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Determination of copper, lead, cadmium and zinc content in commercially valuable fish species from the Persian Gulf using derivative potentiometric stripping analysis

S. Siavash Saei-Dehkordi *, Aziz A. Fallah

Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord 115, Iran
Research Institute of Zoonotic Diseases, Shahrekord University, Shahrekord 115, Iran

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ABSTRACT

This study was performed to determine the concentrations of cadmium, lead, copper and zinc in the edible muscle of pelagic (*Scomberomorus commerson, Chirocentrus dorab, Sphyraena jello, Rachycentron conadum, Thunus tonggol,* and *Tenualosa ilisha*) and demersal (*Nemipterus japonicas, Epinephelus coioides, Platycephalus indicus, Psettodes erumei, Pomadasys argenteus,* and *Acanthopagrus latus*) fish species from the Persian Gulf during winter and summer. The samples were analyzed by the derivative potentiometric stripping technique; and the results were expressed as μg/g of wet weight. The obtained range of metals in fish species was 0.024–0.111 μg/g for cadmium, 0.057–0.471 μg/g for lead, 0.799–4.790 μg/g for copper and 3.226–11.390 μg/g for zinc. The study revealed that seasonal variation influenced the concentration of metals in the samples. The highest concentration of cadmium, lead, copper and zinc was found in *Platycephalus indicus* (0.147 μg/g), *Acanthopagrus latus* (0.534 μg/g), *Psettodes erumei* (5.294 μg/g) and *Psettodes erumei* (13.528 μg/g) in winter, respectively. Moreover, demersal fish species had higher cadmium, lead and zinc concentrations, but lower copper content than pelagic ones. Our study demonstrated that the estimated daily and weekly intakes of lead, copper and zinc, and estimated monthly intake of cadmium via consumption of fish flesh were below the PTDI, PTWI and PTMI values established by FAO/WHO.

1. Introduction

Fish is considered a very valuable food, rich in valuable nutritional constitutes such as proteins, minerals and vitamins. Moreover, beneficial effects of fish consumption such as the reduction of human cardiovascular diseases and different disorders have also been attributed to the presence of polyunsaturated fatty acids, particularly omega-3 and omega-6 fatty acids [1–3].

Heavy metals from anthropogenic activities or natural sources have polluted the aquatic ecosystems along the time and fish as a major inhabitant, bioaccumulated metals [4–8]. Therefore, exposure to the different heavy metals through fish consumption as an important food is obvious. Investigations regarding the existence of heavy metals in fish have increased in latter decade worldwide. However, few studies on the evaluation of heavy metals present in fish in Iran have been carried out and published in authentic international publications. Heavy metal fluctuations in fish tissues might be influenced by several factors such as seasonal variations, nourishment source and biological differences [9,10].

According to the Environmental Protection Agency (EPA), lead, cadmium, copper and zinc are some of the most common heavy metals inducing pollution [11]. Heavy metals could be attributed to many human body disorders and health threatening abilities in consequence to their accumulation in organs such as the liver and the kidney in long time periods. It is found that the main targets of lead and cadmium are the nervous system and the kidney, respectively [12,13]. The role of heavy metals like copper and zinc to human health can be significantly related to their essential role for many enzymatic functions; however, their intake of more than the safe recommended levels may produce toxic effects [4,14,15].

Regarding the semi-enclosed formation of the Persian Gulf and the continual discharges of heavy metals by surrounding industries, regular assessment of metal pollution in seafood, especially fish caught from the Gulf, is necessary.

Considering the existing scientific literature on heavy metal content in foodstuffs, the lack of comprehensive published data concerning the burden of metal accumulation in fish species from the Persian Gulf is noticeable. Hence, this study is focused on the accumulation of lead, cadmium, copper and zinc in the flesh of twelve of the most valuable pelagic and demersal fish species from the Persian Gulf, Iran; and assessment of seasonal and biological variations on concentration levels.

* Corresponding author. Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord 115, Iran. Tel./fax: +98 381 4424427.
E-mail addresses: saei.siavash57@yahoo.com, smazdak@yahoo.com (S.S. Saei-Dehkordi).

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2. Materials and methods

2.1. Sample collection

The study focused on most dietary and economically important marketed fish in Iran (Table 1). Because of the most marketability, the samples were selected according to average weights and lengths. The fish samples were collected from Bandar Abbas (one of the most important fishing ports of the Persian Gulf) during July to September of 2009 and January to March of 2010. Among the collected fish, six species: narrowbarred Spanish mackerel (*Scomberomorus commerson*), dorab wolf herring (*Chirocentrus dorab*), pick handle barracuda (*Sphyraena jello*), cobia (*Rachycentron conadum*), long tail tuna (*Thunus tonggol*) and hilsa shad (*Tenualosa ilisha*) belonged to the pelagic habitat and the rest: Japanese threadfin bream (*Nemipterus japonicas*), orangespotted grouper (*Epinephelus coioides*), bartail flathead (*Platycephalus indicus*), Indian spiny turbot (*Psettodes erumei*), silver javelin (*Pomadasys argenteus*) and yellowfin seabream (*Acanthopagrus latus*) pertained to the demersal habitat (http://fish.mongabay.com/data/ecosystems/Saltwater_Persian_Gulf.htm). The fish samples were placed in clean plastic bags on ice and transported to the laboratory. Total length (cm) and body weight (kg) of the samples were measured and recorded (Table 1) before dissection. Then, the muscle samples were taken, placed in polyethylene pouches and stored at −18 °C prior to analysis.

2.2. Reagents

All reagents were of analytical grade unless otherwise stated. Double deionised water applied throughout this study was of ultrapure quality (conductivity >18 MΩ/cm). Super pure nitric acid (67–69%) and super pure sulfuric acid (94–98%) were obtained from Romill, UK. Hg(II) (1000 mg/kg, 1 M in hydrochloric acid) standard solution was purchased from Steroglass (Steroglass, S. Martino in Campo, Perugia, Italy) and Cu(II), Pb(II), Cd(II) and Zn(II) (1000 mg/kg, 0.5 N in nitric acid) standard solutions were obtained from Panreac (Panreac Quimica Sa, Barcelona, Spain). $Ga(NO_3)_3 \cdot 3H_2O$ (5 g, 99.9%) was purchased from Aldrich Chem. Co. (Milwaukee, WI, USA). The certified reference material for quality control of metal analysis was DORM-3 (Dogfish Muscle Certified Reference Material).

