



## Heavy Metals (Zn, Cu, Pb, Cd and Hg) Contents and Fatty Acids Ratios in Two Fish Species (*Scomberomorus commerson* and *Otolithes ruber*) of the Persian Gulf

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### Abstract

This study was conducted to determine the concentrations of metals (Zn, Cu, Pb, Cd and Hg) in two commercial fish species of the Persian Gulf (coast of Bushehr Province in Iran), as well as in the sea waters. The concentrations of metals were determined by using differential pulse anodic stripping voltammetry (DPASV). Two selected species were narrow-barred Spanish mackerel and tiger tooth croaker. The same fish were also used to determine the ratios of fatty acids including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). Fish and water samples were collected from three different areas, namely Bushehr (station 1), Tangestan (station 2) and Dayyer (station 3). Results showed that all metals contents had lower concentration of the mean values than the permissible limits set by FAO/WHO. It can be concluded that both fish species studied are safe to be consumed. In the present study, for the first time correlation of metals concentrations with fatty acids ratios in both species was statistically analyzed and showed no correlation except for Pb mean value which had negative correlation with PUFA% in tiger tooth croaker species.

*Keywords:* Fatty acids ratios; Heavy metals; Persian Gulf; Spanish mackerel; Tiger tooth croaker.

*Received:* January 12, 2011; *Accepted:* April 1, 2011.

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### 1. Introduction

Heavy metals are considered the most important form of pollution in the aquatic environment because of their toxicity and

accumulation by marine organisms [1]. Heavy metals contents in aquatic environment have been increased by increased activities in industrial, domestic, and agricultural sections [2, 3].

Heavy metals can enter from contaminated water into fish body by different routes and accumulate in organisms [4, 5]. Some of the

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metals found in fish might be essential as they play important roles in biological systems of the fish as well as in the human being. The common heavy metals that are found in fish are copper, iron, zinc, manganese, mercury, lead, and cadmium [6]. Iron, copper, zinc, and manganese are essential metals while mercury, lead, and cadmium are toxic metals [7]. Essential metals can have adverse and toxic effects at high concentrations [8]. Since, fish are highly consumed by human beings and may accumulate large amounts of some metals from the water, it is important to determine the concentration of heavy metals in commercial fish in order to evaluate the possible risk of fish consumption for human health [9].

During the last two decades, polyunsaturated fatty acids (PUFA) have attracted a great interest among scientists for their medicinal and nutritional properties [10]. The abundance of the unsaturated fatty acids in fat is the most valuable characteristics of the fish [11]. Polyunsaturated fatty acids of the  $\omega$ -6 and especially of  $\omega$ -3 family are recognized as the essential biochemical components of the human diet [12]. Fish tissue is the main source of long chain polyunsaturated fatty acids especially the  $\omega$ -3 and  $\omega$ -6. These fatty acids have particular importance in human diet since their consumption contributes to the reduction of the appearance of the cardiovascular diseases [13] as well as the improvement of learning ability [14, 15].  $\omega$ -3 Essential fatty acids help in reducing the cholesterol level [16], and stopping blood platelets from clinging to one another [17]. The inclusion of fish in the diet is the only certain safe way to increase the intake of these fatty acids [18]. Thus, it is important to clarify the fatty acids ratios of all commonly used fish species.

*Scomberomorus commerson* (narrow-barred Spanish mackerel) and *Otolithes ruber* (tiger tooth croaker) are benthic economical fishes in the Persian Gulf. Both of these fish

species were selected for this study. Also, the contents of metals (Zn, Cu, Pb, Cd and Hg) in fish muscle and sea water samples of each station were determined. Along the coast of Bushehr Province, there are aquaculture farms and industrial plants. Due to heavy aquaculture, agriculture, and industrial activities in the region, biomonitoring of metals, especially heavy metals, is essential to assess the ecosystem.

