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Trace Element Level in Different Tissues of *Rutilus frisii kutum* Collected from Tajan River, Iran

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Abstract Tajan River is among the most significant rivers of the Caspian Sea water basin. In this study, the concentration of Cr, Cu, Fe, Mn, Ni, Pb, Cd, and Zn were determined in brain, heart, liver, gill, bile, and muscle of *Rutilus frisii kutum* which has great economic value in the Mazandaran state. Trace element levels in fish samples were analyzed by means of atomic absorption spectrometry. Nearly all non-essential metals levels (Ni, Pb, Cd) detected in tissues were higher than limits for fish proposed by FAO/WHO, EU, and TFC. Generally, non-essential metals (Ni, Pb) were so much higher in muscle than the essential metals (Cu, Zn, and Mn) except Fe, which was higher than other metals in nearly all parts, except in gills. Fe distribution pattern in tissues was in order of heart > brain > liver > muscle > bile > gill. Distribution patterns of metal concentrations in the muscle of fish as a main edible part followed the sequence: Fe > Pb > Ni > Cu > Mn > Zn > Cd.

Keywords Heavy metals · *Rutilus frisii kutum* · Atomic absorption · Non-essential metals · Lead

Introduction

Manufacture, traffic, utilization, and disposal of many modern products cause trace metal release into the aquatic environment, and increasing attention is paid on how humans will be affected by this. Bio-monitoring of trace elements is essential to assess ecosystem health. Heavy metals can be categorized as: potentially toxic (Al, Cd, Pb, Hg), probably essential like Ni and Co, and essential like Cu, Zn, and Se [1]. The essential metals can also be toxic when in excess; elevated intake is of concern. There has been a growing interest to find out the heavy metal contamination level of public food supplies, particularly fish and fishery

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products, because they accumulate contaminants from the aquatic environment [1]. The Caspian Sea with 386,400 km^2 area is the biggest land-locked body of water bordered by five countries: Azerbaijan, Iran, the Russian Federation, Kazakhstan, and Turkmenistan. It has a rich source of natural resources and raw materials hosting a unique variety of living species and a developed natural economic system [2]. It has five major inlet rivers but no outlets and acts as a watershed reservoir for the region [3]. Tajan River is among the most significant rivers of the Caspian Sea water basin. It has a Shahid Rajaee Dam, which is located about 25 km south of Sari, the capital city of Mazandaran province. This river originates from Hezarjarib and Poshtcouh mountains and comprises the three main streams of Tajan, Zaromroud, and Sefidroud. At the beginning of its path, Tajan River undergoes a mountainous gravel bed, while, approaching the estuary, it flows over the sandy bed of northern Iran plains. The average flow of this permanent river is around 20 m^3 per second [2, 4]. Kutum (*Rutilus frisii kutum*, family Cypinidae) live in the Caspian Sea near the coast, from the Terek River in the north to the southern part of the Caspian Sea. This species is a migratory anadromous fish spawning in rivers in March–April. It has a group-synchronous, single-spawning behavior [5], spawning on aquatic weeds, graveled, and sandy substrates in rivers and lagoons [6]. This is a very valuable commercial fish in the southern part of the Caspian Sea and has a great demand, due to its good taste and culinary customs of the local people, and is consumed all year round. It is typically a medium-sized fish, reaching 45–55 cm in length, rarely 70 cm, and weighing up to 4.00 kg, rarely 5.00 kg [7]. The average annual catch of Kutum in Iran was about 9,600 t in 1991–2001 [8]. It is also known as Caspian White Fish. Caspian Roach is a medium-sized, bony, fresh water and brackish water fish native to the Caspian Sea, and it is abundant and commercially valuable species in the northern province of Iran specially Mazandaran and Guilan. Metal absorption in fish is carried out via two uptake routes: digestive tract (diet exposure) and gill surface (water exposure). Metals are further transferred via blood to other target organs, such as the liver and kidney [9]. Liver and muscle are usually used as the target tissues for the analysis of metal concentrations. Studies carried out with different fish species have shown that trace metals accumulate mainly in liver, where metals are stored for detoxification through metallothioneins. Although muscle is not an active tissue for accumulating the heavy metals, except mercury, the study of potential metal accumulation in this part of the body is justified because it is the edible part of the fish for humans [10].

