0.02±0.01	0.01±0.01	0.06±0.01
Cr (mg kg^{-1})	0.30±0.02	1.26±0.06	0.56±0.10	1.45±0.11	0.30±0.01	1.35±0.11
Cu (mg kg^{-1})	1.30±0.07	0.94±0.01	1.66±0.07	1.23±0.60	1.13±0.11	1.31±0.21
Fe (mg kg^{-1})	6.51±2.58	6.39±0.48	16.8±0.18	7.02±1.45	9.69±0.96	8.45±0.06
Hg (mg kg^{-1})	0.01±0.01	0.27±0.01	0.25±0.01	0.11±0.00	0.18±0.01	0.05±0.01
Mn (mg kg^{-1})	0.76±0.16	0.47±0.11	1.60±0.38	1.82±1.79	0.44±0.05	0.42±0.18
Se (mg kg^{-1})	0.68±0.01	0.87±0.03	0.82±0.06	0.60±0.05	0.67±0.01	0.5±0.03
Pb (mg kg^{-1})	0.01±0.00	0.03±0.01	0.03±0.01	0.01±0.02	BDL	0.03±0.04
Zn (mg kg^{-1})	15.3±0.44	11.9±0.21	16.9±0.98	9.67±0.47	14.3±0.68	9.17±1.03

BDL: Below detection level

Table 4. Based on formula (1) calculated annual element intake per adult in Kocaeli Province via ingesting fish (i.e. *O.mykiss, D.labrax,* and *S.aurata*) between December 2011 and January 2013.

Element	*O. mykiss*	*D. labrax*	*S. aurata*
	Akyazı	Aydın offshore	
Ca (g year^{-1})	3.41 ± 0.53	13.5 ± 5.82	5.70 ± 1.19
K (g year^{-1})	61.0 ± 25.8	28.7 ± 39.5	53.8 ± 32.7
Mg (g year^{-1})	4.73 ± 0.82	5.07 ± 0.95	5.90 ± 0.91
Na (g year^{-1})	13.6 ± 3.75	10.3 ± 13.8	10.4 ± 0.21
As (mg year^{-1})	4.20 ± 2.42	6.03 ± 4.31	5.78 ± 1.48
Cd (mg year^{-1})	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
Co (mg year^{-1})	0.13 ± 0.03	0.19 ± 0.19	0.15 ± 0.13
Cr (mg year^{-1})	2.95 ± 2.57	3.43 ± 2.01	3.35 ± 2.99
Cu (mg year^{-1})	4.24 ± 0.95	5.04 ± 1.3	4.96 ± 0.48
Fe (mg year^{-1})	24.4 ± 0.28	42.0 ± 26.1	36.9 ± 3.82
Hg (mg year^{-1})	0.54 ± 0.69	0.64 ± 0.37	0.47 ± 0.38
Mn (mg year^{-1})	2.33 ± 0.77	5.92 ± 0.25	1.75 ± 0.07
Se (mg year^{-1})	2.93 ± 0.52	2.48 ± 0.66	2.38 ± 0.51
Pb (mg year^{-1})	0.07 ± 0.06	0.07 ± 0.06	0.06 ± 0.08
Zn (mg year^{-1})	51.3 ± 9.05	46.5 ± 19.9	47.8 ± 15.1

The highest Cr concentration was determined in *D. labrax* (i.e. 1.45 mg kg^{-1}) in January 2013 while the lowest Cr concentrations were found in *O. mykiss* (i.e. 0.30 mg kg^{-1}) in December 2011. In contrast to all other elements, approximately four times higher Cr concentrations were determined in January 2013 for all species. In a previous study 0.12 mg kg^{-1} - wet weight Cr was determined in cultured *D. labrax* from Mediterranean Sea (Ersoy et al. 2006). Similar with As, Cd, Cr, and Pb, the highest Co, Cu, Fe, Mn and Zn concentrations were determined in *D. labrax* (i.e. 0.09 mg kg^{-1}, 1.66 mg kg^{-1}, 16.8 mg kg^{-1}, 1.60 mg kg^{-1}, and 16.9 mg kg^{-1} respectively in December 2011). On the other hand, the lowest concentrations of Cu and Fe were determined in *O. mykiss* in January 2013 (i.e. 0.94 mg kg^{-1}, and 6.39 mg kg^{-1} respectively) while the lowest Mn and Zn concentrations were determined in S. aurata in January 2013 (i.e. 0.42 mg kg^{-1}, and 9.17 mg kg^{-1}). In a previous study Cu, Fe, Mn and Zn concentrations were determined in similar concentrations (i.e. 1.3 mg kg^{-1}, 10.3 mg kg^{-1}, 0.5 mg kg^{-1}, and 15.9 mg kg^{-1} respectively) in cultured *S. aurata* in Mediterranean Sea (Minganti et al. 2010). On the other hand it should be noted that, element concentrations in the wild marine fish from the Turkish coast, has much higher element concentrations which were determined in fish muscle tissue (Ergül and Aksan, 2013).