## 2. Material and methods

### 2.1. Place of fish and water collection

Bushehr Province is located in the southwest of Iran. Three fishery stations were selected: station 1 in Bushehr (N 28°58', E 50°40'), station 2 in Tangestan (N 28°34', E 50°56'), and station 3 in Dayyer (N 27°40', E 51°33'). Marine fish samples were obtained from these stations. In each station, 6 tiger tooth croaker and 6 narrow-barred Spanish mackerel samples were collected from May to June, 2010. In addition, five sea water samples were collected from each station. A water sampler of 1000 ml capacity was used to collect water (depth: 3-15 m). Total length and weight was recorded for all fish specimens. All fish were dissected for its muscle tissue by plastic knife. The muscles of all fish samples were stored in plastic bags and freezed. A 500 mg sample from each muscle was digested by concentrated nitric acid and perchloric acid (2:1 V/V) (Merck) at 60 °C followed by the dilution of all samples to 25 ml with double distilled water. For water analysis, the samples were prepared using the method of American Public Health Association (APHA) [19].

### 2.2. Measurement of heavy metals

Metal concentrations in fish and water samples were measured by differential pulse anodic stripping voltammetry (polarograph metrohm-797). Zn, Cu, Pb, Cd and Hg concentrations were measured in each water and fish sample three times.

**Table 1.** Metal concentration recorded as means  $\pm$  standard deviation in fish samples of each station. (\*Statistically significant from other samples,  $p < 0.05$ ).

| Site | specie   | N | Cd (ppm)           | Pb (ppm)          | Hg (ppm)          | Cu (ppm)        | Zn (ppm)         |
|------|----------|---|--------------------|-------------------|-------------------|-----------------|------------------|
| 1    | mackerel | 6 | 0.003 $\pm$ 0.032  | 0.070 $\pm$ 0.261 | 0.001 $\pm$ 0.002 | 0.64 $\pm$ 6.70 | 2.47 $\pm$ 19.65 |
|      | croaker  | 6 | 0.003 $\pm$ 0.039  | 0.080 $\pm$ 0.243 | 0.002 $\pm$ 0.002 | 0.90 $\pm$ 7.22 | 2.88 $\pm$ 19.35 |
| 2    | mackerel | 6 | 0.003 $\pm$ 0.037  | 0.031 $\pm$ 0.264 | 0.003 $\pm$ 0.003 | 0.42 $\pm$ 7.38 | 1.62 $\pm$ 19.36 |
|      | croaker  | 6 | 0.002* $\pm$ 0.043 | 0.038 $\pm$ 0.272 | 0.001 $\pm$ 0.004 | 0.66 $\pm$ 7.27 | 2.42 $\pm$ 19.61 |
| 3    | mackerel | 6 | 0.008* $\pm$ 0.045 | 0.072 $\pm$ 0.291 | 0.002 $\pm$ 0.006 | 0.46 $\pm$ 7.25 | 0.86 $\pm$ 17.87 |
|      | croaker  | 6 | 0.002* $\pm$ 0.048 | 0.032 $\pm$ 0.303 | 0.001 $\pm$ 0.006 | 0.50 $\pm$ 6.62 | 2.08 $\pm$ 17.47 |

### 2.3. Extraction of lipid

Extraction of the fish fillet lipids were done according to the method of Bligh and Dyer (1959) [20]. Each 100 g muscle sample was homogenized in a mixer blender for 2 min with a mixture of 100 ml chloroform and 200 ml methanol. To the mixture, was then added 100 ml chloroform and after blending for 30 sec, 100 ml distilled water was added and blending continued for another 30 sec. The homogenate was filtered. The filtrate was transferred to a 500 ml graduated cylinder. After allowing a few min for complete separation and clarification, the alcoholic layer was removed by aspiration and chloroform layer (lower separated layer) contains purified lipid. The extraction of lipids was done separately for each fish sample in each station. Subsequently, the oil was saponified to isolate the free fatty acids (FFA). The oils were analyzed for fatty acid profile by using gas chromatograph (Shimadzu-A17, Japan) with a flame ionization detector (FID) and attached to an integrator. The injected sample was 1 ml with carrier gas He (flow: 1.2 ml/min.), column temperature 170 °C (50m $\times$ 0.32mm $\times$ (ID-BPX 70 $\times$ 0.25mm) cyanopropyle siloxan column), injection port temperature 210 °C, and detection port temperature 230 °C.

