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-RESEARCH ARTICLE-

Bioaccumulation of Some Heavy Metals on Silver-Cheeked Toadfish (*Lagocephalus sceleratus*) from Antalya Bay, Turkey

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Abstract

In the present study, the heavy metal concentration (Cd, Cu, Pb, Ag, Co, Cr, Fe, Mn, Ni & Zn) in skin and muscle were studied in silver-cheeked toadfish *Lagocephalus sceleratus* collected from Antalya Bay. The heavy metals concentration ranges in muscle tissue were Cu (0.276-0.518 µg/g); Fe (5.996-21.367 µg/g); Mn (0.601-2.633 µg/g); Zn (51.472-86.635 µg/g); Cd (0.045-0.139 µg/g); Co (0.541-0.833 µg/g); Cr (0.205-0.361 µg/g); Ni (0.108-0.765 µg/g) and Pb (1.464-2.560 µg/g). The heavy metal concentration ranges in skin were Cu (0.168-0.209 µg/g); Fe (1.738-4.467 µg/g); Mn (0.012-0.414 µg/g); Zn (3.337-6.451 µg/g); Cd (0.113-0.217 µg/g); Co (0.432-0.739 µg/g); Cr (0.101-0.148 µg/g); Ni (0.038-0.217 µg/g) and Pb (0.342-0.584 µg/g). The concentrations of Zn and Pb in the muscle tissues exceeded the acceptable levels for a food source and are not safe for human consumption. Further, this is the first report on distribution of heavy metals of *Lagocephalus sceleratus* from Antalya Bay, northern Levantine Sea, in the Eastern Mediterranean Sea south of Antalya Province, Turkey.

Keywords: Pufferfish, *Lagocephalus sceleratus*, Heavy metals, Antalya Bay

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Introduction

Heavy metals are natural components and environmentally ubiquitous in the earth's crust that readily dissolved in, taken up by aquatic organisms and cannot be degraded or destroyed. They are dangerous elements due to their bioaccumulation and toxicity can threaten aquatic living organisms (Lenntech, 2004). Since the metals are actively used in agriculture, medicine and industry, they could not be ignored from daily life (Permatasari, 2006). Metals may exert beneficial or harmful effects on life, depending on their concentration. Some heavy metals like mercury, cadmium and lead have no known role in aquatic organisms while some others such as copper, zinc, iron and manganese are essential for fish metabolism. These essential metals always function in combination with organic molecules, usually proteins. But the essential metals being above the threshold bioavailable level are also toxic to aquatic organisms and humans (Küçüksezgin et al., 2006). Fish and their some tissues can also be considered as the most significant indicators in water systems for the estimation of metal pollution level (Henry et al., 2004). For these reasons, it is important to determine the concentrations of heavy metals in fish in order to evaluate the possible risks relating both aquatic environment and human health. Therefore, the commercial and edible species have been widely investigated in order to check for those hazards (Begum et al., 2005).

Heavy metals released to aquatic environments result from both natural sources, such as atmospheric deposition and erosion of the geological matrix, and anthropogenic sources, such as industrial effluents and mining wastes (Alam et al., 2002). In agricultural areas, some heavy metal concentrations such as copper and zinc were found highly in fish tissues (Rashed, 2001; Dural et al., 2007). Antalya region is an important touristic area. Hence, there has not heavily industrial activities in the Antalya Bay. However, due to suitable climate of this region, it has been carried out intensive agricultural activities in the region and has been used a lot of fertilizers and pesticides which may lead to water and fish contamination with heavy metals.

Puffers are marine fish species that are distributed in tropical and subtropical areas of the Atlantic, Indian and Pacific Ocean. Puffers include 28 genera and approximately 184 species in all over the world marine waters within the Tetraodontidae family (Matsuura, 2015; Farrag et al., 2016), among which at least ten are found in the eastern Mediterranean (Farrag, 2014). The silver-cheeked toadfish *Lagocephalus sceleratus* (Gmelin, 1789) is a reef-associated pufferfish (Tetraodontidae) distributed in the tropical Indo-West Pacific Ocean (Smith and Heemstra, 1986), which entered the Mediterranean Sea via the Suez Canal and was reported as a confirmed record from the Gökova Bay in Turkish marine waters (Akyol et al., 2005). Subsequently, this Lessepsian invasive species has established large populations along the coasts of many countries of the eastern basin such as Israel, Lebanon, Turkey (Mediterranean and Aegean coasts), Cyprus and Greece (Aegean and Ionian coasts), while still rapidly expanding westwards along the coasts of Egypt, Libya, and along the entire Tunisian coastline (Ben-Soussi et al., 2014).

