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Elemental composition of pufferfish species from Northeastern Mediterranean Sea

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Abstract This study aimed at comparing the elemental levels of muscle and liver tissues of four pufferfish (*Lagocephalus sceleratus*, *Lagocephalus spadiceus*, *Lagocephalus suezensis*, *and Torquigener flavimaculosus*), which are common in the Northeastern Mediterranean Sea (Mersin Bay), in terms of sex and seasonal variables. Na, Mg, P, K, and Ca levels in the muscle tissues of pufferfish range from 1848.4 to 16495.7, 824.4 to 2684.5, 6936.2 to 20486.6, 13019.3 to 36165.5, and 276.4 to 5566.5 μg g^{-1}, respectively. Na, Mg, P, K, and Ca levels in the liver tissues of pufferfish range from 531.8 to 6136.1, 63.8 to 899.1, 773.1 to 6677.5, 1151.4 to 10165.4, and 47.4 to 1607.7 μg g^{-1}, respectively. The levels of macroelements in the muscle and liver tissues can be represented as K > P ≥ Na > Ca ≥ Mg and K ≥ P ≥ Na > Ca ≥ Mg, respectively. Pufferfish muscle tissue has more abundant macroelement levels than liver tissue. Fe, Cu, and Zn levels in pufferfish muscle tissues range from 21.53 to 219.42, 0.73 to 6.54, and 25.38 to 100.47 μg g^{-1}, respectively. Fe, Cu, and Zn levels in pufferfish liver tissues range from 157.8 to 1368.6, 0.86 to 11.23, and 23.17 to 507.75 μg g^{-1}, respectively. Levels of trace elements in the muscle and liver tissues can be represented as Zn ≥ Fe > Cu and Fe ≥ Zn > Cu, respectively. The pufferfish liver tissue is richer than muscle tissue in terms of trace element levels. Cd, Pb, As, and Cr levels in pufferfish muscle tissues range from 0.66 to 6.30, 2.17 to 20.76, 41.90 to 258.37, and 0.34 to 3.70 μg g^{-1}, respectively. Cd, Pb, As, and Cr levels in the liver tissues of pufferfish range from 0.22 to 2.34, 0.17 to 2.27, 9.35 to 61.93, and 0.14 to 2.10 μg g^{-1}, respectively. Levels of metal in the muscle and liver tissues can be represented as As > Pb > Cd ≥ Cr and As > Cd ≥ Pb ≥ Cr, respectively. Pufferfish muscle tissue has a higher association with liver tissue due to metal level accumulation. All pufferfish are contaminated by Cd, Pb, As, Cr, Fe, and Zn metals.

Keywords Pufferfish · Northeastern Mediterranean · Macroelements · Trace elements · Heavy metal

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Introduction

While some Lessepsian species that settle in the Mediterranean Sea are economically important, others are called invasive, harmful species (Zenetos et al. 2012; Kosker et al. 2015). Pufferfish stand out among other alien species due to their deleterious effects on economic fish species, fisheries, and community health (Streftaris and Zenetos 2006; Eisenman et al. 2008). Among the species belonging to the *Tetraodontidae* family living in the Mediterranean Sea, the most

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common ones are *Lagocephalus sceleratus* (Gmelin 1789), *Lagocephalus spadiceus* (Richardson 1845), *Lagocephalus suezensis* (Clark and Gohar 1953), and *Torquigener flavimaculosus* (Hardy and Randall 1983).

L. sceleratus is the most common pufferfish in the Turkish coastal waters in terms of biomass (Bilecenoglu 2010). The natural habitats of this fish are the Indian Ocean and the Pacific Ocean. It has been reported that this fish can be found at depths of 25–200 m (Özbek et al. 2017); furthermore, it can reach a maximum length of 110 cm and weight of 7 kg (Nader et al. 2012). The European Union and the Republic of Turkey Ministry of Food, Agriculture, and Livestock have banned the landing and sale of *L. sceleratus* and *L. spadiceus* (EC 2004a, 2004b; Anonymous 2016). Although *L. spadiceus* has been settled in the Mediterranean Sea for a longer period than other species of pufferfish, there is no reported case of poisoning yet. One scientific study has been conducted on the toxicity of this fish in the Mediterranean Sea (Kosker et al. 2019). Moreover, it has been reported that it is not toxic and can be consumed in many studies of this fish in different countries (especially in Asian countries) (Berry and bin Hassan 1973; Yamaguchi et al. 2013; Ngy et al. 2008; Yu and Yu 1997; Brillantes et al. 2003; Chulanetra et al. 2011). In parallel with this research, *L. spadiceus* has been reported to be consumed in many eastern Asian countries, including Thailand (Brillantes et al. 2003; Kaewnern et al. 2013), Hong Kong (Yu and Yu 1997), Malaysia (Berry and bin Hassan 1973), and Japan (Yamaguchi et al. 2013). Approximately 90% of the pufferfish imported by Japan, according to 2006 trade data, are *L. spadiceus*, and almost all are imported from China (Yamaguchi et al. 2013). *L. suezensis*, also known as the Suez puffer, is an endemic species of pufferfish native to the Red Sea. Studies on the biological properties and toxicity levels of this fish are limited. *T. flavimaculosus*, also known as the yellowspotted puffer, is known as dwarf pufferfish in Turkey. *T. flavimaculosus* is a highly toxic pufferfish (Kosker et al. 2018b). This species of carnivorous fish is fed with small benthic crustacean in the family *Tetraodontidae*. Sabour et al. (2014) reported that it could reach a maximum length of 16 cm, and Golani et al. (2006) reported that it could reach a maximum size of 11 cm (Golani 1987; Bilecenoğlu 2005; Corsini et al. 2006; Sabour et al. 2014).

Pufferfish have negative effects on other aquatic products and humans. However, in terms of their location in the food chain, they can provide information on the metal levels of the ecosystem. The determination of the metal contents of pufferfish, which are prohibited for fishing and are located in the upper trophic zones of the food web, is of great importance in reflecting the level of metals in the marine environment. Metals such as Cu, Zn, and Fe can be defined as essential metals. These metals are necessary for metabolism at normal concentrations, but they may show toxic metabolic effects at high concentrations. Non-essential metals are thought to have no metabolic role. Especially at low concentrations, Hg, Pb, and Cd can be extremely toxic and dangerous for human health (Firat et al. 2008; Durmuş 2018). The heavy metal levels in seafood captured from natural habitats are important indicators of the potential risks of heavy metal contamination in their habitats and human health. In this study, the following aims were pursued:

- Determination of the elemental composition (Na, Mg, P, K, Ca, Fe, Cu, Zn, Cd, Pb, As, and Cr) of the muscle and liver of *L. sceleratus* (Gmelin 1789), *L. spadiceus* (Richardson 1845), *L. suezensis* (Clark and Gohar 1953), and *T. flavimaculosus* (Hardy and Randall 1983) with common distribution in the Mediterranean Sea;
- Determination of the effect of sexual differences on tissue elemental composition;
- Determination of the effect of seasonal differences on tissue elemental compositions.

Materials and methods

Fish collection and measurements

Pufferfish sampling (*L. sceleratus*, *L. spadiceus*, *L. suezensis*, *T. flavimaculosus*) was carried out between the Berdan River (36° 43′ 31.8″ N 34° 54′ 27.0″ E) and Yeşilovacık Bay (36° 08′ 53.6″ N 33° 39′ 40.7″ E) using a commercial trawler (Fig. 1). The sampling was conducted in four seasons between December 2015 and October 2016, and fish samples were provided in sufficient quantities (except some liver samples) for each species. A total of 80 individuals were used for each species including 10 male and 10 female fish in each season.

The lengths and weights of the specimens

Seasonal length and weight measurements of pufferfish species were performed, and sex determination was performed with a microscope. The maximum weight of the individuals of *L. sceleratus* caught during the study was 3661.02 g for females and 2746.21 g for males, with 65.20 cm in maximum length for females and 57.04 cm for males. The maximum length of *L. spadiceus* was 36.60 cm for females and 37.60 cm for males, while the maximum weight for females was 832.18 g, and for males it was 808.68 g. Female *L. suezensis* individuals had lengths and weights of 12.2–20.5 cm and 24.27–92.75 g, respectively, while male individuals were 12.6–21.0 cm and 25.50–96.62 g, respectively. Although the maximum lengths measured in *L. sceleratus* and *L. spadiceus* are similar to those found in the literature, the maximum length measured in *L. suezensis* individuals is different from that in the literature. Golani et al. (2006) reported that the maximum length of *L. suezensis* was 18 cm; but the maximum lengths of the *L. suezensis* caught in this study were measured as 20.5 cm for males and 21 cm for females. Female *T. flavimaculosus* individuals had lengths and weights of 8.3–12.8 cm and 11.18–37.88 g, respectively, while male individuals were 7.4–12.1 cm and 7.41–31.3 g, respectively.

Metal analyses

For each season, the muscle and liver tissues of 10 male and 10 female individuals belonging to each pufferfish species were removed. The samples were dried at 105 °C to reach stable weights. Then, perchloric acid (1 mL, Merck, Germany) and nitric acid (2 mL, Merck, Germany) were added to the tissues, and all samples were placed on a hot plate (150 °C) until all samples were dissolved (Canli and Atli 2003).

