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-RESEARCH ARTICLE-

Determination of Heavy Metal Concentrations in Lessepsian Suez Puffer (Lagocephalus suezensis Clark and Gohar, 1953) from North-Eastern Mediterranean

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Abstract

In the present study, the heavy metal concentrations in different organs (skin and muscle tissue) were studied in Lessepsian suez puffer (Lagocephalus suezensis Clark and Gohar, 1953) collected from Iskenderun Bay, North-eastern Mediterranean. Heavy metals (Cd, Cu, Pb, Co, Cr, Fe, Mn, Ni & Zn) were analyzed in different organs for the above mentioned species. The maximum heavy metals concentrations in muscle tissues of the species were Cu 1.750 mg/kg, Fe 18.096 mg/kg, Mn 0.606 mg/kg, Zn 228.571 mg/kg, Cd 2.00 mg/kg, Co 3.571 mg/kg, Cr 0.952 mg/kg, Ni 1.500 mg/kg and Pb 18.095 mg/kg. Zn was detected higher in all the samples followed by Fe, Pb, Co, Cd, Cu, Ni, Cr and Mn. In the present study, heavy metal concentrations were found high in muscle tissues when compared to skin. The Pb, Cd and Zn concentration over the recommended limits was found in *Lagocephalus suezensis*. Further, this is the first report on distribution of heavy metals and proximate compositions of commercialized important edible pufferfishes from Iskenderun Bay, Turkey.

Keywords:

Lessepsian Suez Puffer, *Lagocephalus suezensis*, Heavy metals, Iskenderun Bay

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Introduction

Marine environments naturally contain many metals. Metals like iron, copper, zinc and manganese, are essential metals because of playing an important role in biological systems. However, mercury, lead and cadmium are toxic and non-essential metals. Actually, they are natural trace ingredient in aquatic environment, but industrial, agricultural and mining activities are increased their levels. Therefore, all heavy metals are dangerous to aquatic organisms at some grade of exposure and absorption (Rayment & Barry, 2000).

Heavy metals are known to bio-accumulate in organisms including plants and fish (Mziray & Kimire, 2016; Govers et al., 2014; Yi et al., 2011). They can also biomagnify along the food chain—resulting into myriad ecological damages and health risks to ecosystems and humans respectively (Mziray & Kimire, 2016; Govers et al., 2014; Leung et al., 2014; Weber et al., 2013; Yi et al., 2011; Chi et al., 2007). The risks to humans are most notable when contaminated fishes are consumed beyond the allowed/recommended daily intake levels (Ahmad et al., 2010). Metal bioaccumulations in fish, as in most organisms, is dependent on environmental factors such as pH, temperature, and alkalinity, pollutant type, sampling location, and species-specific physiological and ecological characteristics – such as feeding habits, age, size, sex, habitats, and trophic level (Mziray & Kimire, 2016; Weber et al., 2013).

Metals absorbed by fish are distributed differentially in fish organs (e.g. liver, gills, muscle, and gonads) thereby causing variations of metal accumulations in these organs (Chi et al., 2007). Liver, gills and muscle are mostly used as bio-indicators in metal analysis of fish due to their different roles in metal bioaccumulation process and their potential in human diet (Al-Yousuf et al., 2000; Henry et al., 2004; Agusa et al., 2005; Ploetz et al., 2007; Turan et al., 2009). Muscle is the main edible part of fish by human and thus forms the most preferred tool for the assessment of public health risks associated with metal pollution in fish (Mziray & Kimire, 2016).

Iskenderun Bay is situated on the North Eastern Mediterranean coast of Turkey. This area, in which there are large quantities of midtreated industrial and domestic sewages, has one of the most polluted coastal waters in Turkey. Since the bay is a semiclosed zone, settling time for contaminants is relatively long and this may be one of the reasons for increasing amounts of pollutants. Therefore, the bay region is occasionally monitored for heavy metal contamination in aquatic organisms and the levels measured in tissues of these organisms can reflect the past exposure (Duysak & Uğurlu, 2017; Gökkuş & Türkmen, 2016; Dural et al., 2011; Turan et al., 2009; Türkmen et al, 2008,2009). Some concern arose from previous studies, particularly in terms of safety for human consumption.