The levels of contaminants, especially heavy metals, in fish are of particular interest because of the potential risk to humans who consume them [1, 10, 11]. The heavy metal levels of fish are widely documented in the literature [1, 4, 12–14]. The aim of the present study was to evaluate the heavy metal levels (Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in muscle, liver, bile, heart, brain, and gills of *R. frisii kutum* from Tajan River. The results obtained from this study will provide information for the background levels of metals in common fish species of this river.

Material and Methods

Tajan River is among significant rivers of the Caspian Sea water basin and is 170 km long. Recently, agricultural and industrial developments as well as increase in pollution substantially increased the contamination of fish with heavy metals [7]. This might be crucial for the future exploitation of this resource, and available data are urgently needed on this issue. Thirty specimens of *R. frisii kutum* were collected from three sampling locations (I-Farrah abad; II-Panbe chulleh; III-Ghadikola) at Tajan River in 2009 (Fig. 1). Fish were transferred to the laboratory to record sex, age, total body length, and total wet weight. The

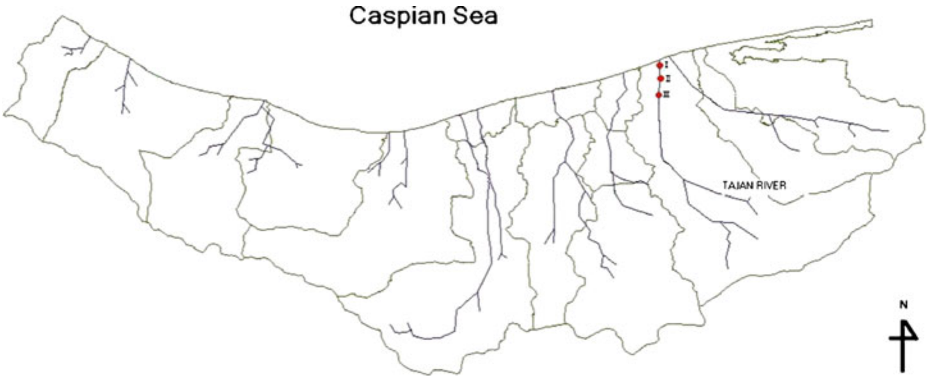

Fig. 1 Sampling locations (I-Farrah abad; II-Panbe Chulleh, III-Ghadikola) in Tajan River, north of Sari, Mazandaran province, Iran

results are given in Table 1. The age of *R. frisii kutum* was determined from computation of annual growth rings on fish (between 1 and 2 years).

Analytical Procedure

Trace element levels were measured using atomic absorption spectroscopy as described in the 1984 Perkin–Elmer manual. Briefly, 0.1 g of samples were weighed and placed in metal-free glass tubes (washed with hydrochloric acid). The samples were dried in an oven at 115°C for 5 h; 0.3 ml of concentrated sulphuric acid was added, and the samples were digested for 2 days. Subsequently, 0.1 ml of concentrated nitric acid and 50 μl of hydrogen peroxide were added, and the freed metal salts were determined using atomic absorption spectroscopy (Perkin–Elmer AAS 100 Wellesley, MA). The results are expressed as micrograms of element per gram of tissue wet weight [15]. The digestion and analytical procedures were checked by analysis of standard reference material (DORM-2 and DOLT-3 National Research Council Canada, Ottawa, ON, Canada). The results were given in Table 2.

Detection Limits of the Investigated Elements

Sixteen blank control solutions were used to estimate the detection limits of the investigated elements following the same analytical procedures. Three times the standard deviation was used as detection limit (Table 3).

Statistical Analysis

Descriptive statistical analysis was used. Student Newman–Keuls test was employed for the comparison of means. The significance was set at $p<0.05$.