ICP-MS (Agilent, 7500ce Model, Japan) was used to identify the metal levels. The device conditions were the following: carrier gas flow rate (L min^{-1}), 1.1; plasma gas flow rate (L min^{-1}), 15; auxiliary gas flow rate (L min^{-1}), 1; radio frequency (RF) (W), 1500; sample depth (mm), 8.6; nebulizer pump (rps), 0.1; sample introduction flow rate (mL min^{-1}), 1; and extract lens (V), 1.5. All analyses were carried out in triplicate for each metal. Macroelements (Na, Mg, P, K, and Ca), trace elements (Cu, Zn, and Fe), and potentially toxic metals (Cd, Cr, Pb, and As) were investigated in the muscle tissue. The levels of Cr, Cu, Cd, and Pb were not evaluated, as their levels were below the detection limit of the device. The element levels in the all tissues were determined as micrograms of metals per gram of dry weight. The standard used for metal analysis is The High Purity Multi Standard (Charleston, SC 29423). Solutions for toxic metals had an arsenic content of between 1 and 50 ppb (0.001–0.050 mg L^{-1}).

Statistical analysis

IBM SPSS STATISTICS 22 statistical program was used for statistical analysis of the data. The effects of season and sex on the metal levels for each pufferfish were evaluated by ANOVA.

Results and discussion

Macroelement compositions of pufferfish species

The changes in macroelement (Na, Mg, P, K, and Ca) levels in muscle and liver tissues of pufferfish were determined according to season and sex (Tables 1, 2). Na, Mg, P, K, and Ca levels in the muscle tissues of pufferfish species range from 1848.4 to 16495.7, 824.4 to 2684.5, 6936.2 to 20486.6, 13019.3 to 36165.5, and 276.4 to 5566.5 μg g^{-1}dw respectively. The levels of macroelements in the muscle tissues for all species can be described as K > P ≥ Na > Ca ≥ Mg.

The maximum levels of the macroelements K, P, Na, Mg, and Ca, investigated during four seasons in the muscle tissues of *L. sceleratus*, were as follows: 8488.04, 2928.00, 953.54, 250.16, and 108.02, respectively (μg g^{-1}, ww). The maximum levels of K, P, Na, Mg, and Ca in the muscle tissues of *L. spadiceus* were as follows: 5423.32, 2910.44, 2811.98, 465.96, and 397.03, respectively (μg g^{-1}, ww). The maximum levels of K, P, Na, Mg, and Ca in the muscle tissues of *L. suezensis* were as follows: 7510.90, 3503.36, 2025.50, 449.88, and 512.41, respectively (μg g^{-1}, ww). The maximum levels of K, P, Na, Mg, and Ca in the muscle tissues of *T. flavimaculosus* were as follows: 7607.5, 4546.0, 3691.7, 638.1, and 1323.2, respectively (μg g^{-1}, ww).

The levels of Na, Mg, P, K, and Ca in the liver tissues of the pufferfish species are 531.8–6136.1, 63.8–899.1, 773.1–6677.5, 1151.4–10165.4, and 47.4–1607.7 μg g^{-1}, dw, respectively. The levels of

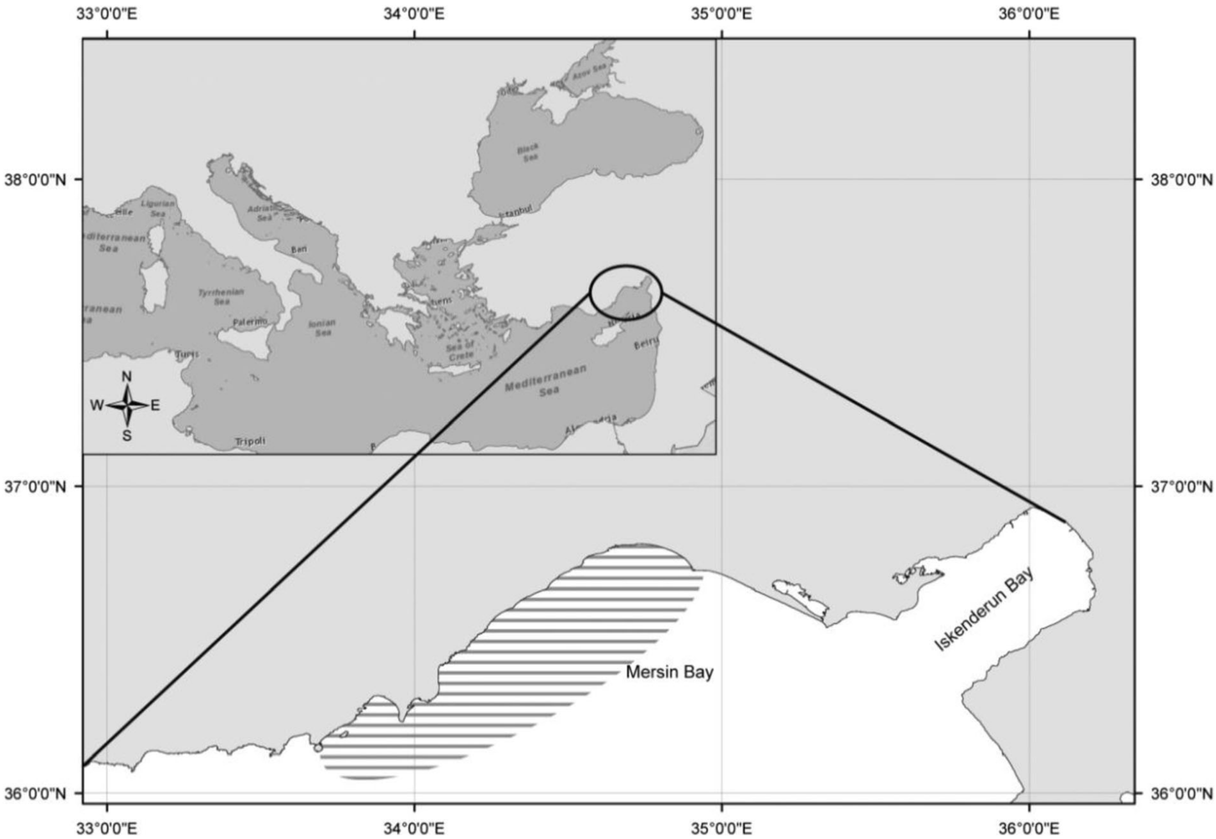

Fig. 1 Sampling location map

macroelements in the liver tissues can be represented as K ≥ P ≥ Na > Ca ≥ Mg (Tables 1, 2). Muscle tissue in the pufferfish has more abundant macroelements than liver tissue. The muscle tissue needs to store energy; accordingly, mineral content in muscles is higher than in other parts of fish. Muscle tissue is a storage organ for macroelements.

Metal studies on pufferfish species are limited. However, the findings of the present study are supported by the findings of previous studies (Thiyagarajan et al. 2012; Kosker et al. 2018a; Ayas and Köşker 2018).

Trace element compositions of pufferfish species

Seasonal and sexual differences in trace element (Fe, Cu, and Zn) levels of muscle and liver tissues of pufferfish were determined (Tables 3, 4).

Fe, Cu, and Zn levels in pufferfish muscle tissues range from 21.53 to 219.42, 0.73 to 6.54, and 25.38 to 100.47 μg g^{-1}, dw, respectively. The levels of trace elements in the muscle tissues can be represented as Zn ≥ Fe > Cu. Fe, Cu, and Zn levels in pufferfish liver tissues range from 157.8 to 1368.6, 0.86 to 11.23, and 23.17 to 507.75 μg g^{-1}, dw, respectively. The levels of trace elements in the liver tissues can be represented as Fe ≥ Zn > Cu. The pufferfish liver tissue is richer than the muscle tissue in terms of trace element levels (Tables 3, 4). The liver tissues of pufferfish are especially rich in Fe. Iron is an important metal involved in liver metabolism.

The maximum Cu levels in pufferfish muscle tissues were 0.82 (μg g^{-1}, ww) for male individuals and 1.56 (μg g^{-1}, ww) for female individuals in autumn. The Cu limit levels reported by FAO (1983) and the Turkish Food Codex are 30 and 20 (μg g^{-1}, ww), respectively. In the present study, it was observed that the Cu levels obtained from pufferfish muscle tissues were below the stated limit levels.