The pufferfish that belongs to the *Tetraodontidae* family consists of approximately 120 species, of which seven are found in the Mediterranean Sea. Six are in this area of which four are Lessepsian migrants (Golani et al., 2006; Sabrah et al., 2006). With the opening of the Suez Canal, the puffer fish quickly spread into the Mediterranean and the Sea of Marmara. For the first time these species have spread to a wide area in the seas around Turkey since first being noted in the early 2000s (Akyol et al., 2005; Bilecenoglu et al., 2002). 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 coastlineq (Ben-Soussi et al., 2014). There is very limited information about puffer fish at the present time, including the systematic, zoogeography and growth in this area (Başusta et al., 2013; Kalogirou, 2013; Karunanidhi et al., 2017; Eken et al., 2017; Ayas & Köşker 2018; Manaşırlı et al., 2017).

To the best of our knowledge, present study is the first to investigate bioaccumulation of some heavy metals of *L. suezensis* individuals caught by commercial fisheries from the Iskenderun Bay, North-Eastern Mediterranean. In this study, it was aimed to analyze the bioaccumulations of some heavy metals (Cadmium (Cd), Copper (Cu), Lead (Pb), Silver (Ag), Cobalt (Co), Chromium (Cr), Iron (Fe), Manganese (Mn), Nickel (Ni) and Zinc (Zn)) in muscle and skin tissues of *L. suezensis*.

Materials and Methods

Lessepsian Suez Puffer (*Lagocephalus suezensis*) samples were collected from Iskenderun Bay (latitude 36°35'46; longitude 36°03'18), the North-eastern Mediterranean part of Turkey during the period of Spring 2017 (Figure 1).

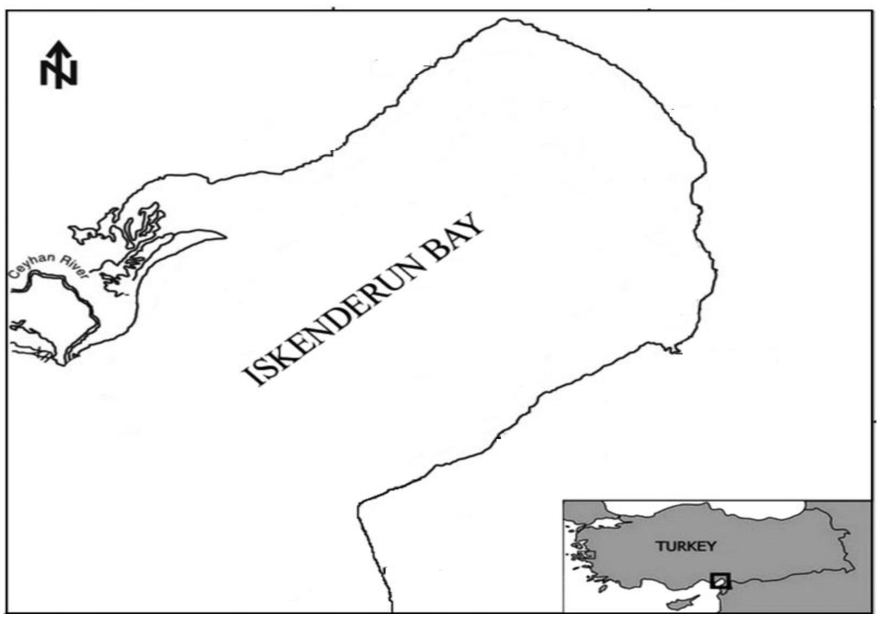

Figure 1. Location of the study area.

