G.U. Journal of Science
23(1): 41-48 (2010)

www.gujs.org

Calcium, Magnesium, Iron, Zinc, Cadmium, Lead, Copper and Chromium Determinations in Brown Meagre (*Sciaena Umbra*) Bone Stone by Flame and Electrothermal Atomic Absorption Spectrometry

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Received: 18.05.2009 Revised: 04.09.2009 Accepted: 18.09.2009

ABSTRACT

The concentrations of Ca, Mg, Fe, Zn, Cd, Pb, Cu and Cr in head bone stones of brown meager (*Sciaena umbra*) fishes and Bone ash 1400 standard reference material were determined by flame and electrothermal atomic absorption spectrometry. Flame atomic absorption spectrometer was used for the determination of Ca, Mg, Fe and Zn in samples by using lanthanum + potassium mixture. Electrothermal atomic absorption spectrometer was used for the determination of Cd, Pb, Cu and Cr in samples by using lanthanum + palladium + ammonium dihydrogen phosphate as chemical modifier mixture. Limits of detection obtained are 2.5, 0.2, 11, 18, 1.1, 0.12, 1.21, 1.09, 0.12 $\mu g\ L^{-1}$ for Ca, Mg, Fe, Zn, Cd, Cu, Pb and Cr, respectively. Recovery tests for analytes in Bone ash 1400 and bone stone sample solutions were studied. Percent recoveries of analytes obtained are in the range of 96 – 102%.

Key Words:Trace metals, Bone Stones of Brown Meager Fish, FAAS and ETAAS, La+Pd+$NH_4H_2PO_4$.

1. INTRODUCTION

In recent years, determination of metal contaminations in sea foods such as fish has been important for marine organisms and human health [1-3]. Metal accumulation levels of different fish species such as Brown Meagre (*Sciaena umbra*) in coastal sea waters of Turkey have been carried out on toxic effects of essential and non-essential metals because the fish are the major parts of the human diet [1-8]. Essential metals such as iron, copper and zinc play important roles in biological systems, whereas, non-essential metals such as lead and cadmium are toxic, even in traces. The essential metals can also produce toxic effects when the metal intake is excessively elevated [1-5]. Cd and Pb have cancerogenic properties and Cu is associated with anemia [9]. Brown meagre (*Sciaena umbra*) fish from sciaena family which generally lives in the Marmara Sea and Black Sea has two white bone stones behind its eyes and over the brain. Some people drink the solution of these stones dissolved in natural lemon water plus olive oil in order to extract or reduce their kidney stones without surgical operation. Dusts of these samples are commercially available and they are used for diseases of human kidney stones and urine ways. The bone stones of *Sciaena umbra* Fish have not been analyzed until now in terms of metal toxicity and the relations between these stones and human kidney disease are not known. Human kidney stones formed in the body have been analyzed and identified by various methods, such as instrumental neutron activation analysis and x-ray diffraction. Three main types of urinary stones which

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are urate calculi, phosphate calculi and oxalate calculi have been identified [10-12]. The removal of them is very important for human health and disease.

Flame atomic absorption spectrometry (FAAS) and electrothermal atomic absorption spectrometry (ETAAS) are suitable and popular techniques for the determination of trace metals such as Cd, Pb, Fe and Zn in various samples such as human teeth and bones, and biological samples due to their high sensitivities, selectivities and low detection limits [7, 13-19]. However, some interference effects from anions and cations, high background signals and volatilization of analyte together with organic compounds may occur in the direct determinations. The influence of a complicated matrix greatly affects the analytical results. Therefore, La + K mixture [19, 20] with FAAS and La + Pd + $NH_4H_2PO_4$ modifier mixture [17, 21, 22] with ETAAS were used to reduce these background signals and chemical interferences. In FAAS, La reacts with phosphorous (P) in bone samples to reduce interferences from P and free atoms of other analyte elements are obtained in the flame [23]. Potassium is also used to prevent the ionizations of the analytes in samples [13, 20]. In ETAAS, La, Pd and $NH_4H_2PO_4$ are used as modifiers.