Table 1 Some morphometric and biological characteristics (mean ± SD) of *R. frisii kutum* from Tajan River

Sex	Number	Age (years)	Fork length (mm)	Length (mm)	Body weight range (g)
♂♂	30	1–2	43.33±5.77 (40–50)	401.67±33.29 (385–440)	733.33±152.7 (680–900)

Explanations: values in parentheses indicate minimum–maximum values

Table 2 Certified metal concentration in reference material

Reference material	Element	Certified ($\mu g\ g^{-1}$)	Found ($\mu g\ g^{-1}$)	RSD (%)
DOLT-3	Cu	31.2±1.0	33.7±0.4	+8
	Zn	86.6±2.4	94.3±0.2	+9
	Ni	2.72±0.35	2.52±0.18	−7
	Cd	19.4±0.6	17.8±0.8	−8
DORM-2	Fe	142±10	138±8	−3
	Cu	2.34±0.16	2.68±0.2	+7
	Mn	3.66±0.34	3.96±0.24	+8

Explanations: data are mean ± SD of five determinations at 95% confidence

Results

The concentrations of trace element (micrograms per gram wet tissues) in muscle, liver, brain, heart, bile, and gill of *R. frisii kutum* are summarized in Table 4. Fe, Zn, Cu, Pb, Mn, Cd, and Ni were detected in all samples. Fe levels are higher than other metals, except gills. Distribution patterns of metal concentrations in the muscle, liver, gill, heart, bile, and brain of *R. frisii kutum* follows the sequence: Fe > Pb > Ni > Cu > Mn > Zn > Cd; Fe > Zn > Cu > Pb > Ni > Cd > Mn; Zn > Pb > Cd > Ni > Fe > Mn > Cu; Fe > Ni>Pb>Cu > Zn > Mn > Cd; Fe > Cu > Pb > Cd > Ni > Zn > Mn; and Fe > Pb > Ni > Cu > Zn > Cd > Mn, respectively. The distribution patterns of Fe in tissues of *R. frisii kutum* follows the order: heart > brain > liver > muscle > bile > gill; of Zn following the order: gill > liver > heart > brain > muscle > bile; of Cu following the order: brain > heart > bile > muscle > liver > gill; of Mn following the order: muscle > heart > gill > brain > liver > bile; of Ni following the order: heart > brain > muscle > gill > bile > liver; of Pb following the order: brain > muscle > heart > gill > bile > liver; of Cd following the order: gill > brain > muscle > heart > bile > liver. Heavy metal contents (Pb, Cd, and Ni) were higher in the brain and gill compared with other metals. Heavy metal levels varied significantly in different tissues of the same species.

Table 3 Operating conditions of the Atomic Absorption Spectrometry (AAS 100)

Elements	Detection λ_{max} (nm)	Drying temp. (°C)	Melting point (°C)	Detection limits ($\mu g\ g^{-1}$)
Cu	324.8	120	1,085	0.033
Fe	248.3	120	1,535	0.047
Zn	213.9	120	450	0.035
Mn	279.5	120	1,246	0.009
Ni	232.0	120	1,455	0.056
Cr	357.9	120	1,900	0.018
Pb	283.4	120	327.46	0.020
Cd	228.8	120	321.07	0.007

For all elements, the slit width is 0.2 nm. The air and acetylene flow rates were 4.0 and 0.5 liters per minute, respectively

Table 4 Heavy metal concentrations (μg g^{-1} wet wt) in tissues of *R. frisii kutum* from Tajan River

Tissues	Liver	Heart	Bile	Brain	Muscle	Gill
Fe	7.120±0.306	58.280±2.380	4.460±0.173	9.200±0.404	5.600±0.240	2.080±0.076
Cu	1.350±0.048	2.020±0.078	1.990±0.066	2.940±0.138	1.680±0.054	0.140±0.005
Ni	0.963±0.039	3.320±0.136	1.470±0.057	3.300±0.153	2.650±0.094	2.149±0.088
Zn	1.450±0.044	0.897±0.041	0.399±0.018	0.800±0.037	0.633±0.029	2.429±0.095
Pb	1.020±0.042	2.900±0.145	1.730±0.051	3.450±0.132	3.120±0.122	2.420±0.100
Cd	0.090±0.002	0.300±0.011	0.158±0.005	0.432±0.015	0.325±0.016	2.373±0.089
Cr	ND	ND	ND	ND	ND	ND
Mn	0.070±0.001	0.760±0.034	0.035±0.000	0.260±0.009	0.780±0.031	0.700±0.026

