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NATURAL WATERS

Potential Usage of Pufferfish Dentin as a Metal Accumulation Indicator

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Abstract—The pufferfish are non-indigenous species in the Mediterranean Sea that do not have predators in the food chain. Therefore, the determination of heavy metal accumulation in pufferfish species can be considered as natural chelation for the aquatic environment. The present study, the potential usage of dentin tissue as an indicator of heavy metal bioaccumulation was examined in pufferfish species. The accumulation of Co, Cu, Zn, Fe, Mo, Ni, Se, Al, Cd, Pb, As, Cr in dentin, liver, kidney and muscle tissues of four pufferfish species (*Lagocephalus sceleratus*, *Lagocephalus spadiceus*, *Lagocephalus suezensis*, and *Torquigener flavimaculosus*) were captured in the Göksu River and Yeşjlovacık coasts of Turkey by a commercial trawler. The different bioaccumulation levels were found in each species in terms of metals concentrations and tissues. The metal levels in dentine tissue were found to be similar to the brain. Aluminium had the highest accumulation among these tissues. The liver and gill tissues showed the same bioaccumulation properties with each other. Zn had the highest accumulation among these tissues ($P < 0.05$). The muscle was different from metabolically active tissues in terms of metal accumulation that arsenic had the highest bioaccumulation ($P < 0.05$). Fe, Se, As, Zn, Cu, Cr and Al were accumulated in all tissue types, however, only Se was not detected in dentin tissue. On the other hand, Co, Ni, Mo, Cd and Pb were not detected in any tissue types. The relative accumulation of metals in dentin tissue was determined as Al > Zn > Cr > Fe > As > Cu. The present study for the first time revealed that dentin tissue can be used as an indicator tissue for toxicology studies that metal accumulations in dentin were determined in parallel with other examined tissues of pufferfish species.

Keywords: dentin, pufferfish, metal accumulation, indicator

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INTRODUCTION

Heavy metal input to the environment is caused by natural events and anthropogenic effects [1]. Heavy metals are distributed in aquatic ecosystems at the different chemical forms according to their weight and participate. Since metal involvement is more anthropogenic, monitoring its changing effect is inevitable for environmental health [2].

Some of the heavy metals such as Cu, Zn, Fe, Cr, Se are essential for an animal, and others such as Cd, Pb, As, Ni are never needed for life which has a toxic effect on a certain concentration following accumulation that causes metabolic and physiological changes and mortality [2]. Fish are used as a bioindicator in pollution monitoring studies in order to be an important protein source in human consumption and to reflect the state of the environment and food chain in aquatic ecosystems [3].

In bioaccumulation studies carried out with various fish species, heavy metal concentration is examined in tissues mainly metabolically active such as gills respiration and osmoregulation, liver detoxification, kidneys excretion, brain reflecting the nerve physiology. Metabolically inactive muscle tissue is also used because of its consumption by a human [4].

Dentin is a stable tissue and used in metal accumulation studies in mammalian as it reflects the interaction of the individual with the heavy metal during the developmental stage [5, 6]. However, dentin tissue in fish has not been used for this purpose, therefore, there is a need to determine whether the dentin tissue can be an indicator of metal accumulation compared to other tissues.

Therefore, in the present study, the accumulation of heavy metals (Co, Cu, Zn, Fe, Mo, Ni, Se, Al, Cd, Pb, As, Cr) in dentin, liver, gill, muscle, and brain were analyzed from four pufferfish species (*Lagocephalus sceleratus*, *Lagocephalus spadiceus*, *Lagocephalus suezensis* and *Torquigener flavimaculosus*) to elucidate whether dentin can be used as an indicator tissue type for bioaccumulation of heavy metals to fish.

METHODS AND MATERIALS

Four individuals for each pufferfish species *L. sceleratus*, *L. spadiceus*, *L. suezensis*, and *T. flavimaculosus* were captured in the between of Goksu River and Yeşjlovacık coasts by a commercial trawler. Dentin, muscle, gill, liver and brain tissues from the 4 individuals for each pufferfish species were dissected and preserved in the laboratory.

The soft tissues (muscle, gill, liver, and brain) (0.05 g dry weight) used for metal analysis were dried at 105°C to reach constant weights and then concentrated with nitric acid (1 mL, Merck) and perchloric acid (0.5 mL, Merck), which were put on a hot plate set to 150°C until all tissues were dissolved as described.