The purpose of this study was to determine the metal levels (Cd, Pb, Cu, Cr, Ca, Mg, Fe and Zn) in bone stones collected from ten different *Sciaena Umbra* Fish samples and Bone Ash 1400 Standard Reference Material (SRM) from NIST by using FAAS with K + La and ETAAS with La + Pd + $NH_4H_2PO_4$ modifier mixture. Recovery tests of the analytes were also studied by analyzing the Bone ash SRM 1400 and a bone stone sample solution for the method validation.

2. Materials and methods

2.1. Apparatus

All atomic absorption measurements of analytes were carried out with Varian Models of 240 FS FAAS and 240Z ETAAS. Varian pyrolytically coated graphite tubes and peak area mode were used to measure absorbance values with ETAAS throughout. Absorbance measurements with FAAS were obtained by using integrated mode. Single element hollow cathode lamps of the analytes were used as radiation source. Instrumental analytical conditions and operating parameters recommended by the manufacturer for the analytes were summarized and given in Table 1. Argon (99.98%, Oksan, Ankara) was used as a carrier gas during all stages except for atomization in ETAAS. An acetylene-air or acetylene-nitrous oxide flame was used for the determination of analytes by FAAS. Optimized graphite furnace temperature program for the determination of Cd, Pb, Cu and Cr by ETAAS with La + Pd + $NH_4H_2PO_4$ modifier mixture were also given in Table 1. Bruker D8 discovery X-ray diffractometer was used for the determination of morphology of the Brown Meager bone stone sample. The experimental conditions of XRD measurement were as follows: CuKα radiation; tube voltage/current, 20 kV/30 mA; scanning range (2θ), 5 – 80°, scanning rate, 5°/min.

2.2. Reagents

All reagents used were of analytical grade or better. All aqueous solutions were prepared with deionized water obtained by using ultra pure water system (Human power I^+, resistivity 18.3 MΩ cm^{-1}, Human Corporation, Korea). HNO_3 (65% m/m) and H_2O_2 (35% m/m) from Merck supra-pure grade were used to dissolve the samples and dilution. Plastic bottles, autosampler cups, PTFE vessels, vials used for collecting samples and glassware were cleaned by soaking in HNO_3 (10 % v/v) for a day, rinsing four times with ultra pure water and drying in an oven at 40°C. All solutions prepared were stored in high-density polypropylene bottles. Autosampler washing solution containing HNO_3 (0.1% v/v) plus Triton X-100 (0.1% v/v) was used to avoid clogging of the autosampler sampling capillary tip and to improve dispersion of sample solution onto the graphite tube [16, 24].

Stock standard solutions of analytes (1 g L^{-1} each) obtained from SCP Science and Merck were used. Working standard solutions were freshly prepared by diluting the stock standard solutions to the desired calibration ranges in 0.2% HNO_3 as diluent.

Stock standard solution of Pd (2 g L^{-1}) was prepared from 0.50 g of Pd $(NO_3)_2.2H_2O$ (Merck) dissolved in 2 mL of HNO_3 and diluted to 100 mL with ultra pure water. $NH_4H_2PO_4$ (2 g L^{-1}) was prepared by dissolving 0.20 g of $NH_4H_2PO_4$ (Merck) in 1 mol L^{-1} HNO_3 and diluting to 100 mL with ultra pure water. La (50 g L^{-1}) was prepared by dissolving 5.86 g La_2O_3 (Merck) in 10 mL of HNO_3 + H_2O mixture (1:1) and diluting to 100 mL with ultra pure water. K stock solution (30 g L^{-1}) was prepared by dissolving 7.76 g KNO_3 (Merck) in HNO_3 (1%, v/v) and diluting to 100 mL with ultra pure water.

2.3. Collection of samples

Ten different Brown meager (*Sciaena umbra*) fishes were collected from the Marmara and the Black Sea waters by fishermen. The bone stones behind eyes and over brain of fish were extracted by stainless steel knife, cleaned with water, dried and stored in polyethylene capsules until analysis. Bone ash SRM 1400 taken from NIST was used.