2.3. Digestion procedure

Two grams of each fish sample was weighed accurately, then digested with 10 ml of a mixture of nitric and sulfuric acid (mixture: four parts nitric acid and one part sulfuric acid). In order to complete the digestion procedure, the temperature was adjusted to 200 °C. The digested material was evaporated to dryness, re-dissolved in 20 ml double deionised water, and filtered through a 0.22 μm acid-resistant cellulose nitrate membrane. However, to eliminate the influence of any external organic material in the analyzed matrix interfering with the analysis procedures, the filtrate was re-filtered on an activated carbon column [16]. At the end of the digestion procedure, the solution was transferred to a 50 ml volumetric flask and diluted with double deionised water.

2.4. Apparatus and analytical procedure

To minimize the risk of contamination, all glassware was soaked with 10% (v/v) super pure nitric acid for 24 h, followed by rinsing with ultrapure water. Measurements of Pb, Cd, Cu and Zn were carried out by derivative potentiometric stripping analysis method using a potentiometric stripping analyzer, PSA ION 3 (Steroglass, S. Martino in Campo, Perugia, Italy), coupled with a compatible personal computer using NEOTES software package to control all functions and analysis procedures. The analyzer was equipped with a three-electrode cell: a working electrode or glassy carbon electrode plated with a grey integrated thin mercury film; an Ag/AgCl electrode (3 M KCl) as the reference electrode and a platinum wire as the counter or auxiliary electrode. The potential of mercury plating on working electrode surface by electrolyzing 20 ml of an Hg(II) (1000 mg/kg) standard solution was adjusted at −950 mV for Cu, Pb and Cd, and −1250 mV for Zn for 1 min with a pre-programmed stirring speed by an electrical borosilicate glass stirrer.

In general, the metal content measurement procedures in potentiometric stripping analyzer are carried out by electrolysis firstly and stripping, secondly. Cd(II), Pb(II) and Cu(II) were analyzed simultaneously and Zn(II) separately.

In order to determine Cd(II), Pb(II) and Cu(II) simultaneously, and Zn(II) separately, 5 ml of limpid sample, 20 ml of ultrapure water, 1 ml of 1000 ppm Hg(II) standard solution and 0.5 ml of 10 μg Ga(III)/ml were placed into an analysis cell. Experimental analysis conditions for the determination of elements are demonstrated in Table 2. Moreover, Fig. 1 represents derivative potentiometric stripping curves for a typical analysis of a given sample.

2.5. Determination of limits of detection

Limits of detection (LODs) were determined according to Palmieri et al. [17] for each metal. Briefly, the LODs were estimated using the

Table 1
Characteristics of fish samples, collected from the Abbas port, Persian Gulf (mean ± SD; n = 6).

Fish species	Summer		Winter	
	Fresh weight (kg)	Length (cm)	Fresh weight (kg)	Length (cm)
Pelagic				
Scomberomorus commerson	3.613 ± 0.711	95 ± 4.515	3.145 ± 0.621	85 ± 3.662
Chirocentrus dorab	0.918 ± 0.409	67 ± 3.124	1.110 ± 0.316	71 ± 4.124
Sphyraena jello	0.927 ± 0.219	71 ± 5.221	0.819 ± 0.463	64 ± 2.913
Rachycentron conadum	1.422 ± 0.529	78 ± 3.871	1.220 ± 0.337	75 ± 3.273
Thunus tonggol	1.014 ± 0.331	54 ± 2.809	1.431 ± 0.725	59 ± 2.711
Tenualosa ilisha	1.123 ± 0.251	45 ± 2.114	1.243 ± 0.215	47 ± 2.217
Demersal				
Nemipterus japonicus	0.141 ± 0.029	25 ± 2.096	0.162 ± 0.048	27 ± 2.332
Epinephelus coioides	1.316 ± 0.633	44 ± 5.718	1.108 ± 0.416	41 ± 4.177
Platycephalus indicus	0.428 ± 0.032	36 ± 3.129	0.517 ± 0.042	39 ± 3.527
Psettodes erumei	0.927 ± 0.111	39 ± 4.151	1.099 ± 0.211	42 ± 2.945
Pomadasys argenteus	1.142 ± 0.419	51 ± 5.00	0.951 ± 0.333	47 ± 3.319
Acanthopagrus latus	1.343 ± 0.538	42 ± 4.417	1.127 ± 0.519	38 ± 5.147

Table 2
Experimental analysis conditions for the determination of elements.

	Copper	Lead	Cadmium	Zinc
Potential range (mV)	−10 to −800	−10 to −800	−10 to −800	−500 to −1200
Element integration range (mV)	−90 to −325	−330 to −500	−520 to −700	−720 to −980
Stripping peak potential (mV)	−200	−420	−625	−870
Electrolysis potential (mV)	−1200	−1200	−1200	−1200
Electrolysis time (s)	120	120	120	120
Electrode cleaning potential, time (mV), (s)	50, 5	50, 5	50, 5	50, 5
Stripping time (s)	10	10	10	10
Sampling time (μs)	300	300	300	300
End analysis potential (mV)	0	0	0	0
Volume and concentration of standard additions (μl and μg/g)	200, 5	200, 1	200, 1	200, 5
Stirrer speed (turns/s)	2	2	2	2
Number of cycles and standard additions	3, 2	3, 2	3, 2	3, 2

