

# Relationship Between Trace Element Content in the Brain of Bony Fish Species and Their Food Items in the Southwest of the Caspian Sea Due to Anthropogenic Activities



Masoud Sattari<sup>1,2\*</sup>, Mohammad Forouhar Vajargah<sup>1</sup>, Mehdi Bibak<sup>1</sup>, Shima Bakhshalizadeh<sup>2</sup>

<sup>1</sup>Department of Fisheries, Faculty of Natural Resources, University of Guilan, Sowmeih Sara, Iran

<sup>2</sup>Department of Marine Sciences, Caspian Sea Basin Research Center, University of Guilan, Rasht, Iran

## \*Correspondence to

Masoud Sattari,  
Email: [msattari@guilan.ac.ir](mailto:msattari@guilan.ac.ir)

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## Abstract

The trace elements (TEs) in aquatic environments due to anthropogenic activities are readily available to aquatic organisms. There are 153 fish species inhabiting the Caspian Sea. However, little is known about TE (TE) concentrations in these species. In this study, we investigated the effects of TEs levels in the brain of some bony fish species inhabiting the Caspian basin. Totally, 405 fish from nine species with different feeding behaviors including *Leuciscus aspius* (n = 20) and *Perca fluviatilis* (n = 37) as piscivores, *Rutilus kutum* (n = 27) and *Rutilus caspius* (n = 71) as carnivores, *Vimba persa* (n = 56), *Ponticola caspia* (n = 25) and *Tinca tinca* (n = 31) as mollusivores, and *Alburnus chalcoides* (n = 77) and *Alosa braschnikowi* (n = 61) as zooplanktivores were collected from the southwest of the Caspian Sea basin from September 2017 to June 2018. The ICP-OES was used to measure TEs in the brain tissue of fish. The variability of TEs concentrations in the brain tissues of these fishes by principal component analysis (PCA) was decreased to 63.59% and 17.68% for PC1 and PC2, respectively, exhibiting that 81.27% of the total variability is associated with K, Mg, P, S, Zn, and Al. The two-dimensional diagrams showed the weight of each component in PCA. The PC1 was mostly influenced by P, Mg, K, and Zn, while the greatest value in PC2 belonged to Al. Furthermore, the entire number of elements determined in this study was found to be a suitable indicator for the distinction between fish species based on their feeding items.

**Keywords:** Fish, Caspian Sea basin, Trace elements, Bony fish, Feeding behavior

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## 1. Introduction

The role of nutritional behavior in mental activity, especially neuroscience is an apparent inter-disciplinary field of investigation seeking to understand the impact of feeding items on brain health across the lifespan (1). Research in this field has recently prospered and illustrated aspects of food from entire food items to specific elements affecting brain function and structure, and so have important roles in understanding the life history events (2). The influences of dietary elements on the neuronal function that are responsible for the action of food items on the brain have been proved (3). However, the diet has traditionally been classified as a means to provide the body with energy and building material. The role of micronutrients in the function of the brain has received the consideration of many researchers over the history of nutrition science (4). Some TEs including Zn, Mn, and Co are necessary for the function and growth of the human brain, while some others such as Hg and Pb create adverse effects on them (3,4), although these effects

have not yet been studied in fish.

On the other hand, for estimating the risk of environmental pollutants in the aquatic organism, trace element (TE) accumulations via ingestion, adsorption, and respiration should be assessed (5,6). The TEs accumulations are elevated by anthropogenic activities in marine environments (7,8). TEs can be classified as toxic (nickel, mercury, cadmium, arsenic, lead, etc.), necessary including cobalt and vanadium and also essential such as iron, manganese, copper, zinc, and selenium (6,9). Aquatic organisms such as fish are assessed as an appropriate index for the long-term accumulation of TEs in the aquatic environments. Thus, numerous authors have examined TEs accumulation in various fishes (8).