In total, ten specimens were collected and transported to the laboratory on ice in an instulated box. The specimens were thawed to room temperature for morphometric study and weighted. Species identification was carried out according to Golani et al. (2006). Standart lenght and weight ranges of the analysed samples were between 12.2-14.5 and 22.36-40.86, respectively. All the specimen were measured to the nearest cm, whereas weights were recorded with the use of electronic balance to the nearest 0.01 g, individually and stored in -20°C for further heavy metal analysis.

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.

Health risk assessment

The metal pollution index (MPI) was employed to examine total heavy metal accumulation in various tissues of different fish species. The MPI was calculated using the equation as follows (Usero et al. 1997): MPI=(CCu×CFe×CMn×CZn×CCd×CCo×CCr× CNi × CPb)1/9 where CCu (mg/kg ww) is the wet weight concentration of Cu in a tissue sample and so on.

Muscle is the main tissue in fish consumption for local inhabitants. Therefore, the health risk assessment was evaluated for the muscle and skin tissue in this study.

Results and Discussion

The mean concentration (±standard deviation) of heavy metals (Cu, Fe, Mn,Zn, Cd, Co, Cr, Ni, Pb) in the muscle and skin tissues of *Lagocephalus suezensis* collected from Iskenderun Bay are summarized in Table 2. Other descriptive results such as minimum and maximum concentrations of heavy metals are also given in Table 1.

Table 1. Mean (±SD) concentrations of heavy metals (μg/g wet weight) in some organs of *Lagocephalus suezensis* collected from Iskenderun Bay.

Metals (μg/g)	**Tissues**			
	Muscle		**Skin**	
	Mean (±SD)	**Min-Max**	**Mean (±SD)**	**Min-Max**
Cu	1.394±0.047	1.667-1.750	0.272± 0.163	0.408-0.735
Fe	14.930±0.736	16.628-18.096	2.217±1.518	3.074-6.092
Mn	0.369±0.180	0.250-0.606	0.024±0.064	0.051-0.172
Zn	122.083±93.298	53.030-228.571	7.994±3.489	5.312-12.206
Cd	1.205±0.430	1.212-2.000	0.208±0.097	0.102-0.294
Co	1.829±0.883	1.818-3.571	0.687±0.183	0.517-0.882
Cr	0.670±0.106	0.750-0.952	0.357±0.313	0.204-0.805
Ni	0.480±0.710	0.238-1.500	0.060±0.011	0.051-0.074
Pb	12.584±4.140	10.758-18.095	1.437 ±1.303	2.908-5.368

As shown, mean concentrations of the heavy metals in the muscle tissue were 1.394 μg/g for Cu, 14.930 μg/g for Fe, 0.369 μg/g for Mn, 122.083 μg/g for Zn, 1.205μg/g for Cd, 1.829μg/g for

Co, 0.670 µg/g for Cr, 0.480 µg/g for Ni, 12.584 µg/g for Pb; mean concentrations of the heavy metal in the skin were 0.272µg/g for Cu, 2.217 µg/g for Fe, 0.024 µg/g for Mn, 7.994 µg/g for Zn, 0.208 µg/g for Cd, 0.687 µg/g for Co, 0.357µg/g for Cr, 0.060 µg/g for Ni, 1.437 µg/g for Pb. Among the muscle tissue samples, Zn was detected as highest followed by Fe, Pb, Co, Cu, Cd, Cr, Ni and Mn (Fig 2)

