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- RESEARCH ARTICLE -

The effects of age and individual size on metal accumulation of *Lagocephalus sceleratus* (Gmelin, 1789) from Mersin Bay, Turkey

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Abstract
In this study, heavy metal levels in muscle and liver tissues of different age groups of Lagocephalus sceleratus caught in the Mersin Bay were investigated. Six different age groups were determined among fishes caught using commercial trawlers from the Mersin Bay. The lengths of fish were measured between 17 cm and 68 cm, the weights were measured between 265 g and 3955 g. Cadmium (Cd), Lead (Pb), Chromium (Cr) and Arsenic (As) levels were investigated using ICP-MS. The heavy metal concentrations in the liver and muscle tissues in L. sceleratus were found to be As>Cr>Cd>Pb and As>Cr>Pb>Cd, respectively. It was observed that with the increased ages of pufferfish, the levels of heavy metals determined in both muscle and liver tissues were increased.

Keywords:
Lagocephalus sceleratus, Pufferfish, Heavy Metals, Age, Metal Accumulation

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Introduction

Environmental pollution is one of the major problems of our global society. Metal pollution is an important element of environmental pollution. The increasing use of metals throughout the world over the past century has caused people and the environment to be commonly exposed to metal pollution (Nordberg et al., 2015). Under normal conditions metals, like many other natural elements, are held in balance by biological and geological cycles. However, human activities such as industrial activities, mining, etc., have an impact on the natural life and cause disruption in the

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cycling of natural life. Although anthropogenic activities cause direct mobilization and emission of metals in the environment, in some cases they may indirectly lead to situations where metals are activated. For example, some metals can be found in nature; and they can cause metal pollution due to mixing with groundwater and marine sediment (Bjerregaard et al., 2015). In particular, heavy metal contamination poses a significant risk to human health and nutrition, but negative effects can also be observed on the natural balance of ecosystems that are increasing by metal accumulation.

Heavy metals are elements that are considered to be toxic for a long time and that are harmful to human health or ecosystems if limit values are exceeded. Heavy metal contamination is a problem that has had a very negative impact on human nutrition and human health (Kulcu et al., 2014). Although environmental elements such as water, air and soil can be influenced by exposure to metals, people are most exposed to metals with food. Epidemiological studies have confirmed the carcinogenic effects of heavy metals such as nickel, chromium, arsenic, cadmium and beryllium on animals and humans (Nordberg et al, 2015). Despite the intense industrial activity in developed countries, government controls are being tightened and efforts are being made to reduce the impact of metal contamination on humans. However, as a general belief, seas are still seen as a compensatory system of pollution (Bjerregaard et al., 2015, Nordberg et al., 2015). This situation has led to dramatic levels of metal contamination, especially in marine ecosystems. Marine organisms consumed by humans can act as a source of nutrients, but they are also effective in spreading the effect of pollution. For this reason, the heavy metal levels of living things collected from natural habitats are an important indicator of the potential risks for human health and heavy metal contamination in living areas.

Pufferfish can be considered as indicator species because they are located on the upper trophic level of the food chain. Detection of heavy metal levels using indicator species is necessary not only for human health but also for the continuity of ecosystems. That’s why, the toxic metal levels in fish species being above acceptable limits is an important indicator for the pollution level of the ecosystem which marine species live in (Authman et al., 2015; Cresson et al., 2015). Determination of the metal levels of individuals in different age groups of indicator species will help to better understand the pollution. Because the size of the individual and the age increase, the accumulation in the individual can be excessive. One of the species that can be used as an indicator species in determining the metal pollution of the ecosystem that lives in the upper trophic levels of the food chain is the *L. sceleratus*.

L. sceleratus is popular fish in recent years in all Mediterranean countries, which often find themselves in the written and visual media. This popularity is due to the toxicity they contain. Pufferfish contain tetrodotoxin (TTX), the most powerful marine toxin known in the world (Köşker et al., 2015).

