Journal of Applied Biological Sciences 10 (2): 29-34, 2016
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

# Metal Accumulation in Tissues of Capoeta bergamae Karaman, 1969 (Cyprinidae, Teleostei) from Köyceğiz Lagoon System, South-West Turkey

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Received: March 07, 2016
Accepted: April 19, 2016

**Abstract**

The present study investigated metal accumulation in tissues of Capoeta bergamae and its relation to individual mean (multi-metal) bioaccumulation index (IMBI). Metals in fish were analyzed by ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrometry). Metal accumulation of C. bergamae was investigated three seasons on 2011. The average maximum concentrations of Cd (0.592±0.32), Co (0.786±0.53), Cu (71.682±66.01) and Fe (159.569±102.48) were determined high in liver on spring, while Zn (86.875±93.76) and Se (2.297±2.00) concentration was found high in liver on winter. On the other hand, Mn (16.354±8.87), Cr (5.789±8.36) and Al (91.796±47.46) concentrations were found highly in muscle on winter. Generally, higher concentrations of the measured metals were found in the spring compared with those during autumn and winter. IMBI values varied from 0.07 to 0.41, 0.03 to 0.31 and 0.04 to 0.34 in liver, muscle and gill respectively. Generally, mean IMBI values calculated in liver (0.18±0.09) were higher than other tissues (0.14±0.08 for muscle and 0.16±0.09 for gill). Average Cd and Pb concentrations in the fish muscle were higher the maximum allowed concentrations for human consumption.

**Keywords: Metal, Capoeta bergamae, bioaccumulation index, Köyceğiz, Turkey**

# INTRODUCTION

Metal contaminants are naturally present in the aquatic systems but can be increased through industrial, domestic and agricultural activity and pollution [1]. Metal pollution in fish has become an important worldwide concern due to the threat it poses to both aquatic food chains and human health [2]. Fish provide a necessary source of protein throughout the world; fishing is increasing worldwide [3]. Fish can accumulate heavy metals from water via gills and through consumption of contaminated food [4]. Therefore, consumption of fish can be a major route for human exposure to metals [5,6].

Metals enter fish through five main routes (food or non-food particles, gills, water, and skin), follow into the blood, and are carried to either a storage point or to the liver for its transformation or storage [7] .

Most of the studies related to the metal pollution in fish have been mainly focused on the muscle tissue, which is not surprising, as it is the main fish part consumed by humans [2,8]. Some tissues are able to accumulate significantly higher metal concentrations than the muscle tissue [9,10]. In general, accumulation of metals in fish is observed in various tissues, mainly in liver and in the gills. Little accumulation has been observed in muscle. The concentrations of metals in the gills reflect the concentrations of metals in the waters where the fish live, whereas the concentrations in liver represent storage of metals [11]. Liver and gills in fish are more often recommended as environmental indicator organs of water pollution than any other fish organs.

Lake Köyceğiz, located in southwestern Turkey, is among the "Specially Protected" areas because of its natural beauty and ecological and archeological importance [12]. The lake is connected to the Mediterranean Sea coast, Iztuzu, where endangered sea turtle species, Caretta caretta, nest and breed [13]. Köyceğiz Lagoon System has also an economic importance for fishery. The region is under the effect of pressure of pollution originating from agriculture, touristic activities, boat and shipping traffic [14]. Therefore, information on elemental concentration in fish species in Köyceğiz Lagoon System becomes of great importance.

The aims of the present study were twofold: (1) to study temporal influences (i.e. three seasons, autumn, winter and spring) on metal distribution in liver, muscle and gill tissue of Capoeta bergamae and (2) performed IMBI values to compare metal accumulation.

# MATERIALS and METHODS

### Sampling Sites

Lake Köyceğiz is situated at the Lycian coast of the province of Muğla, SW Turkey (Figure 1). The surrounding of Lake Köyceğiz, one of the largest coastal lakes in Turkey, is characterized by a variety of biotopes on a relatively small area [15]. Typical Mediterranean climate with hot, dry summers and mild, wet winters prevails in the area. Based on the long term average data, the mean annual precipitation over the basin is 1202 mm. The mean annual temperature is about 18°C [13].