The Caspian Sea basin as the largest lake of the world has characteristics common to both seas and lakes but is not a freshwater one (10-12). It is bordered by the Republic of Azerbaijan, Russia (Astrakhan Oblast, Dagestan, Kalmykia), Turkmenistan, Iran (Mazandaran, Guilan, and Golestan provinces), and Kazakhstan. It is

highly polluted due to growing urbanization, agricultural production, and industrial activities in most riparian countries around the basin (13).

Various studies were carried out on TE contaminations in aquatic resources of Iran including those on inshore sediments (14), on two sturgeon species, i.e., *Acipenser stellatus* and *Acipenser persicus* (15) and also on *Mugil auratus* in the southern part of the Caspian Sea (16). Other studies were also carried out by Abtahi et al (17) on *Liza aurata* and by Ebrahimzadeh et al (18) on *Liza saliens* from the southern part of this sea. Additionally, another study was performed on *Liza abu* obtained from the Karoon and Karkheh watershed (19,20). Furthermore, Alipour and Banagar (21) examined some fish collected from the southeast Caspian basin in the Gorgan bay and also *Silurus glanis* from Anzali lagoon (22). TEs levels were also investigated in the liver and muscle tissues of *Tinca tinca* and *Perca fluviatilis* in Anzali Wetland (23) as well as *R. kutum* by Mirzajani et al (24) but there is no study on the brain of fish from this basin. Therefore, the main objective of this investigation is to distinguish the concentrations of some TEs in the brain tissue of fish from the southwest of the Caspian basin and compare these levels in the brain tissue of fish with different food items.

## 2. Materials and Methods

This experiment was carried out at three fisheries, including Anzali, Kiashahr, and Astara in the south-west of the Caspian Sea basin (Fig. 1). Samples were obtained from September 2017 to January 2018, and totally 405 fish from nine species with different feeding behaviors were sampled including *Leuciscus aspius* (n = 20), and *Perca fluviatilis* (n = 37) as piscivores, *Rutilus kutum* (n = 27) and *Rutilus caspius* (n = 71) as carnivores, *Vimba persa* (n = 56), *Ponticola caspia* (n = 25) and *Tinca tinca* (n = 31) as molluscivores, and *Alburnus chalcoides* (n = 77) and *Alosa braschnikowi* (N = 61) as zooplanktivores. The samples were transferred to the laboratory using a cooler box at 4°C. The specimens were washed with pure water, the brain of samples was separated and oven-dried at 80°C for 18 hours (8). Ten millilitres of 65% nitric acid was used to digest 0.5 g of each fish tissue in a microwave and to extract TEs. Digested tissues were filtered through Whatman filter paper number 40 and finally were diluted with pure water to reach the exact volume. To determine the concentration of TEs in the specimens, ICP-OES (Zarazma Co. Tehran, Iran) was applied with megapixel charge coupled device detector arrays, facilitating simultaneous monitoring of TEs emission. To check the precision of the analytical method, a multi-element standard solution was applied for the calibration. Standard stock solutions of TEs at the concentration of 1000 mg L<sup>-1</sup> were used to produce the solutions with suitable dilution. Standard liquids were of analytical grade (Merck, Darmstadt, Germany). Distilled deionized

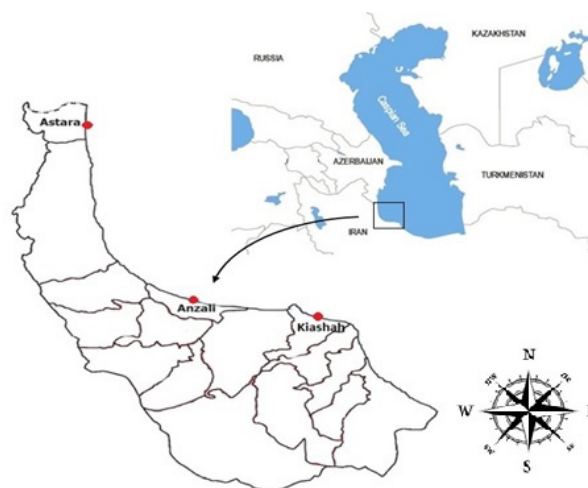


Fig. 1. Fish Sampling Sites in the Southwest of the Inshore Water of Caspian Basin.

water was applied in all dilution procedures. Instrument detection limits were 0.1 mg kg<sup>-1</sup> for major elements such as Fe and Al, and 0.02 mg kg<sup>-1</sup> for TEs.