The natural habitat of *L. sceleratus* is the Indian Ocean and the Pacific Ocean, and it is the most common pufferfish species on the Turkish coast nowadays (Bilecenoğlu, 2010). This species is reported to be found at depths of 25-200 m (Özbek et al., 2017). This fish species can reach a maximum length of 110 cm and weight of 7 kg (Nader et al., 2012). The first maturation length of the *L. sceleratus* is 40 cm and the breeding season is between spring and summer (Aydın, 2011; Boustany et al., 2015). This species on the upper trophic level of the food chain is carnivorus and they are mostly fed with crustacean and mollusc species (Aydın, 2011; Kalogirou et al., 2012).

The individuals living in the Mediterranean of *L. sceleratus* include toxins known as tetrodotoxin (TTX) (Katikou et al., 2009, Kosker et al., 2016). Besides its toxic dangers, *L. sceleratus* are also notable for their adaptability, and they are among the most dangerous invasive species in the Mediterranean. This species has reached the coasts of Italy, Spain and Algeria from the Eastern Mediterranean within a little over a decade (Izquierdo-Muñoz & Izquierdo-Gomez, 2014; Azzurro et al., 2014; Kara et al., 2015). One of the areas where this animal lives intensively on the Turkish coast is the Bay of Mersin which is located in the Northeastern Mediterranean.

There are various factors affecting the pollution in the marine ecosystem in the Mersin Bay. The researchers have reported the wastes of artificial fertilizers and pesticides, domestic wastes, chrome, plastics, fertilizer, glass industrial facilities and intensive marine traffic originating from the port as the main pollution sources in the Mersin Bay (Kalay et al., 2004). The investigation of metal pollution in this area is important for both continuity of ecosystem and possible health risks against people living in this region. *L. sceleratus* has been selected as an indicator animal to determine the metal pollution of the Mersin Bay. In this study, heavy metal levels in muscle and liver tissues of individuals of different age groups of *L. sceleratus* were investigated.

Materials and methods

The *L. sceleratus* specimen used in the study were caught with bottom trawls from the Mersin Bay in Northeastern Mediterranean (Figure 1) in autumn season in 2017.