Teeth were washed with 30% H_2O_2 solution in sterile plastic containers for 1 h. Soft tissues and blood tissue on the teeth were removed using a brush and H_2O_2 solution. The teeth were rinsed with deionized water. Teeth were mechanically pulverized using a grinder. The powdered teeth were washed several times with 30% H_2O_2 solution in sterile plastic cups and then filtered. The powdered teeth were weighed (0.08–0.10 g) and placed in glass tubes using a plastic spoon. The HCl (2 mL, Merck, Hydrochloric acid fuming 37%), HNO_3 (6 mL, Merck, Nitric acid 65%) and H_2O_2 (2 mL, Merck, Hydrogen peroxide 30%) were added to the samples, which were put on a hot plate set to 150°C until all tissues were dissolved. Then dilutions and pH adjustments of the samples were made.

Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent, 7500ce Model) was used to determine metals. ICP-MS operating conditions were as 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. The elements (Co, Cu, Zn, Fe, Mo, Ni, Se, Al, Cd, Pb, As, Cr) in the samples were detected as μg metal per gramm of dry weight. High Purity Multi-Standard (Charleston, SC 29423) was used for the determination of the metal analyses. Standard solutions for calibration curves were prepared by dilutions of the elements. The prepared standard solutions had a content of lead, cadmium, arsenic, nickel, aluminium, molybdenum, cobalt and chromium in the range of 1–50 ppb (0.001–0.050 mg/L) for the metals, and content of copper, iron, and zinc in the range of 1–50 ppm (1–50 mg/L) for the trace elements. Variance analysis techniques were used for the experimental data using the SPSS statistical package program.

RESULTS AND DISCUSSION

Fe, Se, As, Zn, Cu, Cr, Al elements were found in tissues of *L. sceleratus*, *L. spadiceus*, *L. suezensis* and *T. flavimaculosus* at a significant level ($P < 0.05$) while Co, Ni, Mo, Cd, Pb were found under detection limits in all tissues ($P > 0.05$). In the dentin tissue, Al and Zn metals were found to be higher in *L. spadiceus*, and Cr and As metals were higher in *T. flavimaculosus* compared to other species (Table 1).

The comparisons of heavy metal levels in dentin and soft tissues are shown in Table 1. The metal levels in dentine tissue were found to be similar to the brain. Aluminium had the highest accumulation in these tissues. The liver and gill tissues showed the same bioaccumulation properties with each other. Zn had the highest accumulation among these tissues ($P < 0.05$). The muscle was different from metabolically active tissues in terms of metal accumulation that arsenic had the highest bioaccumulation ($P < 0.05$).

The relative accumulation of elements in the dentin, liver, gill, muscle and brain tissues was found to be Al > Zn > Cr > Fe > As > Cu, Zn > Fe ≥ As > Al > Cu > Cr ≥ Se, Zn > As ≥ Fe ≥ Al > Cr ≥ Se ≥ Cu, As > Zn > Al ≥ Fe ≥ Cr ≥ Se ≥ Cu, Al > Zn > Cr ≥ Fe ≥ Cu > As ≥ Se, respectively (Table 1).

Deposition levels of each metal examined in tissues are compared in Fig. 1 that Cr, Al, and Cu were accumulated more in dentine and brain tissues than that other tissues. Although muscle tissue showed the lowest deposition, As and Se were at a higher concentration than other metals examined in muscle tissue. Se was not detected in dental tissue. Cu, Cr and As were higher in *T. flavimaculosus*, and Zn and Se were higher in *L. spadiceus* than in other species (Fig. 1).

Aquatic ecosystems are the main receptors where pollutants are discharged to the environment [1]. For this reason, aquatic organisms are preferred as biological material in environmental monitoring studies