Apart from several large species used for human consumption as a delicious food in few countries, particularly in China, Korea, Japan and Taiwan (Makoto et al., 2000), most pufferfish species have not commercial value. Besides the small size of most species, the family is renowned for the occurrence of a powerful toxin in their skin and organs called tetrodotoxin (TTX). Tetrodotoxin is a very potent neurotoxin and one of the strongest marine paralytic toxins known (El-Sayed et al., 2003; Tsang et al., 2007). In this study, it was aimed to analyze the bioaccumulations of some heavy metals (Cd, Cu, Pb, Ag, Co, Cr, Fe, Mn, Ni & Zn) in muscle and skin tissues of *L. sceleratus* and also to discuss the allowable limit of these heavy metals via fish consumption.

To the best of our knowledge, present study is the first to investigate bioaccumulation of some heavy metals of *L. sceleratus* individuals caught by commercial fisheries from the Antalya Bay, Eastern Mediterranean.

Material and Methods

One species of marine puffer fishes from Tetraodontidae family namely silver-cheeked toadfish, *Lagocephalus sceleratus* (Fig. 1) were collected from Antalya Bay (latitude 36°50’04”; longitude 30°37’74’’), in the Eastern Mediterranean Sea south of Antalya Province, Turkey during the period of spring (Fig. 2). In total, 10 specimens of species were collected and the samples were transported to the laboratory within 5–6 h in ice packed condition in a storage box. The specimen was thawed to room temperature for morphometric study and weight was examined for all the collected specimen. All the specimen were measured to the nearest mm, whereas weights were recorded with the use of electronic balance to the nearest 0.01 g and stored in −20°C for further heavy metal analysis. Species identification were carried out according to Golani et al. (2006).

Figure 1. *Lagocephalus sceleratus* sampled from Antalya Bay

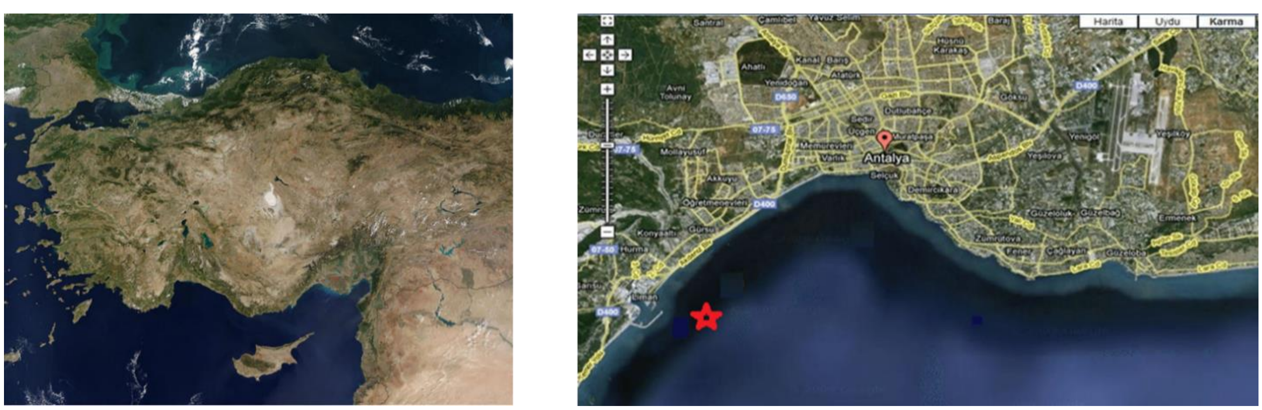

Figure 2. Location of the study area and its surroundings.

After catch, fish samples were made the species identification (Golani et al., 2006), refrigerated and transported to Iskenderun Technical University Marine Sciences and Technology

Faculty's laboratory where they were instantly frozen (-18^{O}C) for later analyze procedure. Weight for skates and disc width (DW) and weight estimations for rays were made to the closest 0.1 cm, and gram, individually (Table 1).

