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A Comparison of Heavy Metal Concentrations in Skin and Muscle Tissues of Wild and Cultured Carp (*Cyprinus carpio*) in the Southeastern Caspian Sea Area of Iran

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Abstract

Metal concentrations in commercial and non-commercial fish were surveyed to assess risks of fish consumption to human health and importantly, assess contamination in the food chain. In this study, lead (Pb), Zinc (Zn) and Copper (Cu) concentrations were determined in muscle and skin tissues of wild and cultured *Cyprinus carpio* (common carp) from the southeastern Caspian Sea area and a nearby fish farm in November 2007. Metal concentrations were determined by atomic absorption. Pb and Cu concentrations in all of the samples were below detection limits and there were no statistically significant differences of Zn concentrations in muscle and skin tissue between wild and cultured carp. But Zn concentrations in the skin tissue were significantly higher than in muscle tissue in both groups; this suggests that more studies about skin tissue as a site of bioaccumulation are necessary. None of the concentrations exceeded WHO safety standards. Our results have suggested that heavy metal contents in carp are negligible and that its consumption should pose no health problems for consumers of either the wild or farmed fish.

Key words: Heavy metal, Aquatic Pollution, *Cyprinus carpio,* Caspian Sea, Bioaccumulation.

مقایسه میزان فلزات سنگین در بافت عضله و پوست ماهی کپور وحشی و پرورشی در منطقه جنوب شرقی دریای‌خزر

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چکیده

ماهی کپور از جمله ماهیان تجاری مهم منطقه جنوب‌شرقی خزر است که هم نوع وحشی و هم نوع پرورشی آن قسمت عمده مصرف ماهی مردم منطقه را به خود اختصاص می‌دهد. در این مطالعه میزان ۳ فلز سنگین: سرب، روی و مس در عضله و پوست ماهیان کپور معمولی و کپور پرورشی که از منطقه جنوب‌شرقی دریای‌خزر و یک مزرعه پرورش ماهی در همان منطقه در پاییز ۸۶ صید شده بودند، مورد بررسی قرار گرفت. پس از بیومتری و آماده‌سازی نمونه‌ها میزان فلزات سنگین بااستفاده از دستگاه جذب اتمی شعله‌ای اندازه‌گیری شدند. غلظت سرب و مس برای تمامی نمونه‌ها زیر حد تشخیص دستگاه و میانگین غلظت روی در پوست و عضله کپور وحشی به ترتیب ۱.۷۰ ± ۱۰.۹۵ و ۰.۵ ± ۲.۷۸ ppm و در پوست و عضله کپور پرورشی به ترتیب ۱.۲۰± ۹.۷۰ و ۰.۷۹ ±2.79 ppm به دست آمد. هیچ‌گونه همبستگی معنی‌داری بین طول و وزن ماهیان با غلظت فلزات سنگین در عضله و پوست مشاهده نشد. تجمع فلز سنگین روی در پوست هر دو گروه ماهی کپور به طور معنی‌داری بیشتر از تجمع این فلز در عضله آن‌ها بود که لزوم توجه به بافت پوست به عنوان بافتی مستعد جهت تجمع زیستی فلزات سنگین پیشنهاد می‌شود. هیچ‌گونه اختلاف معنی‌داری بین غلظت فلزات سنگین در عضله و پوست ماهیان وحشی و عضله و پوست ماهیان پرورشی یافت نشد. تمامی مقادیر به دست آمده پایین‌تر از حد مجاز تعیین شده از سوی سازمان بهداشت جهانی (WHO) می باشد. با این حال لزوم پایش مکرر و ضرورت پالایش پساب‌های کارخانجات حوضه آبریز توصیه می‌شود.

کلمات کلیدی: فلزات سنگین، آلودگی آبزیان، تجمع زیستی، دریای خزر، *Cyprinus carpio*

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Introduction

Heavy metals are natural trace components of an aquatic environment, but their levels have increased due to industrial, agricultural and mining activities (Karadede and Unlu, 2000). As a result, aquatic animals are exposed to elevated levels of heavy metals (Jalali and Aghazadeh, 2006). Some heavy metals such as zinc, copper and cobalt are essential, in trace amounts, for normal growth and development; however others, such as mercury, cadmium and lead, have no biological importance (Langston, 1990). Studies have shown that fish are able to accumulate and retain heavy metals from their environment and transfer these, through the upper classes of the food-chain, to humans (Langston, 1990).