### 2.4. Statistical analysis

Statistical analysis of data was carried out with SPSS software (version 16) and independent samples test or One Way Analysis of Variance (ANOVA) followed by LSD post test were performed for statistically significant difference. Differences in mean values were accepted as

being statistically significant if  $p < 0.05$ .

## 3. Results and discussion

### 3.1. Heavy metals contents in fish species

The mean concentrations of Zn, Cu, Pb, Cd, and Hg in the muscle of Spanish mackerel and tiger tooth croaker samples in each station are shown in Table 1. Both fish species in each station were found to contain Zn concentration much below FAO/WHO limit which is 150 ppm, and lower than reported by Irwandi and Farida, but higher than Zehra, Al-Bader, and Castro *et al.* [21-25].

In all three stations, copper concentration mean in both species were lower than FAO/WHO limit (10 ppm) and results of Irwandi and Farida, but higher than Zehra, Castro *et al.* Dobaradaran *et al.*, and Ronagh *et al.* Pb contents in both species were lower than FAO/WHO limit (1.5 ppm) and results of Irwandi and Farida, Dobaradaran *et al.*, Ronagh *et al.* and Zehra but greater than Al-Bader and Sireli *et al.* [21-28].

The mean values of Cd in both species were lower than FAO/WHO limit (0.2 ppm) and reported by Dobaradaran *et al.*, Zehra and Irwandi and Farida, but higher than Sireli *et al.* Finally the mean values of Hg in both species were below FAO/WHO limit (0.14 ppm) and results reported by Al-Bader, Irwandi and Farida for Indian and Spanish mackerel species and Ubalua *et al.* [21-29].

### 3.2. Heavy metals contents in seawater samples

Table 2 shows the metal concentrations in the sea water samples collected from three stations. Mean values of Pb, Cd, and Hg were greater than WHO and EPA limits (0.05, 0.01,

**Table 2.** Heavy metal concentration in water samples from each station.

| Site | N | Cd (ppm)     | Pb (ppm)     | Hg (ppm)    | Cu (ppm)  | Zn (ppm)  |
|------|---|--------------|--------------|-------------|-----------|-----------|
| 1    | 5 | 0.087±0.008  | 4.939±1.328  | 0.007±0.001 | 1.74±0.38 | 0.41±0.29 |
| 2    | 5 | 0.066±0.018* | 4.390±0.824  | 0.005±0.002 | 1.78±0.33 | 0.44±0.07 |
| 3    | 5 | 0.086±0.004  | 6.941±1.920* | 0.007±0.001 | 1.41±0.44 | 0.22±0.15 |

Data are shown as means±standard deviation.; \*Statistically significant from other samples ( $p<0.05$ ).

and 0.001 ppm, respectively) for potable water. The highest mean values of Pb and Hg were observed in station 3. Cd concentrations in station 1 and 3 were higher in comparison to station 2. In each station, mean values of Zn and Cu were lower than WHO limits (5 and 10 ppm, respectively) for potable water. In the present study, Zn in each station was greater than the result of Irwandi and Farida [22] for surface waters and Al-Kahtani [30]. In each station, Cu concentration was greater than the result of Ozturk et al. and Al-Kahtani [30, 31], but lower than Irwandi and Farida [22]. Also, Cd in each station was higher than reported by Irwandi and Farida [22], Ozturk et al and Al-Kahtani [30, 31]. Pb level in each station was much greater than the results of Ozturk et al. and Al-Kahtani [30, 31]. Hg concentrations were a little more or the same as the reported concentrations by Irwandi and Farida [22]. The comparison between the metal concentrations in seawater (Table 2) and fish (Table 1) showed that Pb was much higher in seawater, whereas, Cu and Zn had higher levels in fish. Both fish and water had Hg in almost equal concentrations as Irwandi and Farida [22]. Cd concentration was a little more in seawater.

