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RESEARCH ARTICLE

Heavy metal levels in *Euthynnus affinis* (Cantor 1849) Kawakawa fish marketed at Karachi Fish Harbour, Pakistan and potential risk to human health

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Abstract

Heavy metals (Fe, Mn, Ni, and Pb) in muscles and liver of *Euthynnus affinis* (mean length of 61.02±3.618cm and mean weight of 2600±586.647g) from Karachi Fish Harbour of Pakistan during different seasons from October 2010 to September 2011 were determined. The metal concentrations in the muscles of *E. affinis* were always lower than those in livers within different seasons. Iron was the highest and lead was the lowest in both muscle and liver. The highest Fe and Mn concentrations were 47±12.5 and 10.4±3.2 µg/g dry wt. in muscles during South-west monsoon, respectively. The maximum concentrations of Fe (660±141 µg/g dry wt.), Mn (47.4±12.3 µg/g dry wt.) and Ni (2.8±0.8 µg/g dry wt.) were also recorded in liver during South-west monsoon season. The maximum Pb concentration in muscle and liver of Kawakawa were 0.4958±0.13641 and 1.5950±0.3045 µg metal g^{-1} dry wt., respectively. In general, it was found that the levels of heavy metals studied were lower than the maximum permissible limit of the food regulations of international standards. These data have provided a useful baseline for future reference.

Keywords: Heavy metal, *Euthynnus affinis,* Karachi Fish Harbour

Introduction

Rapid number of industrialization and economic growth in Karachi has resulted in increased water pollution in the coastal areas. This issue has been the focus of numerous studies. Pollutants deposited into water cause severe changes which in turn directly or indirectly influence the ecological balance of the environment, creating extensive damage and even mass mortality to the life and activities of aquatic organisms because of their high toxicity and accumulative behaviour

(Matta *et al*. 1999). Heavy metal contamination of the coastal environment continues to attract the attention of environmental researchers because of its increasing input to coastal waters, especially in developing countries. In fact, in recent decades, industrial and urban activities have contributed to the increase of heavy metal contamination in the marine environment and have directly influenced coastal ecosystems (Ong and Kamaruzzaman 2009). Anthropogenic activities have increased the release of harmful heavy metals into the aquatic environment (Agusa *et al*. 2005 and 2007; Hajeb *et al*. 2009).

Fish is considered one of the most important foods to humans and is used in a variety of diets; it is a good source of digestible protein, vitamins, minerals, and polyunsaturated fatty acids (Carvalho *et al*. 2005) which support healthy living (Ikem and Egiebor 2005). However, fishes are good indicators of heavy metal contamination in aquatic systems because they occupy different trophic levels and are of different sizes and ages (Burger *et al*. 2002). The heavy metal intakes by fish in a polluted aquatic environment vary depending on ecological requirements, metabolism, and other factors, such as salinity, water pollution level, food, and sediments. Fish accumulates metals in its tissues through absorption, and humans can be exposed to these metals via the food web. The consumption of contaminated fish causes acute and chronic effects to humans (Nord *et al*. 2004).

Mackerel tuna *Euthynnus affinis* (called Kawakawa in the local language) is an Indo-West Pacific species and is widespread and abundant in the Indian and western Pacific Ocean. It is caught in commercial fisheries, primarily as by catch. It is marketed in a variety of products, and reported worldwide landings are increasing. This pelagic and oceanodromous fish occurs in open waters, but it always remains close to the shoreline. The young may enter bays and harbours. It is a highly opportunistic predator feeding indiscriminately on small fishes, on squids, crustaceans and zooplankton. This species is generally marketed canned and frozen. It is also utilized dried, salted, smoked and fresh. It is important in highly commercial fisheries. This species is also used in pet food for dogs and cats (Collette and Nauen 1983; Collette *et al*. 2001 and 2011).

The objective of the present study to determine heavy metals (Fe, Mn, Ni, and Pb) in muscles and liver of *E. affinis* from Karachi Fish Harbour of Pakistan in various seasons from October 2010 to September 2011. Further, their hazardous levels were compared with available certified safety guidelines proposed by EU, World Health Organization (WHO) and Food and Agricultural Organization (FAO) for human consumption.

Materials and Methods

Collection of fish samples
A total of 55 *E. affinis* individuals were collected at the Fish Harbour West

Wharf Karachi (Figure 1) directly from local fishermen during different seasons (autumn inter-monsoon, North-east monsoon, spring inter-monsoon, South-west monsoon) from October 2010 to September 2011 and were kept in a cool box for metal analysis. The total length (cm) and weight (g) of the samples were measured before dissection. The length (L) of the fish was measured from the tip of the anterior part of the mouth to the caudal fin to the nearest 0.1 cm. Fish weight (W) was measured after blot drying with a piece of clean towel to the nearest 0.01 g.

