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DETERMINATION OF SOME TRACE ELEMENT LEVELS IN DIFFERENT SEASONS IN MUSCLE, LIVER AND BRAIN TISSUES OF *CLARIAS GARIEPINUS* (BURCHELL, 1822)

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ABSTRACT

African sharp-tooth catfish known as *Clarias gariepinus* in international literature is the most common fish species in among freshwater fishes. We investigated levels of Al, Cu, Zn, Fe, Mn and Cr elements in muscle, liver and brain of *C. gariepinus* in different seasons. The fishes used in experiment was obtained in Ceyhan river basin where is situated the south of Turkey. This study was divided as winter group (n=4, female fish) and summer group (n=4, female fish). The organs used in experiment that include muscle, liver and brain tissues were incised. These organs were extracted and were analyzed by AAS (Flame Atomic Absorption Spectrometer) for determination of elements. The accumulation of Al element in summer is lower than winter in muscle tissue, but it is higher in the brain ($p<0.05$). The accumulation of Mn element in summer is higher than winter in brain tissue ($p<0.05$). The accumulation of Fe element in summer is lower than winter in only muscle tissue ($p<0.05$). However, the accumulation of Cr element wasn't evaluated statistically due to below the detection limit. According to obtained results from evidences, accumulation of determined trace elements in various tissues were determined statistically differentness as seasonal. As a result, we suggest that trace element levels may be changed in *C. gariepinus* tissues because of the change of its habitat conditions.

KEYWORDS:
Clarias gariepinus, trace element, muscle, liver, brain, seasonal variation.

INTRODUCTION

C. gariepinus known as African sharp – tooth catfish [1] is the most common fish species in among freshwater fishes [2]. *C. gariepinus* has faster growing ratio, an omnivorous nutrition habit and a resistance against environmental stress in high level in the recent years [3, 4]. African catfishes usually feed with insect larva and various invertebrates [5]. Moreover this species feeds with dead birds, rotten fruits and plant's seed [6]. Copper (Cu) that our studied one of the elements is found as +1 and +2 valency in biological systems and plays a role in the events of the oxidoreduction [7]. Cu is an element that is found very common in nature and is needed in trace amount for organisms [8]. It functions in osteogenesis, development of heart, myelination of spinal cord and tissue pigmentation [9]. Zinc (Zn) is biotic and significant element for humans, animals and plants. It is an essential element for growing, skin coherence and function, ovum maturing, immune system power, wound healing and carbohydrate, lipid (fatty), protein, nucleic acid synthesis [10]. In addition to, this element usually is found trace levels in aquatic environment and concentration of zinc increasing with originating from anthropogenic factor of industrial, mining and agricultural activities [11]. Iron (Fe) element is required for heme protein production in oxygen transport [12]. Fe is bioaccumulation of high level element in *C. gariepinus* species [13]. Aluminum (Al) causes to acute ion regulation, respiratory disorders and accumulation Al^{+3} in freshwater fishes [14]. Manganese (Mn) is found as +2 and +3 valency that has function as component of both enzyme and metalo enzyme [7]. These enzymes have a significant role in detoxification of unchained superoxide radicals in organisms [15]. Chrome (Cr) element has some form that is found as natural in rocks, animals, plants, soil, volcanic dust and gases. Most common of these element forms are Cr^0, Cr^{+3}, Cr^{+6} [16]. Behavior of chrome in alive organisms depends to oxidation level, chemical features in oxidation level and physical structure in its environment [17]. Cr level in air and water usually is low. Because of this, nutrients like fruits, plants, meat and ferment creates a significant kind of chrome which took by humans [16].

TABLE 1
Al, Cu, Zn, Fe, Mn and Cr quantities were quantified different in muscle, liver and brain tissues of African sharp-tooth catfish in winter (µg/g) (n=4).

		ELEMENTS					
TISSUES	VALUES	Al	Cu	Zn	Fe	Mn	Cr
Muscle	Min.	6,40	BDL	3,80	7,05	BDL	BDL
	Max.	12,00	0,07	14,20	10,89	0,72	BDL
	Mean	8,9000	0,0175	8,1500	8,6375	0,1975	-----
	SE	1,18181	0,01750	2,22467	0,81053	0,17495	-----
Liver	Min.	5,00	6,72	20,20	61,80	1,35	BDL
	Max.	7,40	68,44	125,80	1401,80	5,00	BDL
	Mean	6,4500	27,2875	50,0500	530,0800	2,7275	-----
	SE	0,58523	14,04735	25,45864	307,43143	0,85511	-----
Brain	Min.	8,95	BDL	1,76	3,78	BDL	BDL
	Max.	18,20	BDL	12,11	14,99	BDL	BDL
	Mean	12,1825	-----	4,9200	8,0300	BDL	-----
	SE	2,14873	-----	2,42243	2,49113	BDL	-----

*BDL: Below Detection Limit, SE: Standard Error.