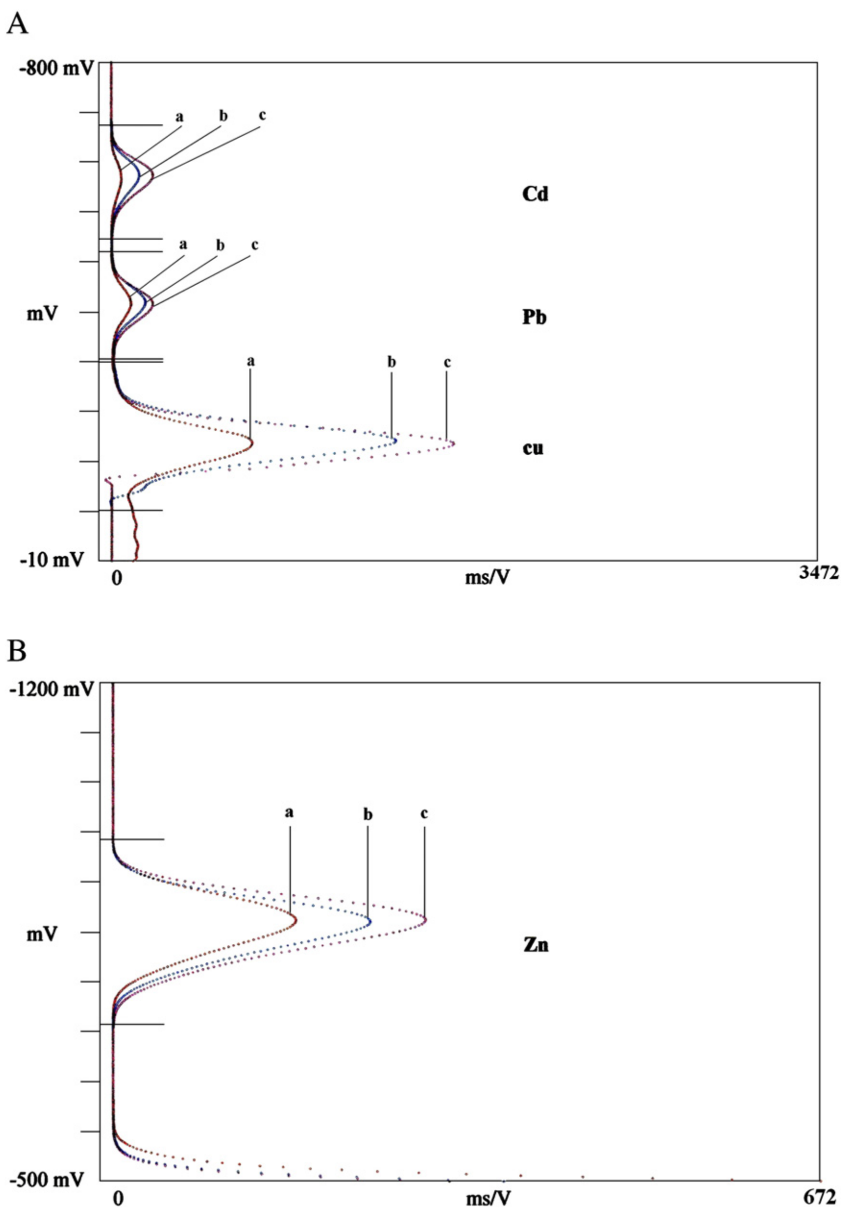

Fig. 1. Stripping curves of simultaneous determination of Cu, Pb and Cd (A), and Zn determination (B); a, sample curve; b, first standard addition curve; c, second standard addition curve. The experimental conditions are given in Table 2.

expression $3S_{blank}/S$, where S_{blank} is the standard deviation of at least five replicate measurements of blanks and S is the slope of the calibration curve. LOD values of Cu, Pb, Cd and Zn were found to be 2.2, 2.5, 1.1 and 1.7 ng/g, respectively.

2.6. Analytical quality assurance

Recovery of the procedures was evaluated by spiking increasing amounts of lead, cadmium, copper and zinc to the homogenized samples of different fish species and also using certified reference material (National Research Council, Canada), DORM-3 (Fish Protein Certified Reference Material). The mean recovery percentages (five replicates) are demonstrated in Table 3.

The instrumental precision and repeatability of the method were assesed as the mean percent relative standard deviation (RSD) and the total percent RSD, respectively. The precision and repeatability were tested on a fish sample by extracting it three times and by quantative measuring three times for each metal in the same extract. As shown in Table 4 the precision and repeatability are acceptable.

2.7. Statistical analysis

Data analyses were carried out using the GraphPad InStat 3 statistical package. The *t*-test was used for comparisons and the results were defined as statistically significant for a given level of $P<0.05$.

3. Results and discussion

3.1. Lead, cadmium, copper and zinc concentrations

The results of the concentrations of the four metals in twelve fish species from the Iranian market are shown in Table 5. As it can be seen, the following sequence of metal concentration was observed in all muscular samples of pelagic and demersal fish species: Zn > Cu > Pb > Cd.

The lead concentration can be ranged as follows: *A. latus* > *P. erumei* > *E. coioides* > *T. tonggol* > *S. commerson* > *P. indicus* > *P. argenteus* > *R. conadum* > *S. jello* > *N. japonicus* > *C. dorab* > *T. ilisha*. The highest concentration

Table 3
Recoveries of heavy metals from fish samples (mean ± SD; *n* = 5).

Metal	Spiking			Certified reference material		
	Spiked concentration (μg/g)	Recovery concentration (μg/g)	Recovery (%)	Certified concentration (μg/g)	Measured concentration (μg/g)	Recovery (%)
Lead	0.025	0.0228 ± 0.003	91	0.395	0.376 ± 0.18	95
	0.050	0.0475 ± 0.002	95			
	0.100	0.0945 ± 0.004	94			
Cadmium	0.025	0.0241 ± 0.004	96	0.290	0.269 ± 0.14	93
	0.050	0.0459 ± 0.004	92			
	0.100	0.0938 ± 0.003	94			
Copper	0.250	0.232 ± 0.025	93	15.5	14.93 ± 1.1	96
	0.500	0.465 ± 0.065	93			
	1.00	0.972 ± 0.040	97			
Zinc	0.250	0.231 ± 0.015	92	51.3	49.98 ± 1.5	97
	0.500	0.449 ± 0.040	91			
	1.00	0.962 ± 0.030	96			

Table 4
Instrument precision and method repeatability calculated for derivative potentiometric stripping analysis determination of lead, cadmium, copper and zinc in fish.