### 2.1. Statistical Analysis

The TEs of the brain were examined for the homogeneity of variances and normality of the data. Then, one-way analysis of variances was performed to assess possible variability of TEs concentrations. Tukey's test was used to compare the differences between means. The Kruskal-Wallis test was employed for non-parametric cases (25). Principal component analysis (PCA) was applied for reduction of the number of variables without any data missing (26). The values of cumulative variances and also the number of principal components against eigenvalues were given to determine the important elements and principal components. The exact place of each specimen was determined by discriminant function analysis (DFA). Based on mean linkage method, cluster dendrogram using Euclidian distance was drawn by Ward's approach. This method was performed just as a complementary procedure to DFA. All statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL).  $\alpha = 0.05$  was considered statistically significant.

## 3. Results and Discussion

In general, human activities such as the establishment of factories, industrial centers, residential houses and the existence of commercial and fishing ports cause the entry of heavy metals into aquatic environments (27-29). The use of metal tools such as the hulls of commercial and fishing vessels and the entry of agricultural effluents containing pesticides are only a small part of human destructive activities in the environment, especially aquatic environments. Therefore, it is very important to study the amount of these harmful elements and metals in fish that directly affect human health.

Totally, 405 fish from nine species with different feeding

behaviors were dissected and their brain tissue was tested for the presence of 36 elements including antimony (Sb), arsenic (As), silver (Ag), aluminum (Al), bismuth (Bi), Barium (Ba), beryllium (Be), cesium (Ce), cobalt (Co), calcium (Ca), copper (Cu), iron (Fe), chromium (Cr), cadmium (Cd), lithium (Li), potassium (K), manganese (Mn), thorium (Th), magnesium (Mg), vanadium (V), lanthanum (La), rubidium (Rb), phosphorus (P), nickel (Ni), molybdenum (Mo), uranium (U), sodium (Na), lead (Pb), scandium (Sc), yttrium (Y), silicon (Si), sulfur (S), strontium (Sr), tin (Sn), titanium (Ti), tungsten (W), and zinc (Zn).

Calcium (Ca) exhibited different levels in brain tissues of the carnivorous, molluscivorous, piscivorous, and zooplanktivorous fish species ( $P < 0.05$ ; Table 1), displaying distinct amounts between molluscivores and zooplanktivores as well as between zooplanktivores and carnivores (Table 2). The variation of TE accumulations in the brain of the above-mentioned dietary fish groups by PCA was decreased in two components (PC1 = 63.59%, PC2 = 17.68%) (Figs. 2 and 3), which determined that 81.27% of the total variability was correlated with TEs such as K, Mg, P, S, Zn, and Al. The two-dimensional diagrams showed the weight of any component in PCA which was mostly affected by Mg, K, P, and Zn, in PC1 while the highest value belonged to Al in PC2 (Figs. 2 and 3, Table 3).

The matrix composed of element concentrations in brain tissue of fish species with different food items was described with two discriminant components (Wilk's Lambda = 0.06,  $\chi^2 = 25.49$ ,  $df = 6$ , and  $P < 0.05$ ) (Fig. 4). Cross-validated grouping in DFA was 77% more successful than simple replacement law. The dendrogram (Fig. 5) discriminated molluscivores from other feeding types. Although zooplanktivores were observed in two sub-groups, carnivores were assigned only to one group

which was distinct from piscivores and zooplanktivores.