### Sample collection and preparation

During the study period, three fishing expeditions were carried out in the Köyceğiz Lagoon in autumn, winter and summer in 2010. The samples of C. bergamae were collected from the various sites of Köyceğiz Lagoon (figure 1). Thirty three C. bergamae were caught with gill-net and samples were collected by electro fishing, using Deca-Lord-12 V generator which produces 250–600 V. Fish caught also every season but from different area in lagoon system. The Standard length (L) and weight (W) of all fish were immediately measured and estimated (mean and standard deviation) as 33.09 cm and 224.19 g respectively. After caught, the samples were placed in an ice box, transported to the laboratory, washed with distilled water and kept in a freezer (-20 $^{o}$C) before analysis. The liver, muscle and gill tissues were dissected using stainless steel knife which had been cleaned with acetone and distilled water prior to use. The samples were then oven dried to constant weight at 90

°C. The Teflon vessel were cleaned, soaked in %5 $HNO_3$ for more than 1 day than rinsed with ultrapure water and dried. For metal analysis, 0.5 g of tissue and 20 mL water sample was treated with 7 mL 70 % $HNO_3$ acid and 3 mL 30% $H_2O_2$ in a closed Teflon vessel and was digested microwave digestion system (Berghof speedway MWS-3+). The digestion flasks were then put on a microwave digestion unit to 120°C (gradually increased) until all the materials were dissolved. The digested solution was then filtered by using Filter papers (Sartorius-Stedim, particle retention=2-3µm) and stored in 25 mL polypropylene tubes.

All samples were analyzed simultaneously two times for Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn, Al, Hg, As, and Se by ICP-AES Optima 2000-Perkin Elmer (Inductively Coupled Plasma-Atomic Emission Spectrometry). Detection limits (µg l-1) were as follows: Cd (0.001), Co (0.001), Cr (0.007), Cu (0.014), Pb (0.001), Zn (0.006), Ni (0.004), Mn, (0.005), Fe (0.003), Al (0.028), Hg (0.061), As (0.053) and Se (0.075). Standard reference materials for fish DORM-3 (National Research Council Canada, Ottawa Ontario, Canada) were used and replicate analysis of these reference materials showed good accuracy, with recovery rates for metals between 91% and 109% for fish.


**Figure 1:** Köyceğiz Lagoon System

**Data analyses**

Kruskal-Wallis and Man-Whitney tests were used to access whether metal concentrations varied significantly between tissues and season, possibilities less than 0.05 ($P<0.05$) were considered statistically significant. Pearson's test also used to correlate metals accumulation.

All statistical calculations were performed with SPSS 20.0 for Windows while Origin 8.0 was used to draw. The individual mean (multi-metal) bioaccumulation index (IMBI) was calculated as:

$$\mathrm{IMBI} = \frac{\sum_{i=1}^{n} C_i / C_{i\max}}{n}$$

With N the total member of metals, Ci the individual metal concentration of heavy metal I, Cimax the maximal observed concentration of heavy metal I and 0<IMBI<1 [16].

# RESULT and DISCUSSION

**Metal concentration in tissue**

Mean concentrations of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn, Al, Hg, As and Se accumulation on autumn, winter and spring are given Table 1.

The accumulation of investigated heavy metals in liver tissue was in order as; Fe> Cu> Zn> Al> Mn> Cr> Ni> Pb> Se> Co> Cd> As> Hg.

In muscle tissue the order of accumulation of the investigated heavy metals was as; Fe > Al> Zn> Mn> Cr> Cu> Ni> Pb> Se> Cd> Co> As> Hg.

In gill tissue the order of the accumulation of the investigated heavy metals was: Fe > Al> Zn> Mn> Cu> Cr> Ni> Pb> Cd> Co> Se> As> Hg.