The common carp *(Cyprinus carpio*) is one of the most extensively cultivated fish species in the world. Cultured carp consume artificial diets, generally commercial pellets containing 32% protein and 3.5% lipid. However, in a natural sea environment the major food source for wild carp is from sediment. Omnivorous, sediment-dwelling, fish species such as carp may therefore accumulate heavy metals more readily than pelagic species as a result of exposure to the generally higher metal content of sediment compared with water (Celechovska *et al.*, 2007).

No detailed study has yet been undertaken to compare and contrast concentrations of heavy metals in cultivated and wild carp in the south eastern Caspian Sea area and nearby farms, despite the fact that fish are considered an essential part of the local diet Concentrations of lead, zinc and copper were measured in the skin and muscle tissue of fish in the southeastern Caspian Sea area and this research could be used to establish a baseline for future studies of heavy metal pollution.

Materials and Methods

Area information

The Caspian Sea is the largest body of brackish water on earth with no natural connection to the ocean. Iran is located to the South of the Caspian Sea and has a shoreline of 650 km. In this study wild carp samples were caught from the southeastern area of the sea, with the geographical location of 37˚ 40'N and 54˚ 22'E. The Gorganrood is the most important river to flow into this area and it discharges agricultural, urban and industrial waste into the southeastern Caspian Sea. Cultured carp samples were caught from a fish farm near this area, located at 38˚ 40' N and 54˚ 22' E. The source of water to the farm was from the Gorganrood.

Collection of samples

30 Adult *C.carpios* were purchased from fishermen in the area and caught from the farm in November 2007. The average length and weight of the wild carp was 34.26 ±2.1cm and 514.52± 91.70g and 37.5±0.55cm and 695.60±101.248g were the respective length and weight averages for the cultured carp.

The fish samples were transferred to the laboratory in ice-packs and were kept frozen there pending analysis.

Digestion of samples

The samples were digested in open beakers on a hot plate and 2g of each tissue sample (muscle and skin) were weighed out in an open beaker and 10 ml of freshly prepared 1:1 nitric acid; hydrogen peroxide was added. The beaker was covered with a watch glass until the initial reaction subsided after about one hour. The beaker was placed in a water bath on a hot plate and the temperature was gradually allowed to rise to 160° C and the contents boiled gently for about 2 hours to reduce the volume to between 2-5 ml. The digests were allowed to cool and were then transferred to 25ml volumetric flasks and made up to mark with de-ionized water (FAO/SIDA, 1993). The digests were kept in plastic bottles for later analysis.

Sample Analysis

Heavy metal contamination levels were determined using an atomic absorption spectrophotometer (AAS) (PERKIN-ELMER-560) by adopting a specific cathode-ray tube for each heavy metal.

Determination of Recovery

The actual concentration of each metal was calculated using the formula below:

Actual concentration of metal in sample = PPmR × dilution factor

Where PPmR = AAS Reading of digest.

Dilution factor = volume of digest used/Weight of sample digested

The concentrations were calculated on a wet weight basis.

Statistical Analysis

Statistical Analysis of the data was carried out using SPSS statistical package programs. Normality of the data was checked by the Shapiro-Wilk test. Logarithmic data instead of the real data was used to establish metal concentrations in the muscles. The bioaccumulation of heavy metals in wild and cultured carp was compared with independent T-test. A one-way analysis of variance (ANOVA) was performed, followed by Scheffe post hoc comparisons between weights/lengths and metal concentrations. Differences in mean values were accepted as being statistically significant if $p<0.05$.

Results

The average size and weight values of each sample group were measured and are shown in Table 1. No statistically significant correlation ($p<0.05$) between carp length and metal concentrations in muscle and skin tissue was found. Also, there was no statistically significant correlation ($p<0.05$) between carp weight and metal concentration in muscle and skin tissue. There was a significant difference, however, between the length to weight ratios of fish due to independent T-tests, which found that the cultured carp were larger than the wild carp.

Mean concentrations of metals in muscle and skin tissues of wild and cultured fish are given in Table 2.

Mean concentrations of zinc in the skin tissue statistically were significantly higher than that of the muscle tissue in both of the sample groups as shown in graph 1. Although the wild carp and the cultured carp had differences in their habitats, food and size they didn't show any significant differences in zinc concentrations of the muscle and skin tissue between the two groups. This is an indication that they accumulate metals in the same manner.