ND not detected

Values are mean ± SD

Discussion

The heavy metals are the most important forms of pollution, and they may accumulate in the tissues of fish which are often at the top of the aquatic food chain. They are accumulated in human tissues and may be the cause of some diseases [11, 16, 17]. Fish may concentrate large amounts of metals from the water, and they might be toxic for human consumption [18]. The effects of trace metals on human health are of great interest today, especially for aquatic food products. Therefore, estimation of the trace metals in fish became important to estimate freshwater pollution and the risk potential of human consumption [11, 18, 19]. The present study focused on the accumulation of heavy metals in *R. frisii kutum* and showed differences in heavy metals accumulation in the different tissues. Specially, muscle tissue, liver, and the gills were analyzed because muscle is main edible part and liver and gills play a role in the bioaccumulation process. The heavy metals accumulate mainly in metabolically active tissues such as the liver. In the liver, metals are bound to metallothioneins, low molecular weight proteins with high cysteine content [11, 20]. It is the major organ involved in xenobiotic metabolism, while the gills are the primary site of metal uptake from the water, especially if metals are bound to particulate matter [21].

Zinc is an essential element involved in many metabolic activities, and its deficiency can lead to loss of appetite and growth, skin damages, and immunological abnormalities. Samples had lower levels of Zn when compared with the corresponding Turkish Food Codes (TFC) limits of 50 mg kg^{-1} [1, 22]. Copper is also an essential element for good health, but the elevated intake can cause adverse health problems [23]. The Cu levels of all samples were below the TFC limits of 20 mg kg^{-1} [1, 22]. They were far below the permissible limits for human consumption in comparisons with the Canadian food standards (Cu, 100 mg kg^{-1}; Zn, 100 mg kg^{-1}) [10]. It demonstrates that the content of these metals in the edible part of the examined fish is lower than the guidelines mentioned above.

Some trace elements are essential to all cells, and deficiencies of essential metals may also cause disease. For example, in diabetes, chromium, copper, and zinc have important roles in the insulin secretion process from beta cells of the Langerhans islets and reinforcing insulin action [24, 25].

Chromium (III) is another essential element that influences the carbohydrate, lipid, and protein metabolism. However, Cr (VI) is highly carcinogenic [1]. There is little evidence of chromium accumulation in fishes [10]. In the literature, chromium levels in fish have been reported in the range of 0.06–2.63 mg kg^{-1} for muscles and 0.20–3.97 mg kg^{-1} for livers of fish from Turkish seas [9]. No limits were established for Cr concentrations in fish and fishery products in TFC. No chromium was found in fish in our study.

Cadmium accumulates in human body and may induce kidney dysfunction, skeletal damage, and reproductive deficiencies [1]. In fishes, Cd can cause very adverse effects because this metal blocks thiol group (SH) in enzymes and competes for binding sites that are essential for normal enzyme functioning [10, 26]. The limit value for cadmium in the edible part of fish, proposed by the European Commission is 0.1 mg kg^{-1} wet weights [27]. The maximum cadmium level permitted for fishes is 0.1 mg kg^{-1} according to TFC [22]. In this study, all samples analyzed presented concentrations exceeding the proposed limits.

Lead poisoning is generally ranked as the most common environmental health hazard. Impairment of hearing ability, anemia, renal failure, weakened immune system, low birth weights, still births and miscarriages, premature births, elevated blood, and urine lead levels are the most common symptoms of lead poisoning [1, 28]. The European Community established threshold values of non-essential metal concentration of fish muscles as micrograms per gram (milligrams per kilogram) wet weight and is 0.2 for lead [29]. The maximum lead level permitted for fishes is 0.4 mg kg^{-1} according to TFC [22]. The concentration of lead measured in fish was so much higher than the levels issued by FAO and TFC limits [10]. Such high values were reported earlier in fish species of different lakes [22, 30].