Average metal accumulation of C. bergamae was investigated three seasons; the highest accumulation of heavy metal for each season was Fe. The maximum concentrations of Cd (0.592±0.32), Co (0.786±0.53), Cu (71.682±66.01) and Fe (159.569±102.48) were determined high in liver on spring, while Zn (86.875±93.76) and Se (2.297±2.00) concentration was found high in liver on winter. Lowest Se concentrations were detected in all tissues on autumn and spring whereas in winter Se concentration was 2.297± 2.00 µg/g in liver and 1.061± 0.89 µg/g in muscle. On the other hand, Mn (16.354±8.87), Cr (5.789±8.36) and Al (91.796±47.46) concentrations were found highly in muscle on winter. Highest total Pb concentration was found in all tissues on autumn and spring while concentration of Pb was determined lowest on winter. The lowest As concentration determined on autumn and winter but increase of As concentrations was observed in all tissues on summer.

**Table 1.** Metals concentration in tissues of Capoeta bergamae

| | Tissues | Cd | Co | Cr | Cu | Fe | Mn | Ni | Pb | Zn | Al | Hg | As | Se |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Autum | Liver (n=13) | 0,142± 0,152 | 0,218± 0,724 | 2,588± 3,288 | 35,613± 26,682 | 33,382± 55,404 | 4,784± 5,511 | 4,673± 2,660 | 2,176± 1,132 | 25,620± 11,651 | 13,840± 22,868 | 0,065± 0,103 | 0,058± 0,080 | BDL |
| | Muscle (n=13) | 0,134± 0,124 | BDL | 0,494± 0,594 | 2,119± 2,374 | 24,331± 87,726 | 1,802± 1,909 | 4,149± 2,284 | 1,274± 0,786 | 28,175± 12,230 | 8,403± 7,392 | 0,052± 0,062 | 0,023± 0,041 | BDL |
| | Gill (n=13) | 0,199± 0,196 | BDL | 2,893± 3,273 | 2,444± 2,789 | 14,040± 35,339 | 11,994± 7,532 | 3,651± 1,825 | 1,950± 1,103 | 30,212± 9,020 | 44,656± 59,088 | 0,025± 0,041 | 0,037± 0,058 | BDL |
| Winter | Liver (n=9) | 0,062± 0,089 | 0,025± 0,054 | 3,946± 3,893 | 39,370± 27,045 | 134,581± 59,284 | 4,913± 3,140 | 0,489± 0,530 | 0,025± 0,060 | 86,875± 93,769 | 69,490± 47,350 | BDL | 0,022± 0,066 | 2,297± 2,002 |
| | Muscle (n=9) | BDL | 0,006± 0,012 | 5,789± 8,364 | 2,969± 0,645 | 61,732± 36,661 | 16,354± 8,878 | 0,509± 0,591 | 0,079± 0,105 | 38,093± 25,079 | 91,796± 47,468 | BDL | 0,044± 0,088 | 1,061± 0,898 |
| | Gill (n=9) | BDL | 0,088± 0,221 | 2,686± 1,518 | 2,181± 0,836 | 50,443± 45,499 | 7,167± 6,391 | 0,657± 0,711 | 0,144± 0,246 | 24,779± 25,863 | 73,460± 45,856 | BDL | 0,056± 0,092 | 0,662± 0,634 |
| Spring | Liver (n=11) | 0,592± 0,320 | 0,786± 0,535 | 3,119± 2,181 | 71,682± 66,011 | 159,569± 102,482 | 4,534± 1,645 | 1,852± 0,932 | 2,412± 2,078 | 20,703± 16,048 | 24,763± 23,969 | 0,248± 0,261 | 0,222± 0,673 | 0,177± 0,586 |
| | Muscle (n=11) | 0,556± 0,317 | 0,697± 0,563 | 3,145± 1,882 | 2,279± 0,937 | 55,945± 41,750 | 7,020± 7,472 | 1,473± 0,926 | 2,426± 1,838 | 14,857± 29,368 | 39,780± 43,241 | 0,315± 0,367 | 0,313± 0,618 | 0,109± 0,363 |
| | Gill (n=11) | 0,575± 0,329 | 0,724± 0,554 | 3,333± 0,718 | 6,619± 13,431 | 136,967± 93,378 | 12,931± 8,223 | 1,673± 0,499 | 2,019± 1,737 | 25,952± 14,637 | 31,765± 23,002 | 0,258± 0,313 | 0,469± 0,578 | 0,222± 0,737 |
| Average | Liver | 0,278± 0,317 | 0,364± 0,615 | 3,171± 3,088 | 49,502± 46,548 | 107,538± 93,218 | 4,733± 3,699 | 2,457± 2,412 | 1,635± 1,725 | 41,659± 57,835 | 33,872± 39,011 | 0,111± 0,194 | 0,106± 0,403 | 0,730± 1,492 |
| | Muscle | 0,238± 0,304 | 0,234± 0,458 | 2,822± 4,842 | 2,404± 1,619 | 45,069± 63,719 | 7,510± 8,592 | 2,264± 2,199 | 1,332± 1,464 | 26,441± 23,841 | 41,606± 48,224 | 0,126± 0,250 | 0,126± 0,374 | 0,326± 0,674 |
| | Gill | 0,270± 0,321 | 0,265± 0,467 | 2,983± 2,197 | 3,764± 7,980 | 64,944± 81,275 | 10,990± 7,644 | 2,175± 1,753 | 1,481± 1,451 | 27,310± 16,448 | 48,215± 47,725 | 0,0960± 0,212 | 0,186± 0,386 | 0,255± 0,586 |