2.4. Decomposition and preparation of samples

A bone stone sample was put into a glass beaker and dried in an oven at 80 ºC for 2 h and weighed. Each sample (the mass range being from 0.5 to 1.5 g) or a portion of bone ash SRM 1400 (0.2 - 1.0 g) was put into a PTFE beaker with a watch glass and a 3 mL of mixture of HNO_3 (65% w/w) plus H_2O_2 (30% v/v) (2:1) [25, 26] and 2 mL of H_2O were added to each sample. The sample was left overnight at laboratory temperature to dissolve without heating [13, 27]. Samples were decomposed by heating on a hot-plate at about 140 °C and waited for sample evaporation nearly to 2 mL [27]. After the sample was decomposed completely, it was transferred into 25 mL volumetric flask by washing interior surface of beaker with HNO_3 (0.5% v/v) three times and diluted to the mark. A Brown Meagre Fish bone stone dissolved in 50 mL volumetric flask was divided into two equal volumes in two 50 volumetric flasks in order to perform a recovery test. One mL of 30 mg L^{-1} Fe, 20 mg L^{-1} Zn, 1 mg L^{-1} Pb and Cu, 0.5 mg L^{-1} Cr and 20 µg L^{-1} Cd standard aqueous solutions, respectively, were added into one of the flasks. Ca and Mg were not added because the concentrations of them were very high in the solution. Two 50 mL volumetric flasks were diluted to the mark again. One mL of 0.5 mg L^{-1} Cr was also added to powder Bone Ash SRM 1400 before dissolving in 25 mL volumetric flask for the recovery study. Blank solutions were prepared by using the procedure given above in order to check the contaminations from reagents and the containers in contact with the sample solution.

3. Results and discussion

3.1. Chemical modification

The main purpose of using chemical modification in ETAAS is to stabilize the analyte element to pyrolysis temperature as high as possible by forming chemical compounds or intermetallic phases [28], to obtain best recoveries and to remove most of the matrix efficiently without loss of analyte mass. Mass and mass ratios of La + Pd + $NH_4H_2PO_4$ modifier mixture and pyrolysis temperatures of analytes given in previous works [22, 26] were used for the determination of Cd, Pb, Cu and Cr in ETAAS. Masses of modifier mixture components studied and used were 20 µg La, 4 µg Pd and 50 µg $NH_4H_2PO_4$. Pyrolysis, atomization and cleaning temperatures of analytes studied in the presence of La + Pd + $NH_4H_2PO_4$ were given in Table 1. Optimum concentration ratio of La + K modifier mixture (3.0 g L^{-1} La/ 2.0 g L^{-1} K) on the absorbance values of analytes studied in previous works [19, 20] was used in the flame atomic absorption determinations of Ca, Mg, Zn and Fe. Standards, blanks, reference material and bone stone samples were analyzed by using the modifier mixtures given above.

3.2. X-ray diffraction pattern of a sample

An X-ray diffraction pattern of a bone stone of Brown Meager fish solid sample was given in Figure 1. As can be seen in Figure 1, concentration of $CaCO_3$ compound in sample is high range. XRD data for aragonite peak values in the figure were compared with the 2 theta values obtained from the database for all three crystalline structures present in calcium carbonate in previous work [29]. The main diffraction peaks of calcium carbonate (aragonite) at approximately 2θ = 26.2, 27.5 and 33.5 are in good agreement with the reference pattern of calcium carbonate (aragonite) given in literature (JCPDS 24-0025) [30].

3.3. Analytical characteristics

Calibration graph method was used for the determination of analytes in bone ash SRM 1400 and Brown meager bone stone samples by FAAS or by ETAAS. Linear ranges of calibration graphs and calibration equations for aqueous standard solution of analytes in the presence of modifier mixtures were given in Table 2. As can be seen in Table 2, correlation coefficients (R^2) of all calibration graphs for analytes obtained are higher than 0.99.

Limit of detection (LOD, concentration of analyte related to the three times standard deviation of blank solution) and characteristic mass (m_o) are important factors for the analytes and they might be influenced by instrumental parameters. The limits of detection obtained from ten consecutive measurements were given in Table 2. LOD and m_o obtained for the analytes were compared with previous works [16, 17, 31, 32] and some values were given in Table 2. Small differences were observed.