Macronutrient elements i.e. Ca, K, Mg and Na were determined in considerable levels in all fish species. While the highest Ca and Mg concentrations were determined in *D. labrax* the lowest levels of these elements were determined in *O. mykiss*. On the other hand, in contrast to these element concentrations the highest K and Na concentrations were determined in *O. mykiss* while the lowest levels were determined in *D. labrax* (Table 3). In a previous study which was carried out in food pellets that used in Aegean Sea fish farms (Kalantzi et al. 2013) reveals that, average Ca, Mg and Na concentrations in fish muscle tissue in the present study (i.e. 2.10 g kg^{-1}, 1.40 g kg^{-1}, and 3.10 g kg^{-1} respectively) were lower than Ca, Mg and Na levels in food for farm fish (i.e. 13.0 g kg^{-1}, 2.00 g kg^{-1}, and 6.00 g kg^{-1} respectively) except potassium which were determined in average as 12.6 g kg^{-1} in the present study while it is reported as 7.00 g kg^{-1} in food for farm fish.

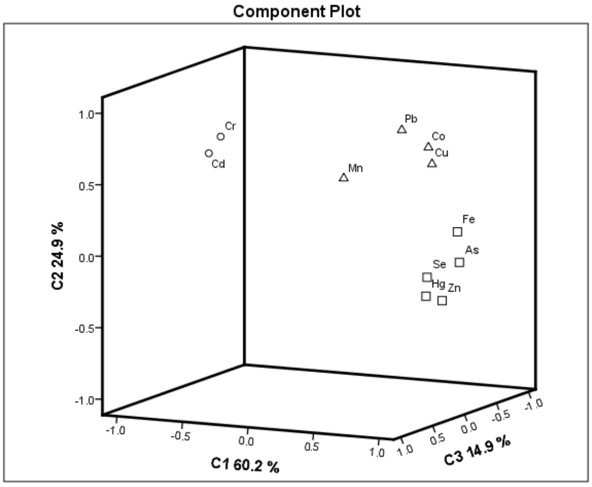

Figure 1: Based on Principal Component Analysis, there components explain total variance for As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb, Se, and Zn in cultured marine fish.

Except macronutrients (i.e. Ca, K, Mg, and Na) Principal Component Analysis (PCA) revealed that elements can be grouped under three components in cultured marine fish muscle tissue (Fig 1). First group contains As, Hg, Fe, Se and Zn that explain 60.2 % of total variance. In this group, significant correlations were determined between Hg and Se ($r = 0.99$, $p < 0.05$). Because it is known that Se and Hg have binding affinity for each other (Paulsson and Lundbergh 1991), this result indicates that Se has a protective effect on health against Hg. Also significant correlations were determined between Hg and Zn ($r = 0.97$, $p < 0.05$) and between As and Fe ($r = 0.98$, $p < 0.05$) in this group. Second group contains Co, Cu, Mn, and Pb that explain 24.9 % of total variance. Although there were no significant correlations ($p > 0.05$) among the elements in this group, Co, Cu, Mn, and Pb are known as transition and post-transition metals and similar chemicals behavior is expected in the tissue. Third group contains Cd and Cr that explain 14.9 % of total variance. Also significant correlation were determined between these elements ($r = 0.98$, $p < 0.05$). Cd and Cr are known by their affinity for proteinaceous tissue (Mathews and Fisher 2009), presence of these metals with high correlation is an expected result in the fish muscle tissue.

CONCLUSIONS

In the present study, element concentrations, including essential and non-essentials, in the muscle tissue of highly consumed farm fish were determined for two consecutive years in muscle tissue and average annual intake of these elements were calculated for adults. To our knowledge, this research is the first element assessment study in cultured fish species in Turkey. None of measured elements exceed threshold levels or recommended values for consumption in fish. Also, comparisons with the previous studies reveal that, metal levels were found much lower than those results in wild marine fish. However, non-essential elements (i.e. As, Cd, Hg, and Pb) were determined in all samples and it is suggested that monitoring of these elements can be useful for further studies. Also in the future studies element measurements in food pellets and nearby surface sediments could supply valuable information to evaluate the results.

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