In this study, we evaluated TEs accumulation in the fish brain as an indicator for different fish food items. Habitat, biological, physiological, and environmental parameters seem to be the reasons for different accumulation levels of

**Table 2.** Pai-wise Comparison (Mann-Whitney U-test) of the Ca Level Among the Four Feeding Types of Fish

Feeding type	M-W	Z	P
Piscivores-Molluscivores	0.00	-1.73	0.08
Piscivores-Carnivores	1.00	-1.39	0.17
Piscivores-Zooplanktivores	3.00	-0.46	0.64
Molluscivores-Carnivores	4.00	-0.71	0.48
Molluscivores-Zooplanktivores	0.00	-2.12	<0.05
Carnivores-Zooplanktivores	0.00	-2.31	<0.05

M-W and Z are grouping variable scores for Mann-Whitney and Kruskal-Wallis tests.  $P < 0.05$  was considered statistically significant.

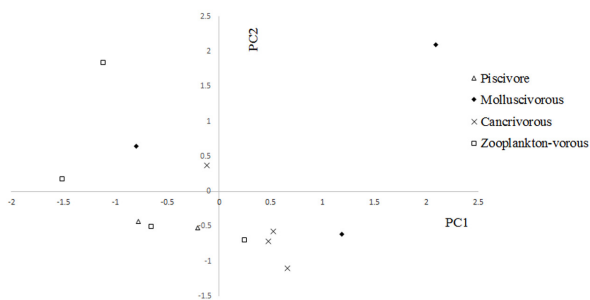
**Table 3.** Characteristic Loadings for PC1 and PC2 Resulting from PCA for Trace Elements Concentrations of the Brain in Different Feeding Types of Fish in the Southwest of the Caspian Basin

Elemental Variables	PC1	PC2
Al	0.033	0.819
Ca	0.827	0.472
Cr	0.876	-0.070
Fe	0.624	-0.212
K	0.908	-0.367
Mg	0.915	0.349
Na	0.766	-0.502
P	0.931	-0.265
S	0.919	-0.355
Si	0.761	0.181
Sr	0.654	0.628
Zn	0.908	0.219

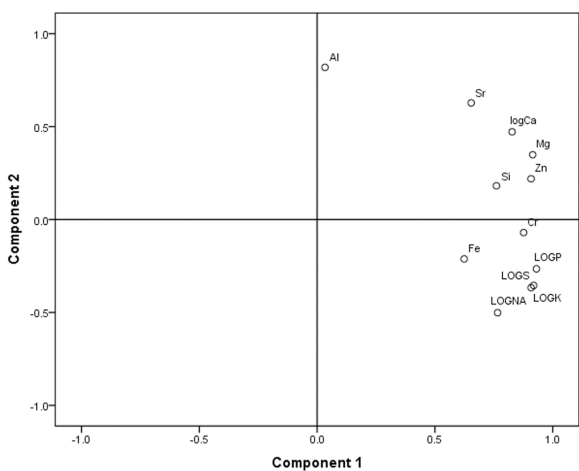
**Table 1.** Concentration of Trace Elements in the Brain of Different Feeding Types of Fish in the Southwest of the Caspian Basin

