# Determination and Comparison of Heavy Metals (Hg, Cd, Pb and As) of *Barbusgrypus* and *Capoetacapoeta* in Heleh River from Iran

Laleh Roomiani, Fatemeh Mashayekhi and Mansoreh Ghaeni

*Abstract*—**This Study was carried out to investigate contamination of heavy metals Hg, Cd, Pb and As in muscle of *Barbusgrypus* and *Capoetacapoeta* in Heleh river from Iran. Heavy metal levels in fish samples were analyzed by Perkin Elmer 4100 ZL atomic absorption. The highest concentration of Hg, Cd, Pb and As were measured in muscle of *Barbusgrypus* and the lowest concentration of Hg, Cd, Pb and As in muscle of *Capoetacapoeta*. Concentrations of heavy metals Cd, Pb and As in muscle of *Barbusgrypus* and *Capoetacapoeta* were showed significant difference ($P < 0.05$), but for Hg there was no significant difference ($P > 0.05$). Heavy metal concentrations were higher in the *Barbusgrypus*, whereas compared with *Capoetacapoeta*.**

***Index Terms*—*Barbusgrypus, Capoetacapoeta*, heavy metal, Heleh River, Persian Gulf.**

## I. Introduction

Fish are a major part of the human diet and it is therefore not surprising that numerous studies have been carried out on metal pollution in different species of edible fish [1]. In the recent years, world consumption of fish has increased simultaneously with the growing concern of their nutritional and therapeutic benefits. The American Heart Association recommended eating fish at least twice per weak in order to reach the daily intake of omega- 3 fatty acids. Fish lipids have also assumed great nutritional significance, because of their high polyunsaturated fatty acid levels and good source of digestible protein, vitamins and minerals. However, fish are relatively situated at the top of the aquatic food chain; therefore, they normally can accumulate heavy metals from food, water and sediments [2].

Trace metals in aquatic environments may be of natural origin from rocks and soil or from human activities [2], e.g. industry [3], urban and agricultural discharge [4], mine runoff, solid waste disposal and atmospheric deposition [5]. Heavy metals (HMs) pollution of aquatic environment has become a great concern in recent years. HMs can have toxic effects on organs [6]. Heavy metals have the tendency to accumulate in various organs of marine organisms, especially fish, which in turn may enter into the human metabolism through consumption causing serious health hazards. Iron, copper, zinc and manganese are essential metals while, mercury, lead and cadmium are toxic metals [7]. Heavy metals still play an important role as pollutants affecting aquatic systems [8]. Metal enrichment of coastal waters mainly originates from river inputs. Exposure to pollutants is therefore of prime concern for biota inhabiting coastal areas, particularly during their early development, which is very sensitive to toxic effects of trace elements [9].

The content of toxic heavy metals in fish can counteract their beneficial effects; several adverse effects of heavy metals to human health have been known for long time. This may include serious threats like renal failure, liver damage, cardiovascular diseases and even death. Therefore many international monitoring programs have been established in order to assess the quality of fish for human consumption and to monitor the health of the aquatic ecosystems [10].

Some of the metals found in the fish might be essential as they play important role in biological system of the fish as well as in human being, some of them may also be toxic as might cause a serious damage in human health even in trace amount at a certain limit. The common heavy metals that are found in fish include copper, iron, copper, zinc and manganese, mercury, lead and cadmium [9]-[11]. Toxic elements can be very harmful even at low concentration when ingested over a long time period. The essential metals can also produce toxic effects when the metal intake is excessively elevated [12].

In the last few decades, the concentrations of heavy metals in fish have been extensively studied in different parts of the world. Most of these studied concentrated mainly on the heavy metals in the edible part. However, other studied reported the distribution of metals in different organs like the liver, kidneys, heart, gonads, bone, digestive tract and brain [4].

This matter that, importance of the heavy metals measuring relate to two important subjects which are aquatics ecosystem management and human health, the present study was carried out to determine the level of Hg, Cd, Pb and As in muscle samples of *Barbusgrypus* and *Capoetacapoeta*inHeleh River from Iran. The fish and fish products for the people in those ports are generally catch and carried by local vehicles from the Heleh River. It should be noted that fish species are considered to be a heavy metals part of the diet in the region. The main objective of this study was to determinate the contents of Hg, Cd, Pb and As in liver and muscle samples *Barbusgrypus* and *Capoetacapoeta*in Heleh River, in order to assess fish quality and to assess the health risk for humans.