Table 1. Length–weight of *Lagocephalus sceleratus*, Antalya Bay, Turkey

Species	N	Size range (cm)	Weight range (g)
Lagocephalus sceleratus	10	19.7 -25.2	85.92-222.31

Throughout the study, all acids and chemicals used were analytical grade. For acid digestion, various parts namely, skin and muscle tissue of fish samples were dissected using sterile stainless knife and scissor. The segments of the skin and muscle tissues from the examples were evacuated, homogenized and around 1.0± 0.2 g was taken for investigation. 10 ml of nitric acid is added to the sample and kept overnight at room temperature. Afterwards the examples were processed, utilizing a water bath at 60^{O}C for 3 days. Then, the samples were cooled to room temperature, filtered and was completed to 50 ml (UNEP, 1984).

After dilution, metal contents of tissue measured on a inductively coupled plasma atomic emission spectrometry (ICP-AES) (Varian model, Liberty Series II; Palo Alto, USA) and metal concentration in the tissue was presented as µg/g. For calibration ICP-AES was used as a High Purity Multi Standard. All digested samples were analyzed three times for the each metals. Blank samples were prepared in the same manner as the samples and the same acid matrix was used in the standard solution.

Results and Discussion

Puffer fishes are bottom living, carnivorous, slow swimmer and non-target fish species. Nowadays, consumption of puffer fishes as an alternative food to meet out the increasing food demand due to growing population and day-by-day collapse of natural resources. The main reasons for the consumption of puffer fishes are due to easily available nature and low marker price. The concentrations of heavy metals Cu, Fe, Mn, Zn, Cd, Co and Cr in skin and muscle tissues of puffer fishes were showed in Table 2.

As shown, mean concentrations of the heavy metals in the muscle tissue were Cu (0.409 µg/g), Fe (12.719 µg/g), Mn (1.393 µg/g), Zn (63.989 µg/g), Cd (0.078 µg/g), Co (0.696 µg/g), Cr (0.284 µg/g), Ni (0.407 µg/g), Pb (2.047 µg/g); in the skin were Cu (0.122 µg/g), Fe (2.731 µg/g), Mn (0.273 µg/g), Zn (4.746 µg/g), Cd (0.170 µg/g), Co (0.591 µg/g), Cr (0.124 µg/g), Ni (0.157 µg/g), Pb (0.189 µg/g). Among the muscle tissue samples, Zn was detected as higher, followed by Fe, Pb, Mn, Co, Ni, Cr and Cd.

Table 2. Mean (±SD) concentrations of heavy metals (µg/g wet weight) in some organs of *Lagocephalus sceleratus* collected from Antalya Bay.

Metals (µg/g)	Tissues			
	Muscle		Skin	
	Mean (±SD)	**Min-Max**	**Mean (±SD)**	**Min-Max**
Cu	0.409±0.122	0.276-0.518	0.122±0.076	0.168-0.209
Fe	12.719±7.863	5.996-21.367	2.731±0.105	1.738-4.467
Mn	1.393±1.087	0.601-2.633	0.273±0.226	0.012-0.414
Zn	63.989±19.650	51.472-86.635	4.746±1.577	3.337-6.451
Cd	0.078±0.052	0.045-0.139	0.170±0.052	0.113-0.217
Co	0.696±0.146	0.541-0.833	0.591±0.153	0.432-0.739
Cr	0.284±0.078	0.205-0.361	0.124±0.023	0.101-0.148
Ni	0.407±0.313	0.108-0.765	0.157±0.103	0.038-0.217
Pb	2.047±0.051	1.464-2.560	0.189±0.035	0.342-0.584

Turkish legislation establishes maximum levels for four of the metals studied, above which human consumption is not permitted as; 0.1 µg/g for Cd, 1.0 µg/g for Pb, 20.0 µg/g for Cu, 50 µg/g for Zn (Anonymous, 1996). Food and Agricultural Organization limits for Cd and Pb 0.5 µg/g, for Cu and Zn 30 µg/g (FAO, 2000). The concentrations of Zn and Pb measured in the muscle of our species studied were higher than the levels issued by FAO and Turkish legislation. Yet, Cd concentrations in the muscle tissues were at the limit levels set by law.