3.4. Recovery studies

The bone ash SRM 1400 and a spiked sample solution were analyzed by using FAAS with La + K and ETAAS with La + Pd + $NH_4H_2PO_4$ modifier mixture for the validation. The results of analytes found in Bone Ash SRM 1400 and a bone stone sample solution by using the optimum parameters in Table 1 and chemical modifiers are given in Table 3. They are represented as average ± confidence interval (5 degrees of freedom (n-1) at 95% confidence level). As can be seen in Table 3, the results of analytes obtained with La + K or with La + Pd + $NH_4H_2PO_4$ are in good agreement with bone ash SRM 1400 certified values. Cr was added to the bone ash SRM 1400 bone since there is no Cr value in it. Percent recoveries of analytes obtained in bone ash SRM 1400 and a sample solution are in the range of 96-102%.

3.5. Sample analysis

Ten Brown Meagre (*Sciaena umbra*) Fish stone samples were analyzed by using FAAS with La + K and ETAAS with La + Pd + $NH_4H_2PO_4$ modifier mixture. The parameters given in Table 1 were used. Results of analytes found in samples were given in Table 4. The results are also represented as average ± confidence interval as given above.

As can be seen in Table 4, the Ca and Mg concentrations are higher than the other analytes in samples. There is no information in the literature about maximum permissible Ca and Mg concentrations in fish and fishery products.

The maximum concentration levels of analytes obtained from samples were compared with the literature reported for muscle tissues and for liver tissues of *Sciaena umbra* from North Aegean Sea (NAS), Turkey [1]. They were given in Table 5. The permissible analyte concentrations for fish according to Turkish Food Codex Regulation [33] and the maximum permissible analyte levels for fish according to Nauen [34] were also given in Table 5.

Table 1. Instrumental conditions of investigated analytes

	FAAS			
Parameters	**Ca**	**Mg**	**Fe**	**Zn**
Acetylene, L min^{-1}	6.35 + 11.0^{a}	2.0	2.0	2.0
Air, L min^{-1}	-	13.5	13.5	13.5
Wavelength, nm	422.7	285.2	248.3	213.9
Lamp current, mA	10.0	4.0	5.0	5.0
Slit with, nm	0.5	0.5	0.2	1.0
	ETAAS			
	Pb	**Cu**	**Cd**	**Cr**
Argon flow, mL min^{-1}	0.3	0.3	0.3	0.3
Sample volume, µL	20	20	20	20
Lamp current, mA	10	4	4	7
Heating program temperature ºC (ramp time (s), hold time (s))				
Drying 1	60 (5, 10)	60 (5, 20)	60 (5, 20)	60 (5, 15)
Drying 2	95 (15, 15)	95 (15, 15)	95 (15, 15)	95 (10, 15)
Drying 3	150 (15, 15)	150 (15, 15)	150 (15, 15)	150 (15, 15)
Pyrolysis	1100 (15, 10)	1100 (15, 10)	800 (10, 15)	1250 (10, 15)
Atomisation	2100 (1, 3)	2300 (1, 3)	1800 (1, 3)	2600 (1, 3)
Cleaning	2500 (1, 2)	2500 (1, 2)	2500 (1, 2)	2700 (1, 2)

[a] 11.0 L min^{-1} N_2O was used for Ca determination.

