



## Determination of Trace Elements in Muscle Tissue of Caspian Roaches (*Rutilus rutilus caspicus*) Collected in Iranian Coastal Waters of the Caspian Sea

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(Received: Oct 7, 2010; Accepted: Jan 15, 2011)

**Abstract:** In the present study concentrations of 6 trace elements (Mn, Pb, Cr, Cu, Zn and Cd) were determined in muscle of Caspian roaches collected from coastal waters of southern part of the Caspian Sea, by flame and graphite furnace atomic absorption spectrometry after microwave digestion. The accuracy of the method was corrected by standard material. The contents of investigated trace metals in fish samples were found to be in the range 16.80-22.40 for zinc, 0.381-2.890 for manganese, 0.28-1.10 for chromium, <0.001-0.765 for copper, <0.001-0.038 for cadmium and <0.001-0.039 for lead  $\mu\text{g/g}$  dry wt., respectively. In this study, the relationship between the concentration of the elements and biological characteristics has been examined. The significant length and weight dependent relationships were observed for some elements. The results were compared with the literature values. According to the results of this study, the consumption of Caspian roaches were collected from coastal waters of the southern part of Caspian Sea can be safe for human health in spite of possible contamination with heavy metals.

**Key words:** Trace elements % Caspian roaches % *Rutilus rutilus caspicus* % Coastal waters of Caspian Sea

### INTRODUCTION

The Caspian Sea is the largest continental body of water in the world, is bordered by the states of Azerbaijan, Islamic Republic of Iran, Federation of Russia, Kazakhstan and Turkmenistan [1].

As a closed-environment without an outlet, various pollutants due to effluents from coastal catchments areas and leakage from offshore oil production and land-based sources have accumulated in the Caspian Sea [2]. Several pollutants such as heavy metals are very toxic, stable and not easily biodegradable [3]. Amongst food, fish are constantly exposed to heavy metals present in polluted water. Thus, fish have been found to be good bio-indicators of water pollution. These heavy metals can accumulate in their tissues in different amounts depending on the size and age of fish [4, 21]. As compared to red meat, fish flesh is easily digestible because it contains long muscle fibers. In addition, fish is a good source of fluorine and iodine required for the development of strong teeth and prevention of goiter in man [5]. Consumption of fish is very popular amongst people all around the world because it has high protein content, low saturated fatty acids and high omega fatty acids content [3].

Bony fishes such as Caspian roaches (*Rutilus rutilus caspicus*) are abundant and commercially valuable species in the Caspian Sea. They are distributed throughout the Caspian Sea and important prey items for sturgeons and Caspian Seals. It is therefore necessary to examine the geographical variation of trace elements in these fishes and to understand their tropic in the Caspian Sea ecosystem [6]. Levels of heavy metals in fish and canned fish samples have been widely reported in the literatures. However, data on trace metal levels in this fish in Iran are very limited. The aim of this study was to determine the levels of trace heavy metals in Caspian roaches samples.

In this study, we examined the total concentrations of 6 trace elements in muscle of Caspian roaches collected from coastal waters of southern part of the Caspian Sea, by flame and graphite furnace atomic absorption spectrometry after microwave digestion. It is expected that the results of this research will assist in acquiring information about the level of toxic metals in this region.

### MATERIAL AND METHODS

**Apparatus:** A Perkin Elmer 2380 atomic absorption spectrophotometer equipped with a deuterium



Fig. 1: Sampling locations of fishes in Caspian Sea

background corrector was used for the determination of heavy metals, lead, chromium and cadmium concentrations were determined by a graphite furnace atomic absorption spectrophotometer 110 employing pyrolytic platform graphite tubes. Hydride generation was with a Varian model 77 with quartz tubes.

All the plastic and glassware were cleaned by soaking over night in 10% (V/V) nitric acid, followed by washing with 10% (V/V) hydrochloric acid and rinsed with double distilled water and dried before using.