	Lead (μg/g)	Cadmium (μg/g)	Copper (μg/g)	Zinc (μg/g)
First extraction				
Mean ± SD	0.185 ± 0.012	0.068 ± 0.006	2.282 ± 0.151	7.077 ± 0.167
RSD%	6.486	8.823	6.573	2.359
Second extraction				
Mean ± SD	0.194 ± 0.016	0.064 ± 0.006	2.253 ± 0.124	7.540 ± 0.423
RSD%	8.240	9.375	5.503	5.610
Third extraction				
Mean ± SD	0.187 ± 0.009	0.061 ± 0.002	2.149 ± 0.159	7.654 ± 0.463
RSD%	4.81	3.278	7.398	6.049
Precision (Mean RSD%)	6.512	7.128	6.491	4.672
Total mean ± SD	0.189 ± 0.012	0.064 ± 0.005	2.228 ± 0.140	7.724 ± 0.419
Repeatability (total RSD%)	6.349	7.812	6.283	5.643

of lead was observed in *A. latus* (0.471 ± 0.109 μg/g), while *T. ilisha* by 0.057 ± 0.017 μg/g, showed the lowest concentration (Table 5). Agah et al. [18] analyzed the lead concentration of some collected fish species in 2004 from the Persian Gulf. According to their study the concentration range of lead was reported as 2–25, 0.2–17 and 2–9 ng/g of wet weight in *Pomadasys* sp., *Haemulidae*; *Platycephalus* sp., *Platycephalidae* and *Epinephelus tauvina*, respectively. It seems that the mean concentrations of lead of the current study comparing to those of Agah et al. [18] are higher both in similar and dissimilar fish species, remarkably. In another study, trace metal analysis was carried in fleshy tissues of several fish species collected from one of the southwestern regions of the Persian Gulf along the coast of Kuwait in 1990 [19]. Accordingly, the lead concentrations of *A. latus* and *P. erumei* were 0.3 to 0.8 μg/g of dry weight (approx. 0.075 to 0.2 μg/g of wet weight) and 0.6 μg/g of dry weight (approx. 0.15 μg/g of wet weight), respectively. These results are somewhat closer and more comparable to our obtained data in similar fish species.

The mean concentrations of cadmium in the analyzed fish species ranged between 0.024 ± 0.008 and 0.111 ± 0.078 μg/g of wet weight (Table 5). The highest to lowest concentration rank was observed as follows: *P. indicus* > *T. tonggol* > *P. erumei* > *S. commerson* > *E. coioides* > *A. latus* > *S. jello* > *P. argenteus* > *R. conadum* > *C. dorab* > *N. japonicus* > *T. ilisha*. Pourang et al.'s [20] investigation on collected sediments, water and some fish species from the northern part of the Persian Gulf was performed in 2003. Among the analyzed fish, *E. coioides* and *P. erumei* had cadmium concentration equal to 0.111 and 0.105 μg/g of wet weight that are comparable to the same species of the present study. Also, results of a survey for determination of cadmium concentration in several fish species consumed in Rayong province of Thailand showed that the element levels were 0.009, 0.021, 0.009 μg/g of wet weight in *S. commerson*, *T. Tonggol* and *Epinephelus tauvina*, respectively [21]. In addition, Agah et al. [18] reported cadmium concentrations of 0.1–0.2, 0.3–12 and 0.4–13 ng/g of wet weight in *Pomadasys* sp., *Haemulidae*; *Platycephalus* sp., *Platycephalidae* and *Epinephelus tauvina* (sampled in 2004), respectively. In contrast to the present study, Agah et al. [18] and Kerdthep et al. [21] reported cadmium at a remarkable lower concentrations than our study.

The concentration of copper and specially zinc, in the samples varied obviously. The concentrations of copper and zinc in the analyzed samples ranged from 0.799 ± 0.212 to 4.790 ± 0.798 μg/g and 3.226 ± 1.098 to 11.390 ± 3.002 μg/g, correspondingly. In addition, the sequence of concentration levels from highest to the lowest for copper was detected as follows: *P. erumei* > *S. commerson* > *E. coioides* > *T. tonggol* > *S. jello* > *C. dorab* > *A. latus* > *R. conadum* > *T. ilisha* > *N. japonicus* > *P. indicus* > *P. Argenteus*; and for zinc as: *P. erumei* > *T. tonggol* > *A. latus* > *S. commerson* > *P. indicus* > *P. argenteus* > *E. coioides* > *C. dorab* > *S. jello* > *N. japonicus* > *R. conadum* > *T. ilisha*. Comparison of the concentration range of present study with another Iranian investigation [18] showed a much higher burden of copper, however in case of zinc, in spite of the existence of a higher concentration; the difference is more inconspicuous.

3.2. Effect of season and habitat on fish muscular metal concentration

Heretofore, to our knowledge, no comprehensive report or information is available on lead, cadmium, copper and zinc concentrations of fish from the Persian Gulf with regard to the seasonal or habitat variations. Also, our previous results on total arsenic and mercury supported the influence of season and habitat parameters on their concentrations [22]. Different seasonal dependent conditions such as water temperature, dietary factors and growth and reproductive cycles are effective on heavy metal fluctuations

Table 5
Total lead, cadmium, copper and zinc concentrations (μg/g of wet weight) in fish species from Iranian market (mean ± SD; n = 6).