**BDL:** Below the detection limit.

The present study focused on the accumulation of heavy metals in C. bergamae and showed differences in the accumulation of heavy metals during the seasons. The significant differences were found for Fe, Ni, Pb, Al, Hg and As in liver and muscle also there is significances were found for Cd and Cr in muscle between autumn and winter ($p<0.05$). Furthermore; Fe, Mn and Ni levels in gill on autumn differed significantly from winter ($p<0.05$). However, significant differences for Cd, Co, Ni and Fe in liver were identified between autumn and spring ($p<0.05$). Cu, Pb, Al and As levels in muscle on autumn not different from spring ($p<0.05$). The significant differences for Ni, Fe, Co and Cd were found in gill between autumn and spring. Trace metals such as, Mn, Fe, Cu and Cr were not show significantly differences in liver and muscle between winter and spring whereas other metals show significantly differences (except Mn for muscle and Fe for gill, $p<0.05$). As exhibited were not significant differences in all tissues between winter and spring.

During the rainy season, Köyceğiz Lagoon is heavily flooded and the lake is drastically affected causing mixing of polluted and unpolluted waters. This leads to a decrease in metal concentration whereas the increase in the concentration of metals observed during spring season could be due to drought and decreased water level [17].

It is important to note that accumulation of metals in muscles is relatively lower than in other tissues because muscles do not directly contact with metals as they are fully covered externally by the skin [18]. Another explanation for that is that muscles are not an active site for detoxification, and hence for transport of metals from other tissues to muscles[10]. Metals mainly accumulate in metabolically active tissues [19,20]. Liver tissue is highly active in the uptake and storage of metals, and it is well known that large amount of metallothionein induction occurs in fish liver tissues [21].

**Correlation matrix**

Pearson's correlation analysis was applied to test the relationship among the metals analyzed and showed in Table 2. The correlation matrix for tissues showed that Cd were highly correlated with Co, Pb, Hg and Fe showing a strong positive association (Cd-Co= 0.84, Cd-Pb= =0.65, Cd-Hg= 0.58, Cd-Fe= 0.37; $p<0.001$). Pb was negatively correlated with Zn, Al, Se (0.28, 0.31 and 0.39 respectively; $p<0.01$). The high correlation obtained between Co-Fe (0.45, $p<0.001$), Co-Pb (0.53, $p<0.001$) Co-Hg (0.52, $p<0.001$) in tissues of C. capoeta. Only Ni was negatively correlated with almost all metals except Cd (0.20, $p<0.05$) and Pb (0.31, $p<0.01$). Significantly positive relationships were found between Fe-Cu (0.54, $p<0.001$) and also was found positive correlation with other metals and Fe but correlation was not significantly important. Other trace metals showed weaker relationships. However, different organs showed significant correlation in metals bioaccumulation, which may be different in aquatic environment, uptake, sex and age of the fish [22].