The maximum Zn levels in the pufferfish muscle tissues were 22.49 (μg g^{-1}, ww) for male individuals and 20.11 (μg g^{-1}, ww) for female individuals in autumn. The Zn limit levels reported by FAO (1983) and Turkish Food Codex (2011) are 30 and 50 (μg g^{-1}, ww), respectively. Trace elements are

Table 1 The effects of season and sex on macroelement levels of muscle tissues of the pufferfish species (μg g^{-1})

Macroelement	Winter	Spring	Summer	Autumn	Species	Sex
Na	2281.4 ± 101.7^{b,xy} (535.45)	1987.0 ± 79.3^{a,x} (469.33)	3325.8 ± 103.2^{a,z} (796.53)	2467.0 ± 87.1^{a,y} (573.08)	LSC	♂
	1848.4 ± 89.3^{a,x} (419.59)	2974.7 ± 111.0^{b,z} (678.53)	4016.6 ± 44.5^{b,q} (953.54)	2579.5 ± 91.2^{a,y} (603.60)	LSC	♀
	5450.0 ± 112.4^{c,y} (1225.71)	3450.3 ± 85.7^{c,x} (758.03)	6665.3 ± 124.0^{d,z} (1497.03)	6234.3 ± 173.5^{b,z} (1378.4)	LSP	♂
	6329.9 ± 197.3^{d,z} (1431.19)	3229.3 ± 99.3^{c,x} (717.55)	4633.3 ± 222.4^{c,y} (1027.20)	12,723.9 ± 441.5^{d,q} (2811.98)	LSP	♀
	6634.2 ± 256.4^{d,y} (1514.59)	5206.9 ± 172.6^{d,x} (1120.52)	9456.1 ± 464.2^{ef,q} (2025.50)	7762.1 ± 173.1^{c,z} (1812.45)	LSU	♂
	7123.1 ± 399.2^{d,y} (1604.83)	8630.2 ± 335.9^{b,z} (1889.15)	6391.8 ± 374.5^{d,x} (1422.18)	6567.7 ± 621.2^{bc,xy} (1547.35)	LSU	♀
	6623.0 ± 123.4^{d,x} (1406.1)	8177.4 ± 543.6^{e,y} (1814.6)	8957.4 ± 66.8^{e,y} (2138.1)	16,495.7 ± 444.2^{e,z} (3691.7)	TFL	♂
	9214.7 ± 459.0^{e,y} (2081.6)	5470.9 ± 471.6^{d,x} (1234.8)	10,235.9 ± 327.0^{f,y} (2357.3)	15,288.1 ± 144.2^{e,z} (3634.0)	TFL	♀
Mg	872.6 ± 23.6^{a,xy} (204.80)	824.4 ± 31.1^{a,x} (194.72)	1059.0 ± 64.2^{a,y} (253.63)	998.6 ± 73.1^{a,xy} (231.98)	LSC	♂
	847.0 ± 44.6^{a,x} (192.27)	984.6 ± 35.9^{ab,x} (224.59)	1138.0 ± 34.5^{a,y} (250.16)	1069.9 ± 61.2^{a,xy} (250.36)	LSC	♀
	1304.4 ± 56.4^{bc,y} (293.36)	1044.9 ± 72.6^{b,x} (229.57)	1261.6 ± 34.8^{ab,y} (283.36)	1297.8 ± 33.1^{b,y} (286.94)	LSP	♂
	1188.0 ± 99.2^{b,x} (268.61)	965.4 ± 53.4^{ab,x} (214.51)	1117.4 ± 64.1^{a,x} (247.73)	2108.4 ± 66.7^{c,y} (465.96)	LSP	♀
	1406.1 ± 116.1^{c,x} (321.01)	1161.7 ± 99.6^{bc,x} (250.00)	1923.4 ± 104.5^{c,y} (411.99)	1926.7 ± 41.2^{c,y} (449.88)	LSU	♂
	1513.9 ± 109.2^{cd,xy} (341.08)	1751.1 ± 155.7^{d,y} (383.32)	1358.3 ± 91.6^{b,x} (302.22)	1794.3 ± 117.9^{c,y} (422.74)	LSU	♀
	1056.3 ± 256.3^{b,x} (224.3)	1886.2 ± 182.1^{d,xy} (418.6)	2166.7 ± 321.9^{c,yz} (517.2)	2613.4 ± 241.4^{d,z} (584.9)	TFL	♂
	1699.8 ± 59.5^{d,y} (384.0)	1261.8 ± 31.4^{c,x} (284.8)	1831.8 ± 87.9^{c,y} (421.9)	2684.5 ± 39.6^{d,z} (638.1)	TFL	♀
P	11,170.1 ± 168.3^{cd,y} (2621.62)	10,342.9 ± 125.7^{bc,x} (2442.99)	11,686.8 ± 333.2^{bc,y} (2798.99)	11,803.5 ± 312.2^{ab,y} (2741.95)	LSC	♂
	10,206.5 ± 210.0^{cd,x} (2316.88)	12,019.4 ± 219.0^{de,y} (2741.63)	12,196.6 ± 125.3^{c,y} (2895.47)	12,512.8 ± 99.7^{b,y} (2928.00)	LSC	♀
	11,881.5 ± 432.7^{d,yz} (2672.15)	12,521.7 ± 111.7^{e,z} (2751.02)	11,473.2 ± 121.3^{b,xy} (2576.88)	11,068.1 ± 134.5^{a,x} (2447.16)	LSP	♂
	9828.9 ± 76.7^{b,x} (2222.31)	10,367.5 ± 44.7^{b,y} (2303.66)	12,101.5 ± 279.8^{c,z} (2682.90)	13,169.4 ± 412.2^{bc,z} (2910.44)	LSP	♀
	10,186.7 ± 165.4^{cd,x} (2325.62)	9934.2 ± 88.9^{a,x} (2137.84)	13,399.1 ± 219.7^{d,y} (2870.09)	15,003.7 ± 76.4^{d,z} (3503.36)	LSU	♂
	11,190.1 ± 401.2^{cd,yz} (2521.13)	12,148.8 ± 45.6^{de,z} (2659.37)	10,290.3 ± 341.2^{a,x} (2289.59)	14,752.3 ± 201.4^{d,q} (3475.64)	LSU	♀
	6936.2 ± 55.4^{a,x} (1472.6)	20,486.6 ± 328.6^{f,q} (4546.0)	15,035.2 ± 219.9^{e,z} (3588.9)	13,859.3 ± 88.7^{c,y} (3101.7)	TFL	♂
	10,479.1 ± 221.4^{bc,x} (2367.2)	10,718.3 ± 115.4^{c,x} (2419.1)	12,113.4 ± 447.2^{c,y} (2789.7)	14,705.4 ± 101.9^{d,z} (3495.5)	TFL	♀
K	36,165.5 ± 1056.2^{e,y} (8488.04)	20,533.1 ± 720.4^{b,x} (4849.92)	23,148.3 ± 1064.1^{c,x} (5544.02)	23,655.1 ± 3073.6^{abc,x} (5495.08)	LSC	♂
	19,523.0 ± 1009.3^{cd,x} (4431.72)	25,056.2 ± 635.1^{c,y} (5715.32)	24,184.7 ± 340.7^{c,y} (5741.45)	24,191.1 ± 161.4^{b,y} (5660.72)	LSC	♀
	21,515.9 ± 1005.1^{d,x} (4838.93)	24,685.1 ± 345.9^{c,y} (5423.32)	21,285.4 ± 764.0^{bc,x} (4780.70)	19,717.7 ± 1111.7^{a,x} (4359.58)	LSP	♂
	17,930.3 ± 799.9^{b,x} (4054.04)	19,155.8 ± 1432.1^{ab,xy} (4256.42)	23481.5 ± 345.8^{c,y} (5205.85)	22,009.3 ± 478.8^{ab,y} (4864.06)	LSP	♀

Table 1 (continued)

Macroelement	Winter	Spring	Summer	Autumn	Species	Sex
	17,830.7 ± 761.4^{b,x} (4070.75)	17,288.2 ± 144.7^{a,x} (3720.42)	23,328.1 ± 784.4^{c,y} (4996.88)	32,166.6 ± 2403.3^{d,z} (7510.90)	LSU	♂
	19,171.6 ± 645.2^{cd,y} (4319.36)	21,387.4 ± 561.1^{b,z} (4681.70)	16,311.2 ± 455.6^{a,x} (3629.24)	26,970.4 ± 449.9^{c,q} (6354.23)	LSU	♀
	13,019.3 ± 884.4^{a,x} (2764.0)	24,329.4 ± 3401.1^{bc,y} (5398.7)	31,870.4 ± 334.9^{d,z} (7607.5)	29,832.0 ± 1373.2^{d,z} (6676.4)	TFL	♂
	19,723.7 ± 261.1^{cd,y} (4455.6)	16,496.4 ± 554.3^{a,x} (3723.2)	24,836.3 ± 1134.5^{c,z} (5719.8)	29,629.3 ± 1601.1^{d,q} (7042.9)	TFL	♀
Ca	417.6 ± 16.4^{b,xy} (98.01)	276.4 ± 22.1^{a,x} (65.29)	451.0 ± 14.1^{b,y} (108.02)	330.9 ± 33.1^{a,xy} (76.87)	LSC	♂
	279.5 ± 19.2^{a,x} (63.45)	355.7 ± 15.1^{bc,y} (81.14)	364.7 ± 14.0^{a,y} (86.58)	312.8 ± 11.9^{a,xy} (73.20)	LSC	♀
	861.0 ± 25.2^{c,y} (193.64)	397.2 ± 12.0^{c,x} (87.27)	1067.8 ± 34.9^{d,z} (239.83)	830.0 ± 33.1^{b,y} (183.51)	LSP	♂
	977.3 ± 39.9^{c,z} (220.97)	447.4 ± 13.4^{c,x} (99.41)	602.5 ± 33.5^{c,y} (133.57)	1796.5 ± 61.6^{c,q} (397.03)	LSP	♀
	855.7 ± 27.6^{c,y} (195.36)	638.3 ± 44.3^{d,x} (137.36)	2392.2 ± 55.4^{e,q} (512.41)	2014.5 ± 53.5^{d,z} (470.39)	LSU	♂
	1576.5 ± 37.8^{e,z} (355.19)	1471.4 ± 77.4^{e,yz} (322.09)	1198.8 ± 104.4^{d,y} (266.73)	886.3 ± 39.0^{b,x} (208.81)	LSU	♀
	1169.2 ± 78.3^{d,x} (248.2)	1669.5 ± 76.6^{e,yz} (370.5)	3257.4 ± 54.4^{f,z} (777.5)	2531.8 ± 365.5^{e,z} (566.6)	TFL	♂
	2311.1 ± 99.9^{f,x} (522.1)	3737.8 ± 88.4^{f,y} (843.6)	2182.3 ± 109.8^{e,x} (502.6)	5566.5 ± 321.9^{f,z} (1323.2)	TFL	♀