Fish species	Season	Metal concentrations			
		Lead	Cadmium	Copper	Zinc
Pelagic					
Scomberomorus commerson	Summer	0.158 ± 0.066[a]	0.053 ± 0.035[a]	4.219 ± 0.782	8.112 ± 1.541[a]
	Winter	0.289 ± 0.160[b]	0.102 ± 0.048[b]	4.880 ± 0.900	11.243 ± 1.627[b]
	Means of summer and winter	0.223 ± 0.135	0.078 ± 0.048	4.552 ± 0.876	9.677 ± 2.226
Chirocentrus dorab	Summer	0.093 ± 0.028	0.033 ± 0.014	2.567 ± 0.371[a]	5.195 ± 1.113
	Winter	0.081 ± 0.040	0.046 ± 0.009	3.827 ± 0.702[b]	6.467 ± 1.402
	Means of summer and winter	0.087 ± 0.033	0.039 ± 0.013	3.197 ± 0.848	5.831 ± 1.342
Sphyraena jello	Summer	0.113 ± 0.035[a]	0.049 ± 0.024	2.864 ± 0.497[a]	4.223 ± 0.628[a]
	Winter	0.192 ± 0.076[b]	0.057 ± 0.021	4.245 ± 0.720[b]	7.100 ± 0.915[b]
	Means of summer and winter	0.152 ± 0.070	0.053 ± 0.022	3.554 ± 0.931	5.661 ± 1.679
Rachycentron conadum	Summer	0.142 ± 0.039	0.026 ± 0.006[a]	1.552 ± 0.428[a]	2.643 ± 0.562[a]
	Winter	0.178 ± 0.048	0.055 ± 0.016[b]	2.546 ± 0.458[b]	4.000 ± 0.811[b]
	Means of summer and winter	0.160 ± 0.045	0.040 ± 0.019	2.047 ± 0.667	3.317 ± 0.975
Thunus tonggol	Summer	0.194 ± 0.144[a]	0.083 ± 0.009[a]	3.176 ± 0.502[a]	8.962 ± 2.310[a]
	Winter	0.277 ± 0.147[b]	0.128 ± 0.060[b]	4.637 ± 1.260[b]	13.456 ± 2.405[b]
	Means of summer and winter	0.235 ± 0.135	0.106 ± 0.047	3.907 ± 1.191	11.209 ± 3.250
Tenualosa ilisha	Summer	0.068 ± 0.017[b]	0.017 ± 0.006[a]	0.899 ± 0.133[a]	3.362 ± 1.402
	Winter	0.046 ± 0.009[a]	0.031 ± 0.004[b]	1.608 ± 0.535[b]	3.170 ± 0.816
	Means of summer and winter	0.057 ± 0.017	0.024 ± 0.008	1.253 ± 0.524	3.226 ± 1.098
Demersal					
Nemipterus japonicus	Summer	0.091 ± 0.025[a]	0.023 ± 0.009 [a]	0.830 ± 0.142[a]	3.743 ± 0.891
	Winter	0.149 ± 0.039[b]	0.039 ± 0.008 [b]	1.490 ± 0.533[b]	4.285 ± 0.502
	Means of summer and winter	0.120 ± 0.043	0.031 ± 0.011	1.160 ± 0.507	4.014 ± 0.745
Epinephelus coioides	Summer	0.227 ± 0.084[a]	0.061 ± 0.019 [a]	3.427 ± 0.813[a]	6.229 ± 1.124
	Winter	0.367 ± 0.090[b]	0.092 ± 0.016 [b]	4.463 ± 0.490[b]	6.800 ± 1.604
	Means of summer and winter	0.297 ± 0.111	0.076 ± 0.023	3.945 ± 0.838	6.514 ± 1.354
Platycephalus indicus	Summer	0.211 ± 0.035	0.076 ± 0.015	0.989 ± 0.144	7.445 ± 1.399[a]
	Winter	0.173 ± 0.039	0.147 ± 0.101	1.121 ± 0.267	10.780 ± 1.564[b]
	Means of summer and winter	0.192 ± 0.041	0.111 ± 0.078	1.056 ± 0.215	9.112 ± 2.244
Psettodes erumei	Summer	0.223 ± 0.102[a]	0.104 ± 0.016	4.285 ± 0.687[a]	9.252 ± 2.474[a]
	Winter	0.397 ± 0.049[b]	0.091 ± 0.012	5.294 ± 0.566[b]	13.528 ± 1.653[b]
	Means of summer and winter	0.310 ± 0.119	0.097 ± 0.015	4.790 ± 0.798	11.390 ± 3.002
Pomadasys argenteus	Summer	0.186 ± 0.071	0.034 ± 0.007 [a]	0.833 ± 0.222	6.850 ± 1.710[a]
	Winter	0.143 ± 0.041	0.066 ± 0.018 [b]	0.765 ± 0.215	9.149 ± 1.364[b]
	Means of summer and winter	0.164 ± 0.060	0.050 ± 0.020	0.799 ± 0.212	8.022 ± 1.918
Acanthopagrus latus	Summer	0.408 ± 0.079[a]	0.060 ± 0.020 [a]	2.961 ± 0.543[b]	8.131 ± 1.918[a]
	Winter	0.534 ± 0.102[b]	0.085 ± 0.013 [b]	2.010 ± 0.310[a]	12.430 ± 1.462[b]
	Means of summer and winter	0.471 ± 0.109	0.072 ± 0.021	2.485 ± 0.651	10.280 ± 2.772

[a,b]Means ± SD indicate significant differences ($p < 0.05$) between summer and winter.

[23,24]. The higher metal content in winter might be a result from considerable rainfall which washed down the wastes [22,25]. Table 5 shows the highest and the lowest concentration of lead in *A. latus* (0.534 ± 0.102 μg/g) and *T. ilisha* (0.046 ± 0.009 μg/g) in winter, respectively.

Considering the existing legislated regulations on permissible limits of lead, we observe that there are some differences among them. According to Regulation No. 78/2005 of EU [26], the maximum permitted limit (MPL) has been reported at 0.2 mg/kg of wet weight, whereas the 37th Codex Committee on Food Additive and Contaminants (CCFAC) has considered a maximum level for lead to be in a range between 0.2 and 0.5 mg/kg of wet weight for all fish species [27]. Also for example, the maximum limit of lead has been reported: 0.5, 2, 1, 0.4 and 0.5 mg/kg by Australian [28], Malaysian [29], Turkish [30], Lithuanian [31] and Chinese [32] standards and regulations, respectively. The lead concentration of *A. latus* in winter is slightly higher than the legislated maximum limit (0.5 mg/kg of wet weight) according to the 37th CCFAC [27]. The results showed statistically significant differences ($P < 0.05$) between winter and summer concentrations of lead in eight analyzed fish species (Table 5). However, approximately more than 66% of all fish species had higher lead content in winter. For cadmium concentrations in the flesh of fish species, the highest extent (0.147 ± 0.101 μg/g) belonged to *P. indicus* in winter, while concentration was the lowest (0.017 ± 0.006 μg/g) for *T. ilisha* in summer. In view of the fact that MPL of the European Union [26] is 0.05 mg/kg of wet weight, cadmium concentrations (Table 5) were higher in the eight species, particularly in winter. Moreover, the maximum permissible limit of cadmium in marine products has been reported to be 0.1 μg/g of wet weight in Croatia, 0.5 μg/g of wet weight in Saudi Arabia [33], 0.1 μg/g of wet weight in Turkey [30], 1 μg/g of wet weight in Malaysia [29], 0.5 μg/g of wet weight in China [32] and 0.5 μg/g of wet weight in FAO limits [23]. According to our study, it seems that, even with the excess burden of cadmium in several fish species comparing to the EU limit as the most fastidious legislator, the case could be considered as having no problem according to the other mentioned limits. Meanwhile, about 91% of all fish species had higher cadmium level in winter.