Elemental Variables (ppm)	Mean $\pm$ SE				P Value
	Piscivores	Molluscivores	Carnivores	Zooplanktivores	
Al	0.05 $\pm$ 0.05	0.19 $\pm$ 0.05	0.05 $\pm$ 0.03	0.13 $\pm$ 0.13	0.31
Ca	11.18 $\pm$ 5.08	220.95 $\pm$ 198.54	92.60 $\pm$ 68.11	9.86 $\pm$ 2.33	<b>0.04*</b>
Cr	0.00 $\pm$ 0.00	0.05 $\pm$ 0.03	0.05 $\pm$ 0.01	0.01 $\pm$ 0.01	0.08
Fe	0.41 $\pm$ 0.08	1.86 $\pm$ 1.15	0.61 $\pm$ 0.20	0.34 $\pm$ 0.18	0.18
K	34.70 $\pm$ 9.30	72.27 $\pm$ 31.36	62.76 $\pm$ 15.92	18.28 $\pm$ 7.96	0.22
Mg	3.35 $\pm$ 0.65	10.67 $\pm$ 4.36	8.46 $\pm$ 1.84	3.38 $\pm$ 0.99	0.13
Na	23.75 $\pm$ 12.25	28.87 $\pm$ 9.43	80.00 $\pm$ 23.74	21.15 $\pm$ 9.59	0.23
P	45.00 $\pm$ 24.90	110.80 $\pm$ 55.63	120.92 $\pm$ 44.21	25.73 $\pm$ 11.34	0.26
S	24.25 $\pm$ 5.25	59.43 $\pm$ 25.54	51.02 $\pm$ 13.59	14.35 $\pm$ 6.63	0.25
Si	0.00 $\pm$ 0.00	0.21 $\pm$ 0.05	0.15 $\pm$ 0.04	0.09 $\pm$ 0.06	0.12
Sr	0.10 $\pm$ 0.10	1.92 $\pm$ 1.71	0.49 $\pm$ 0.28	0.09 $\pm$ 0.03	0.13
Zn	0.55 $\pm$ 0.05	1.17 $\pm$ 0.41	0.84 $\pm$ 0.22	0.40 $\pm$ 0.07	0.19

\* $P < 0.05$  was considered statistically significant.



**Fig. 2.** Principal Component Analysis (PCA) of Trace Elements (TE) Concentrations in the Brain of Different Feeding Types of Fish from the Southwest the Caspian Basin. The scatter plots show fish scores for PC1 vs. PC2, which together describe the total variance of 81.27%.



**Fig. 3.** Traits Loadings for PC1 and PC2 Resulting from Principal Components Analysis (PCA) of Multi-trace Elemental Investigation of the Brain of Different Feeding Types of Fish from the Southwest of Caspian Basin.

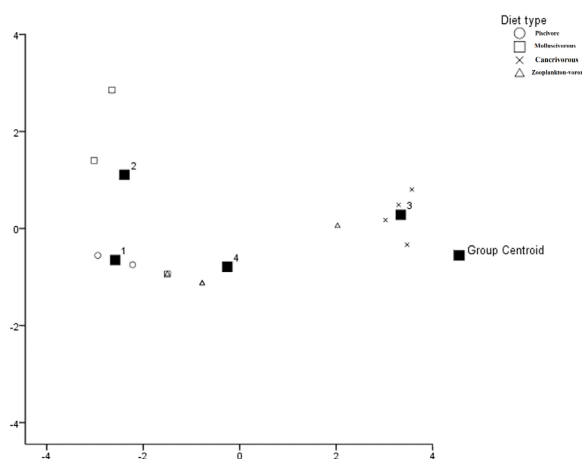
TEs in different types of fish food items (30,31). Overall, some parameters such as food items, age, size, genetics, and habitat influence TE accumulation in different tissues such as the organism’s brain (32,33). Most TEs enter the body via consumption of food items, which is an important way of TE accumulation inside the tissues (34,35). The TE concentrations in these tissues depend on so many parameters such as the kind of feed, biological characteristics (such as metabolic rate, swimming behavior, species, etc.), adsorption and disposal chemical characteristics, kinetics, TE bioavailability, environmental circumstances (alkalinity, temperature, salinity, etc.) and TE concentration in sediments and water (36). The bioavailability of TEs due to feeding is mostly dependent upon the feed structure and considerably varied among different fish species (37).