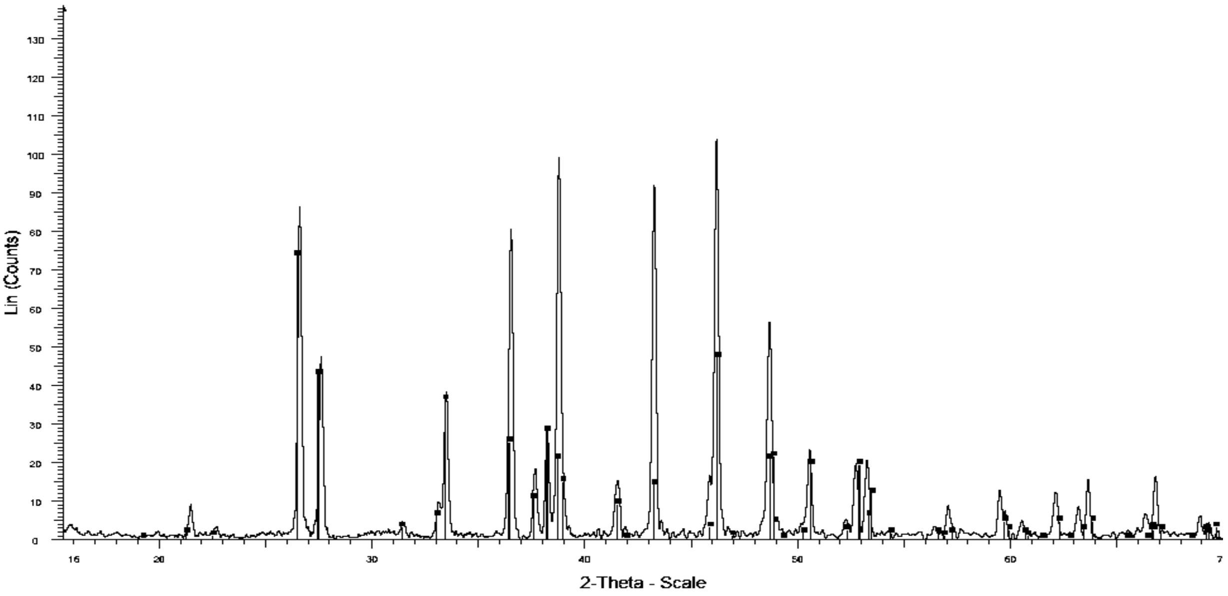

Figure 1. X-ray diffraction pattern of a bone stone of *Sciaena umbra* Fish sample.

Table 2. Parameters of analytes determined by FAAS and ETAAS

	FAAS			
Parameters	**Ca**	**Mg**	**Fe**	**Zn**
Limits of detection, $\mu g\ L^{-1}$	2.5	0.2	18	1.1
Calibration range, $mg\ L^{-1}$	0.4 - 2.4	0.1 - 0.8	1 - 5	0.1 - 0.8
Calibration equation	$0.1722C^{a} + 0.052$	$2.2141C^{a} + 0.0071$	$0.0958C^{a} + 0.0016$	$0.6752C^{a} + 0.0665$
R^2	0.9979	0.9996	0.9983	0.9985
	ETAAS			
	Pb	**Cu**	**Cd**	**Cr**
Limits of detection, $\mu g\ L^{-1}$	1.09 (This study)	1.21 (This study)	0.12 (This study)	0.12
	1.4 [30]	2.2 [30]	0.08 [30]	
Characteristic masses, pg	21.6 (This study)	11.4 (This study)	4.12 (This study)	4.49
	20.8 [31]	16.0 [17]	3.6 [31]	
Calibration range, $\mu g\ L^{-1}$	5-60	10-40	0.1-0.8	5-20
Calibration equation	$0.0038C^{b} + 0.0260$	$0.0029C^{b} + 0.0267$	$0.0124C^{b} + 0.0212$	$0.0218C^{b} + 0.0195$
R^2	0.9996	0.9994	0.9984	0.9996

[a] C is the concentration of the analytes in $mg\ L^{-1}$. [b] C is the concentration of the analytes in $\mu g\ L^{-1}$.

Table 3. Recovery tests for analytes in Bone Ash SRM 1400 and a sample solution by using FAAS with La + K mixture and ETAAS with La + Pd + $NH_4H_2PO_4$

