# Determination of total mercury in common carp, *Cyprinus carpio*, large scaled barb, *Barbus grypus*, tiger tooth croaker, *Otolithes ruber* and from surface waters in Khuzestan, Iran

**Nanvazadeh H.[1, 2]; Khodayar M.J.[1, 2*]; Jahangiri A.[3, 4]; Rahbar N.[4]; Behfar A.[5]; Dehbashi F.[1, 2]**

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1-Department of Toxicology and Pharmacology, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, P.O. Box 6135733184, Ahvaz, Iran.
2-Toxicology Research Center, Department of Toxicology and Pharmacology, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, 6135733184, Iran.
3-Nanotechnology Research Centre and Department of Medicinal Chemistry, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, 6135733184, Iran.
4-Department of Medicinal Chemistry, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, 6135733184, Iran.
5-Department of Food and Drug Control, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, 6135733184, Iran.
Corresponding Author's Email: jKhodayar@ Yahoo.com

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## Introduction

Fishes are a valuable source of dietary protein and minerals for seafood consumers and are also used in pharmaceutical products (Mozaffarian, 2009). Over the past several decades aquatic ecosystems have been extensively contaminated with pollutants released from domestic and industrial waste waters and other anthropogenic activities in Khuzestan, Iran. Heavy metals such as mercury are considered as one of the four most important pollutants of aquatic life because of their high toxicity and ability to bioaccumulate in tissue of aquatic organisms (Hajeb *et al.*, 2009). The toxicity of Hg is highly dependent on its chemical form. Organic mercury compounds are more toxic than inorganic mercury salts which may bioaccumulate to levels that could increase health risks to seafood consumers (Syversen and Kaur, 2012). Since most mercury in fish tissue is present as methyl mercury (Me-Hg), it has been recommended that T-Hg in tissues can serve as a surrogate for Me-Hg (Kannan *et al.*, 1998).

Although there are several studies that have been done before, separately (Agah *et al.*, 2010, Sary and

Mohammadi, 2012) for the first time T-Hg concentrations were analyzed in three regions of Khuzestan Province simultaneously. The main objective of this study was to determine the mercury concentrations in the muscle tissue of three fish species in three different water sources of Khuzestan to assess the influence of regional differences and pollutant sources such as petrochemical industries on total mercury (T-Hg).

## Materials and methods

One hundred and fifty samples of three fish species were analyzed for mercury determination (fifty numbers for each species in 10 composites). Samples were collected between September/November of 2011 from 3 regions of Khuzestan province that is located in the southwest of Iran including *Cyprinus carpio* (common carp) from aquaculture in Ahvaz, *Barbus grypus* (large scaled barb) from Karun River in Shushtar and *Otolithes ruber* (tiger tooth croaker) from coastal areas of the Persian Gulf in Mahshahr seaport (namely Khor; near the petrochemical power plant) (Fig. 1).


**Figure 1: Map of Karun River and area of this study in Khuzestan Province, Iran.**

Sample preparation and treatment was performed according to United States Environmental Protection Agency (EPA) instruction (U.S.EPA, 1991). Briefly, after biometric measurement, fish were washed with deionised water, placed individually in plastic bags, labelled and placed in the refrigerator. Then, after scarping and grasping the fish skin, dorsal muscle tissue of one side of the fish was dissected with a stainless steel knife. After that, the fillets were rinsed with double deionised water. Finally, five samples that were similar in length and weight were mixed completely with a stainless steel blade mixer. All the composites were stored in plastic containers and were frozen at ≤ -20°C (U.S.EPA, 1991). For each fish species, water and fish samples were collected at the same time and location. Three composite samples of water from one meter depths were gotten from each location. The sampling flask was washed with water from the sampling site. Water samples were filtered through a 0.45μm membrane, to separate the water-insoluble mercury. The filtrate solution was collected through amber glass bottles and mercury free nitric acid was added as a preservative solution until pH reached 2-3 (IAOAC, 1990).T-Hg analysis was performed with a Direct Mercury Analyzer-80 (DMA-80, Milestone, Italy). Briefly, 0.12-0.17 g of homogenized composite of each fish sample was accurately weighed onto metal boat, and then was introduced to the system. For water analysis, 100 μL of water sample was loaded. The accuracy was checked based on a standard curve data. Calibration of the DMA-80 was performed using mercury standard for AAS (1000 mg Hg/L in nitric acid, Trace CERT®, Fluka, USA). All sample analyses were performed in triplicate. The DMA-80 produces accurate results with a detection limit of 0.005 ng Hg.

*Statistical analysis*

The statistical analysis was done using SPSS16 software and one way ANOVA with post hoc Tukey's test $p<0.05$ was considered to indicate statistically significant.