Table 1. The comparison of metals with the tissues of pufferfish

Tissue	Metals, μg/g	L. sceleratus	L. spadiceus	L. suezensis	T. flavimaculosus
Dentin	Al	738.91	819.16	614.98	705.88
	Zn	390.18	557.82	352.6	277.49
	Cr	135.33	168.75	132.02	261.68
	Fe	64.06	92.75	74.73	88.45
	As	37.47	39.43	30.91	61.98
	Cu	17.66	23.99	24.74	24.67
	Se	0	0	0	0
Liver	Al	13.97	10.95	9.1	51.11
	Zn	634.83	263.18	377.36	229.35
	Cr	5.35	3.33	3.39	13.16
	Fe	36.62	55.78	123.81	123.74
	As	116.01	60.26	30.54	61.23
	Cu	10.5	4.65	3.95	32.2
	Se	5.6	4.87	3.2	4.18
Gill	Al	74.77	89.16	166.26	243.9
	Zn	125.57	3396	2292.4	893.66
	Cr	11.51	15.45	23.49	64.96
	Fe	98.48	116.96	88.93	95.13
	As	153.9	145.87	105.94	259.4
	Cu	3.79	7.21	14	14.48
	Se	4.64	10.13	5.09	5.57
Muscle	Al	12.21	13.58	12.99	11.7
	Zn	36.48	42.66	36.94	73.92
	Cr	4.43	6.74	4.81	30.26
	Fe	4.92	4.98	3.77	14.09
	As	53.26	119.39	85.4	168.55
	Cu	1.11	1.54	1.37	4.99
	Se	2.38	4.16	2.12	2.34
Brain	Al	738.91	819.16	614.98	705.88
	Zn	203.48	137.77	207	279.65
	Cr	78.84	117.95	11.28	250.39
	Fe	63.23	69.4	44.54	85.08
	As	11.42	107.76	79.16	153.57
	Cu	13.83	20.97	15.1	57.16
	Se	9.14	12.19	0	0

[7]. The pufferfish are non-indigenous species in the Mediterranean that do not have predators in the food chain. Therefore, the determination of heavy metal accumulation in these fish tissues can be considered as natural chelation for the aquatic environment.

The present study for the first time revealed that dentin tissue can be used as an indicator for toxicology studies that metal accumulations in dentin were determined in parallel with other examined tissues of pufferfish species. Cr and Al were accumulated in dentin tissue of pufferfish species at the highest level while Se was not detected. This is due to the fact that the passage of Cr and Al through the Ca channels is more difficult than in other metals. On the other hand, calcium-rich dentine tissue may chelate these met-