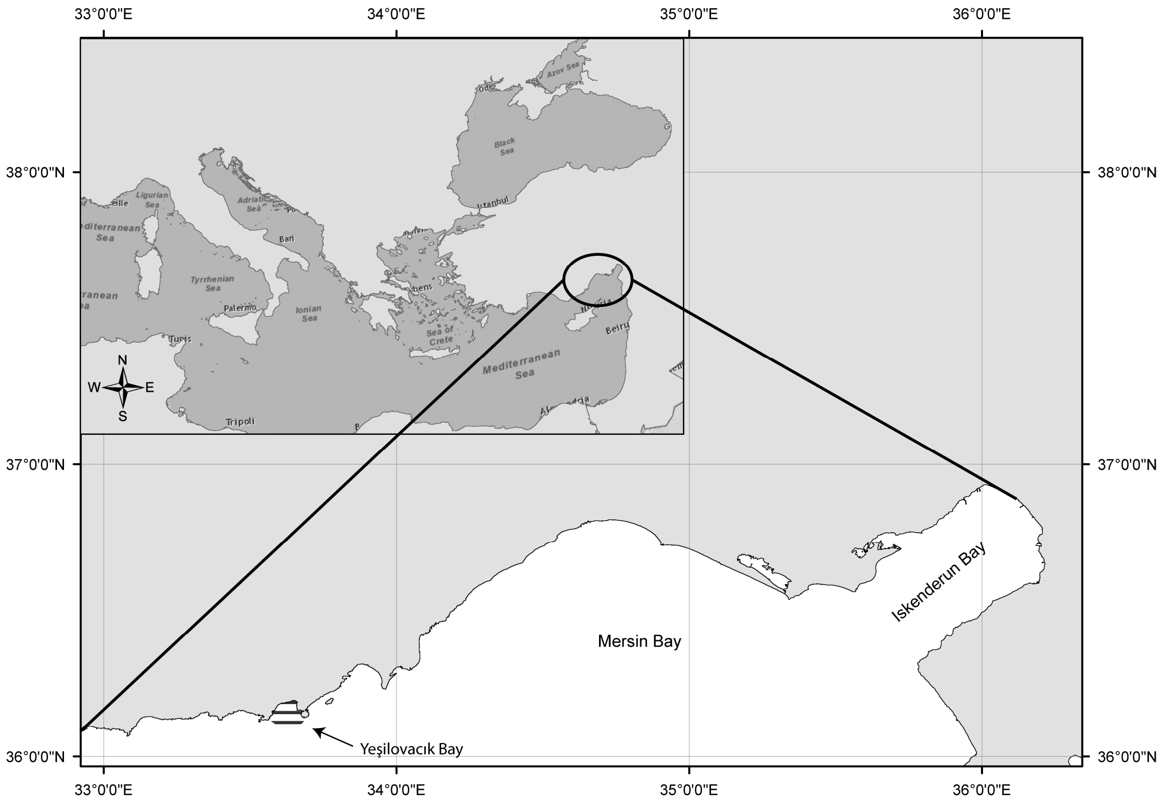

Figure 1. Sampling location map. (The shaded region (Yeşilovacık Bay) is the sampling area)

Age of *L. sceleratus* specimens were calculated by reading of the vertebrae. Before otolith reading, third and fourth vertebrae was carefully removed and then were cleaned with 4% NAOH and stayed in 70% ethyl alcohol, and then placed in boiling distilled water for 5-6 minutes. Every opaque ring on the vertebrae as an annuli were determined with using a binocular microscope (Farrag et al., 2015).

The weight and size of all age groups (1+ - 6+) were measured and given in Table 1. While the lengths of fish were measured between 17 cm and 68 cm, the weights were measured between 265 g and 3955 g (Table 1).

Table 1. Length and weight at age groups of *L. sceleratus*

Ages	N	Mean TL (cm)	Range (cm)	Mean Weight (cm)	Range (g)
0+	0	0	0	0	0
1+	7	19.86	17-24	284.57	265-351
2+	5	33.20	29-37	331.80	285-401
3+	21	44.38	40-51	1004.90	867-1421
4+	14	53.36	52-59	1389.86	1353-1451
5+	3	61	59-62	2135.33	1654-2753
6+	2	67.5	67-68	3923.00	3891-3955

Metal analysis

The samples (0.1 g dry weight) used for metal analysis were dried at 105°C to reach constant weights, and then concentrated nitric acid (2 mL, Merck, Darmstadt, Germany) and percholoric acid (1 mL, Merck, Darmstadt, Germany) were added to the samples, and they were put on a hot plate set to 150°C until all tissues were dissolved (Canli & Atli, 2003).

Inductively coupled plasma mass spectrometer (ICP-MS, Agilent, 7500ce Model, Japan) was used to determine metals. ICP-MS operating conditions were the following: radio frequency (RF) (W),1500; plasma gas flow rate (L min^{-1}),15; auxiliary gas flow rate (L min^{-1}), 1; carrier gas flow rate (L min^{-1}), 1.1; spray chamber T (°C), 2; sample depth (mm), 8,6; sample introduction flow rate (mL min^{-1}), 1; nebuliser pump (rps), 0.1; extract lens (V), 1.5. All digested samples were analysed three times for each metals. All chemicals and standard solutions used in the study were obtained from Merck and were of analytical grade. The levels of potential toxic metal (Cd, Pb, Cr, As) in samples were detected as μg metal g^{-1} dry weight. High Purity Multi Standard (Charleston, SC 29423) was used for determination of the metal analyses. Standard solutions for calibration curves were prepared by dilutions of the potential toxic metals. Solutions have prepared for the toxic metals had a content of lead, cadmium, arsenic and chromium in the range of 1-50 ppb (0.001 to 0.050 mg/L).