Table 1. The average wet weight and total lengths of fish.

	W (g)	L (cm)
Wild carp	514.52± 91.70	34.26 ±2.1
Cultured carp	695.60±101.248	37.5±0.55

Table 2. Mean concentrations of heavy metals (μg/g W Wt) in wild and cultured fish.

Fish Species	Tissues	**Pb**	**Zn**	**Cu**
Wild *Common carp*	Muscle	N.D.	2.78±0.5	N.D.
	Skin	N.D.	10.95±1.7	N.D.
Cultured *Common carp*	Muscle	N.D.	2.79±0.79	N.D.
	Skin	N.D.	9.70±1.2	N.D.

N.D.= Not detected

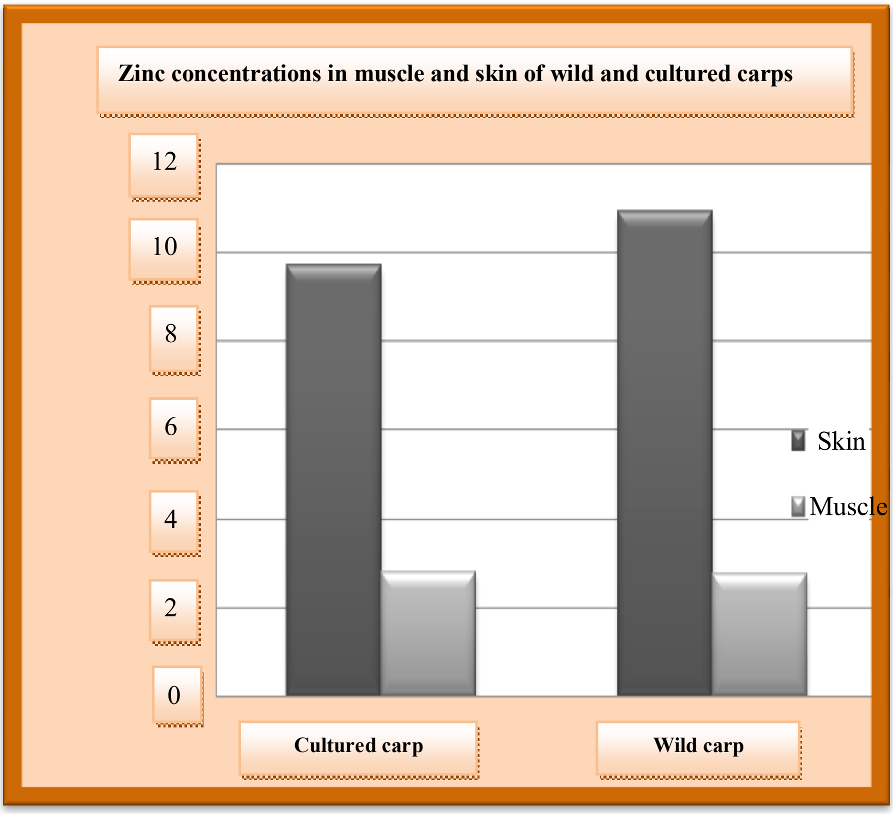

Figure 1. Mean concentrations of Zn in tissues of carps.

Copper and lead are known to be among the heavy metals that are toxic to aquatic organisms. Lead poisoning causes teratogenic effects and also inhibits the synthesis of hemoglobin; dysfunction in the kidneys, joints and reproductive systems, cardiovascular system and acute and chronic damage to the central nervous system (PNS) and peripheral nervous system

(PNS) (Ogwuebgu and Muhanga, 2005) both lead and copper were found to be below detectable levels of 1 µg/g in our AAS.

Zinc is considered to be relatively non-toxic, especially if taken orally. However, an excessive amount can cause a system dysfunction that results in impaired growth and reproduction (Inecar, 2000).

The levels of metals found in tissues of *Cyprinus carpio* were compared to the different safety standards, as shown in Table 3.

Table 3. Different safety standards in fish are shown.

No.	***Standards***	**Zn (ppm/W.Wt)**	**Pb (ppm/W.Wt)**	**Cu (ppm/W.Wt)**
1	WHO	____	10	10
2	NHMRC	150	1.5	10
3	(U.K)MAFF	50	2	20
4	Results in this Study	Muscle: 2.78 Skin: 10.95	Under 1 ppm	Under 1 ppm

1- World Health Organization

2- Australian National Health and Medical Research Council

3- Ministry of Agriculture, Fisheries and Food

Due to the fact that these standards are set at higher levels than the levels detected in the laboratory, it can be assumed that the samples were safe for human health.