Statistical analysis showed Cd mean value of seawater in station 2 (N=5) had a significant difference from other stations ( $p<0.05$ ). Pb content of water in station 3 (N=5) had a

significant difference from other stations ( $p<0.05$ ). In station 3, Cd mean value of croaker (N=6) had a significant difference ( $p<0.05$ ) from other stations. Also in station 2, Cd concentration of croaker (N=6) had a significant difference ( $p<0.05$ ) from station 1 (N=6). Cd concentration of Spanish mackerel (N=6) in station 3 had a significant difference in comparison to other stations ( $p<0.05$ ). Statistical analysis of stations without consideration of the type of fish species indicated that Cd and Hg contents of fish in station 3 (N=12) had a significant difference ( $p<0.05$ ) from other stations. In station 3, Pb content in fish (N=12) had a significant difference ( $p<0.05$ ) from station 1. In station 3, Zn mean value in fish (N=12) had a significant difference ( $p<0.05$ ) from other stations.

Higher concentrations of nonessential metals in fish and seawater samples in station 3 were probably because of its vicinity to the petrochemical and natural gas industrial complexes.

Independent sample test for species (N=18) without the consideration of stations showed no significant difference ( $p<0.05$ ) in metal contents except for Cd. Tiger tooth croaker (a bottom feeder) had higher Cd concentration in comparison to Spanish mackerel (a surface feeder). It is well known that the bottom feeders accumulate more metals than the surface feeders [23].

**Table 3.** Fatty acids ratio of both species in each station.

| Site | specie   | N | % PUFA   | % MUFA   | % SFA    |
|------|----------|---|----------|----------|----------|
| 1    | mackerel | 6 | 25.3±1.6 | 33.9±1.6 | 40.8±2.5 |
|      | croaker  | 6 | 23.9±1.7 | 33.6±1.4 | 42.6±1.4 |
| 2    | mackerel | 6 | 24.9±0.9 | 35.8±1.0 | 39.3±0.6 |
|      | croaker  | 6 | 23.0±1.7 | 36.1±1.3 | 41.0±0.9 |
| 3    | mackerel | 6 | 25.5±1.6 | 33.1±2.1 | 41.4±1.2 |
|      | croaker  | 6 | 22.4±0.5 | 34.2±0.7 | 43.4±1.0 |

Data shown as means±standard deviation.

### 3.3. Fatty acids ratios

Fatty acids ratios of both species in each station are shown in Table 3. In each station Spanish mackerel had higher PUFA% and lower SFA% in comparison to tiger tooth croaker. The percentage of total unsaturated fatty acids was high in both species.

In this study, fatty acids ratios of Spanish mackerel specie were close to the results of Tawfik [12] and Nazemroaya et al. [32]. Also, PUFA percent in Spanish mackerel was higher than the results reported by Hedayatifard and Jamali [33] for Sander lucioperca species. In each station, PUFA% in croaker was greater than the results of Alvarez [34] for *Micropogon undulates* but lower than Agren et al. [35] for *Otolithes argenteus*, *Acanthoparus cuvieri*, *Oreochromis spirulus*, and *Epinephelus suillis* species.

Independent samples test for fish species showed a significant difference ( $p < 0.05$ ) between Spanish mackerel (N=18) and tiger tooth croaker species (N=18) in PUFA% and SFA%. Statistical analysis showed no correlation between fatty acids ratios and metal contents except for Pb concentration which had negative correlation ( $r = -0.507$  spearman) with PUFA% in tiger tooth croaker species (N=18).

### 4. Conclusion

The nonessential heavy metals in muscles of fish samples could be attributed to the industrial activities and other anthropogenic metal sources affecting aquatic habitats in Bushehr Province. Although the results revealed that both fish species are safe from the human health point of view. It is suggested that monitoring studies be periodically performed to examine the metal concentrations especially in commercial fish. Also, Spanish mackerel and tiger tooth croaker species have considerable unsaturated fatty acids content and, therefore, more studies should be done to find other valuable high PUFA containing fish species in the Persian

Gulf.

### Acknowledgment

Financial support (grant number 89-5188) by Shiraz University of Medical Sciences, Shiraz, Iran is highly appreciated. The research was a part of student thesis.

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