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## II. Material and Methods

60 samples of *Barbusgrypus* and *Capoetacapoeta* were collected from Heleh River of Bushehr Province. After capture, fishes were placed in plastic bags with ice and transported to the laboratory. Samples were cut into pieces and labeled and then all sampling procedures were carried out according to internationally recognized guidelines [13].The procedure for the extraction heavy metals was based on Standard Method 3052 (Microwave – assisted total heavy metal digestion, USEPA, 1996). TORTE 2 (Lobster Hepatopancreas Reference Material for Trace metals) (National Research Council, Canada) was used as Standard Reference Material for the digestion of fish, to verify the accuracy of metal determination. The recovery rates were 90%± 10%. All of the digests were filtered through Whatman 5cc filter paper and stored at 4℃. The concentration As, Cd and Pb in sample solutions were determined by graphite furnace atomic absorption spectrometry (GFAAS) and hydride generation atomic absorption spectrometry (HGAAS). The spectrometer (Analyst 800 coupled to FIAS 100, PerkinElmer, USA) uses transversally heated graphite tubes and applies the Zeeman effect for background correction.

The concentration of Hg was analysed by cold vapour atomic absorption spectrometry (FIMS 100, PerkinElmer, USA) at a wavelength of 253.7 nm. Generation of hydrides for HGAAS (As, Cd) and reduction of $Hg^{+2}$ to $Hg^{o}$ was achieved by reaction of the sample solution with HCl (1 M) and $NaBH_4$ (6 $gl^{-1}$As, Cd, 2$gl^{-1}$Hg). Working standard solutions for system calibration and control of analytical accuracy were obtained by dilution of stock solutions (1000 mg/ l, Merck, Germany) using purified water (18 MΩ $cm^{-1}$) and analytical grade $HNO_3$ (GFAAS) or HCl (HGAAS, cola vapour AAS). Precision for determination of concentration was better than 10% (Table I).

TABLE I: Operating Conditions of Atomic Absorption Spectrometry

| Element | Cd | As | Pb |
|---|---|---|---|
| Sample | F+W | F+W | F+W |
| Method | GFAAS | HGAAS | GFAAS |
| Wavelength, nm | 228.2 | 193.7 | 283.3 |
| T drying[a], °C | 110–130 | 400 | 110–130 |
| T pyrolysis, °C | 500 | - | 850 |
| T atomization, °C | 1,700 | 2,100 | 1,600 |
| T clean out, °C | 2,450 | 2,300 | 2,450 |
| Matrix modifier | NH4H2PO4+Mg(NO3)2 | Ir | NH4H2PO4+Mg(NO3)2 |

W water, F fish, GFAAS graphite furnace atomic absorption spectrometry, HGAAS hydride generation atomic absorption spectrometry
[a] Temperature of trapping and drying for HGAAS

All reagents were of analytical reagent grade unless otherwise stated. Double distilled water was used for the preparation of solution. All the plastic and glass ware were soaked in nitric acid for 15 min and rinsed with deionized water before use. The stock solutions of metals (1000 mg/ L) were obtained by dissolving appropriate salts of the corresponding metals (E. merk) and further diluted prior to use. High purity Argon was used as inert gas prior to use. The samples were solubilized using high-pressure decomposition vessels, commonly known as a digestion bomb. A sample (1g) was placed in to Teflon container and 5 ml of concentrated $HNO_3$ was added. The system was heated to 130 ℃ for 90 min and finally diluted to 25 mL with deionized water. The sample solution was clear. A blank digest was carried out in the same way. Mercury, cadmium, lead and copper metals were determined against aqueous standards.

T-test was run for all the collected data for fish samples different using SPSS (17 version) computer programs. Mean values of each parameter were compared using Fisher's protected least tests with significance levels of 5% were conducted on each metal to test for significant differences between sites (Table I). All statistical analyses were conducted using the Office Excel 2003 software package.

## III. Results and Discussion

Concentration levels of metals Hg, Cd, Pb and As in muscle of *Barbusgrypus* and *Capoetacapoeta*were measured and presented in Table II. Concentrations of metals are presented in mg/ kg dry weight unless otherwise mentioned. The highest concentrations of Hg, Cd, Pb and As in tissues were measured in muscle of *B. grypus* and the lowest concentration of Hg, Cd, Pb and As were showed in muscle of *C. capoeta* (Fig. 1). Concentrations of heavy metals Cd, Pb and As in muscle of *Barbusgrypus* and *Capoetacapoeta* showed significant difference ($P < 0.05$) but concentration of Hg not showed significant difference ($P > 0.05$). Heavy metal concentrations were higher in the *Barbusgrypus*, when compared with *Capoetacapoeta*.