LSC, *Lagocephalus sceleratus*; *LSP*, *Lagocephalus spadiceus*; *LSU*, *Lagocephalus suezensis*; *TFL*, *Torquigener flavimaculosus*. Different letters (x, y, z, q) in the same rows for each season indicate significant differences ($p < 0.05$). Different letters (a, b, c, d, e, f) in the same columns for each metal indicate significant differences ($p < 0.05$). In the parentheses are given values over the wet weight of macroelements

important metals for metabolic activities. However, if they are above certain limits, they may have negative effects on the organism. In the present study, it was observed that the Zn levels obtained in the pufferfish muscle tissues were below the stated limit levels. Metals such as Cu, Zn, and Fe are important for metabolic activities. Although these metals are necessary for metabolism at regular concentrations, they may show toxic impacts at high concentrations for metabolism.

Toxic element compositions of pufferfish species

The changes in toxic element (Cd, Pb, As, and Cr) levels in pufferfish muscle and liver tissues were determined according to season and sex (Tables 5, 6).

Cd, Pb, As, and Cr levels in the muscle tissues of pufferfish range from 0.66 to 6.30, 2.17 to 20.76, 41.90 to 258.37, and 0.34 to 3.70 μg g^{-1}, dw, respectively. Cd, Pb, As, and Cr levels in pufferfish liver tissues range from 0.22 to 2.34, 0.17 to 2.27, 9.35 to 61.93, and 0.14 to 2.10 μg g^{-1}, dw, respectively. The levels of metal in the muscle tissues can be represented as As > Pb > Cd ≥ Cr and As > Cd ≥ Pb ≥ Cr for liver tissues. Pufferfish muscle tissue has a higher association with liver tissue due to the nature of metal accumulation. The liver is the place where metals are metabolized. If excess metal is taken in, it is stored in other parts of the body such as muscle and bone. If metal accumulates in the muscle tissue, it means that the fish has been exposed to these metals for a very long time. This can be expected for the pufferfish, which is in the upper part of the food chain.

No previous metal studies were found relating to any of the four pufferfish species investigated in the present study, with the exception of *L. sceleratus*. In the previous study, Cd levels in the muscle tissue of

Table 2 The effects of season and sex on macroelement levels of liver tissues of the pufferfish species (μg g^{-1})

Macro element	Winter	Spring	Summer	Autumn	Species	Sex
Na	2017.9 ± 76.4^{b,z}	1155.1 ± 43.6^{b,x}	1395.6 ± 44.0^{a,y}	1414.2 ± 27.1^{a,y}	LSC	♂
	1423.1 ± 33.1^{a,y}	531.8 ± 11.9^{a,x}	2972.3 ± 59.8^{c,z}	1545.3 ± 50.4^{a,y}	LSC	♀
	2304.7 ± 56.1^{bc,y}	1849.0 ± 35.6^{c,x}	3624.1 ± 110.0^{d,z}	3275.9 ± 123.6^{c,z}	LSP	♂
	3653.5 ± 63.4^{d,y}	3620.2 ± 78.0^{d,y}	2341.6 ± 89.6^{b,x}	5237.4 ± 99.7^{d,z}	LSP	♀
	1520.1 ± 27.7^{a,x}	3006.9 ± 47.3^{d,y}	6136.1 ± 92.9^{e,z}	–	LSU	♂
	2609.1 ± 51.9^{c,y}	1708.1 ± 20.0^{c,x}	3537.8 ± 90.9^{d,z}	2680.1 ± 54.3^{b,y}	LSU	♀
	–	–	–	–	TFL	♂
	–	–	–	–	TFL	♀
Mg	211.8 ± 7.9^{a,y}	149.8 ± 8.2^{b,x}	220.2 ± 14.2^{a,y}	286.6 ± 13.9^{b,z}	LSC	♂
	169.3 ± 11.6^{a,y}	63.8 ± 5.0^{a,x}	246.4 ± 11.5^{a,z}	187.6 ± 8.3^{a,y}	LSC	♀
	370.9 ± 19.5^{c,x}	311.7 ± 12.9^{c,x}	829.9 ± 23.4^{d,z}	515.2 ± 22.0^{c,y}	LSP	♂
	682.5 ± 47.6^{e,y}	427.6 ± 23.4^{d,x}	392.0 ± 17.2^{b,x}	755.1 ± 17.9^{d,y}	LSP	♀
	288.1 ± 8.3^{b,x}	621.3 ± 28.6^{e,y}	899.1 ± 14.3^{d,z}	–	LSU	♂
	469.0 ± 19.7^{d,xy}	352.8 ± 25.1^{cd,x}	695.3 ± 31.4^{c,z}	496.8 ± 24.9^{c,y}	LSU	♀
	–	–	–	–	TFL	♂
	–	–	–	–	TFL	♀
P	3140.5 ± 77.4^{b,y}	2104.7 ± 105.1^{b,x}	2045.8 ± 131.2^{a,y}	4169.6 ± 120.4^{d,y}	LSC	♂
	1994.7 ± 88.3^{a,x}	773.1 ± 109.1^{a,y}	2724.6 ± 105.7^{b,y}	2309.0 ± 109.3^{a,y}	LSC	♀
	4073.6 ± 121.3^{c,yz}	3296.0 ± 18.5^{c,z}	5093.4 ± 102.6^{c,y}	3281.5 ± 43.5^{b,y}	LSP	♂
	4535.8 ± 67.2^{c,x}	4554.5 ± 76.5^{e,y}	3129.5 ± 179.0^{b,z}	4685.9 ± 32.6^{e,y}	LSP	♀
	1831.9 ± 16.1^{a,x}	3994.4 ± 48.0^{d,x}	6677.5 ± 290.1^{d,y}	–	LSU	♂
	2988.9 ± 51.2^{b,yz}	2449.0 ± 41.4^{b,z}	4833.2 ± 45.0^{c,x}	3652.4 ± 28.6^{c,q}	LSU	♀
	–	–	–	–	TFL	♂
	–	–	–	–	TFL	♀
K	3977.1 ± 102.5^{b,y}	3061.2 ± 81.7^{b,x}	2698.8 ± 68.1^{a,x}	4332.5 ± 103.9^{b,y}	LSC	♂
	2742.9 ± 44.7^{a,y}	1151.4 ± 65.9^{a,x}	4112.9 ± 38.4^{b,d}	3331.7 ± 141.4^{a,z}	LSC	♀
	5274.2 ± 115.6^{c,y}	4114.5 ± 79.8^{c,x}	5851.8 ± 202.4^{c,y}	4227.8 ± 42.5^{b,x}	LSP	♂
	5766.3 ± 79.6^{c,y}	5360.6 ± 115.4^{d,y}	4045.7 ± 88.8^{b,x}	5587.0 ± 203.4^{c,y}	LSP	♀
	2766.6 ± 71.8^{a,x}	5424.1 ± 69.8^{d,y}	10,165.4 ± 53.3^{d,z}	–	LSU	♂
	4112.5 ± 81.6^{b,y}	3273.2 ± 41.7^{b,x}	5856.2 ± 55.3^{c,z}	5986.2 ± 99.8^{c,z}	LSU	♀
	–	–	–	–	TFL	♂
	–	–	–	–	TFL	♀
Ca	139.3 ± 2.6^{a,x}	117.9 ± 7.3^{b,x}	317.3 ± 11.1^{b,y}	313.8 ± 7.9^{b,y}	LSC	♂
	124.2 ± 3.4^{a,x}	47.4 ± 5.6^{a,y}	218.6 ± 7.5^{a,y}	151.9 ± 10.1^{a,xy}	LSC	♀
	228.9 ± 11.4^{b,x}	319.0 ± 6.7^{c,y}	1375.5 ± 44.7^{d,q}	600.9 ± 14.9^{c,z}	LSP	♂
	702.1 ± 15.9^{e,y}	344.2 ± 8.7^{c,x}	714.7 ± 23.0^{c,y}	1383.2 ± 42.8^{d,z}	LSP	♀
	625.0 ± 9.9^{d,x}	1607.7 ± 55.5^{e,y}	1471.2 ± 21.1^{d,y}	–	LSU	♂
	498.2 ± 27.5^{c,x}	788.0 ± 66.4^{d,y}	1504.8 ± 14.1^{d,z}	654.5 ± 17.7^{c,y}	LSU	♀
	–	–	–	–	TFL	♂
	–	–	–	–	TFL	♀