Statistical analysis

Prior to the analyses, all data were checked for outliers and homogeneity of variance was also tested. Statistical analysis of data was carried out with the IBM SPSS STATISTICS 22 statistical program. ANOVA (Analysis of Variance) was used to evaluate the effect of age on the metals levels.

Results

Heavy metal levels (Cd, Pb, As and Cr) in the liver and muscle tissues of pufferfish are shown in Table 2 and Figure 2.

Table 2. Levels of some heavy metals in different age groups of *L. sceleratus* (µg g^{-1})

Tissues	Cd $\bar{X} \pm S_{\bar{X}}$	Pb $\bar{X} \pm S_{\bar{X}}$	Cr $\bar{X} \pm S_{\bar{X}}$	As $\bar{X} \pm S_{\bar{X}}$	Ages
Muscle	0.51±0.06^{a}	0.66±0.10^{a}	0.99±0.11^{a}	32.78±0.67^{a}	1+
	0.67±0.11^{ab}	0.61±0.16^{a}	0.95±0.17^{a}	34.01±0.56^{a}	2+
	0.71±0.05^{ab}	0.72±0.17^{a}	1.33±0.10^{ab}	33.27±1.16^{a}	3+
	0.94±0.07^{bcd}	0.94±0.05^{ab}	1.61±0.16^{abc}	44.01±0.56^{b}	4+
	0.96±0.07^{bcd}	0.96±0.07^{ab}	1.39±0.28^{ab}	44.96±1.51^{b}	5+
	1.10±0.04^{cd}	1.19±0.16^{bc}	1.95±0.06^{bcd}	46.23±1.67^{b}	6+
Liver	0.84±0.06^{bc}	0.84±0.07^{ab}	1.78±0.11^{bcd}	34.80±0.80^{a}	1+
	0.89±0.12^{bc}	0.94±0.06^{ab}	1.89±0.12^{bcd}	34.50±0.52^{a}	2+
	0.94±0.06^{bcd}	1.00±0.11^{ab}	1.72±0.16^{bc}	44.27±1.51^{b}	3+
	0.99±0.02^{bcd}	1.48±0.04^{c}	2.44±0.43^{d}	46.94±0.95^{b}	4+
	1.22±0.23^{ed}	1.50±0.28^{c}	1.50±0.06^{abc}	59.84±4.28^{c}	5+
	1.51±0.07^{e}	1.50±0.06^{c}	2.12±0.34^{cd}	63.90±2.89^{c}	6+

Values in same column with different letters (a-e) are significantly different ($P<0.05$). $\bar{X} \pm S_{X}$: Average±Standard eror

The heavy metal concentrations of liver and muscle tissues in *L. sceleratus* was found to be As>Cr>Cd>Pb and As>Cr>Pb>Cd, in order.