Nickel is a problematic element for human life [1, 31]. The maximum daily intake for an adult man (a 70-kg person) is 25 μg [32]. The concentration of Ni measured in the muscle of fish was higher than the permissible limit for human. There is no information about maximum nickel levels in fish samples in Turkish standards [22].

Mn plays a vital role in biochemical processes and improvement of impaired glucose tolerance and has an indirect role in the management of diabetes mellitus [25, 33]. Manganese can protect against skeletal deformities and gonadal dysfunction [34]. The daily mineral requirement of an adult man (a 70-kg person) is 2.8 mg [31].

The reason for the presenting Pb in *R. frisii kutum* tissues is probably due to the increase in agricultural and industrial activities as well as in the pollution to Tajan River. The range of international standards for Pb in fish is 0.5–10 mg kg^{-1}wet weight depending on the types of fish [27, 35, 36]. In this study, Zn and Cd levels of the gill metals were higher than in other tissues. The level of Zn was high in gill and liver because of its role in the enzymatic process. Metal levels in the gill could be due to element complexion with the mucus, which is impossible to be completely removed from the lamellae before tissues are prepared for analysis. The adsorption of metals onto the gill surface, the first target for pollutants in water, may also influence the total metal levels of the gill [11]. In addition, the liver represents storage of metals [11, 17]. Thus, the liver and gill in fish are more often recommended as environmental indicator organs of water pollution than other fish organs [11, 37]. In this study, the gill metal concentrations were so high. Gills are critical organs in fish for respiratory, osmoregulatory, and excretory functions. A high rate of absorption capacity for elements through the gills also makes fish vulnerable to its exposure [38]. In this study, the highest bio-concentration of Zn and Cd and high level of accumulation of Ni and Pb were observed in the gills, potentially affecting their respiratory capacity while also influencing the oxidative phosphorylation [39].

There are very limited data about trace element determination in *R. frisii kutum*. Cu, Fe, and Zn concentrations in our samples were so much lower than those reported by Zeynali et al. [40]. Of the six tissue types analyzed, humans consume only muscle. Furthermore, the sampling and preparation of muscle tissue is easy. Thus, unless fish muscle cannot be considered to be a metal-accumulating tissue, it is the ideal tissue for use in monitoring programs [21].

Metal concentrations found in the tissues of *R. frisii kutum* varied considerably, and levels of non-essential metals (Cd, Ni, Pb) were higher in muscle than liver (Table 4). The concentrations of these elements were higher than minimum limit of detection for the males. Numerous studies have shown that concentrations of trace elements are usually elevated in liver when compared with muscle tissue [41–43]. Despite their antagonistic biochemical roles, some metals are capable of inducing the synthesis of metal-binding proteins such as metallothioneins in hepatic tissue. The higher concentrations of these metals in the muscles examined in this work could reflect the high levels of these binding proteins in the muscle tissue [44]. On the other hand, the increase in copper liver content was likely due to the fact that this metal could be bio-accumulated by some fish species without any toxic effect [10, 45].

Conclusion

Nearly all non-essential metals (Cd, Ni, Pb) levels detected in tissues were higher than limits for fish proposed by Food and Agriculture Organization /World Health Organization FAO/WHO, European Union (EU), and TFC. *R. frisii kutum* was associated with enhanced non-essential metal (Cd, Ni, Pb) contents in the muscle and was not within the safe limits for human consumption. Levels of essential metals (Cu, Zn, and Mn) were below the limits proposed by EU and FAO/WHO and TFC for fish. Because of high levels of heavy metals, a potential danger may emerge in the future depending on the domestic wastewaters and agricultural activities in this region. In relation to this, it is recommended that monitoring studies be periodically performed to assess the human exposure to these toxic elements through fish and fishery product consumption. These results can be used to test the chemical quality of fish in order to evaluate the possible risk associated with their consumption by humans. The present study shows that precautions need to be taken in order to prevent heavy metal pollution. Otherwise, these pollutions can be dangerous for fish and human health.

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