LSC, *Lagocephalus sceleratus*; *LSP*, *Lagocephalus spadiceus*; *LSU*, *Lagocephalus suezensis*; *TFL*, *Torquigener flavimaculosus*. Different letters (x, y, z, q) in the same rows for each season indicate significant differences ($p < 0.05$). Different letters (a, b, c, d, e, f) in the same columns for each metal indicate significant differences ($p < 0.05$)

Table 3 The effects of season and sex on trace element levels of muscle tissues of the pufferfish species (μg g^{-1})

Trace element	Winter	Spring	Summer	Autumn	Species	Sex
Fe	$40.39 \pm 3.41^{b,z}$ (9.48)	$26.38 \pm 2.62^{a,xy}$ (6.23)	$30.61 \pm 3.01^{a,yz}$ (7.33)	$21.53 \pm 1.17^{a,x}$ (5.00)	LSC	♂
	$26.92 \pm 2.23^{a,xy}$ (6.11)	$29.83 \pm 1.90^{a,y}$ (6.80)	$22.39 \pm 1.57^{a,x}$ (5.32)	$23.06 \pm 1.23^{a,x}$ (5.40)	LSC	♀
	$43.35 \pm 1.49^{b,y}$ (9.75)	$27.42 \pm 2.56^{a,x}$ (6.02)	$69.84 \pm 4.27^{c,z}$ (15.69)	$56.44 \pm 3.14^{bc,z}$ (12.48)	LSP	♂
	$38.83 \pm 2.45^{b,yz}$ (8.78)	$27.39 \pm 0.91^{a,x}$ (6.09)	$43.59 \pm 3.57^{b,z}$ (9.66)	$70.86 \pm 6.34^{cd,q}$ (15.66)	LSP	♀
	$41.68 \pm 1.56^{b,x}$ (9.52)	$37.13 \pm 1.99^{b,x}$ (7.99)	$116.18 \pm 6.23^{d,y}$ (24.89)	$55.59 \pm 2.75^{e,z}$ (12.98)	LSU	♂
	$46.93 \pm 3.67^{bc,xy}$ (10.57)	$67.17 \pm 0.67^{d,z}$ (14.70)	$46.65 \pm 0.99^{b,x}$ (10.38)	$52.85 \pm 4.44^{bc,y}$ (12.45)	LSU	♀
	$54.90 \pm 1.01^{c,x}$ (11.66)	$88.72 \pm 3.89^{f,y}$ (19.69)	$172.85 \pm 10.45^{e,z}$ (41.26)	$219.42 \pm 9.92^{e,q}$ (49.11)	TFL	♂
	$78.85 \pm 7.67^{d,y}$ (17.81)	$47.65 \pm 2.45^{c,x}$ (10.75)	$177.60 \pm 4.51^{e,z}$ (40.90)	$98.09 \pm 8.89^{d,y}$ (23.32)	TFL	♀
Cu	$1.16 \pm 0.07^{b,y}$ (0.27)	$0.81 \pm 0.09^{a,x}$ (0.19)	$1.34 \pm 0.14^{b,y}$ (0.32)	$0.80 \pm 0.10^{a,x}$ (0.19)	LSC	♂
	$0.73 \pm 0.06^{a,x}$ (0.17)	$0.89 \pm 0.11^{a,x}$ (0.20)	$0.82 \pm 0.06^{a,x}$ (0.20)	$0.84 \pm 0.04^{a,x}$ (0.20)	LSC	♀
	$1.65 \pm 0.16^{cd,z}$ (0.37)	$1.06 \pm 0.08^{ab,x}$ (0.23)	$1.36 \pm 0.11^{b,yz}$ (0.31)	$1.26 \pm 0.07^{b,xy}$ (0.28)	LSP	♂
	$1.36 \pm 0.17^{bc,x}$ (0.31)	$1.09 \pm 0.09^{ab,x}$ (0.24)	$1.18 \pm 0.17^{ab,x}$ (0.26)	$2.18 \pm 0.20^{c,y}$ (0.48)	LSP	♀
	$1.65 \pm 0.20^{cd,xy}$ (0.38)	$1.26 \pm 0.13^{b,x}$ (0.27)	$2.13 \pm 0.07^{c,y}$ (0.46)	$3.53 \pm 0.14^{e,z}$ (0.82)	LSU	♂
	$1.68 \pm 0.11^{cd,x}$ (0.38)	$2.69 \pm 0.30^{c,y}$ (0.59)	$1.41 \pm 0.17^{b,x}$ (0.31)	$2.50 \pm 0.33^{cd,y}$ (0.59)	LSU	♀
	$1.53 \pm 0.11^{c,x}$ (0.33)	$1.83 \pm 0.14^{c,x}$ (0.41)	$2.57 \pm 0.19^{c,y}$ (0.61)	$3.24 \pm 0.19^{d,y}$ (0.73)	TFL	♂
	$2.21 \pm 0.21^{d,y}$ (0.50)	$1.30 \pm 0.17^{bc,x}$ (0.29)	$2.82 \pm 0.43^{c,y}$ (0.65)	$6.54 \pm 0.56^{e,z}$ (1.56)	TFL	♀
Zn	$92.72 \pm 8.11^{c,z}$ (21.76)	$31.09 \pm 3.56^{a,xy}$ (7.34)	$42.57 \pm 3.85^{ab,y}$ (10.20)	$25.38 \pm 2.10^{a,x}$ (5.90)	LSC	♂
	$29.34 \pm 3.45^{a,x}$ (6.66)	$42.31 \pm 2.89^{ab,y}$ (9.65)	$29.39 \pm 4.10^{a,x}$ (6.98)	$30.25 \pm 3.00^{ab,x}$ (7.08)	LSC	♀
	$59.24 \pm 5.12^{b,y}$ (13.32)	$56.03 \pm 2.19^{c,y}$ (12.31)	$37.50 \pm 1.38^{a,x}$ (8.42)	$36.54 \pm 2.76^{b,x}$ (8.08)	LSP	♂
	$59.54 \pm 4.70^{b,y}$ (13.46)	$40.78 \pm 3.54^{ab,x}$ (9.06)	$41.12 \pm 3.94^{ab,x}$ (9.12)	$90.98 \pm 4.90^{d,z}$ (20.11)	LSP	♀
	$53.91 \pm 6.11^{b,xy}$ (12.31)	$45.79 \pm 2.33^{b,x}$ (9.85)	$63.19 \pm 4.23^{bc,yz}$ (13.54)	$76.70 \pm 4.98^{c,z}$ (17.91)	LSU	♂
	$56.12 \pm 2.62^{b,y}$ (12.64)	$78.09 \pm 5.31^{d,z}$ (17.09)	$51.01 \pm 5.18^{b,xy}$ (11.35)	$44.21 \pm 1.88^{b,x}$ (10.42)	LSU	♀
	$53.58 \pm 6.01^{b,x}$ (11.38)	$78.33 \pm 4.31^{d,y}$ (17.38)	$66.78 \pm 3.44^{bc,xy}$ (15.94)	$100.47 \pm 15.81^{d,z}$ (22.49)	TFL	♂
	$59.94 \pm 5.89^{b,xy}$ (13.54)	$40.38 \pm 6.00^{ab,x}$ (9.11)	$70.95 \pm 2.39^{c,y}$ (16.34)	$74.64 \pm 8.80^{c,y}$ (17.74)	TFL	♀

LSC, *Lagocephalus sceleratus*; *LSP*, *Lagocephalus spadiceus*; *LSU*, *Lagocephalus suezensis*; *TFL*, *Torquigener flavimaculosus*. Different letters (x, y, z, q) in the same rows for each season indicate significant differences ($p < 0.05$). Different letters (a, b, c, d, e, f) in the same columns for each metal indicate significant differences ($p < 0.05$). In the parentheses are given values over the wet weight of trace elements

Table 4 The effects of season and sex on trace element levels of liver tissues of the pufferfish species (μg g^{-1})