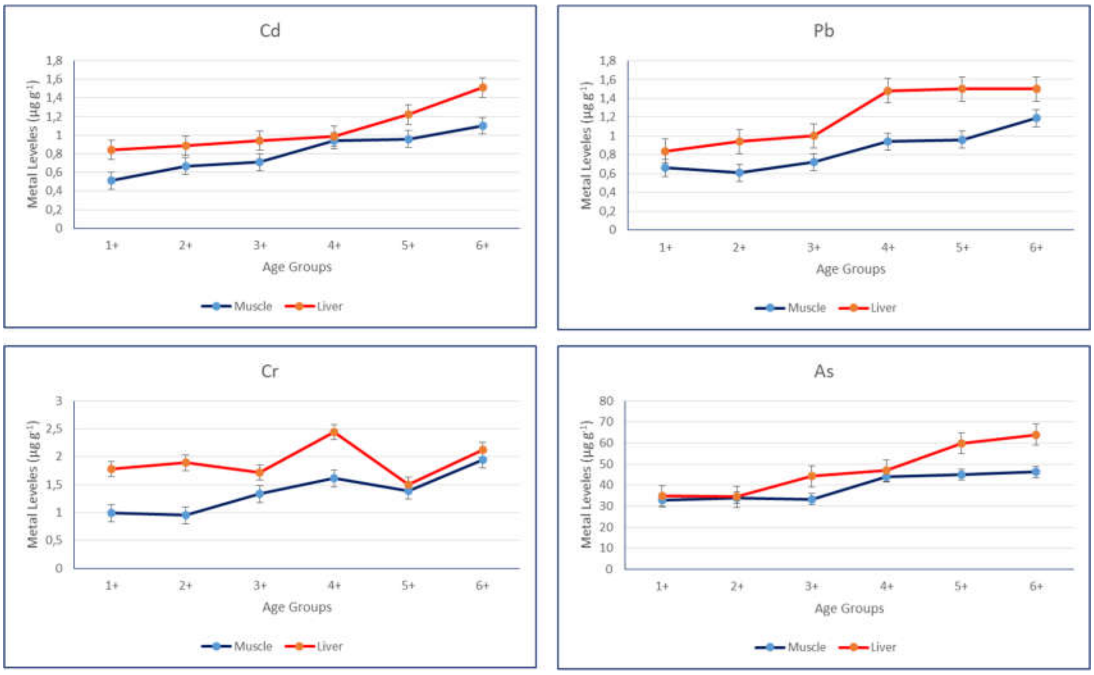

Figure 2. The age-related changes of the metals in muscle and liver tissues of *L. sceleratus*

Cd levels were found to range between 0.51 µg g^{-1}dw and 1.10 µg g^{-1}dw for muscle tissue and between 0.84 µg g^{-1}dw and 1.51 µg g^{-1}dw for the liver in all age groups (Table 2, Figure 2). It was determined that 1+, 2+, 3+ and 6+ age groups showed statistical differences in terms of Cd values in muscle tissues ($P<0.05$). In liver samples, 1+, 2+, 3+ and 4+ age groups were not statistically different ($P> 0.05$) while 6+ age group was statistically different from all other age groups ($P<0.05$).

Pb levels were found to be in the range of 0.61 µg g^{-1}dw to 1.19 µg g^{-1}dw for muscle tissue and 0.84 µg g^{-1}dw to 1.50 µg g^{-1}dw for the liver in all age groups (Table 2, Figure 2). There was no statistical difference between 1+, 2+, 3+, 4+ and 5+ age groups in muscle tissue in terms of Pb levels ($P> 0.05$), while there were statistical differences between 6+ age group and 1+, 2+, 3+ age groups ($P<0.05$). In the liver samples, 1+, 2+, 3+ age groups did not show any statistical differences ($p> 0.05$); similarly, age groups of 4+, 5+, 6+ did not show any statistical differences ($P> 0.05$).

Cr levels were found to range between 0.99 µg g^{-1}dw and 1.95 µg g^{-1}dw for muscle tissue and between 1.50 µg g^{-1}dw and 2.44 µg g^{-1}dw for the liver in all age groups (Table1). There was no statistically significant difference between the muscle tissues of the 1+, 2+, 3+, 4+ and 5+ age groups in terms of Cr values ($P> 0.05$), however statistical differences were observed between 6+ age group and the other age groups ($P<0.05$). In liver samples, 1+, 2+, 3+ and 5+ age groups did not show any statistical differences ($P>0.05$), however statistical differences were observed between the 4+ age group and the other age groups ($P<0.05$).

As levels were found to be in the range of 32.78 µg g^{-1}dw to 46.23 µg g^{-1}dw for muscle tissue and 34.50 µg g^{-1}dw to 63.90 µg g^{-1}dw for liver in all age groups (Table1). In terms of As values, the muscle tissues of the 1+, 2+, 3+ age groups did not show any statistical differences ($P>0.05$), similarly, 4+, 5+, 6+ age groups did not show statistical differences ($p>0.05$). In liver samples, 1+, 2+ age groups did not show any statistical difference ($P>0.05$), similarly, 3+, 4+ age groups did not show statistical differences ($P>0.05$). And also, 5+ and 6+ age groups did not show statistical differences ($P>0.05$).