MATERIALS AND METHODS

In this study, African sharp-tooth catfish (*Clarias gariepinus*) has been obtained four numbers in both winter and summer from drainage canals in Ceyhan-Adana/Turkey river basin.

Preparation of Fish Sample to Trace Element Determination

Muscle, liver and brain tissues of *C. gariepinus* was incised with the help of a sterile scalpel. Afterward, incised samples were left to dry in 45 degree incubator for 4 – 5 days. Seared samples were weighed in assay balance and weights of these samples were standardized to 0.5 gram. Then, weighed samples were placed to Teflon® (polytetrafluoroethylene) tubes conjunction with 1 ml concentrated perchloric acid (Sigma-Aldrich, Germany) and 5 ml concentrated nitric acid (Sigma-Aldrich, Germany). Next, this acid mixture was waited one hour in order to interpenetrate to samples. Thereafter, Teflon® tubes had been placed to microwave solubilization device and had been interacted with 450 watt microwave in 15 minutes four periods. After these processes, solutions are filtered and 1 ml filtered solution is incased cylindrical graduates. Then, 9 ml distilled water is added on 1 ml filtered solution. After, prepared solutions are analyzed in flame AAS (Flame Atomic Absorption Spectrometer) [18].

Statistical Analysis

IBM SPSS 21 program was used in statistical calculation. One – Way Anova Test was made to experiment results. On the other hand, Kruskal – Wallis Test was made for determine to relation between metal accumulations and seasons. Then, “t” test was applied for determine to difference degree of heavy metal values among studied organs [19].

RESULTS

Research Findings. Al, Cu, Zn, Fe, Mn, and Cr quantities were showed respectively in muscle, liver and brain tissues of *C. gariepinus* species in tables. According to “Table 1”, most accumulation was in brain, and least accumulation is in liver in Al element. On the other hand, most accumulation in Cu element is in liver, but least accumulation is muscle tissue. Most Fe accumulation is in liver, but least accumulation is in muscle tissue and brain. However, Cr can’t be determined due to it was below determine limit in any tissues.

According to “Table 2”, most Al accumulation is in brain, but least accumulation in muscle tissue. On the other hand, most Cu accumulation is in liver, but least accumulation muscle and brain tissues. Least Zn accumulation is in brain. Least Fe accumulation is in muscle tissue and brain. Most Mn accumulation is in brain, but least accumulation is in muscle tissue. However, Cr element can’t be determined due to it was below determine limit in any tissues.

According to “Table 3”, the accumulation of Al element in summer is lower than winter in muscle tissue, but it is higher in brain and these quantities are significant statistically ($p<0.05$). Moreover the accumulation of Mn element in summer is higher than winter in brain that this quantity is significant statistically ($p<0.05$). The accumulation of Fe element in summer is lower than winter in only muscle tissue and this quantity is significant statistically ($p<0.05$). However, the accumulation of Cr element can’t be determined.

TABLE 2
Al, Cu, Zn, Fe, Mn and Cr quantities were quantified different in muscle, liver and brain tissues of African sharp-tooth catfish in summer (µg/g) (n=4).

		ELEMENTS					
TISSUES	VALUES	Al	Cu	Zn	Fe	Mn	Cr
Muscle	Min.	4,98	BDL	2,97	2,45	BDL	BDL
	Max.	5,20	BDL	3,20	2,62	BDL	BDL
	Mean	5,0750	-----	3,0875	2,5300	-----	-----
	SE	0,05188	-----	0,05963	0,03629	-----	-----
Liver	Min.	5,93	5,10	15,30	54,12	0,86	BDL
	Max.	6,32	5,23	15,80	56,80	1,13	BDL
	Mean	6,1125	5,1650	15,5675	55,6150	0,9850	-----
	SE	0,08976	0,03227	0,10274	0,57454	0,05545	-----
Brain	Min.	19,97	BDL	2,15	3,38	2,56	BDL
	Max.	20,34	BDL	2,34	3,67	2,70	BDL
	Mean	20,1950	-----	2,2275	3,4900	2,6350	-----
	SE	0,08026	-----	0,04029	0,06646	0,02901	-----

*BDL: Below Detection Limit, SE: Standard Error.

TABLE 3
Trace element quantity of muscle tissue, liver and brain (µg/g) in winter and summer so comparing of these seasonal quantity (p < 0.05).

		ELEMENTS					
	TISSUES	Al	Cu	Zn	Fe	Mn	Cr
WINTER (mean)	Muscle	8,9000	0,0175	81,5000	8,6375	0,1975	BDL
	Liver	6,4500	27,2875	50,0500	530,0800	2,7275	BDL
	Brain	12,1825	BDL	4,9200	8,0300	BDL	BDL
SUMMER (mean)	Muscle	5,0750	BDL	3,0875	2,5300	BDL	BDL
	Liver	6,1125	5,1650	15,5675	55,6150	0,9850	BDL
	Brain	20,1950	BDL	2,2275	3,4900	2,6350	BDL
p value	Muscle	0,018*	0,356	0,063	0,000*	0,302	-----
	Liver	0,589	0,166	0,224	0,174	0,088	-----
	Brain	0,010*	-----	0,309	0,118	0,000*	-----

(*) This symbol signs statistical significant difference of each parameter in tissues, BDL: Below Detection Limit.