As shown in Table 5, the highest burden of both copper and zinc was detected in *P. erumei* in winter and was equal to 5.294 ± 0.566 and 13.528 ± 1.653 μg/g of wet weight, respectively. The lowest concentration of copper was measured in *P. argenteus* (0.765 ± 0.215 μg/g of wet weight) in winter; and the lowest concentration of zinc was detected in *R. conadum* (2.643 ± 0.562 μg/g of wet weight) in summer. Considering Table 5, it can be revealed that ten species had the highest extent for both copper and zinc. Concentrations of copper were below the adopted limits by MAFF [34], 30 mg/kg, Turkish Food Standard, 20 mg/kg [30], FAO, 30 mg/kg [23] and Malaysian Food and Drug Regulation, 30 mg/kg [29]. Also, MPLs of zinc in fish according to FAO [35] standard, Turkish Food Standard [30], Malaysian Food and Drug Regulation [29], are 30, 50, 100 mg/kg, respectively. Therefore, none of the studied metals, copper and zinc, exceeded the maximum permissible concentration.

The habitat is one of the main factors influencing heavy metal burdens of marine inhabitants [22]. Lead, cadmium, copper and zinc

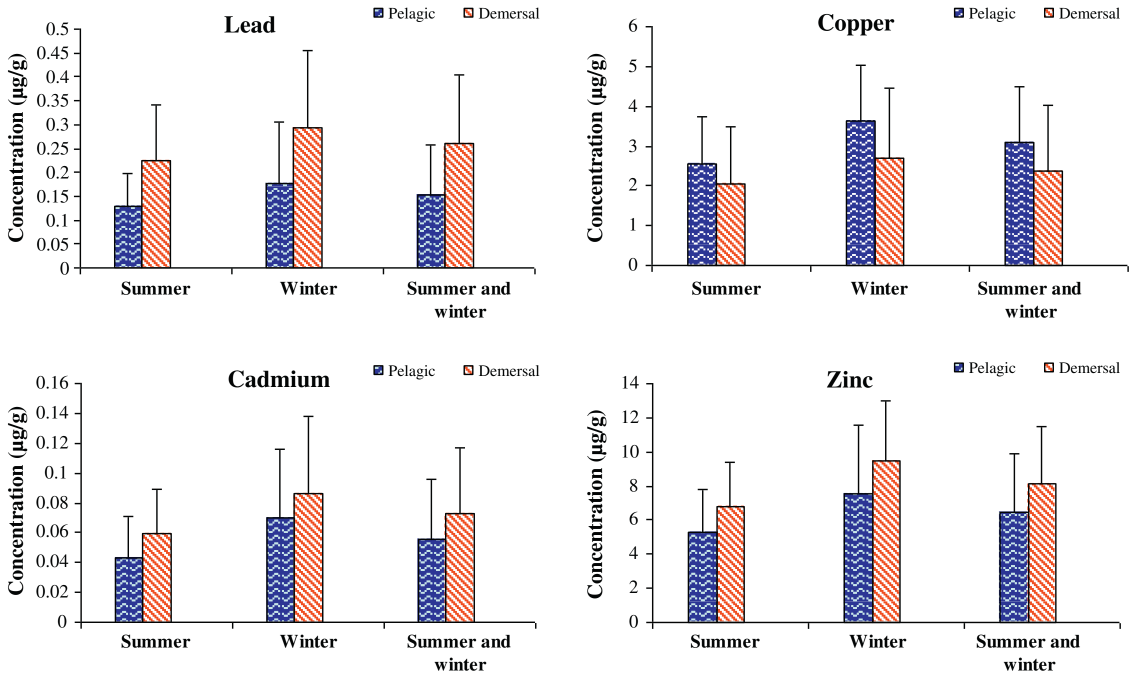

Fig. 2. Lead, cadmium, copper and zinc concentrations of pelagic and demersal fish.

concentrations in pelagic and demersal fish species in summer, winter and summer and winter are presented in Fig. 2. With the exception of copper, the higher concentration of lead, cadmium and zinc belonged to the demersal habitat. The results (in case of lead, cadmium and zinc) are comparable to studies by other authors [9,22,36]. In general, results acquired from studies by some researchers have indicated that demersal or bottom-dwelling species can accumulate a higher proportion of heavy metals than the pelagic ones [22,36]. The level of cadmium and zinc of pelagic fishes from Kerguelen Islands [37] and the strontium of pelagic fishes [9] is reported higher in comparison to demersal or benthic ones. This characteristic might be related to food preferences, organism mobility or other attributes of behaviour with respect to the environment [36].

3.3. Assessment of exposure to heavy metals due to fish consumption

Dietary exposure assessment for metals through fish consumption was carried out by evaluating the PTWIs or PTMIs in summer, winter and summer and winter. According to the Annual Fishery Statistics of Iran [38] the annual per capita fish consumption has been reported at 7.62 kg in 2009. Therefore, the flesh of fresh fish consumption per day and week in Iran has been considered at 21 and 147 g in 2009, respectively. PTWI depends on the amount and period of consumption and contamination level of consumed food [39].

According to the summary of evaluations performed by Joint FAO/WHO Expert Committee on Food Additives (JECFA), PTWI of lead and cadmium is 25 and 7 μg/kg body weight/week or 250 and 70 μg/day for a subject of 70 kg of body weight, respectively [40]. However, the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) that was held in Geneva, Switzerland, June 2010 announced that because of the long half-life of cadmium, daily ingestion in food has little or even an insignificant effect on the overall exposure. Thereby, dietary intake and tolerable intake assessment of long or short-term health risks due to cadmium exposure should be considered over months or one month, respectively. Hence, in view of this fact the PTWI of cadmium should be expressed by provisional tolerable monthly intake (PTMI), preferentially. The committee appointed a PTMI of 25 μg/kg of body weight [41]. The estimated daily and weekly intakes (EDI and EWI) of lead and estimated monthly intake (EMI) of cadmium for an adult of 70 kg body weight are given in Table 6.