**Individual metal bioaccumulation index and human consumption**

IMBI can explain distribution of total metal load seasonally and showed figure 2. IMBI has been used in many investigations for European eel Anguilla anguilla, Blue crab Callinectes sapidus, European chub Squalius cephalus and fresh water mussels Unio sp. as a good monitoring tool [16,23,24,25,26]. The distribution of total metal load values ranged from 0.07 to 0.41, 0.03 to 0.31 and 0.04 to 0.34 in liver, muscle and gill respectively. According to [27] Maes et al., (2005) index value assessment was defined before 0.22 "low" and after 0.25 "high" polluted individuals. Usually average IMBI values determined on higher in liver (0.18±0.09) than other tissues (0.14±0.08 for muscle and 0.16±0.09 for gill). The liver is the main site of accumulation, biotransformation, and excretion of pollutants in fish (Shinn et al., 2009). Furthermore, average IMBI values

**Table 2.** Pearson's correlation matrices of metal concentration in tissues (n=97)

| | Cd | Co | Cr | Cu | Fe | Mn | Ni | Pb | Zn | Al | Hg | As | Se |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cd** | - | | | | | | | | | | | | |
| **Co** | 0.84*** | - | | | | | | | | | | | |
| **Cr** | 0.05 | 0.13 | - | | | | | | | | | | |
| **Cu** | 0.27** | 0.28** | 0.09 | - | | | | | | | | | |
| **Fe** | 0.37*** | 0.45*** | 0.17 | 0.54*** | - | | | | | | | | |
| **Mn** | 0.1 | 0.07 | 0.28** | -0.11 | 0.19 | - | | | | | | | |
| **Ni** | 0.20* | 0.1 | -0.15 | 0.1 | -0.17 | 0.11 | - | | | | | | |
| **Pb** | 0.65*** | 0.53*** | -0.02 | 0.19 | 0.11 | -0.05 | 0.31** | - | | | | | |
| **Zn** | -0.18 | -0.18 | 0.02 | 0.06 | 0.19 | 0.04 | -0.05 | -0.28** | - | | | | |
| **Al** | -0.14 | -0.12 | 0.40*** | -0.05 | 0.15 | 0.43*** | -0.13 | -0.31** | 0.19 | - | | | |
| **Hg** | 0.58*** | 0.52*** | -0.05 | 0.1 | 0.17 | -0.02 | -0.02 | 0.43** | -0.09 | -0.05 | - | | |
| **As** | 0.13 | 0.15 | -0.08 | -0.01 | 0.1 | 0.04 | -0.06 | 0.08 | 0.12 | 0.06 | 0.24* | - | |
| **Se** | -0.35** | -0.23* | 0.32** | 0.14 | 0.12 | 0.08 | -0.35** | -0.39** | 0.1 | 0.43*** | -0.17 | 0.09 | - |

also in spring (0.22±0.09) calculated higher than autumn (0.12±0.07) and winter (0.13±0.06). The seasonal behavior of metals can be explained by the impact of wastewaters that cause high levels of metals during the spring, and by intense agricultural activities in spring.

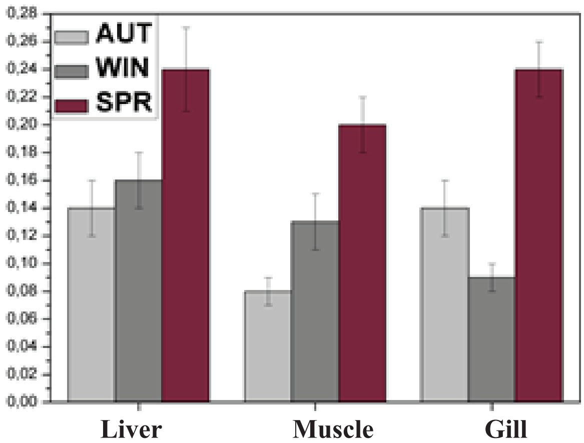


**Figure 2:** The seasonal distribution of individual mean bioaccumulation index (IMBI) values in tissues of Capoeta bergamae

Unfortunately, there is no uniform source of guidance or standards for most metal residues in aquatic ecosystems, especially in fish tissue. We could not find single references for acceptable levels of most metals in marine or fresh water fish, self-caught or commercial ones. Results were compiled from Agriculture Organization and the World Health Organization, European Commission, Turkish guidelines and public research (Table 3).