**Reagents:** All chemical used were of analytical reagent grade. Standard stock solutions were prepared from Titrasol (1000mg/l) and were diluted to the corresponding metal solution. The working solution was freshly prepared by diluting an appropriate aliquot of the stock solutions. Double deionized water was used for all dilutions.

**Sampling:** Thirty fish samples used for this study were collected in 2008 from three stations of Iranian coast (Figure 1); Noshahr (Station 1), Babolsar (Station 2) and Turkman (Station 3). Body weight and length of fishes were measured prior to dissection. Muscle sample was taken and stored at  $-20^{\circ}\text{C}$  until chemical analyses.

**Digestion and Chemical Analysis:** The procedure used for measuring concentrations of trace elements has been described previously [7]. Freeze-dried and homogenized samples ( $1\pm 0.001\text{g}$ ) were digested with 6 ml of concentrated  $\text{HNO}_3$  (Suprapure, Merk) and 2 ml of concentrated  $\text{H}_2\text{O}_2$  (Suprapure, Merk) in a microwave oven with a Teflon PTFE tube. The solution was allowed to cool, transferred into a 50ml volumetric flask and diluted to the mark with double deionized water. Concentration of cadmium, chromium and lead were determined by graphite furnace and other elements were determined in an air-acetylene flame.

**Validation of Methods:** Caspian roaches fish (Freeze-dried and homogenized) samples were spiked with various concentrations of heavy metals for the recovery repeatability tests and for verifying the analytical methodology. For each run, triplicate samples, spiked samples and blanks were carried through the digestion reaction. The results are shown in Table 1.

## RESULTS AND DISCUSSION

The relative standard deviations were less than 8% for all investigated elements. The student *t*-test was used in this study ( $D < 0.05$ ). The recovery value were nearly quantitative (95%) for the microwave digestion method. The accuracy of the method was evaluated by means of trace metals determination in standard reference material. The achieved results were in good agreement with certified values (Table 1). All metal concentrations were determined on a dry weight basis ( $\mu\text{g/g}$  dry wt.).

Concentrations of 6 elements in the muscle of fish samples are shown in Table 2. Zinc was highest in muscle of all the species analyzed in this study, followed by Cr, Cu, Mn, Pb and Cd. Trace elements levels are known to vary in fishes depending on various factors such as its habitat, feeding behavior and migration even in the same area [8, 9, 10]. Caspian roaches are bathypelagic and semi migratory fish, feeding mainly on mollusks. These species-specific characteristics may result in the variation of trace element accumulation between the species from Caspian Sea.

Growth-dependent variations were found in concentrations of several trace elements in muscle of this fish. Since significant positive correlations between body weight and length were found in all fish ( $p < 0.001$ ), only relationships between body weight

Table 1: Recovery of trace elements from Caspian roaches samples

Element	Certified value ( $\mu\text{g/g}$ )	Experimental value ( $\mu\text{g/g}$ )	Recovery (%)
Cu	2.120	2.08 $\pm$ 0.160	98
Zn	20.250	20.65 $\pm$ 0.820	102
Mn	2.340	2.22 $\pm$ 0.110	95
Pb	0.068	0.065 $\pm$ 0.01	95
Cr	30.710	29.45 $\pm$ 2.150	96
Cd	0.046	0.044 $\pm$ 0.00	95

Table 2: Concentrations of trace elements in Caspian roaches ( $\mu\text{g/g}$  dry wt.)

Element	Minimum	Maximum	Mean	Std. Deviation ( $\pm$ )
Cu	0.00	0.77	0.22	0.01
Zn	16.80	22.40	19.45	2.70
Mn	0.38	2.89	1.52	1.08
Pb	0.00	0.04	0.02	0.02
Cr	0.28	1.10	0.63	0.38
Cd	0.00	0.04	0.02	0.01