Trace element	Winter	Spring	Summer	Autumn	Species	Sex
Fe	$1177.8 \pm 70.54^{c,z}$	$359.0 \pm 17.42^{a,x}$	$362.7 \pm 38.85^{ab,x}$	$587.4 \pm 15.61^{d,y}$	LSC	♂
	$385.7 \pm 22.32^{b,y}$	$249.4 \pm 18.89^{a,x}$	$431.1 \pm 25.03^{b,y}$	$398.6 \pm 22.21^{c,y}$	LSC	♀
	$193.9 \pm 10.41^{a,x}$	$294.9 \pm 16.32^{a,y}$	$408.0 \pm 22.42^{b,y}$	$157.8 \pm 10.4^{a,x}$	LSP	♂
	$373.3 \pm 25.01^{b,y}$	$279.8 \pm 21.11^{a,x}$	$290.3 \pm 23.07^{a,xy}$	$274.1 \pm 13.24^{b,x}$	LSP	♀
	$356.3 \pm 12.33^{b,x}$	$1368.6 \pm 69.05^{c,z}$	$1053.5 \pm 44.99^{d,y}$	–	LSU	♂
	$360.8 \pm 14.55^{b,x}$	$568.9 \pm 32.76^{b,y}$	$721.3 \pm 34.09^{c,z}$	$558.5 \pm 37.11^{d,y}$	LSU	♀
	–	–	–	–	TFL	♂
	–	–	–	–	TFL	♀
Cu	$4.36 \pm 0.22^{c,z}$	$3.19 \pm 0.22^{b,y}$	$1.99 \pm 0.11^{a,x}$	$5.87 \pm 0.19^{c,q}$	LSC	♂
	$2.36 \pm 0.11^{a,y}$	$0.86 \pm 0.04^{a,x}$	$2.33 \pm 0.10^{a,y}$	$2.04 \pm 0.22^{a,y}$	LSC	♀
	$2.17 \pm 0.15^{a,x}$	$2.89 \pm 0.17^{b,x}$	$4.89 \pm 0.24^{c,y}$	$11.23 \pm 0.44^{d,z}$	LSP	♂
	$3.13 \pm 0.13^{b,x}$	$3.30 \pm 0.22^{b,x}$	$3.07 \pm 0.13^{b,x}$	$3.26 \pm 0.15^{b,x}$	LSP	♀
	$2.23 \pm 0.04^{a,x}$	$7.67 \pm 0.31^{c,y}$	$6.28 \pm 0.21^{d,y}$	–	LSU	♂
	$2.56 \pm 0.07^{a,x}$	$2.99 \pm 0.18^{b,x}$	$5.45 \pm 0.28^{c,y}$	$6.39 \pm 0.41^{c,y}$	LSU	♀
	–	–	–	–	TFL	♂
	–	–	–	–	TFL	♀
Zn	$100.76 \pm 6.44^{a,x}$	$95.89 \pm 4.79^{b,x}$	$105.65 \pm 5.74^{a,x}$	$83.00 \pm 5.41^{a,x}$	LSC	♂
	$120.43 \pm 9.23^{a,y}$	$23.17 \pm 1.97^{a,x}$	$195.63 \pm 12.54^{b,z}$	$96.52 \pm 4.42^{a,y}$	LSC	♀
	$237.98 \pm 11.15^{b,y}$	$242.08 \pm 20.03^{c,y}$	$296.26 \pm 9.85^{c,y}$	$141.98 \pm 11.47^{b,x}$	LSP	♂
	$344.01 \pm 13.43^{c,y}$	$344.55 \pm 14.85^{d,y}$	$227.42 \pm 5.44^{b,x}$	$234.84 \pm 17.33^{c,x}$	LSP	♀
	$196.28 \pm 8.75^{b,x}$	$363.24 \pm 11.34^{d,y}$	$507.75 \pm 14.59^{e,z}$	–	LSU	♂
	$193.69 \pm 9.99^{b,x}$	$229.07 \pm 20.13^{c,x}$	$406.97 \pm 11.25^{d,y}$	$216.59 \pm 10.19^{bc,x}$	LSU	♀
	–	–	–	–	TFL	♂
	–	–	–	–	TFL	♀

LSC, *Lagocephalus sceleratus*; *LSP*, *Lagocephalus spadiceus*; *LSU*, *Lagocephalus suezensis*; *TFL*, *Torquigener flavimaculosus.* Different letters (x, y, z, q) in the same rows for each season indicate significant differences ($p < 0.05$). Different letters (a, b, c, d, e, f) in the same columns for each metal indicate significant differences ($p < 0.05$)

L. sceleratus were found to be between 0.16 and 0.24 μg g^{-1} (ww) for males and between 0.17 and 0.23 μg g^{-1} (ww) for females; in addition, Pb levels were found between 0.10 and 0.20 μg g^{-1} (ww) for male individuals, and 0.14 and 0.20 μg g^{-1} (ww) for female individuals (Kosker et al. 2018a). The levels of Cd and Pb reported by Kosker et al. (2018a) are lower than those obtained in the present study. This situation is thought to be caused by the difference in the research methods applied by the two studies. While pufferfish were investigated through individuals in the previous study (Kosker et al. 2018a), in this study, a total of 80 individuals for each species were mixed according to season and sex groups, and a homogeneous sample was used.

L. sceleratus

There was no statistically significant difference between the Cd levels of *L. sceleratus* muscle tissues in terms of season and sex ($p > 0.05$). The maximum Cd levels in male and female specimens were 0.19 and 0.17 (μg g^{-1}, ww), respectively. It was observed that the Cd levels in the samples were below the limit value of 0.5 (μg g^{-1}, ww) determined by the FAO (1983). However, all samples were found to contain Cd above the limit value of 0.05 (μg g^{-1}, ww) as determined by EU (EC 2001 and the Turkish Food Codex (2011).

There was no statistically significant difference in the Pb values of *L. sceleratus* between individuals of different sexes caught in the same season ($p > 0.05$).

Table 5 The effects of season and sex on toxic element levels of muscle tissues of the pufferfish species (μg g^{-1})

Toxic element	Winter	Spring	Summer	Autumn	Species	Sex
Cd	$0.79 \pm 0.11^{a,x}$ (0.19)	$0.76 \pm 0.09^{a,x}$ (0.18)	$0.70 \pm 0.08^{a,x}$ (0.17)	$0.66 \pm 0.10^{a,x}$ (0.15)	LSC	♂
	$0.67 \pm 0.06^{a,x}$ (0.15)	$0.75 \pm 0.07^{a,x}$ (0.17)	$0.68 \pm 0.07^{a,x}$ (0.16)	$0.69 \pm 0.12^{a,x}$ (0.16)	LSC	♀
	$1.00 \pm 0.11^{ab,x}$ (0.23)	$0.69 \pm 0.09^{a,x}$ (0.15)	$0.82 \pm 0.04^{a,x}$ (0.18)	$0.75 \pm 0.06^{a,x}$ (0.17)	LSP	♂
	$0.93 \pm 0.11^{ab,x}$ (0.21)	$0.79 \pm 0.10^{a,x}$ (0.18)	$0.79 \pm 0.05^{a,x}$ (0.18)	$1.62 \pm 0.17^{b,y}$ (0.36)	LSP	♀
	$0.94 \pm 0.12^{ab,x}$ (0.22)	$0.72 \pm 0.12^{a,x}$ (0.16)	$2.38 \pm 0.31^{b,y}$ (0.51)	$2.15 \pm 0.25^{c,z}$ (0.50)	LSU	♂
	$0.89 \pm 0.07^{a,x}$ (0.20)	$1.46 \pm 0.09^{b,y}$ (0.32)	$0.83 \pm 0.02^{a,x}$ (0.19)	$1.01 \pm 0.09^{a,x}$ (0.24)	LSU	♀
	$0.96 \pm 0.21^{ab,x}$ (0.20)	$2.00 \pm 0.21^{b,y}$ (0.44)	$4.31 \pm 0.33^{b,z}$ (1.03)	$6.30 \pm 0.40^{c,q}$ (1.41)	TFL	♂
	$1.39 \pm 0.16^{b,xy}$ (0.31)	$0.93 \pm 0.17^{ab,x}$ (0.21)	$3.94 \pm 0.41^{b,z}$ (0.91)	$1.88 \pm 0.19^{b,y}$ (0.45)	TFL	♀
Pb	$15.27 \pm 1.67^{c,y}$ (3.58)	$14.55 \pm 1.49^{c,y}$ (3.44)	$2.30 \pm 0.22^{a,x}$ (0.55)	$2.17 \pm 0.22^{a,x}$ (0.50)	LSC	♂
	$12.86 \pm 1.81^{c,y}$ (2.92)	$14.51 \pm 0.99^{c,y}$ (3.31)	$2.25 \pm 0.21^{a,x}$ (0.53)	$2.27 \pm 0.31^{a,x}$ (0.53)	LSC	♀
	$3.30 \pm 0.33^{ab,x}$ (0.74)	$2.27 \pm 0.40^{a,x}$ (0.50)	$2.69 \pm 0.13^{a,x}$ (0.60)	$2.48 \pm 0.24^{a,x}$ (0.55)	LSP	♂
	$3.06 \pm 0.37^{ab,x}$ (0.69)	$2.59 \pm 0.11^{a,x}$ (0.58)	$2.60 \pm 0.14^{a,x}$ (0.58)	$5.33 \pm 0.59^{ab,y}$ (1.18)	LSP	♀
	$3.08 \pm 0.27^{a,x}$ (0.70)	$2.37 \pm 0.11^{a,x}$ (0.51)	$7.84 \pm 0.89^{b,y}$ (1.68)	$7.07 \pm 0.75^{c,z}$ (1.65)	LSU	♂
	$2.92 \pm 0.30^{a,y}$ (0.66)	$4.81 \pm 0.44^{b,y}$ (1.05)	$2.73 \pm 0.39^{a,x}$ (0.60)	$3.33 \pm 0.48^{a,y}$ (0.79)	LSU	♀
	$3.16 \pm 0.33^{ab,x}$ (0.67)	$6.59 \pm 0.70^{b,y}$ (1.46)	$14.19 \pm 1.45^{c,z}$ (3.39)	$20.76 \pm 2.34^{c,q}$ (4.65)	TFL	♂
	$4.60 \pm 0.44^{b,xy}$ (1.04)	$3.06 \pm 0.34^{a,x}$ (0.69)	$12.97 \pm 1.27^{c,z}$ (2.99)	$6.20 \pm 0.77^{b,y}$ (1.47)	TFL	♀
As	$135.56 \pm 7.41^{c,x}$ (31.82)	$103.07 \pm 7.69^{c,x}$ (24.35)	$188.46 \pm 14.25^{c,y}$ (45.14)	$238.93 \pm 23.11^{d,y}$ (55.50)	LSC	♂
	$105.37 \pm 9.22^{c,x}$ (23.92)	$99.69 \pm 13.94^{c,x}$ (22.74)	$240.35 \pm 24.52^{d,y}$ (57.06)	$258.37 \pm 21.25^{d,y}$ (60.46)	LSC	♀
	$139.05 \pm 12.22^{c,z}$ (31.27)	$83.93 \pm 8.99^{bc,xy}$ (18.44)	$100.54 \pm 10.01^{a,yz}$ (22.58)	$75.73 \pm 3.43^{a,x}$ (16.74)	LSP	♂
	$88.97 \pm 7.56^{bc,y}$ (20.12)	$69.66 \pm 7.01^{b,x}$ (15.48)	$186.42 \pm 9.03^{c,y}$ (41.33)	$75.57 \pm 6.03^{a,x}$ (16.70)	LSP	♀
	$67.95 \pm 6.48^{ab,x}$ (15.51)	$72.21 \pm 7.53^{b,x}$ (15.54)	$126.72 \pm 6.23^{ab,y}$ (27.14)	$86.52 \pm 8.56^{a,x}$ (20.20)	LSU	♂
	$70.96 \pm 6.17^{ab,x}$ (15.99)	$75.89 \pm 7.94^{b,x}$ (16.61)	$148.99 \pm 11.81^{bc,z}$ (33.15)	$114.14 \pm 3.94^{bc,y}$ (26.89)	LSU	♀
	$55.80 \pm 5.10^{a,x}$ (11.85)	$102.56 \pm 13.01^{c,y}$ (22.76)	$109.02 \pm 5.06^{a,y}$ (26.02)	$157.24 \pm 10.01^{cd,z}$ (35.19)	TFL	♂
	$75.51 \pm 9.28^{abc,y}$ (17.06)	$41.90 \pm 3.93^{a,x}$ (9.46)	$109.74 \pm 3.91^{a,z}$ (25.27)	$140.98 \pm 12.45^{cd,z}$ (33.51)	TFL	♀
Cr	$0.87 \pm 0.09^{b,y}$ (0.20)	$0.40 \pm 0.03^{a,x}$ (0.10)	$0.77 \pm 0.05^{b,y}$ (0.18)	$0.41 \pm 0.02^{a,x}$ (0.10)	LSC	♂
	$0.52 \pm 0.05^{a,y}$ (0.12)	$0.48 \pm 0.03^{a,y}$ (0.11)	$0.42 \pm 0.03^{a,xy}$ (0.10)	$0.34 \pm 0.03^{a,x}$ (0.08)	LSC	♀
	$0.49 \pm 0.06^{a,x}$ (0.11)	$0.38 \pm 0.04^{a,x}$ (0.08)	$1.52 \pm 0.06^{c,y}$ (0.34)	$2.01 \pm 0.13^{c,z}$ (0.44)	LSP	♂
	$0.47 \pm 0.07^{a,x}$ (0.11)	$0.40 \pm 0.02^{a,x}$ (0.09)	$0.48 \pm 0.03^{a,x}$ (0.11)	$1.04 \pm 0.17^{e,y}$ (0.23)	LSP	♀