In *Scorpaena porcus*, as a sessile species (38), measuring TEs concentrations and isotopic compounds in its muscle reflects the food items consumed. Predatory fish in comparison with non-predatory fish, tend to accumulate

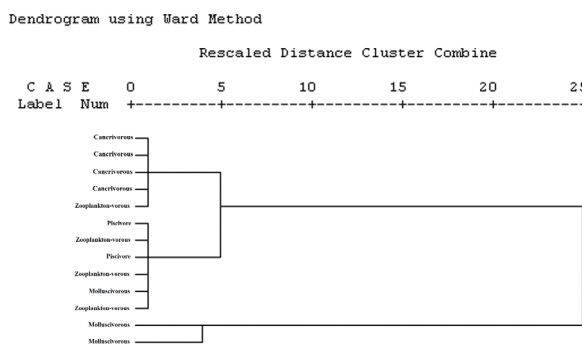
mercury, while benthic fish tend to accumulate cadmium (39,40). Briefly, there is a significant relationship between the food items of the aquatic organisms and the TEs concentration in their bodies (39). The local water contamination with some heavy metals such as mercury (Hg), in turn, leads to an increasing level of some elements in organisms inhabiting these localities (41) because the degree of contamination with the mercury in the sediments of the Caspian Sea was high; therefore, as expected, the concentration of this metal in carps was also high (30).

With the increase of size and age of the aquatic organism, a considerable increase in nitrogen (N) concentration was found, albeit with no detectable carbon (C) elevation. The elevated feeding levels in larger and older aquatic organisms are related to alterations in their food items and increased consumed dietary N (42).

Perga and Gray (43) observed different growth rates between adult and young fish based on morphological and physiological characters. The larger size of the fish mouth allows fish to ingest a wider range of preys with



**Fig. 4.** Scheme of Discriminant Operation 1 and 2 for the Trace Elements Concentrations of the Brain of Different Feeding Types of Fish from the Inshore Water of Caspian Basin.



**Fig. 5.** Dendrogram Derived from Cluster Analysis of Trace Elements Concentrations of the Brain of Different Feeding Types of Fish from the Southwest of the Caspian Basin.

larger sizes such as Brachyura, Caridea, and Teleostei (44-47) to afford their requirements (38), which in turn influence the accumulation of TEs in their body.

In the present study, significant differences in the level of most TEs were found based on fish food items and species. In the literature, gender exhibited no influence on TE accumulation, so we did not consider it. According to previous studies, As and Hg levels were higher in adult fish than in younger ones, while Cd, Ba, Pb, and Cu levels were reported to be higher in younger fish than in adults. Fish age had no significant effect on Ni, Zn, and Cr levels (30). Significant correlations were found between fish age, size as well as total nitrogen and Hg concentration, exhibiting that TE bioaccumulation over their lifespan is related to their feeding level. It was not true for other elements (30). Our results were in line with those studies which indicated the positive correlation between nitrogen level and feeding rate (48-52).

The lack of correlations between some TEs and nitrogen level and also fish age exhibit that these elements did not accumulate via feed consumption during the organism's life span, which is similar to results found by Ikemoto et al (54), Hao et al (55), and Zhang and Wang (56), while other studies revealed accumulation of As, Cd, and Pb in fresh and saltwater fish over their lifespan (57). However, some experimental studies displayed a low level of accumulation and a high level of dissipation of Cd and K, leading to a reduced potential of accumulation (58). However, their levels along with P, S, Zn, and Al exhibited marked alterations based on the fish food items. It was also true for Cd accumulation reported by other authors (59). Similar results were reported about the correlation of As and nitrogen in fishes of the North Atlantic Ocean (60,61).

Genetics besides food items is the other effective parameter influencing TEs accumulations in the organism tissues (62). Few reports are available about the effect of genetic characteristics on the TEs accumulation in humans and other animals, indicating that most of them were related to Cu and Zn. Ecotype influences TE composition in animal tissues. Therefore, their concentrations seem to be controlled by genetic characteristics (63,64). In the tissues of various animal species, the Cu, Mn, F, and Si levels were reported to be different from each other (65).

As mentioned before, gender is one of the useful parameters influencing TE accumulation in the organism tissues. Schepers (66) by injecting tetraethyllead and tetramethyl lead to rats found marked variations in the male and female rats in terms of Pb concentrations in their brain, liver, spleen, kidney, lung, and muscle. The Pb accumulations in all of these tissues (except for muscle) were higher in male rats than in females.