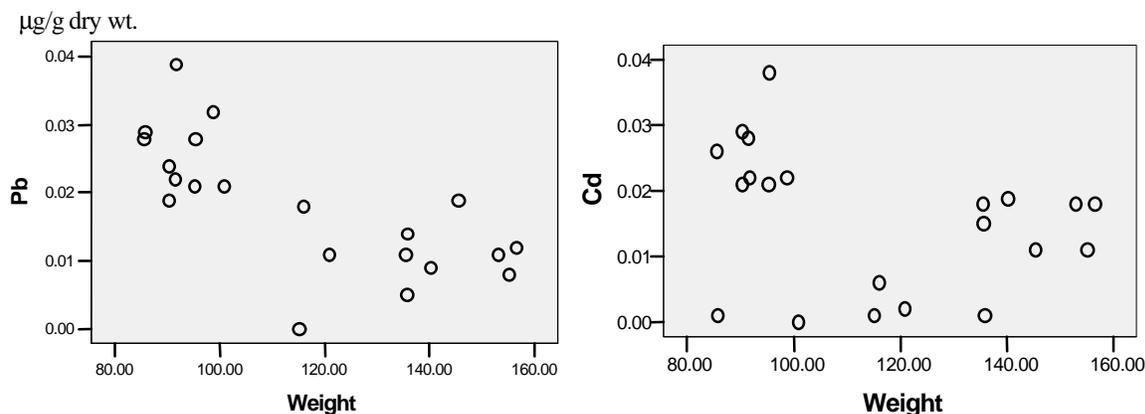


Fig. 2: Growth-dependent variation of some trace element Concentration in the muscle of Caspian roaches

and trace element concentrations are reported here. Significant negative correlation with body weight were found for Pb, Cd ( $p < 0.05$ ), Cr, Cu ( $p < 0.001$ ) and Zn, Mn ( $p < 0.01$ ) (Fig. 2).

Until now, growth (body weight, length and age) dependent decrease in trace elements levels has been reported for many fish species [11, 12]. Generally, the growth-dependent variations of trace element levels are known to be influenced by various factors such as metabolic rate and growth dilution of the elements [13, 14].

According to FAO [15] there is no information on the carcinogenicity of manganese. The range of manganese levels was found to be 0.381-2.890  $\mu\text{g/g}$  dry wt. in Caspian roaches fish in this study. Manganese contents in the literatures have been reported in the range 0.092-9.23  $\mu\text{g/g}$  dry wt. in fishes collected from coastal waters of the Caspian Sea [6], 0.10-0.99  $\mu\text{g/g}$  wet wt. in the muscle tissues in sea foods [16], 0.01-2.55  $\mu\text{g/g}$  in canned fish samples [3], 0.90-2.50  $\mu\text{g/g}$  in canned tuna and anchovy fish samples [18], 1.56-3.76  $\mu\text{g/g}$  dry wt. in fish samples of the middle Black sea [17] and 0.214-2.71  $\mu\text{g/g}$  dry wt. in muscle of sturgeons in the Caspian Sea [19].

Lead is known to induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease in adults [20]. The fact that toxic metals are present in high concentrations in fishes is of particular importance

in relation to the FAO/WHO [15] standards for Pb and Cd as toxic metals. The maximum permissible doses for an adult are 3 mg Pb and 0.5 mg Cd per week, but the recommended doses are only one-fifth of those quantities.

The range of lead levels were found to be <0.001-0.039  $\mu\text{g/g}$  in Caspian roaches samples. In the literatures lead levels have been reported in the range 0.001-0.191  $\mu\text{g/g}$  dry wt. in fishes collected from coastal waters of the Caspian Sea [6], 0.33-0.88  $\mu\text{g/g}$  wet wt. in the muscle tissues in sea foods [16] and <0.001-0.575  $\mu\text{g/g}$  dry wt. in muscle of sturgeons in the Caspian Sea [19]. In canned fish samples, lead levels have been reported in the range 0.09-0.40  $\mu\text{g/g}$  [17], 0.0-0.03  $\mu\text{g/g}$  [3] and 0.0162-0.072  $\mu\text{g/g}$  wet wt. [4]. The maximum lead level permitted for canned fishes is 0.2 mg/kg according to the European communities [20].