Regarding the guidelines provided by JECFA, the PTWIs of copper and zinc were considered equivalent to 3500 and 7000 μg/kg body weight/week, respectively [40]. The EDI and EWI of copper and zinc are shown in Table 6.

Table 6
The estimated daily and weekly intakes of lead, cadmium, copper and zinc and estimated monthly intakes of cadmium for the fish samples from the Iranian market.

Element	PTWI[a]	PTWI[b]	PTMI[c]	PTMI[d]	PTDI[e]	EDI[f]			EWI[g]			EMI[h]		
						Summer	Winter	Summer and winter	Summer	Winter	Summer and winter	Summer	Winter	Summer and winter
Lead	25	1750	–	–	250	3.696	4.935	4.305	25.872	34.545	30.135	–	–	–
Cadmium	–	–	25	1750	–	–	–	–	–	–	–	–	–	–
Copper	3500	245,000	–	–	35000	48.384	66.213	57.288	338.688	463.491	401.016	32.13	49.14	40.32
Zinc	7000	490,000	–	–	70000	127.26	179.277	153.279	890.820	1254.939	1072.953	–	–	–

[a] Provisional tolerable weekly intake (μg/week/kg body weight).
[b] PTWI for an adult person (μg/week/70 kg body weight).
[c] Provisional tolerable monthly intake (μg/month/kg body weight).
[d] PTMI for an adult person (μg/month/70 kg body weight).
[e] Permissible tolerable daily intake (μg/day/70 kg body weight).
[f] Estimated daily intake (μg/day/70 kg body weight).
[g] Estimated weekly intake (μg/week/70 kg body weight).
[h] Estimated monthly intake (μg/week/70 kg body weight).

Results indicated that EDI, EWI of lead, cadmium, copper and zinc and EMI of cadmium could be considered safe in comparison with respective legislation limits (PTWIs and PTMI); thereby there is no health threatening concern due to the consumption of fish in Iran.

4. Conclusions

According to the potential threatening effects to human safety regarding heavy metal concentrations in seafood products, its situation should be evaluated along the time, regularly. The results of the present study provide important information about lead, cadmium and zinc concentrations in twelve of the most commercially valuable pelagic and demersal fish species from the Iranian market. In conclusion, the results of the present study demonstrate that lead, cadmium, copper and zinc contents of fish species were different either seasonally or habitatly. Indeed, the metal concentration was higher in winter than summer. The demersal fish species had a higher content of heavy metals (except for copper) than the pelagic ones. The estimated daily and weekly intakes of lead, copper and zinc and estimated monthly intake of cadmium showed that the quantities are lower than the recommended permissible intakes in the Iranian population. However, although the EDI and EWI of lead, copper and zinc; and EMI of cadmium were lower than the legislated limits; the role of the low capitation consumption of fish per year in Iran should not be overlooked.

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References

[1] S. Miniadis-Meimaroglou, C. Dimizas, V. Loukas, A. Moukas, A. Vlachos, N. Thomaidis, et al., Proximate composition, fatty acids, cholesterol, minerals in frozen red porgy, Chem. Phys. Lipids 146 (2007) 104–110.

[2] U. Gogus, C. Smith, n-3 Omega fatty acids: a review of current knowledge, Int. J. Food Sci. Technol. 45 (2010) 417–436.

[3] Y. Kaya, H. Turan, Comparison of protein, lipid and fatty acids composition of anchovy (*Engraulis encrasicolus* L. 1758) during the commercial catching season, J. Muscle Foods 21 (2010) 474–483.

[4] Y. Keskin, R. Baskaya, O. Özyaral, T. Yurdun, N.E. Lüleci, O. Hayran, Cadmium, lead, mercury and copper in fish from the marmara Sea, Turkey, Bull. Environ. Contam. Toxicol. 78 (2007) 258–261.

[5] J. Kojadinovic, M. Potier, M.L. Corre, R.P. Cosson, P. Bustamante, Bioaccumulation of trace elements in pelagic fish from the Western Indian Ocean, Environ. Pollut. 146 (2007) 548–566.

[6] N. Gokoglu, P. Yerlikayal, M. Gokoglu, Trace elements in edible tissues of three shrimp species (*Penaeus semisulcatus*, *Parapenaeus longirostris* and *Paleomon serratus*), J. Sci. Food Agric. 88 (2008) 175–178.

[7] K. Uysal, Y. Emre, E. Köse, The determination of heavy metal accumulation ratios in muscle, skin and gills of some migratory fish species by inductively coupled plasma-optic emission spectrometry (ICP-OES) in Beymelek Lagoon (Antalya/Turkey), Microchem. J. 90 (2008) 67–70.

[8] I. Jarić, Ž. Višnjić-Jeftić, G. Cvijanović, Z. Gačić, L. Jovanović, S. Skorić, M. Lenhardt, Determination of differential heavy metal and trace element accumulation in liver, gills, intestine and muscle of sterlet (*Acipenser ruthenus*) from the Danube River in Serbia by ICP-OES, Michrochem. J., doi:10.1016/j.microc.2010.11.008.

[9] M.L. Carvalho, S. Santiago, M.L. Nunes, Assessment of the essential element and heavy metal content of edible fish muscle, Anal. Bioanal. Chem. 382 (2005) 426–432.

[10] F. Zeynali, H. Tajik, S. Asri-Rezaei, S. Meshkini, A.A. Fallah, M. Rahnama, Determination of copper, zinc and iron levels in edible muscle of three commercial fish species from Iranian coastal waters of the Caspian Sea, J. Anim. Vet. Adv. 8 (2009) 1285–1288.

[11] H.H. Ang, Lead contamination in *Eugenia dyeriana* herbal preparations from different commercial sources in Malaysia, Food Chem. Toxicol. 46 (2008) 1969–1975.

[12] M. Ursinyova, V. Masanova, Cadmium, lead and mercury in human milk from Slovakia, Food Addit. Contam. 22 (2005) 579–589.

[13] R.K. Sharma, M. Agrawal, F.M. Marshall, Heavy metals in vegetables collected from production and market sites of a tropical urban area of India, Food Chem. Toxicol. 47 (2009) 583–591.

[14] L. Garcia-Rico, J. Leyva-Perez, M.E. Jara-Marini, Content and daily intake of copper, zinc, lead, cadmium, and mercury from dietary supplements in Mexico, Food Chem. Toxicol. 45 (2007) 1599–1605.