Table 5 (continued)

Toxic element	Winter	Spring	Summer	Autumn	Species	Sex
	$0.53 \pm 0.05^{a,x}$ (0.12)	$0.37 \pm 0.04^{a,x}$ (0.08)	$1.32 \pm 0.09^{c,y}$ (0.28)	$1.34 \pm 0.12^{b,z}$ (0.31)	LSU	♂
	$0.58 \pm 0.05^{ab,x}$ (0.13)	$0.81 \pm 0.04^{c,y}$ (0.18)	$0.52 \pm 0.06^{a,x}$ (0.12)	$0.52 \pm 0.05^{a,x}$ (0.12)	LSU	♀
	$0.44 \pm 0.04^{a,x}$ (0.09)	$0.91 \pm 0.09^{c,y}$ (0.20)	$2.53 \pm 0.09^{d,y}$ (0.60)	$3.70 \pm 0.20^{d,z}$ (0.83)	TFL	♂
	$0.63 \pm 0.05^{ab,xy}$ (0.14)	$0.54 \pm 0.03^{b,x}$ (0.12)	$2.31 \pm 0.23^{cd,z}$ (0.53)	$0.81 \pm 0.06^{b,y}$ (0.19)	TFL	♀

LSC, *Lagocephalus sceleratus*; *LSP*, *Lagocephalus spadiceus*; *LSU*, *Lagocephalus suezensis*; *TFL*, *Torquigener flavimaculosus*. Different letters (x, y, z, q) in the same rows for each season indicate significant differences ($p < 0.05$). Different letters (a, b, c, d, e, f) in the same columns for each metal indicate significant differences ($p < 0.05$). In the parentheses are given values over the wet weight of elements

Statistically significant differences were found in Pb values of *L. sceleratus* muscle tissue according to season ($p < 0.05$). The Pb value of the female specimens was 3.31 (μg g^{-1}, ww) in spring, while that of males in winter was found to be 3.58 (μg g^{-1}, ww). The limit value determined by the FAO (1983) and the European Union (EC 2001) for Pb is 0.3 (μg g^{-1}, ww), and the limit value determined by the Turkish Food Codex (2011) is 0.2 (μg g^{-1}, ww). Pb was detected above the legal limits reported in all the samples.

In terms of Cr levels, statistically significant differences were observed in seasonal samples of both sexes ($p < 0.05$) and maximum Cr values were determined in winter for both sexes. The maximum measured Cr levels in male and female subjects were 0.20 and 0.12 (μg g^{-1}, ww), respectively. No limit value was found in the Food Codex for the maximum level of elemental Cr in seafood.

There were statistically significant differences in the As levels in samples in terms of season and sex ($p < 0.05$). The maximum determined As levels for male and female samples in autumn were 55.50 and 60.46 (μg g^{-1}, ww), respectively. It was observed that the As levels obtained in this study were quite high. Humans are exposed to many different forms of arsenic, including inorganic (arsenite and arsenate) and organic (arsenobetain, arsenosugars, and arsenolipid) arsenic compounds (Navas-Acien et al. 2011). Seafood usually contains organic arsenic, which is less toxic than inorganic arsenic (EFSA CONTAM 2009). It is thought that this situation may be due to Mersin Bay's unique conditions.

L. spadiceus

The maximum Cd levels in the samples were 0.23 and 0.36 (μg g^{-1}, ww) in the muscle tissues of male and female individuals, respectively. The Cd levels were below the limit value of 0.5 (μg g^{-1}, ww) that was determined by FAO (1983). However, all samples were found to contain more Cd than the limit value of 0.05 (μg g^{-1}, ww) as determined by EU (EC 2001) and Turkish Food Codex (2011).

The limit value determined by the FAO (1983) and the European Union (EC 2001) for Pb is 0.3 (μg g^{-1}, ww) and the limit value determined by the Turkish Food Codex (2011) is 0.2 (μg g^{-1}, ww). Pb was detected above the legal limits reported in all samples. This situation is thought to be caused by the subjective conditions of Mersin Bay.

The maximum As level found in male individuals was 31.27 (μg g^{-1}, ww) in winter, while the level found in female individuals was 41.33 (μg g^{-1}, ww) in summer. No limit value was found in the food codexes (FAO 1983; EC 2001 and Turkish Food Codex 2011) for the maximum level of elemental As in seafood. However, it was observed that the levels of As detected in the muscle tissues of male and female individuals of the species *L. spadiceus* were high.

Statistical differences were observed in terms of both sex and season between Cr values obtained as a result of analyses made with the examined samples ($p < 0.05$). In both male and female muscle samples, the maximum value of Cr was observed in the autumn season; these were 0.44 and 0.23 (μg g^{-1}, ww), respectively.