DISCUSSION AND CONCLUSIONS

Heavy metals in low concentration get high concentrations with anthropogenic factor effects in natural aquatic environment and this condition causes to habitat changes, collective deaths or changes in biotic events [20]. Biological factors like chemical features, organic compounds, growth rate, diet and habitat selecting affect to accumulation of heavy metal in fishes [21, 22]. Heavy metal accumulation and toxic effects of these elements correlate with biotic and abiotic factors of environment were shown in previous studies [23, 24].

In this study, Al element accumulation of fishes in winter was obtained least in liver. On the other hand, Al accumulation levels among tissues in winter were sorted as "liver < muscle < brain", but were sorted as "muscle < liver < brain" in summer. Moreover according to relation between accumulation of Al element and seasons, muscle showed statistically significant decreasing ($p<0.05$), but brain showed statistically significant increasing ($p<0.05$).

Accumulation of Cu element on below detection limit in brain was accepted least in winter and accumulation of Cu element on below detection limit in muscle and brain were accepted least in summer. On the other hand, Cu accumulation levels among tissues in winter were sorted as "brain < muscle < liver", but were sorted as "muscle ≈ brain < liver" in summer. Moreover according to relation between accumulation of Cu element and seasons, decreasing in muscle and liver were not obtained as statistically significant ($p>0.05$), and Cu element accumulation of brain on below detection limit wasn't calculated statistically. In Asi River, Cu element values of muscle tissue in previous studies were lower than values of this study. These variations in results can be originated from terrestrial environments features, industrial, domestic and agricultural activities in environments of research areas. As a result, low Cu element level in this study shown less pollution in this area water than last time. [16, 25-27]. The accumulation of Zn element in brain was determined least in both winter and summer. In addition, Zn accumulation levels among tissues in winter were sorted as "brain < muscle < liver", but were sorted as "brain < muscle < liver" in summer. On the other hand, according to relation between accumulation of Zn element and seasons, decreasing in any tissues were not obtained statistically significant ($p>0.05$). The accumulation of Fe element in winter was determined least in brain, but in summer was determined in muscle tissue. In addition, Fe accumulation levels among tissues in winter were sorted as "brain < muscle < liver", but were sorted as "muscle< brain < liver" in summer. Moreover according to relation between accumulation of Fe element and seasons, only muscle tissue showed statistically significant decreasing ($p<0.05$), but increasing or decreasing in other tissues showed statistically insignificant ($p>0.05$). Accumulation of Mn element on below detection limit in brain was accepted least in winter and accumulation of Mn element on below detection limit in muscle tissue was accepted in summer. In addition, Mn accumulation levels among tissues in winter were sorted as "brain < muscle < liver", but were sorted as "muscle < liver < brain" in summer. According to relation between accumulation of Mn element and seasons, decreasing in accumulation levels of Mn element in muscle and liver showed statistically insignificant ($p>0.05$). Accumulation of Cr element levels on below detection limit can't be evaluated statistically. In among seasons, the accumulation of element was usually least in summer and most in winter. In among tissues, it was usually least in muscle tissue and most in liver tissue. On the other hand, in last studies, the accumulation of heavy metal in fish tissues was determined least in muscle tissue and most in liver tissue [25, 28, 29].

In this study, most accumulation of element was determined in liver tissue. Moreover according to study of Karadede et al. (1997), the accumulation of Cu, Fe and Zn elements in *Mastacembelus simack* in Atatürk Dam Lake was most in liver tissue [30].

According to study of Mendil and Uluozlu (2007), Fe element was most accumulated element in *Silurus glanis, Capoeta tinca, Leuciscus cephalus, Carassius gibelio* and *Cyprinus carpio* were obtained from Belpinari, Atakoy, Bedirkale, Akin, Boztepe and Avara lakes [31].

In this study, liver showed higher accumulation than muscle tissue in elements were obtained without Cr in both summer and winter. According to study of Dogan (2004) in Hatay, the concentration of heavy metals in *Carasobarbus luteus* were variable in skin, liver and muscle tissue, but metal accumulation in liver was more than the accumulation in muscle tissue [25].

As a result, the accumulation values of Al, Cu, Zn, Fe, Mn and Cr elements in this study are less than acceptable limit values in literature. Therefore, significant accumulation can't be observed in tissues of African sharp - tooth catfish. According to us, trace element accumulation level in same tissues which show statistically difference in different seasons can be originated from dietary value of water which is habitat of fishes and changes in habitat conditions.

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