C. bergamae are important sources of protein for human nutrition in the region. Therefore, the presence of metals in posed a risk for food contamination. According to EU, TFC and FAO/WHO for food, the present finding of Cu Mn and Zn value is below the tolerable levels. The data in this study suggest that some metals in muscle have relatively high levels of contaminants of concern, such as Cd and Pb. According to our results, the examined fish were associated with Cd and Pb content in their muscle and were not safe within the limits for human consumption. Low levels of Cd cause kidney damage; meanwhile, severe exposure causes nephrotoxicity, carcinogenicity, reproductive disorders, coronary atherosclerosis, and neurological disorders [28,29]. Pb is classified as a priority pollutant because it is not required for metabolic activity and can be toxic even at quit low concentrations. Moderate exposure to Pb can significantly reduce human semen quality and is related to many diseases in adults and children alike (e.g. damage to DNA or impairment of the reproductive function) [30]. In addition, chronic exposure to Pb causes dullness, poor attention span, vomiting, coma, encephalopathy, and death [31].Human beings have been exposed to heavy metal

toxins for a measurable amount of time. These toxins contribute to a variety of adverse health effects. Knowledge

**Table 3.** Average metal concentrations (µg/g) in muscle tissue of Capoeta bergamae and Comparison of metals in muscle (µg/g) with different other studies in Mugla part and guidelines.

| | Cd | Cr | Cu | Fe | Mn | Ni | Pb | Zn | References |
|---|---|---|---|---|---|---|---|---|---|
| Present study | 0,238±0,30 | 2,822±4,84 | 2,404±1,61 | 45,069±63,71 | 7,510±8,59 | 2,264±2,19 | 1,332±1,46 | 26,441±23,84 | - |
| Sarıçay Stream,Turkey | 0.01±0.01** | 0.97±0.19 | 1.72±0.15 | 21.32±3.55 | 5.94±0.57 | 1.36±0.19 | 1.48±0.24 | 37.09±6.10 | Oglu et al., 2015 |
| Sarıçay Stream (I and II) | 0.02 and 0.001 | BDL | 0.19 and 0.57 | 4.47 and 4.24 | 0.11 and 1.2 | BDL | 0.07 and 0.30 | 6.35 and 9.66 | Yılmaz et al., (2007) |
| Köyceğiz Lagoon System | 0,223±0,26 | 1,390±1,45 | 8,563±29,26 | 49,734±87,96 | 2,148±2,28 | 2,204±2,15 | 1,076±0,99 | 32,929±41,01 | Yorulmaz et al., 2015 |
| FAO/WHO | 0.50 | - | 30 | - | - | - | 0.50 | 40 | FAO/WHO (1989) |
| EU | 0.10 | - | 10 | - | - | - | 0.10 | - | EU (2006) |
| TFC | 0.1 | - | 20 | - | 20 | - | 1 | 50 | TFC (2002) |

**BDL:** Below the detection limit.

of heavy metal concentrations in fish is important both with respect to nature management and human health.

However, the bioaccumulation of metals in gills and liver was much higher than muscle. It may be also concluded that the edible part of fish muscle is not safe for consumption by human beings. Conspicuously, it is quite evident that higher Cd and Pb accumulate in muscle and if preventive measures are not taken, the condition may get worse in times to come for Köyceğiz Lagoon System.

**Acknowledgments**

The authors are grateful to the Scientific and Technological Research Council of Turkey (TUBITAK) for financial support. This study is a part of TUBITAK CAYDAG project (108Y261).

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