Table 6 The effects of season and sex on toxic element levels of liver tissues of the pufferfish species (μg g^{-1})

Toxic element	Winter	Spring	Summer	Autumn	Species	Sex
Cd	$0.46 \pm 0.04^{b,x}$	$0.40 \pm 0.02^{b,x}$	$0.26 \pm 0.04^{a,x}$	$0.26 \pm 0.04^{a,x}$	LSC	♂
	$0.53 \pm 0.06^{b,y}$	$0.30 \pm 0.03^{ab,x}$	$0.28 \pm 0.03^{a,x}$	$0.24 \pm 0.04^{a,x}$	LSC	♀
	$0.26 \pm 0.02^{a,x}$	$0.25 \pm 0.02^{a,x}$	$0.54 \pm 0.04^{b,y}$	$0.25 \pm 0.04^{a,x}$	LSP	♂
	$0.47 \pm 0.01^{b,y}$	$0.22 \pm 0.01^{a,x}$	$0.49 \pm 0.04^{b,y}$	$1.46 \pm 0.24^{b,z}$	LSP	♀
	$0.29 \pm 0.01^{a,x}$	$1.03 \pm 0.13^{c,y}$	$2.34 \pm 0.31^{c,z}$	–	LSU	♂
	$0.38 \pm 0.03^{ab,x}$	$0.36 \pm 0.04^{b,x}$	$0.50 \pm 0.04^{b,x}$	$1.75 \pm 0.29^{b,y}$	LSU	♀
	–	–	–	–	TFL	♂
	–	–	–	–	TFL	♀
Pb	$0.26 \pm 0.07^{a,x}$	$0.22 \pm 0.09^{a,x}$	$0.25 \pm 0.02^{a,x}$	$0.25 \pm 0.02^{a,x}$	LSC	♂
	$0.30 \pm 0.01^{a,x}$	$0.17 \pm 0.09^{a,y}$	$0.27 \pm 0.01^{a,x}$	$0.31 \pm 0.01^{a,x}$	LSC	♀
	$0.25 \pm 0.03^{a,x}$	$0.24 \pm 0.02^{a,x}$	$0.52 \pm 0.04^{b,y}$	$0.24 \pm 0.04^{a,x}$	LSP	♂
	$0.45 \pm 0.07^{a,xy}$	$0.21 \pm 0.01^{a,x}$	$0.47 \pm 0.04^{b,y}$	$1.42 \pm 0.24^{b,z}$	LSP	♀
	$0.28 \pm 0.07^{a,x}$	$0.99 \pm 0.10^{b,y}$	$2.27 \pm 0.19^{c,z}$	–	LSU	♂
	$0.36 \pm 0.02^{a,x}$	$0.35 \pm 0.04^{a,x}$	$0.48 \pm 0.04^{b,x}$	$1.70 \pm 0.30^{b,y}$	LSU	♀
	–	–	–	–	TFL	♂
	–	–	–	–	TFL	♀
As	$34.23 \pm 3.21^{c,x}$	$35.63 \pm 2.74^{b,x}$	$25.50 \pm 1.55^{a,x}$	$35.51 \pm 3.23^{b,x}$	LSC	♂
	$20.71 \pm 3.42^{a,x}$	$12.91 \pm 1.90^{a,x}$	$56.28 \pm 4.23^{b,y}$	$53.55 \pm 1.85^{c,y}$	LSC	♀
	$29.96 \pm 2.11^{bc,y}$	$16.15 \pm 2.09^{a,x}$	$26.64 \pm 0.94^{a,y}$	$14.71 \pm 1.42^{a,x}$	LSP	♂
	$27.37 \pm 1.09^{ab,y}$	$32.93 \pm 2.91^{b,y}$	$32.90 \pm 3.39^{a,y}$	$17.14 \pm 1.74^{a,x}$	LSP	♀
	$9.35 \pm 0.89^{a,x}$	$19.35 \pm 2.03^{a,y}$	$61.93 \pm 10.22^{b,z}$	–	LSU	♂
	$13.90 \pm 1.78^{a,x}$	$13.18 \pm 1.44^{a,x}$	$42.99 \pm 4.17^{b,y}$	$19.77 \pm 0.95^{a,x}$	LSU	♀
	–	–	–	–	TFL	♂
	–	–	–	–	TFL	♀
Cr	$0.21 \pm 0.02^{a,x}$	$0.20 \pm 0.01^{a,x}$	$0.22 \pm 0.09^{a,x}$	$0.23 \pm 0.03^{a,x}$	LSC	♂
	$0.24 \pm 0.02^{a,x}$	$0.14 \pm 0.04^{a,x}$	$0.25 \pm 0.02^{a,x}$	$0.28 \pm 0.03^{a,x}$	LSC	♀
	$0.23 \pm 0.02^{a,x}$	$0.20 \pm 0.05^{a,x}$	$0.43 \pm 0.04^{a,x}$	$0.20 \pm 0.04^{a,x}$	LSP	♂
	$0.41 \pm 0.03^{a,x}$	$0.17 \pm 0.05^{a,x}$	$0.38 \pm 0.02^{a,x}$	$1.16 \pm 0.10^{b,y}$	LSP	♀
	$0.26 \pm 0.02^{a,x}$	$0.93 \pm 0.08^{b,y}$	$2.10 \pm 0.22^{b,z}$	–	LSU	♂
	$0.30 \pm 0.04^{a,x}$	$0.81 \pm 0.05^{b,y}$	$0.41 \pm 0.02^{a,x}$	$1.43 \pm 0.29^{b,y}$	LSU	♀
	–	–	–	–	TFL	♂
	–	–	–	–	TFL	♀

LSC, *Lagocephalus sceleratus*; *LSP*, *Lagocephalus spadiceus*; *LSU*, *Lagocephalus suezensis*; *TFL*, *Torquigener flavimaculosus*. Different letters (x, y, z, q) in the same rows for each season indicate significant differences ($p < 0.05$). Different letters (a, b, c, d, e, f) in the same columns for each metal indicate significant differences ($p < 0.05$)

L. suezensis

There were statistically significant differences in Cd values in terms of seasonal and sexual changes in the examined fish samples ($p < 0.05$). The maximum Cd levels in males and females were 0.51 and 0.32 (μg g^{-1}, ww), respectively. The Cd limit value reported by FAO (1983) is 0.5 (μg g^{-1}, ww) and that for the EU (EC 2001) and Turkish Food Codex (2011) is 0.05 (μg g^{-1}, ww). The Cd levels of the females were observed below the limit values determined by the FAO (1983) but above the limit values determined by the EU (EC 2001) and Turkish Food Codex (2011).

Pb values in muscle tissues were found to be statistically different between seasons and between sexes ($p < 0.05$). The maximum Pb values measured in male and female samples were 1.68 and 1.05 (μg g^{-1}, ww), respectively. The limit value determined by the FAO (1983) and the European Union (EC 2001) for Pb is 0.3 (μg g^{-1}, ww) and the limit value determined by the Turkish Food Codex (2011) is 0.2 (μg g^{-1}, ww). Pb was detected above the legal limits reported in all samples.

There was a statistically significant difference between the seasonal levels of As in both sex groups ($p < 0.05$). As a result of the comparison between the males and females in the same season, it was observed that there was only a statistical difference in the autumn season ($p < 0.05$). The maximum As levels found were 27.14 (μg g^{-1}, ww) in males and 33.15 (μg g^{-1}, ww) in females, both in summer.

Statistical differences were observed in terms of both sex and season between Cr values obtained as a result of analyses made with the examined samples ($p < 0.05$). In both male and female samples, the maximum value of Cr was observed in the fall season: 0.31 and 0.18 (μg g^{-1}, ww), respectively.

T. flavimaculosus

There was a statistically significant difference between the Cd levels in the muscle tissues of *T. flavimaculosus* in terms of season and sex ($p > 0.05$). The maximum Cd levels in male and female specimens were 1.41 and 0.91 (μg g^{-1}, ww), respectively. It was observed that the Cd levels in the samples were above the limit value of 0.5 (μg g^{-1}, ww) determined by the FAO (1983). All samples were also found to contain Cd above the limit value of 0.05 (μg g^{-1}, ww) as determined by the EU (EC 2001) and Turkish Food Codex (2011).

The maximum Pb values measured in male and female samples were 4.65 and 2.99 (μg g^{-1}, ww), respectively. The limit value determined by the FAO (1983) and the European Union (EC 2001) for Pb is 0.3 (μg g^{-1}, ww) and that determined by the Turkish Food Codex (2011) is 0.2 (μg g^{-1}, ww). Pb was detected above the legal limits in all samples.

The maximum As level was found in male individuals in autumn at 35.19 (μg g^{-1}, ww), while its level in female individuals was 33.51 (μg g^{-1}, ww) in autumn. No limit value was found in the food codexes (FAO 1983; EC 2001; Turkish Food Codex 2011) for the maximum level of elemental As in seafood. However, it was observed that the levels of As detected in the muscle tissues of male and female individuals of *T. flavimaculosus* were high.

Statistical differences were observed in terms of both sex and season between Cr values obtained as a result of analyses made with the examined samples ($p < 0.05$). In male muscle samples, the maximum observed value of Cr was 0.83 (μg g^{-1}, ww) in the autumn season, while its maximum level in females was 0.53 (μg g^{-1}, ww) in the summer season.

Conclusions

Although Pb is naturally present in nature, it is generally increased by anthropogenic activities in pollution effects by industrial activities such as mining, the casting industry, and battery production and wastewater discharges (Alexander et al. 2010). Similarly, the amount of Cd can be increased by industrial and agricultural activities. Compounds containing Cr and Cd are used as corrosion inhibitors in batteries, electronic components, nuclear power plants, and steelmaking. It is believed that the high levels of Cd and Pb are caused by human activities in the bay. Various factors affect the pollution in Mersin Bay's marine ecosystem. Artificial fertilizers and pesticides, household waste, the chromium industry, plastics, fertilizers, industrial waste in the region, and intensive marine traffic due to the presence of the harbor, which is used extensively in agricultural activities, are the main pollution sources in the bay. It is possible that the iron–steel industry activities that are carried out intensively in Iskenderun Bay have a direct negative impact on the Northeastern Mediterranean Sea. Pufferfish found in the upper trophic zones of the food chain contain toxic metals at high levels in various tissues. These fish, which are already limited in industrial use because they contain TTX, are further restricted by metal contamination.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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