Discussion

In various researches heavy metal bioaccumulation in the tissue of aquatic creatures varies according to fish length and weight. (Barghigiani and Ranier ,1992), (Zyadah, 1999). In order to check this association, all of the fish were weighed and their lengths were measured. However, no significant correlation was found between fish size and weight to metal content. This was also verified by the research findings of (Yilmaz *et al*., 2003).

Having a small range of differences in weights and lengths of each group of fish, because of their commercial sizes, may affect this result. Although the cultured carp were significantly bigger than the wild carp. The efficiency of metal uptake from contaminated water and food may differ in relation to ecological need, metabolism and the contamination gradient of water, food and sediment, as well as other contributing factors such as salinity, temperature and interacting agents (Barghigiani and Ranier, 1992), (Yilmaz, 2005).

It was expected that metal concentrations in the tissues of cultured carp would be higher than in wild carp. There were two reasons for this assumption; firstly, that the cultured carp were caught from fresh water that had had a lot of waste water discharged into it; and secondly, because of the artificial diet of the cultured carp, as opposed to the wild carp's diet of feeding on sediment, and of course because of the larger size of the cultivated carp. Although some studies have shown that sediment has a higher heavy metal content compared with a water column (5), our results did not show any significant differences between metal contents in the tissue of wild carp from that of the cultured carp. Similar results were found by Alam *et al*. (2002). Although in their research metal contents in the sediment were significantly higher than those in the water column, contents of the same metals between cultured carp and wild carp had no significant differences. This suggests that both of the samples uptake metals from water. The literature also states that metal uptake from water is much higher than uptake from sediment (Mance, 1987; Langston, 1990). In another research by Alam *et al*. (2002) concentrations of 13 metals in tissue from muscle, liver, kidney and gonad were compared between wild and cultured carp and distribution models of metals between the various tissues were the same for both groups of fish. Our results verify this finding.

Concentrations of Pb and Cu in the tissue of both groups of carp were under detectable amounts. But concentrations of Zn were significantly higher in the skin compared with the muscle. This result was replicated in both groups of fish. It is generally accepted that muscle is not an organ where metals accumulate (Langston, 1990). Similar results were reported from a number of fish species showing that muscle is not an active tissue in accumulating heavy metals (Kalay and Canli, 1998), (Karadede and Unlu, 1998, 2000), (Rajan *et al*., 1995) and (Alam *et al.,* 2002).

A study of distributions of zinc concentrations in different tissues of *Cyprinus carpio* reported by Celechovska *et al.,* (2007) demonstrated the following:

Muscle tissue<Testes<spleen<Liver<Ovaria<Gills<Kidney

In fact, the Zn concentration in the kidney was reportedly 40 times higher than that of the muscle tissue. Although skin is an edible part of fish, unfortunately the bioaccumulation of metals in this tissue has not yet been the subject of much research. Yilmaz (2003) indicated that concentrations of heavy metals were always higher in skin samples than in muscles. Furthermore, Rezaii *et al.* (2007) measured higher concentrations of cadmium and chrome in the skin compared with the muscle. The reason for this high metal concentration in the skin could be due to metal complexion with mucus that makes its complete removal from the tissue before analysis impossible (Yilmaz, 2003). Also, skin has higher fatty acid content and this could be one of the reasons for higher concentrations of heavy metals in skin tissue. In addition, some heavy metals are transferred to the skin through the process of excretion (Jalali and Aghazade, 2006). In fact skin may be useful as an indicator, and has an important role in detecting heavy metal pollutants.

Consequently, it can be concluded from this study that levels of heavy metals in the selected tissues were at acceptable levels for all of the studied samples and that carp reared in the region are not heavily burdened with metal contamination. In addition, despite their dietary differences, the wild and cultured fish accumulate metals in much the same manner.

Efforts should however be concentrated on ensuring that these concentrations are not exceeded. In view of the importance of fish to the human diet, it is necessary that regular biological monitoring of the water and those fish destined for consumption should be carried out to ensure the continuation of food safety. Safe disposal of domestic sewage and industrial effluent should be practiced, wherever possible, to avoid these metals and other contaminants from entering the environment. Furthermore, the legislation enacted to protect our environment should be fully enforced.

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