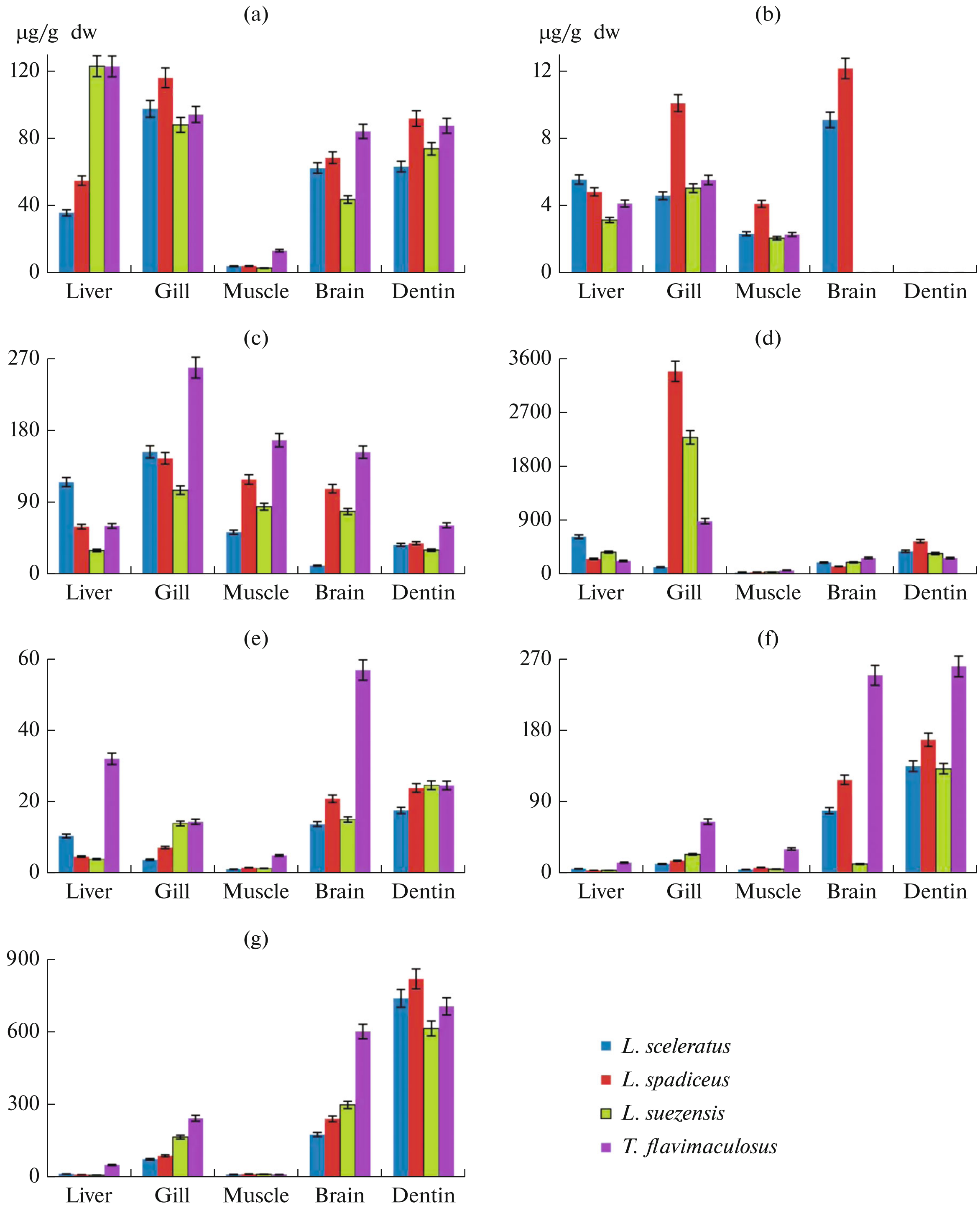

Fig. 1. The comparison of tissues according to the metals: (a) Fe; (b) Se; (c) As; (d) Zn; (e) Cu; (f) Cr; (g) Al.

als. Dentine and brain tissue were similar in terms of metal accumulation which may be related to that brain and bone tissues are the last storage place for heavy metals.

The present finding is supported by studies conducted in mammals [5, 6]. Dentin tissue was used in mammals as an indicator for toxicology studies because it is stable in contrast to other tissues and it can bind some metals in its mineral phase. Accumulation of Ni, Cr, Mn, Pb, Cd, Hg and Zn were determined at high levels in human dentin tissue in pollution monitoring studies [8]. It has been reported that the accumulation of Pb, Cd, Zn, Cu and St in the dentin tissue of *Clethrionomys glareolus*, mice, living in contaminated industrial pollution were high. It is also indicated that the properties of dentin tissue allow the determination of metal accumulation according to the life stages of organisms [6].

Fe is an important trace element for life, having functions in oxygen transfer, respiratory chain reactions, DNA synthesis and the immune system, but, over dose cause to heart disease, cancer, and impaired insulin sensitivity [9]. In this study, Fe was found at the highest level in liver and secondly in gill tissue of pufferfish. The reason for the presence of iron at the highest concentrations in liver and gill tissue can be explained by the metabolic activity of the tissues as well as the essential properties of Fe.

Zn is a trace element that has a second importance in biological systems after Fe and has an active role in carbohydrate, protein, lipid, and nucleic acid metabolisms as well as the immune system, neurotransmission and cell transmission [10]. The limits for Zn concentrations in fish species according to the Food and Agricultural Organization varied between 98 and 303 μg/g [11]. On the other hand, in the present study, Zn was found at the highest level in gill (893.66 μg/g) and liver (634.83 μg/g) tissues. The main intake routes of heavy metals are fish gills and digestive system, thus the highest accumulation of Zn in gill tissue can be explained in this way. Zn has generally been considered to be non-toxic and a minor amount is required for growth and development. However, extremely high intakes of Zn beyond the recommended dietary allowance (15–100 mg/day) can pose to symptoms like nausea, vomiting, lethargy and fatigue [12].

Se is involved in the structure of proteins in fish and produces enzymes called selenoprotein which act as antioxidants. Se has a duty in thyroid metabolism with antioxidant enzymes such as glutathione peroxidase, thioredoxin reductases and with a selenoenzyms such as iodothyronine deiodinases. The toxic effect of Se results from the production of selenomethionine in combination with other sulfur-containing amino acids such as methionine. Nanomaterials with Se content such as Quantum dots have been reported to reach the brain via olfactory bulb in *Micropterus salmoides* that may be the reason for the detected highest accumulation of Se in the brain tissue among the others in the present study [13].