Element	Certified /Added values	Found[d]	Recovery (%)
Bone ash 1400, concentrations			
Ca, % (m/m)	38.18 ± 0.13^{a}	37.9 ± 0.2	99
Mg, % (m/m)	0.684 ± 0.013^{a}	0.69 ± 0.03	100
Fe, $mg\ kg^{-1}$	660 ± 27^{a}	657 ± 24	100
Zn, $mg\ kg^{-1}$	181 ± 3^{a}	185 ± 4	102
Pb, $mg\ kg^{-1}$	9.07 ± 0.12^{a}	9.1 ± 0.4	100
Cu, $mg\ kg^{-1}$	2.3^{b}	2.26 ± 0.06	98
Cd, $\mu g\ kg^{-1}$	30^{b}	28.9 ± 0.9	96
Cr, $\mu g\ L^{-1}$	20^{c}	19.4 ± 0.2	97
Bone stone sample solution, $\mu g\ L^{-1}$			
Fe	-	372 ± 9	
	600^{c}	967 ± 11	100
Zn	-	691 ± 7	-
	400^{c}	1106 ± 17	101
Pb	-	16 ± 1	-
	20^{c}	35 ± 2	97
Cu	-	18 ± 1	-
	20^{c}	39 ± 2	102
Cd	-	0.41 ± 0.02	-
	0.4^{c}	0.78 ± 0.03	96
Cr	-	11.8 ± 0.6	-
	10^{c}	21 ± 2	97

[a] Certified and [b] non-certified values for Bone Ash 1400. [c] Added values for Bone Ash 1400 and a sample solution. [d] Mean of six replicate measurements with 95 % confidence level, $\overline{X} \pm ts/\sqrt{n}$.

Table 4. Results of analytes in stones of brown meager (*Sciaena umbra*) fish samples using FAAS with La + K (for Ca, Mg, Fe and Zn) and ETAAS with La + Pd + $NH_4H_2PO_4$ modifier mixtures (for Pb, Cu, Cd and Cr)

Sample	Concentrations[a] (dry weight basis)							
	Ca (%)	Mg (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Pb (mg kg^{-1})	Cu (µg kg^{-1})	Cd (µg kg^{-1})	Cr (µg kg^{-1})
B-1	24.1±0.1	33.0±0.2	15.1±0.1	1.49±0.01	1.84±0.05	187±4	140±10	820±10
B-2	27.6±0.1	51.7±0.3	14.8±0.2	1.52±0.02	4.61±0.39	286±21	111±2	689±9
B-3	30.5±0.2	38.4±0.8	14.2±0.3	5.30±0.08	4.93±0.13	450±20	123±3	525±16
B-4	23.5±0.2	36.0±0.2	12.6±0.2	1.35±0.06	1.17±0.03	257±15	69±6	121±3
B-5	22.7±0.2	43.2±0.2	12.2±0.2	6.27±0.02	0.30±0.02	378±7	56±4	120±3
B-6	26.3±0.3	31.6±0.1	11.7±0.4	3.19±0.03	0.29±0.02	315±13	62±5	314±8
B-7	25.8±0.2	35.4±0.5	11.4±0.4	2.48±0.10	0.45±0.01	271±18	78±6	259±6
B-8	29.6±0.4	52.1±0.2	16.3±0.4	2.80±0.06	3.83±0.42	290±47	116±7	212±5
B-9	27.9±0.4	37.2±0.3	13.6±0.7	1.58±0.04	2.86±0.02	316±41	76±6	616±8
B-10	28.6±0.3	38.4±0.4	14.3±0.6	1.69±0.03	3.17±0.12	329±36	82±6	683±13

[a] Mean of six replicate measurements with 95 % confidence level, $\overline{X} \pm ts/\sqrt{n}$.

Table 5. Comparison of analyte concentations obtained from samples with other studies and maximum permissible values

Element	Concentrations, mg kg^{-1}				
	This study	Other studies and maximum permissible values			
	Sciaena umbra bone stone (max. values)	*Sciaena umbra* muscle tissues [1]	*Sciaena umbra* liver tissues [1]	Fish[33]	Fish [34]
Cd	0.14 ± 0.01	0.03 ± 0.00	0.13 ± 0.03	0.10	0.05-5.50
Cr	0.82 ± 0.01	0.25 ± 0.06	0.52 ± 0.09	-	1.00
Cu	0.45 ± 0.02	6.48 ± 1.57	14.9 ± 3.5	20.0	10 - 100
Fe	16.3 ± 0.4	40.1 ± 8.7	148 ± 22	-	-
Pb	4.93 ± 0.13	0.54 ± 0.10	1.29 ± 0.17	0.4	0.5 – 6.0
Zn	6.27 ± 0.02	11.6 ± 1.64	28.3 ± 4.12	50	30 - 100

As can be seen in Table 4 and 5, the minimum and maximum Cd contents in analyzed samples are 56 ± 4 µg kg^{-1} and 140 ± 10 µg kg^{-1}. The maximum Cd content in analyzed samples is in agreement with *Sciaena umbra* liver tissues [1] and Nauen [34].