**Results and discussion**

The results of analysis of T-Hg in flesh of fish and water of their ecosystems and bioaccumulation factor are presented in Table 1. Based on bioaccumulation factor, *C. carpio* and *B. grypus* were considered as samples that have no artificial or anthropogenic sources of mercury around their living environment.

Unlike the concentration of mercury in water samples, mercury concentrations between fish species were significantly different ($p<0.0001$). Studies have shown that mercury levels generally present in surface waters were below EPA standards, 140-150 ppb (ATSDR, 1999). Mercury has a high tendency to be adsorbed on sediments, and a large proportion of Hg in the water phase is attached to suspended particles.

**Table 1: Mean concentration of mercury in water and fish and bioaccumulation factor.**

| Sampling site | T-Hg conc. µg/kg in fish | | T-Hg conc. µg/L* in water | Bioaccumulation Factor** |
|---|---|---|---|---|
| Ahvaz (Aquaculture) | (Mean±SEM) | 0.52 ±0.25 | 2.11±0.08 | 0.246445 |
| | Min-Max | ND-2.40 | 2.10-2.12 | |
| Shushtar (Karun River) | (Mean±SEM) | 42.51 ±2.58 | 2.39 ±0.49 | 17.78661 |
| | Min-Max | 34.27-59.82 | 1.80-3.37 | |
| Mahshahr (Coastal area of the Persian Gulf) | (Mean±SEM) | 216.3 ± 12.12 | 1.99±0.01 | 108.6935 |
| | Min-Max | 146.23-258.21 | 1.97-2.01 | |

*Mean concentration of three points of sample site.

**Calculated as the ratio of the mercury concentrations in the muscle of fish to the mean water concentrations.

Meanwhile, elemental mercury can't remain in water for a long time and rapidly changes to methyl mercury. This form of mercury is accumulated in fish from aquatic environments (Langeland, 2015). Regardless of different ecosystems, studies have shown that total mercury levels in water are low relative to those found in fish tissue (Mast and Krabbenhoft, 2010).

In the present study, results indicated that marine (*O. ruber*) and freshwater (*B. grypus*) species have higher mercury concentrations than farmed fish (*C. carpio*). However, Mercury concentrations in all three fish species were below WHO advisory thresholds; mercury standard concentration in fish muscle by WHO is 500 ppb (0.5mg/kg) (ATSDR, 1999). Previous studies have shown that rivers, lakes and seas that have been acidified by acid rain or industrial runoff, favor the methylation of mercury, which biomagnified up the food chain (Regnell and Tunlid, 1991). Since long-established Petrochemical industries are situated near Mahshahr (Salehi and Esmaili-Sari, 2010) and petrochemical industries produce mercurial wastewater (U.S.EPA, 1997), this can lead to excessive amounts of mercury in fish and its bioaccumulation in *O. ruber* species. Our results for Tiger Tooth Croaker in the Persian Gulf were similar to those of the FDA data in the Pacific and Atlantic Ocean. Investigation showed that wild (predatory) fish species contained the highest concentrations of methyl mercury among many commercially available fish, while farmed Atlantic salmon, contained very low levels of mercury (Kelly *et al.*, 2008). Furthermore, it also showed that the T-Hg concentration in oceanic carp was high (295 µg/kg), while the results of aquaculture samples in this study showed that the level of mercury in *C. carpio* is negligible (0.5 µg/kg) (Farias *et al.*, 2005). Accordingly, the concentration of heavy metals in the tissues varied significantly on the location from where the species were collected. In this regard, investigations show that local environmental conditions,such as acidic conditions may make a pond unfavorable or completely unsuitable for farmed fish

(Lavoie *et al*., 2013). By adding lime, gypsum, alum and potassium permanganate the water chemistry of a pond will be adjusted and pH will be increased (Bhatnagar and Devi, 2013).

Studies showed that there is an inverse correlation between pH and Me-Hg formation (Clayden *et al*., 2013, Dong *et al.*, 2016). It can be due to the supply of carbon dioxide by adding lime for photosynthesis and also increasing algal growth. Furthermore, in a pond immobile of water exceed algal growth (Long *et al*., 2011, Devgoswami *et al.*, 2013). Algae take up mercury through the water directly by adsorption and absorption, like zooplankton and phytoplankton, which can result in low levels of mercury in surrounding waters (Diéguez *et al*., 2013). Moreover, the main source of nutrition for farmed fish provided by human is more healthy. As a result, farmed fish are not exposed to high levels of mercury and thus mercury does not enter the food chain, and cannot be considered a serious risk to human health. It seems that this may be responsible for low levels of T-Hg concentration in *C. carpio*. The determined levels of mercury in *B. grypus* and *O. ruber* fish species suggest the more intensive mercury monitoring in these fish that are consumed by humans especially in Mahshahr. Furthermore, this study may be able to offer suitable policies for the consumers especially in pregnant women because of mercury's harmful effects on developing foetuses and infants.

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