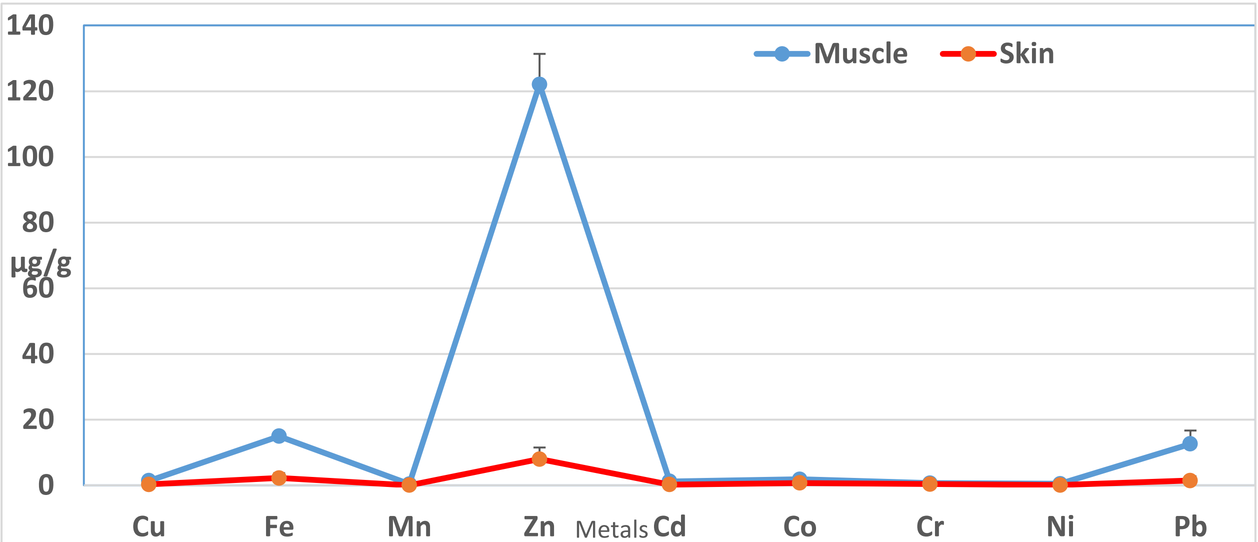

Figure 2. Comparison of the mean Cu, Fe, Mn, Zn, Cd. Co, Cr, Ni and Pb concentrations between muscle and skin of *L. Suezensis.*

The repartition of the trace metals analysed, between the two tissues of *Lagocephalus suezensis* considered (muscle and skin), shows that the trace metals are preferentially accumulated in muscle. This finding is consistent with the work done by Eken et al.(2017) in the Antalya Bay.

Considering the MPI in different tissues, the distribution of the analyzed heavy metals was in the ascending order of Skin < Muscle for all his species. In a study conducted by Jia et al. (2017), MPI values were calculated for 3 freshwater species and MPI values for all species were lower than those for our study. The feeding behavior posed great influence on the heavy metal distribution in different fish species. Heavy metals were transferred and bioaccumulated from primary producers and low trophic levels to higher trophic levels through the food chain (Tao et al. 2012). Referring to studies in general, the variations of heavy metal concentrations in fish species were affected by the habitat site, while the demersal species accumulated more contents of heavy metals than the midwater species (Jia et.al 2017).

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. Nonetheless, Cd concentrations in the muscle tissues were at the limit levels set by law.

Lead is a non-essential element which can cause serious damage to human health at trace level (García-Lestón et al. 2010). The significant variations were observed between the muscle and skin, of *Lagocephalus suezensis* according to the result of analysis. The accumulation pattern of Pb in fish tissues was in the descending order of muscle > skin . It was demonstrated that the gills of fathead minnows (*Pimephales promelas*) could accumulate Pb rapidly when exposed to Pb-contaminated water (Grosell et al. 2006). In our work, high Pb levels can also result in sudden exposure to lead containing wastewater.

Compared to several other metal ions with similar chemical properties, zinc is relatively harmless. Zinc is an essential micronutrient for both animals and humans due to its biological role in transcription factors. Whereas intoxication by excessive exposure is rare, deficiency of Zn can lead to various chronic diseases such as malabsorption, growth retardation, immunological abnormalities, chronic liver and renal diseases, etc. The Zn concentrations in muscles were significantly different from skin. That mean that Zn preferred to be accumulated in the muscle, rather than in the skin of fish.