According to the priority List of Hazardous Substance established by the Agency for Toxic Substances and Disease Registry (ATSDR, 2013) [13], the descending order of heavy metals threatening to human health were As>Pb> Cd> Ni> Zn> Cr> Cu>Mn.

A variable range of different metal concentrations has been observed by various researchers worldwide. The absorption of metals on to the gill surface, as the first target for pollutants in water, could also be an important influence in the total metal levels of the liver [14], [15]. Distribution patterns of metal concentrations in muscle of *Barbusgrypus* and *Capoetacapoeta* from Heleh river follows the sequence: Pb> As> Cd> Hg. There are various studies on the heavy metal levels in fish from different waters. Minimal accumulation and storage of heavy metals in such families on fish (*Liza auratus*) in the southern Atlantic coast of Spain [16], on fish (*Mugilauratus*) in the Black Sea Turkey [17], on fish (*Liza abu*) in Lake Ataturk [18], on fish (*Liza carinata*) [19] is proven. The observed variability of heavy metal levels in different species depends on feeding habits [20], ecological needs, metabolism [21], age, size and length of the fish [22] and their habitats [7]-[23]. Reference [24] studied the heavy metal levels in kidney, liver, gill and muscle of *Torgrypus* and Reference [25] studied the concentration of Zn and Pb in liver of *Cyprinuscarpio*, *C. aculeate* and *C. damasciana* which concentration of Zn were higher than Pb.

Reference [24] studied the heavy metal levels in muscle, liver, gonad, and gill of gilthead seabream (*Sparusaurata*), European sea-bass (*Dicentrarchuslabrax*), and keeled mullet (*Liza carinata*) which concentration of Zn were higher than Pb. The levels of Zn in all tissues were higher than the Pb levels, as Zn is present in many enzymes throughout the fish's body.

TABLE II: THE CONCENTRATIONS OF HEAVY METALS IN MUSCLE OF *BARBUS GRYPUS* AND *CAPOETA CAPOETA*IN HELEH RIVER (MG/ KG)

| Fish | Heavy metal | muscle |
|---|---|---|
| | mercury | 0.072±0.004[a] |
| | cadmium | 0.120±0.022[a] |
| *Barbusgrypus* | lead | 0.248±0.037[a] |
| | arsenic | 0.138±0.025[a] |
| | mercury | 0.063±0.007[a] |
| *Capoetacapoeta* | cadmium | 0.111±0.056[b] |
| | lead | 0.219±0.072[b] |
| | arsenic | 0.115±0.038[b] |

a. non-significant differences at $P < 0.05$. b: significant differences at $P < 0.05$.

In this study, concentrations of Hg, Cd, Pb and As in muscle of *Barbusgrypus* higher than *Capoetacapoeta*. Muscle tissue is the main edible fish part and can directly influence human health. Lead enters into the body with gill cells and especially is accumulated in gills and the later aim organs are liver and muscle [26]. In other studies, concentrations of Hg, As, Cd and Pb in liver of *Liza dussumieri* higher than muscle [27].Level of mercury in liver of *Liza parsia* was higher than muscle [28] also concentration of heavy metals (Hg, Cd, Pb, Cu, Zn, Fe and Mn) in liver of *Liza abu* was higher than muscle [29]. In other study such as *Mugilauratus* [17], *Sparusauratus, Triglacuculus*, *Sardinapilchardus*, *Mugilcephalus*, *Atherinahepsetus*, *Scomberesoxsaurus* [7], *Serranusscriba*, *Epinephelus costae*, *Cephalopholisnigri* and *Pseudupenaeusprayensis* [20], *Scomberomorusguttatus*, *Scomberomoruscommerson* and *Otolithesruber* [30], *Barbusxanthopterus* [31], *Barbusgrypus* and *Barbusxanthopterus* [31] concentrations of heavy metals in liver was higher than muscle.

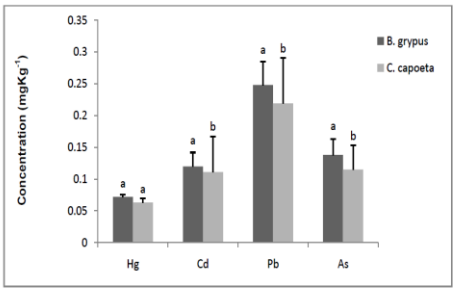


Fig. 1. Comparison of heavy metals in muscle of *Barbusgrypus* and *Capoetacapoeta* in Heleh river (mg/ kg) (a: non-significant differences at $P < 0.05$, b: significant differences at $P < 0.05$).