Discussion

Cd levels were found to range between 0.51 µg g^{-1}dw and 1.51 µg g^{-1}dw in all age groups (Table 2, Figure 2). No studies have been found in the literature regarding the Cd levels of *L. sceleratus*. Turan et al., (2009) reported Cd levels of different fish species caught in the Iskenderun Bay between 0.18 and 1.69 (µg g^{-1} dw). In a similar study, Turkmen et al. (2005) reported that Cd levels ranged from 0.02 to 4.16 (µg g^{-1} dw) in the study conducted in the Iskenderun Bay with different fish species. These findings support our results.

While the age increases, the increase in heavy metal deposits in the tissues is considered normal (Figure 2), indicating that there is a regular Cd entry into the region being investigated. Cd contamination poses risks to human health as well as ecological pollution. The limits for Cd, which can cause serious health problems for humans if accumulated beyond limit levels, is 0.05 (µg g^{-1} ww) for European Commission (EC, 2006) and Turkish Food Codex (TFC, 2011). The amount of Cd can also be increased by industrial activities and agricultural activities. Compounds containing Cd and Cd are used as corrosion inhibitors in batteries, electronic components, nuclear power plants and steelmaking. Cd levels are thought to have originated from human-induced activities in the Mersin Bay.

In the literature, there has been no study of Pb levels of *L. sceleratus*; however, Thiyagarajan et al., (2012) reported that the levels of Pb were below the limits of *L. lunaris* found in the same genus with *L. sceleratus*. Turan et al., (2009) reported Pb levels of different fish species caught in the Iskenderun Bay between 0.06 and 0.56 (µg g-1 dw). In the present study; Pb levels were found to be in the range of 0.61 µg g^{-1}dw to 1.50 µg g^{-1}dw in all age groups. The limit value determined by FAO (1983) and European Union Commission (EC, 2001) for Pb is 0.3 (µg g^{-1} ww) and the limit value determined by Turkish Food Codex (2011) is 0.2 µg g^{-1} ww. This is due to lead pollution in the area where these fish are caught. Although Pb is present in nature, it is generally increased by people via industrial activities such as mining, casting industry, battery production (Alexander et al., 2010). Besides, intensive maritime activities in the Mersin Bay are thought to be effective in increasing the level of Pb pollution.

No studies have been found on the Cr-level in the pufferfish. However, Turkmen et al., (2005) reported Cr levels in different fish species caught in the Iskenderun Bay between 0.04 and 5.43 (µg g^{-1} dw). In a similar study, Turan et al., (2009) reported Cr levels of different fish species caught in the Iskenderun Bay between 0.11 and 1.89 (µg g-1 dw). In the present study, Cr levels were found to range between 0.99 µg g^{-1} dw and 2.44 µg g^{-1}dw. These findings support our results.

There is no regulation in Turkish Food Codex (TFC, 2011) or European Commission (EC, 2006) for As limit values. Humans are exposed to many different forms of arsenic, including inorganic (arsenite, arsenate) and organic (arsenobetaine, arsenosugars and arsenolipid) arsenic compounds (Navas-Acien et al., 2011). In nature, a significant proportion of this metal is present in organic form and is not as toxic as inorganic forms (Castro-González & Méndez-Armenta, 2008). For this reason, it is difficult to evaluate the potential health risk associated with arsenic concentration in fish. Besides, the origin of this metal is the earth's crust. The detection of such high levels of As in *L. sceleratus* may be due to the geology of the region. However, there is a risk of taking up a potentially toxic metal like As.

Conclusion

There was a statistical difference between the different age groups for the As, Cr, Cd, and Pb levels of both tissues. Besides, the levels of the potential toxic metals in the liver tissue were higher than the levels of the muscle tissues and in general displayed variation with age as well. This situation can be explained by the liver being more active in the detoxification metabolism.

The metal levels of the environment is well reflected by this species. That's why, we recommend *L. sceleratus* can be used as a biological indicator.