[15] Ö. Özden, Ş. Ulusoy, N. Erkan, Study on the behavior of the trace metal and macro minerals in *Mytilus galloprovincialis* as a bioindicator species: the case of Marmara Sea, Turkey, J. Verbr. Lebensm. 5 (2010) 407–412.

[16] G. Dugo, L. La Pera, A. Bruzzese, T.M. Pellicanò, V. Lo Turco, Concentration of Cd(II), Cu(II), Pb(II), Se(IV) and Zn(II) in cultured sea bass (*Dicentrarchus labrax*) tissues from Tyrrhenian Sea and Sicilian Sea by derivative stripping potentiometry, Food Control 17 (2006) 146–152.

[17] R.M. Palmieri, L. La Pera, G.D. Bella, G. Dugo, Simultaneous determination of Cd(II), Cu(II), Pb(II) and Zn(II) by derivative stripping chronopotentiometry in *Pittosporum tobira* leaves: a measurement of local atmospheric pollution in Messina (Sicily, Italy), Chemosphere 59 (2005) 1161–1168.

[18] H. Agah, M. Leermakers, M. Elskens, S.M.R. Fatemi, W. Baeyens, Accumulation of trace metals in the muscle and liver tissues of five fish species from the Persian Gulf, Environ. Monit. Assess. 157 (2009) 499–514.

[19] A.H. Bu-Olayan, M.N.V. Subrahmanyam, Trace metals in fish from the Kuwait Coast using the microwave acid digestion technique, Environ. Int. 22 (1996) 753–758.

[20] N. Pourang, A. Nikouyan, J.H. Dennis, Trace element concentrations in fish, superficial sediments and water from northern part of the Persian Gulf, Environ. Monit. Assess. 109 (2005) 293–316.

[21] P. Kerdthep, L. Tongyonk, L. Rojanapantip, Concentrations of cadmium and arsenic in seafood from Muang District, Rayong Province, J. Health Res. 23 (2009) 179–184.

[22] S.S. Saei-Dehkordi, A.A. Fallah, A. Nematollahi, Arsenic and mercury in commercially valuable fish species from the Persian Gulf: influence of season and habitat, Food Chem. Toxicol. 48 (2010) 2945–2950.

[23] B. Ersoy, M. Çelik, Essential elements and contaminants in tissues of commercial pelagic fish from the Eastern Mediterranean Sea, J. Sci. Food Agric. 89 (2009) 1615–1621.

[24] A. Meche, M.C. Martins, B.E.S.N. Lofrano, C.J. Hardaway, M. Merchant, L. Verdade, Determination of heavy metals by inductively coupled plasma-optic emission spectrometry in fish from Piracicaba River in Southern Brazil, Microchem. J. 94 (2010) 171–174.

[25] M. Dural, M.Z.L. Göksu, A.A. Özak, Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon, Food Chem. 102 (2007) 415–421.

[26] EU, Regulation (EC) No. 78/2005. JO L16, 19.01.05, 2005, pp. 43–45.

[27] Codex Alimentarius Commission, Discussion paper on maximum level for lead in fish. Joint FAO/WHO Food Standards Programme, Thirty-eighth Session The Hague, the Netherlands, 2006.

[28] Food Standards Australia, Issue 53, Standard 1.4.1 and 1.4.2., 2002.

[29] A.K. Ahmad, M. Shuhaimi-Othman, M.M. Ali, A temporal study of selected metal concentration in fishes of Lake Chini, peninsular Malaysia, Malaysian J. Anal. Sci. 13 (2009) 100–106.

[30] TKB, Fisheries Laws and Regulations, Ministry of Agriculture and Rural Affairs Conservation and Control General Management, Ankara, Turkey, 2002.

[31] Standards of Hygiene of Ministry of HealthCare of the Republic of Lithuania, Food products, Maximum tolerable limit of residue of contaminants and pesticides, L R HN, 54, 2001.

[32] L. Xiaobo, J. Linzhi, Z. Yunlong, W. Qun, C. Yongxu, Seasonal bioconcentration of heavy metals in *Onchidium struma* (Gastropoda: Pulmonata) from Chongming Island, the Yangtze Estuary, China, J. Environ. Sci. 21 (2009) 255–262.

[33] M.M. Storelli, G.O. Marcotrigiano, Content of mercury and cadmium in fish (*Thunnus alalunga*) and cephalopods (*Eledone moschata*) from the south-eastern Mediterranean Sea, Food Addit. Contam. 21 (2004) 1051–1056.

[34] MAFF, Monitoring and Surveillance of Non-radioactive Contaminants in the Aquatic Environment and Activities Regulating the Disposal of Wastes at Sea, Aquatic Environment Monitoring Report No.44, Directorate of Fisheries Research, Lowestoft, 1995.

[35] FAO, Statistics Division, Food Security Statistics, Food Consumption, 2008, http://www.fao.org/es/ESS/faostat/foodsecurity/index_en.htm.

[36] Z.K. Gaspić, T. Zvonarić, N. Vrgoć, N. Odzak, A. Barić, Cadmium and lead in selected tissues of two commercially important fish species from the Adriatic Sea, Water Res. 36 (2002) 5023–5028.

[37] P. Bustamante, P. Bochet, Y. Cherel, P. Miramand, F. Caurant, Distribution of trace elements in tissues of benthic and pelagic fish from the Kerguelen Islands, Sci. Total Environ. 313 (2003) 25–39.

[38] Annual Fishery Statistics of Iran, Consumption of Fish in Iran, Ministry of Agriculture, 2010, pp. 36–40.

[39] M. Türkmen, A. Türkmen, Y. Tepe, Metal contaminations in five fish species from Black, Marmara, Aegean and Mediterranean Seas, Turkey, J. Chilean Chem. Soc. 53 (2008) 1435–1439.

[40] FAO/WHO, Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1956–2003), (First Through Sixty First Meetings), ILSI Press International Life Sciences Institute, 2004.

[41] FAO/WHO, Joint FAO/WHO Expert Committee on Food Additives, Summary report of the seventy-third meeting of JECFA in the WHO Technical Report Series, Geneva, Switzerland, 2010, pp. 12–13.