The other research, concentrations of heavy metals (mean± SD) Hg, As, Cd and Pb in muscle and liver of *Liza dussumieri* from Boshehr seaport were higher than in *Liza dussumieri* from Deylam seaport ($P < 0.05$), except for concentration of Pb that in muscle of *Liza dussumieri* from Deylam seaport were higher than in muscle of *Liza dussumieri* from Boshehr seaport ($P < 0.05$) [27].

Reference [32] reported that in *Barbusxanthopterus* and *Barbusrajanorummystaceus* in Ataturk Dam Lake, Turkey, heavy metal concentrations in gill and liver were high while these concentrations were the least in muscle. Reference [24] has reported the concentrations of Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in liver and muscle of *Tor grypus* in Ataturk Dam Lake, Turkey. Reference [20] determined the concentrations of Pb, Cd, Zn, Ni, Cu, Cr and Hg in liver and muscle of *Labeorohita* and *Ctenopharyngodonidella* in the Lake of Bhopal, India. It was showed that the concentrations of heavy metals in liver were higher than muscle.

The mean estimated concentrations for Hg, Pb and Cd in the present study were lower than international standards for these metals as declare by [33] and [34]. Concentrations of As in this study were higher than [35] (Table III). Muscle As concentrations in the two fish exceed WHO limited, representing a potential risk for human consumption in case fish were eaten.

TABLE III: COMPARISON OF HG, CD, PB AND CU CONCENTRATIONS (MG/ KG) IN MUSCLE OF *BARBUSGRYPUS* AND *CAPOETACAPOETA* WITH STANDARDS (WORLD HEALTH ORGANIZATION, U.S. FOOD AND DRUG ADMINISTRATION, MINISTRY OF AGRICULTURE, FISHERIES AND FOOD (UK), NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL (AUSTRALIA), FOOD AND AGRICULTURE ORGANIZATION) (MG/ KG)

| Standards | Hg | Cd | Pb | As | References |
|---|---|---|---|---|---|
| WHO | 0.5 | 0.2 | 2 | 0.02 | WHO, 1996 |
| FDA | 0.5 | 2 | 0.5 | - | Chen and Chen, 2001 |
| UK (MAFF) | 0.5 | 0.2 | 2 | - | MAFF, 1995 |
| NHMRC | 1 | 0.05 | 1.5 | - | Chen and Chen, 2001 |
| *B. grypus* | 0.072 | 0.120 | 0.248 | 0.138 | This study |
| *C. capoeta* | 0.063 | 0.111 | | | |

Although rock weathering, atmosphere deposition and phosphate mineral sources are the natural inputs of heavy metals to water systems, different anthropogenic activities such as discharges from electroplating and textile factories, ship antifouling paints, agriculture runoff and vehicle emissions all contribute to heavy metal pollution in marine sediments [36]. These toxic elements consequently accumulate in fish muscle, threatening human health through the consumption of contaminated fish. Such non- essential metals also poses the most harm since continuous exposure of organisms to low concentrations of these metals may result in their bioaccumulation, and their subsequent transfer to human beings via the food chain [37]. In above investigations, fishes have been successfully used as accurate indicators organisms for environmental monitoring programmes because they posses numerous advantages which include: 1) They are typically present in all aquatic systems; 2) there is extensive life- history and environmental response information available for most species; 3) fish communities usually include a range of species that represent a variety of trophic levels and include of foods ob both aquatic and terrestrial origin; 4) they are comparatively stable and therefore provide a long- term record of environmental stress; 5) they contain many life forms and functional guilds and thus are likely to cover all components of aquatic systems affected by anthropogenic disturbance; and 6) they are both sedentary and mobile and thus reflect stressors within one areas as well as providing scientists to give a broader assessment of effects [38]. Therefore, the elucidation of heavy metal levels in the fish species investigated in this study provided an indication of the current environmental conditions of the Heleh River. Metal concentrations measured in fish are directly or indirectly influenced by a large set of biotic and abiotic factors. Fish can uptake trace metals by two main routes, either by absorption from water through the gill, and from food absorbed through the digestive tract. According to the literatures, metal bioaccumulation by fish and subsequent distribution organs is greatly inter- specific. In additions, many factors can influence metal uptake like sex, age, size, reproductive cycle, swimming patterns, feeding behavior and living environment [39].

Metal present in water show different bio- availabilities, both for fish and their prey. Water chemistry features such as dissolved and suspended organic carbon, pH, hardness and alkalinity are important modified of metal bio- availabilities and toxicity to aquatic organisms.

Many factors can be at the region of the difference between fish species collected in the same site, e.g., diet, lifespan and physiological characteristics. Several authors attribute the interspecific differences observed in the metal burden of fish tissues to variation in diet [40].

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