Pb is a nonessential element for living organism and also it possess various adverse effects such as neuro and nephro toxicity, rapid behavioral malfunction, and decreases the growth, metabolism, and survival rate, alteration of social behavior in some mammals (Malik et al., 2010). Rashed (2001) found that elevated Pb level in fishes obtained from fresh water ecosystem affected by extended agriculture, poultry forms, textile, industrial and other activities. So the sediments could be the major sources of Pb contamination and the bottom feeders may directly affects with this deposited element in consequence to their feeding habitat (Garcia et al., 2010). Zn is the essential element for both human and aquatic organisms and they showed productive activity against Cd and Pb toxicity in biological organisms (Sarkar et al., 2016).

The high accumulation of heavy metals in puffer fishes is due to its carnivorous feeding nature and bottom habitat. Even though the puffer fishes are non-target species, peoples are started to consume because of their huge quantity or extended by-catch, low market price and high nutrient value. The present study concluded that the long term consumption of these fishes may leads to potential risk to humans in future. So regular monitoring of marine resources is essential to improve the quality of sea-food against contaminants.

Compared with other studies, the results in the present study were lower than the previously reported values of Cu, Cd in fish species from different and also same area (Table 3) (Kaleshkumar et al., 2017; Mat-Piah, 2011; Nurjanah et al., 2015; Kumagai & Saeki, 1983; Uysal & Emre, 2011; Tepe et al., 2008; Türkmen et al., 2008; Türkmen et al., 2009; Tepe, 2009; Yipel & Yarsan, 2014; Aktan & Tekin-Özan, 2012; Gökkuş & Türkmen, 2016; Duysak & Uğurlu, 2017; Tekin-Özan, 2014; Kayhan et al., 2010).