Soares et al (67) fed rats with different doses of methyl mercury (MeHg) and found that its accumulations in male and female rat were different, indicating that male had higher levels of Hg than female. The effects of gender

on TE composition in the blood and bone of male and female chicken have been studied by Vo et al (68). They found that there are significant differences in terms of the Mg, Ca, Na, and Cl levels in the blood, as well as the concentrations of Ca, Na, Mg, Zn, Fe, Mn, and P in bones between males and females.

The physiological circumstances of aquatic organisms, especially hormones and pregnancy, are among the effective parameters in TE accumulation in their tissues. Many TEs cause alterations in element metabolism inside an organism's tissues.

Injection of estrogen resulted in an increased Cu level and also elevated serum ceruloplasmin in many mammals (69,70). Furthermore, serum Cu levels increased in patients using contraceptive pills (71,72). Johnson et al (73) reported that testosterone leads to an increased serum Cu level in humans. Pregnancy also leads to elevated serum ceruloplasmin and consequently to an increased serum Cu level. Evans (74) suggested that elevated ceruloplasmin during pregnancy is, in fact, an effort to ensure the transmission of Fe and Cu to the growing fetus.

Diseases are also evident from TEs concentrations in the organism tissues. Different stressors lead to elevated TEs accumulations in many different species. However, exercise, inflammatory agents, and diseases are the most effective parameters (74).

Age is another parameter influencing TE accumulation in organism tissues. At present, neural damage due to aging is well known in monkeys and rodents. Many reports, for example, exhibited that TE composition is influenced by aging. Heumann and Leuba (75) reported that neuronal death (decrease in neurons) in the cerebral cortex of mouse occurs with age. It is also known that those mental diseases such as ischemia and its consequences may lead to elevated Ca and reduced K in the related tissue. Therefore, age-dependent alterations in K, Ca, and Rb levels may clearly lead to neural degeneration of the brain due to aging and the kind of food item. The organisms fed with food items belonged to low levels of the food chain than in piscivores which feed on the higher-level ones.

Habitat is also another factor affecting TEs accumulation in organisms. Feeding and also element concentrations are also notable parameters influencing TEs concentration in tissues of aquatic organisms. Little is known about the correlation between TEs accumulation in animal tissues and their concentrations in the ambient environment in the world. Environmental circumstances such as temperature are also effective factors in TEs concentrations inside the organism tissues. Additionally, these conditions exhibit their influences on calcium-containing tissues. Mueller (76) and de Andrade et al (77) reported that increased temperatures result in reduced Ca level in blood serum and consequent increase in Ca deposition inside some tissues. Therefore, at higher and more constant temperatures, aquatic organisms fed on

mollusks or benthivores exhibit higher Ca level in their brain than those consumed zooplanktons and also pelagic preys.

#### 4. Conclusion

In conclusion, while some TEs, including Zn, are necessary for brain function, some others such as Hg have adverse effects. The presence of TEs in different food item types reflects the importance of the TEs monitoring in environmental toxicology, health, and risk assessment. However, further studies on TEs accumulations in the fish brain are essential for the complete understanding of their role in fish brain activity as well as more surveys to understand long-term responses. Our study contributes towards these aims and sheds light on the specific roles of food item which need to be more investigated for the monitoring of aquatic contamination in fish besides genetic factors. Given the importance of human health, it is necessary to measure the amount of harmful metals that affect the health of the consumer. For this reason, it is better to do these studies annually. Accurate identification of contaminant source as well as determining the type of metal that enters the environment by contaminants can be of great help in such studies.

#### Authors' Contributions

MS: Supervision, writing, review, and editing; MFV: Methodology, sample analysis, sampling, sample perpetration, investigation, writing; MB: Methodology, sample analysis, sampling, sample perpetration, investigation, writing; SB: Software, writing, review, and editing.

#### Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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