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References

Alexander, J., Benford, D., Boobis, D., Ceccatelli, S., Cravedi, J.P., Di Domenico, A. & van Leeuwen, R. (2010). Scientific opinion on lead in food. *The European Food Safety Authority Journal,* 8, 1-147.

Authman, M. M., Zaki, M. S., Khallaf, E. A. & Abbas, H.H. (2015). Use of fish as bio-indicator of the effects of heavy metals pollution. *Journal of Aquaculture Research & Development,* 6(4), 1.

Ayas, D., Köşker, A. R., Durmuş, M. & Bakan, M. (2016). Determination of Seasonal Changes on Some Heavy Metal (Cd, Pb, Cr) Levels of Shrimp and Prawn Species from North-Eastern Mediterranean Sea, Gulf of Mersin, Turkey. *Journal of Aquaculture Engineering and Fisheries Research,* 2, 42-49.

Aydın, M. (2011). Growth, Reproduction and Diet of Pufferfish (*Lagocephalus sceleratus* Gmelin, 1789) From Turkey's Mediterranean Sea Coast. *Turkish Journal of Fisheries and Aquatic Sciences,* 11, 589-596.

Azzurro, E., Castriota, L., Falautano, M., Giardina, F. & Andaloro, F. (2014). The Silver-Cheeked Toadfish *Lagocephalus sceleratus* (Gmelin, 1789) Reaches Italian Waters. *Journal of Applied Ichthyology,* 30(5): 1050-1052.

Bilecenoğlu, M. (2010). Alien Marine Fishes of Turkey - An updated review. In: Golani D, Appelbaum-Golani B (eds) Fish Invasions of the Mediterranean Sea: Change and Renewal. Pensoft Publishers, Sofia-Moscow, p.189-217.

Bjerregaard, P., Andersen, C.B. & Andersen, O. (2015). Ecotoxicology of metals—sources, transport, and effects on the ecosystem. In Handbook on the Toxicology of Metals (Fourth Edition) (pp. 425-459).

Boustany, L., El Indary, S. & Nader, M. (2015). Biological Characteristics of the Lessepsian Pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) off Lebanon. *Cahiers de Biologie Marine,* 56, 137-142.

Canli, M. & Atli, G. (2003). The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environmental Pollution,* 121(1): 129-136.

Castro-González, M.I. & Méndez-Armenta, M. (2008). Heavy metals: Implications associated to fish consumption. *Environmental toxicology and pharmacology,* 26(3): 263-271.

Cresson, P., Bouchoucha, M., Miralles, F., Elleboode, R., Mahe, K., Marusczak, N. & Cossa, D. (2015). Are red mullet efficient as bio-indicators of mercury contamination? A case study from the French Mediterranean. *Marine pollution bulletin,* 91(1): 191-199.

EC (European Commission) (2006). European Union Commission Regulation (1881/2006) Official Journal of the European Communities, Maximum levels for certain contaminants in foodstuffs. Official J Eur. Commun L364/5

FAO (Food and Agriculture Organization) (1983). Compilation of legal limits for hazardous substances in fish and fishery products. Food and Agriculture Organization of the United Nations, 464: 5-10.

Farrag, M.M., Elhaweet, A.A.K. & Akel Ekha, M.M. (2015). Stock Status of Puffer Fish *Lagocephalus sceleratus* (Gmelin, 1789) Along the Egyptian Coast, Eastern Mediterranean Sea. *American Journal of Life Sciences,* Special Issue: New Horizons in Basic and Applied Zoological Research, 3(6-1): 83-93.

Izquierdo-Muñoz, A. & Izquierdo-Gomez, D. (2014). First Record of *Lagocephalus sceleratus* (Gmelin, 1789) (Actinopterygii, Tetraodontidae) on the Mediterranean Spanish Coast. *Mediterranean Marine Science,* 15(3): 675-695.

Kalay, M., Koyuncu, C.E. & Dönmez, A.E. (2004). Comparison of Cd levels in the muscle and liver tissues of *Mullus barbatus* and *Sparus aurata* caught from the Mersin Gulf. *Ekoloji Dergisi,* 13(52): 23-27.