Table 3. Heavy metal levels in fish muscles from different locations worldwide

Species	Cu (µg/g)	Cd (µg/g)	Pb (µg/g)	Zn (µg/g)	Cr (µg/g)	Co (µg/g)	Mn (µg/g)	Ni (µg/g)	Fe (µg/g)	Location	References
Takifugu oblongus *Lagocephalus guentheri* *Arothron hispidus* *A. immaculatus* *Chelonodon patoca*	1,80 (mean)	0,24 (mean)	8,31 (mean)	43,37 (mean)						South east coast of India	Kaleshkumar et al. (2017)
Marilyna pleurosticta	4-6	0,11-0,15	0,3-0,5	100-130	4-6		3-6	0,6-1	200-300	New South Wales	Mat-Piah (2011)
Lagochepalus lunaris	0,43±0,01	0,02±0,00	0,51±0,01	73,63±0,60					6,85±0,01	Indonesia, West Java	Nurjanah et al. (2015)
Fugu ruripes (cultured)	0,28	0,21	0,26	6,4		0,056	0,062	0,13		Japan	Kumagai and Saeki (1983)
Diplodus sargus *Siganus rivulatus* *Lithognathus mormyrus* *Liza aurata* *Chelon labrasus*	1,6-1,8 0,8-1 08,-0,9 1,6-1,7 0,4-0,6			5-10 1-4 10-12 1-4 1-3			0,5-1 0-0,5 0-0,5 0-0,5 0,7-1		10-15 8-10 6-9 10-12 10-13	Antalya Bay	Uysal and Emre (2011)
Mullus barbatus	0,68±0,12	0,32±0,09	0,22±0,05	7,35±1,36	0,05±0,01	0,05±0,01	0,87±0,31	0,96±0,34	49,3±22,3	Antalya Bay	Tepe et al. (2008)
Sparus aurata	1,36 ± 0,29	0,16 ± 0,06	0,72 ± 0,24	6,34 ± 0,60	0,65±0,19	0,18 ± 0,03	0,49 ± 0,11	2,03 ±0,58	22,3 ± 6,35	Antalya Bay	Türkmen et al. (2008)
Scyliorhinus canicula *Pomadasys incises* *Uranoscopus scaber* *Scomber japonicas*	0,88±0,23 0,34±0,4 0,70±0,17 0,63±0,17	0,07±0,02 0,39±0,04 0,08±0,02 0,08±0,04	0,27±0,07 0,66±0,08 0,28±0,18 0,25±0,08	10,9±2,93 8,94±1,47 8,56±1,27 7,21±0,67	0,29 ± 0,08 1,48 ± 0,04 0,38 ± 0,04 0,28 ± 0,07	<0,01±0,00 0,45±0,03 0.03 ± 0.00 0,02±0,00	1,04±0,27 0,69±0,05 0,80±0,29 0,36±0,05	0,27±0,07 3,13±0,35 0,69±0,27 0,84±0,24	34,7±9,32 0,34±0,4 31,5±10,0 43,5±20,2	Antalya Bay	Türkmen et al. (2009)
Sardinella aurita *Mullus surmuletus* *Lithognathus mormyrus* *Pagellus erythrinus*	0,37±0,07 0,49±0,25 0,73±0,17 0,73±0,25	0,23±0,08 0,40±0,00 0,30±0,16 0,03±0,01	0,39±0,12 0,93±0,22 0,04±0,02 0,35±0,11	6,99±0,72 6,25±0,97 5,64±0,71 3,94±0,92	0,72±0,23 1,53±0,14 0,51±0,12 0,33±0,03	0,18±0,08 0,43±0,01 0,03±0,01 0,03±0,01	0,54±0,09 0,64±0,01 0,42±0,10 0,43±0,16	2,46±0,58 4,11±0,03 0,29±0,10 0,51±0,07	40,60±30,59 6,89±0,18 22,91±4,28 47,59±19,49	Antalya Bay	Tepe (2009)
Mullus barbatus *Mugil cephalus* *Panaeus semisulcatus*	1,880±1,451 1,293±0,627 7,152±2,140	0,009±0,009 0,016±0,015 0,082±0,087	0,352 ±0,088 0,326±0,055 0,365±0,223	4,781±0,819 7,702±1,594 16,829±5,289						Antalya Bay	Yipel and Yarsan (2014)
Scomber japonicas	4,62±3,17	0,03±0,01		50,98±42,17	0,37±0,12	0,05±0,03	0,55±0,29	0,13±0,11	96,24±43,2	Antalya Bay	Aktan and Tekin-Özan (2012)
Rhinobatos rhinobatos	1,39-1,54	0,06-0,07	0,43-0,59	9,94-11,30	0,22-0,39	0,09-0,10	0,68-0,76	0,56-0,96	41,9-50,8	Antalya Bay	Gokkus and Turkmen (2016)
Sepia officinalis	2,17±0,51	0,68±0,11			0,33±0,16	10,20±6,81		0,33±0,10		Antalya Bay	Duysak and Ugurlu (2017)
Boops boops	11,31±5,26	0,0064±0,0007	0,07±0,05	18,31±3,05	0,47±0,6		0,64±0,06	0,54±0,06	49,88±7,02	Antalya Bay	Tekin-Özan (2014)
Thunnus thynnus (cultured)			0,16±0,001							Antalya Bay	Kayhan et al. (2010)
L. sceleratus	0,276-0,518	0,045-0,139	1,464-2,560	51,472-86,635	0,205-0,361	0,541-0,833	0,601-2,633	0,108-0,765	5,996-21,367	Antalya Bay	This research

When the Zn values were compared, the values in the study of Mat-Piah (2011) were found to be higher than those in our study. The same situation is seen in the value of Pb in the study of Kaleshkumar et al. (2017). Compared with the studies given in Table 3, Co (except Duysak and Uğurlu, 2017), Mn (except Mat-Piah, 2011) and Ni (except Tepe et al., 2008; Türkmen et al., 2008 and 2009 in Antalya Bay) values are generally higher in our study. However, these comparisons should be used with caution due to differences in fish species and habitats between the studies. Although the skin is a consumed part of the fish, it has not been studied in previous works in this area. This study indicated that concentrations of heavy metals were lower in all of the skin samples than in the muscle samples.

This study showed that *Lagocephalus sceleratus* contain high level of Zn and Pb accumulation in muscle tissues organs when compare to the skin. The high accumulation of heavy metals in puffer fishes is due to its carnivorous feeding nature and bottom habitat. Even though the puffer fishes are non-target species, peoples are started to consume because of their huge quantity or extended by-catch, low market price and high nutrient value. The present study concluded that the long term consumption of these fishes may leads to potential risk to humans in future. So regular monitoring of marine resources is essential to improve the quality of sea-food against contaminants. This is first report of heavy metal analysis on recently silver-cheeked toadfish *Lagocephalus sceleratus* on Antalya Bay region and this finding may lead new insight for further research.

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