Chromium concentration ranges in bone stone samples found are from 120 to 820 µg kg^{-1} (Table 4). As can be seen in Table 5, maximum Cr level in samples is higher than the literature [1] and it is lower than the maximum permissible value (1.0 mg kg^{-1}) [34].

The lowest and highest Cu levels in bone stone samples found are 187 ± 4 µg kg^{-1} and 450 ± 20 µg kg^{-1} (Table 4). Cu content in the literature is 0.19 ± 0.01 mg kg^{-1} for *Sciaena umbra* from the East Mediterranean Sea [3]. As can be seen in Table 5, maximum Cu level in samples obtained is lower than Cu contents in literature [1], both maximum permissible values given in Turkish Food Codex Regulation [33] and the levels (10-100 mg kg^{-1}for fish) reported by Nauen [34].

Iron concentration ranges in *Sciaena umbra* bone stone samples found are from 11.4 to 16.3 mg kg^{-1} (Table 4). Fe contents in the literature reported are 7.16-16.5 mg kg^{-1} for muscles and 48.1-384 mg kg^{-1} for livers of fish from Tuzla lagoon, Mediterranean Sea region [8]. As can be seen in Table 5, maximum Fe level obtained in samples is lower than literature values [1, 8]. There is no information about maximum permissible Fe concentrations in fish samples [33, 34].

The minimum and maximum Pb contents in samples found are 0.29 ± 0.02 mg kg^{-1} and 4.93 ± 0.13 mg kg^{-1} (Table 4). Maximum Pb level in samples is higher muscle and liver tissues [1] and Turkish Food Codex Regulation [33], but it is in agreement with the maximum permissible Pb levels [34] (Table 5).

Zn concentration levels in bone stone samples found are from 1.35 to 6.27 mg kg^{-1} (Table 4). Zn content in the literature reported is 2.38 ± 0.15 mg kg^{-1} for *Sciaena umbra* from the East Mediterranean Sea [3]. As can be seen in Table 5, maximum Zn level in samples obtained is lower than the values reported in the literature [1], maximum permissible values given in Turkish Food Codex Regulation [32] and the levels reported by Nauen [34].

The maximum values of Cd, Cr, Cu, Fe, Pb and Zn found in the samples were also compared with metal contents of fish samples taken from different sea sides of Turkey reported in the literatures [4, 5] and our results obtained are lower or in agreement with them. The metal contents obtained in the samples are not sufficient to cause toxicological effects on human health when they are consumed in the diet. Therefore, it can be concluded that these metal levels in samples should pose no health problems for consumers.

4. Conclusion

Calcium, magnesium, iron, zinc, cadmium, lead, copper and chromium determinations in bone stones of Brown meagre (*Sciaena umbra*) and bone ash SRM 1400 by flame and electrothermal atomic absorption spectrometry using La + K and La + Pd + $NH_4H_2PO_4$ modifier mixtures were determined. Recovery tests for bone ash SRM 1400 and a bone stone sample solution were performed for the accuracy. Chemical interferences and ionization effects from the sample matrix are minimal by using the modifier mixtures and an acid mixture of HNO_3 plus H_2O_2 in dissolution of the samples. La + K and La + Pd + $NH_4H_2PO_4$ modifier mixtures can be applied for the determination of analytes in bones such as stones of Brown meagre, teeth and other biological materials. The number of samples analyzed in this study is not enough to reach statistical conclusions. The results of this study supply valuable information about the metal contents in bone stones of *Sciaena umbra* fish from the Marmara Sea and Bleak Sea. These results can also be used to test the chemical quality of the marine food, in order to evaluate the possible risk associated with their consumption by humans. A deeper study applying flame and electrothermal atomic absorption spectrometry using La + K and La + Pd + $NH_4H_2PO_4$ modifier mixtures, well selected and planned collection of samples will provide useful information.

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