Copper is essential for good health but a very high intake can cause adverse health problems, such as liver and kidney damage [3]. The contents of investigated trace metals in Caspian roaches fish samples were found to be in the range <0.001-0.765  $\mu\text{g/g}$  for copper. In the literatures, copper levels have been reported in the range of 0.001- 5.02  $\mu\text{g/g}$  dry wt. in fishes collected from coastal waters of the Caspian sea [6], 0.32-6.48  $\mu\text{g/g}$  wet wt. in the muscle tissues in sea foods [16], 0.617-3.92  $\mu\text{g/g}$  dry wt. in muscle of sturgeons in the Caspian sea [19], in canned

fish samples copper levels have been reported in the range of 0.01-5.33 µg/g [3], 1.10-2.50µg/g [17], 7.1-45.7µg/g [22]. There recommended daily intakes of copper is 1.5mg Cu for adult males and 3.0 mg Cu for adult females [23]. Copper concentration in analyzed Caspian roaches fish samples were below the MAFF guideline value of 30mg Cu/kg [15, 24].

Zinc is known to be involved in most metabolic pathways in humans and zinc deficiency can lead to loss of appetite, growth retardation, skin changes and immunological abnormalities. Zinc is widespread among living organisms, due to its biological significance. The maximum zinc level permitted for fish is 50 mg/kg according to Food Codex [25]. The recommended daily intakes of zinc are 15 mg for adult males and 12 mg for adult females [23].

According to the results (Table 2), concentration of zinc to be in the range 16.80-22.30 µg/g dry wt. In the literatures, zinc levels in fish samples, have been reported in the range of 4.49-11.6 µg/g wet wt. in the muscle tissues in sea foods [16], in canned fish samples, 0.14-97.80µg/g [3], 7.57-34.4 µg/g [17], 33.8-566 µg/g [22], 12.5-201 µg/g dry wt. in fishes collected from coastal waters of the Caspian Sea [6] and 11.6-68.9 µg/g dry wt. in muscle of sturgeons in the Caspian Sea [19].

Chromium (III) is an essential nutrient that potentate's insulin action and thus influences carbohydrate, lipid and protein metabolism. However, Cr (VI) is carcinogenic [17]. In this study, the range of chromium levels were found to be 0.10-1.01 µg/g dry wt. Chromium contents in the literatures have been reported in the range 0.04-1.75 µg/g wet wt. in the muscle tissues in sea foods [16], 0.97-1.70 µg/g [17], 0.0-0.30 µg/g [3], 0.08-1.4 µg/g dry wt. in fishes collected from coastal waters of the Caspian Sea [6] and 0.20-0.95 µg/g dry wt. in muscle of sturgeons in the Caspian Sea [19].

Cadmium may accumulate in the human body and may induce kidney dysfunction, skeletal damage and reproductive deficiencies. The contents of investigated trace metals in Caspian roaches samples were found to be in the range <0.001-0.038 µg/g dry wt. for cadmium. Cadmium contents, in the literatures have been reported in the range 0.02-0.37 µg/g wet wt. in the muscle tissues in sea foods [16], 0.06-0.25 µg/g [17], 0.0-0.05 µg/g [3] and 0.0046- 0.0720 µg/g wet wt.[4], <0.001-0.233 µg/g dry wt. in muscle of sturgeons in the Caspian Sea [19], <0.001-0.355 µg/g in fishes collected from coastal waters of the Caspian Sea [6] and 0.01-4.16 µg/g dry wt. in fish species from Iskenderum Bay, northern east Mediterranean Sea [26].

## CONCLUSION

Dietary standards and guideline applicable in the UK for fish have been summarized by MAFF [24] for Cd (0.2 µg/g), Cu (20 µg/g), Pb (2.0 µg/g), Zn (50 µg/g). In this present study, concentration of Cd, Cu, Pb, Zn, Mn and Cr in Caspian roaches were lower than the guideline for food or within the range of freshwater, marine fishes and fish canned from other areas, to those reported in other regions.

Therefore, according to the results of this study, the consumption of Iranian Caspian roaches fish of the Caspian Sea can be safe for human health in spite of possible contamination with heavy metals.

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