Kalogirou, S., Wennhage, H. & Pihl, L. (2012). Non-Indigenous Species in Mediterranean Fish Assemblages: Contrasting Feeding Guilds of *Posidonia oceanica* Meadows and Sandy Habitats. *Estuarine, Coastal and Shelf Science,* 96: 209-218.

Kara, M.H., Lamine, E.B. & Francour, P. (2015). Range Expansion of an Invasive Pufferfish, *Lagocephalus sceleratus* (Actinopterygii: Tetraodontiformes: Tetraodontidae), to the South-Western Mediterranean. *Acta Ichthyologica et Piscatoria,* 45(1): 103.

Katikou, P., Georgantelis, D., Sinouris, N., Petsi, A. & Fotaras, T. (2009). First Report on Toxicity Assessment of the Lessepsian Migrant Pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) from European Waters (Aegean Sea, Greece). *Toxicon,* 54(1): 50-55.

Köşker, A. R., Özoğul, F., Ayas, D., Durmuş, M. & Uçar, Y. (2015). The new toxin of Mediterranean: Tetrodotoxin. *Ege Journal of Fisheries and Aquatic Sciences,* 32(1): 15-24.

Kosker, A. R., Özogul, F., Durmus, M., Ucar, Y., Ayas, D., Regenstein, J. M. & Özogul, Y. (2016). Tetrodotoxin levels in pufferfish (*Lagocephalus sceleratus*) caught in the Northeastern Mediterranean Sea. *Food chemistry,* 210: 332-337.

Kulcu A.M., Ayas, D. & Kosker A.R. (2014). The investigation of metal and mineral levels of some marine species from the Northeastern Mediterranean Sea. *Journal of Marine Biology & Oceanography,* 3(2): 1-6.

Nader, M.R., Indary, S. & Boustany, L.E. (2012). The Puffer Fish *Lagocephalus sceleratus* (Gmelin, 1789) in the Eastern Mediterranean. EastMed Technical Documents 2012, GCP/INT/041/EC–GRE–ITA; FAO: Rome, Italy.

Navas-Acien, A., Francesconi, K.A., Silbergeld, E.K. & Guallar, E. (2011). Seafood Intake and Urine Concentrations of Total Arsenic, Dimethylarsinate and Arsenobetaine in the US Population. *Environmental research*, 111(1): 110-118.

Nordberg, G. F., Fowler, B. A. & Nordberg, M. (Eds.) (2015). Handbook on the Toxicology of Metals. Academic Press.

Özbek, E.Ö., Çardak, M. & Kebapçıoğlu, T. (2017). Spatio-Temporal Patterns of Abundance, Biomass and Length of the Silver-Cheeked Toadfish *Lagocephalus sceleratus* in the Gulf of Antalya, Turkey (Eastern Mediterranean Sea). *Turkish Journal of Fisheries and Aquatic Sciences*, 17: 725-733.

TFC (Turkish Food Codex) (2011). Türk Gıda Kodeksi Bulaşanlar Yönetmeliği. Accessed Sep. 15, 2016. http://www.mevzuat.gov.tr/MevzuatMetin/yonetmelik/7.5.15692-ek.doc (In Turkish).

Thiyagarajan, D., Dhaneesh, K.V., Kumar, T.T.A., Kumaresan, S. & Balasubramanian, T. (2012). Metals in Fish along the Southeast Coast of India. Bulletin of Environmental Contamination and Toxicology, 88(4): 582-588.

Turan, C., Dural, M., Oksuz, A. & Ozturk, B. (2009). Levels of heavy metals in some commercial fish species captured from the Black Sea and Mediterranean coast of Turkey. *Bulletin of Environmental Contamination and Toxicology,* 82(5): 601-604.

Turkmen, A., Turkmen, M. & Tepe Y. (2005). Heavy metals in three commercially valuable fish species from Iskenderun Bay, Northern East Mediterranean Sea, Turkey. *Food Chemistry,* 91: 167-172.