Cadmium is not a part of natural biochemical processes and is extremely hazardous due to its organ toxicity and carcinogenicity to human beings (Robards and Worsfold, 1991). Significant variations of Cd concentrations were observed between tissues of *Lagocephalus suezensis* (Table 2). The Cd level in muscle was higher than that in the skin for this fish species. This is due to the existence of metallothionein proteins which could bind certain heavy metals such as Cd and Cu for detoxification (Ploetz et al. 2007). The Cd contamination in fish tissues might be derived from anthropogenic sources such as mining and smelting activities.

Compared with other studies, the results in the present study were higher than the previously reported values of Pb, Zn and Cd in fish species from different and also same area (Table 2) (Yılmaz, 2003; Türkmen et al., 2005; Çiçek et al., 2008; Yılmaz et.al., 2010; Dural et.al. 2011; Manaşırlı et al., 2015; Nurjanah et al., 2015; Karunanidhi et. al 2017; Eken Dural et. Al. 2017; Kaleshkumar et al., 2017; Ayas and Kösker, 2018).

Table 2. Heavy metal levels in fish muscles from different locations of 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)
Lagochepalus lunaris	0,43±0,01	0,02±0,	0,51±0,01	73,63±0,60	-	-	-	-	6,85±0,01	Indonesia, West Java	Nurjanah et al. (2015)
Sparus aurata	6.237±1.45	1.255±0.79	3.830 ± 1.445	14.349 ± 2.427	0.573 ± 0.281			762.89 ± 98.27	17.310 ± 3.472	Iskenderun Bay	Dural et.al. (2011)
Mugil cephalus *Trachurus mediterraneus*	1.45 1.29		7.45 1.03	38.23 19.55	1.46 1.28	-	-	1.22 0.94	70.28 41.84	Iskenderun Bay	Yılmaz (2003)
Chame pacifica *Ostrea stentina*	46.93 64.70	7.53 4.27	62.34 6. 21	419.8 1002	3.36 9.17	33.80 8.67	5.79 16.36	22.87 6.92	82.02 270.6	Iskenderun Bay	Türkmen et al. (2005)
Trigla lucerna *Lophius budegassa* *Solea lascaris*	4.19 6.24 5.64	0.01 0.02 0.04	0.14 0.17 0.39	28.2 20.8 27.5	0.65 0.32 0.70		1.77 1.04 1.64	0.72 0.22 1.01	45.5 37.6 76.7	Iskenderun Bay	Yılmaz et al. (2010)
Mullus barbatus, *Pagellus erythrinus* *S. undosquamis*	3.05±2.47 2.46±2.50 1.78±2.37			32.36±21.04 24.10±20.73 20.50±15.62					64.74±41.71 63.82±46.63 37.45±19.21	Iskenderun Bay	Çiçek et al. (2008)
S. undosquamis, *P. erythrinus and* *M. barbatus*	1.50±0.08 1.92±0.85 2.69±0.09	-	-	29.16±3.24 30.28±3.12 33.17±0.86	-	-	-	-	90.22±3.17 79.96±6.32 75.66±5.32	Iskenderun Bay	Manaşırlı et al. (2015)
A. hispidus *T. oblongus* *L. guentheri*	0.42 –6.31	0.01–0.79	5.80–19.87	6.75–65.08	-	-	-	-	-	Iskenderun Bay	Karunanidhi et al. (2017)
Lagocephalus sceleratus	-	0.51±0.06	0.66	-	0.99	-	-	-	-	Iskenderun Bay	Ayas and Köşker (2018)
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	Eken et al. (2017)
L. suezensis	1.394±0.047	1.205±0.43	12.584±4.140	122.083±9.29	0.670±0.106	1.829±0.883	0.369±0.18	0.480±0.71	14.930±0.736	Iskenderun Bay	This research

This study showed that *Lagocephalus suezensis* contain high level of Zn and Pb accumulation and Cd values were also at the limit 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. The present study concluded that the long term consumption of these fishes may leads to potential risk to humans in future.

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