As is found in different chemical forms in nature and its accumulation and toxicity in the organism can easily change due to the influence of environmental factors. As induces epidemiological toxicity, damaging organisms by producing excessive Reactive Oxygen Species (ROS). It has also cytotoxic and genotoxic effect on human [14]. Besides, it is well known that chronic exposure to arsenic can lead to arsenicosis, including skin lesions, blackfoot disease, peripheral vascular disease and cancers [15]. In this study, accumulation was higher in gill and muscle tissue compared to liver tissue. The high level of As in the gill tissue may be due to the fact that this tissue has an excretion mechanism as well as intake mechanism. The biomethylation of As may be the reason for the detected high accumulation in the muscle tissue.

Cu is a basic micronutrient, but in high concentrations becomes toxic to aquatic organisms [16]. In the present study, Cu was found at the highest level in the brain, and less in muscle tissues. It has been reported that the liver tissue accumulates at a higher level of Cu in terms of its functional characteristics. However, it was found in high concentrations in gonad, brain and bone tissues [17]. Papagiannis et. al. [18], reported that copper in four different fish species sampled from Pamwotis Lake was accumulated in high concentrations liver [19]. Cu has a potential neurotoxic effect and its accumulation in the brain tissue of *Oncorhynchus mykiss* revealed inhibition of antioxidant enzymes activity by increasing lipid peroxidation and protein oxidation [20]. In addition, Cu may also cause additive toxic effects in association with Zn [21].

Although Cr is an essential element that functions in carbohydrate, lipid and protein metabolism at low concentrations in the form of +3 in an animal, Cr has poisonous effect for some fish species such as anaemia, zosinophilia and lymphocytosis, bronchial and renal lesions. Cr concentration in rivers and lakes range between 1 to 10 μg/L, and permissible level recommendation according to EPA are between 50 to 100 μg Cr/L for protection of human health and aquatic life. Cr is known for its lesser accumulation in fish bodies while the higher concentrations of Cr damage the gills of fish swimming near point of Cr disposal [22]. In the present study, Cr was found in high concentrations in dentin and second in the brain tissue of four different pufferfish species. Cr has high oxidation ability at high concentrations that DNA damage was detected in the liver and brain under the effect of 24 and 96 h at a concentration of 43.7 mg/L Cr + 6 in *Oreochromis sp.* [23]. Moreover, the effects of Cr^{+6} caused histopathology in gill, liver and kidney tissue, changed in plasma cortisol level and slowing grow that 2 and 4 mg/L in *Channa punctata* [24].

Al is a non-essential element for the animal so accumulation even at very low concentration causes toxic effects. In the present study, Al was accumulated at higher concentrations in the dentin and secondly in brain tissue than that the liver and muscle tissue. The removal of Al from the environment is carried out with ionic or suspended solids that the high concentration of Al in the dental tissue can be explained by the accumulation of ionic Al form in the mineral phase. The high concentration of Al in brain tissue supports the neurotoxic effects of this metal. Different forms of Al in Al contaminated regions were found at high concentrations in the liver, kidney, skeleton and gill tissue of different fish species [1].

Heavy metal toxins contribute to a variety of adverse health effects. There are over 23 different heavy metal toxins that can impact human health and each toxin will produce different behavioural, physiological, and cognitive changes in an exposed individual [25]. Knowledge of heavy metal concentrations in fish is important both with respect to nature management and human health. The toxicity of metals most commonly involves in the brain and the kidney, but other occurred manifestations caused by some metals such as arsenic are capable of causing human cancer [14, 15].

Bioaccumulation was changed depending on biotic and abiotic factors in metal-contaminated regions as well as functional differences between tissues. The liver and gill are metabolically active tissues because of their working properties while muscle and dentin tissues metabolically less active. Therefore, dentin tissue may contribute to time-dependent monitoring studies because of the protection of dentin tissue due to its stable structure. Moreover, the mineral phase of dentin tissue can display a chelation effect for heavy metals.

In conclusion, the different bioaccumulation levels were found in each pufferfish species in terms of analyzed metals and tissues. Accumulation of Fe, Se, As, Zn, Cu, Cr and Al in pufferfish can be effectively used for metal pollution monitoring in the marine environments. The relative accumulation of metals in dentin tissue of pufferfish was determined as Al > Zn > Cr > Fe > As > Cu. Therefore, pufferfish dentin can be used as an indicator tissue for bioaccumulation of heavy metals in fish.

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