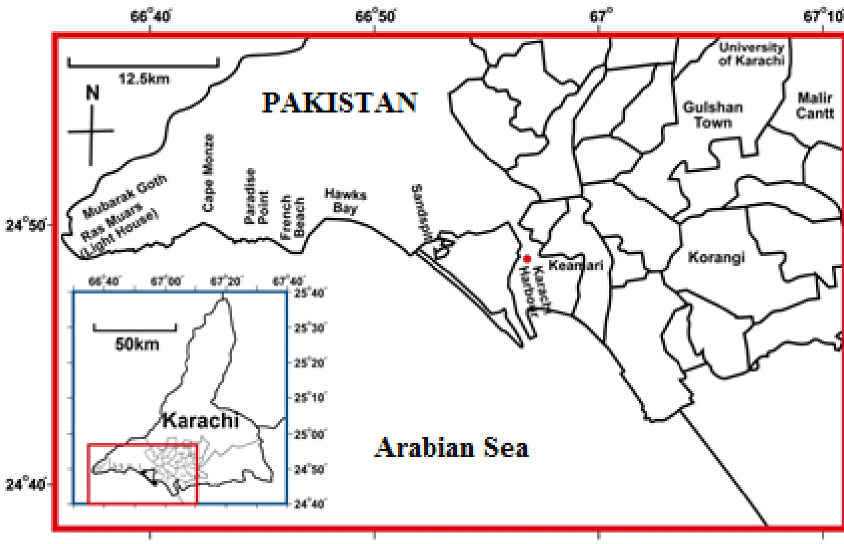

Figure 1. Location of Karachi Harbour

Digestion of fish samples

The edible parts (muscle tissues) and liver of the fish samples was then separated and was washed with deionized water and weighed. The muscle and liver tissues of the fish were dissected for analysis according to the method described by UNEP (1984). The samples were homogenized separately in a blender and five gram homogenate samples of dorsal muscles and entire liver tissues were dried in an oven until constant weight was obtained. Samples were then ground and calcinated at 500°C for 3 hours until it turned to white or grey ash. The ashes were dissolved with 0.1 M HCl according to the method of Gutierrez *et al*. (1978). The ashes were dissolved in 10 ml (HCl) in a beaker and after which the dissolved ash residue was filtered with Whatman filter paper, then diluted to 25 ml distilled water with 1 N HNO_3 (UNEP 1984 and 1985).

Chemical analysis
The concentration of Fe, Mn, Ni and Pb were measured for metal analysis. The equipment AA (Analyst 700) was started and prepared with programme win lab 32 software. Three standards from Fisher Scientific U.K. 1000 ppm stock solution to 2 ppm, 4 ppm and 6 ppm were prepared. The equipment with the above mentioned standards were calibrated. The wavelengths (nm) of Fe, Mn, Ni and Pb were 248.3, 279.5, 232.0 and 283.3, respectively. The samples one by one were aspirated to detect the required metals. The precision of analysis was estimated by variations from the mean value reported and in all experiments several blanks were performed with the reagents used in order to check for possible contamination. Finally the report using the software was prepared. The results are expressed in micrograms of metal per gram of dry fish (μg/g).

Results and Discussion

In the present study a total of 55 *E. affinis* (mean length of 61.02+3.618 cm and mean weight of 2600+586.647 g) were purchased at Karachi Fish Harbour for metal analysis. Highest mean length 64.44±2.706 cm and weight 3300±359.556 g of fish were measured during South-west monsoon season. Lowest length weight 2000±256.455 cm and weight 1400-2300 were recorded in autumn inter-monsoon (Table 1).

The levels of heavy metal in Kawakawa were based on dry weight and the results are presented in Tables 2 and 3. The values are given as mean ± SD.

Table 1. Length and weight of *E. affinis* collected at Karachi Fish Harbour from October 2010 to September 2011

Seasons	**N**	**Length (cm) Mean±SD**	**Weight (g) Mean±SD**
Autumn inter- monsoon	13	56.62± 2.98	2000± 256
North-east monsoon	12	60.33± 0.49	2500± 119
Spring inter- monsoon	12	61.33± 0.49	2700± 74
South-west monsoon	18	64.44± 2.71	3300± 360
Total	55	61.02± 3.62	2600± 587

Muscle and liver tissues of *E. affinis* were chosen as target organs for assessing metal accumulation. The metal concentrations in the muscles of *E. affinis* were always lower than those in livers within different seasons (Tables 2 and 3).

There was no significant difference between seasons in the concentration of heavy metals (Fe, Mn, Ni and Pb) in the fish (ANOVA, p>0/05).

Table 2. Heavy metal levels in the muscle tissues of *E. affinis* collected at Karachi Fish Harbour from October 2010 to September 2011 (μg/g dry wt.)

Seasons	Metals	Mean±SD	Min-Max	S.E.
Autumn inter- monsoon		38.02± 9.10	24.63-52.46	2.52
North-east monsoon	Fe	36.77±13.51	16.64-62.02	3.90
Spring inter- monsoon		35.89±5.69	28.12-44.98	1.64
South-west monsoon		46.96±12.54	22.33-67.16	2.96
Autumn inter- monsoon		6.11±1.90	3.24-9.88	0.53
North-east monsoon	Mn	5.52±0.85	4.66-7.63	0.24
Spring inter- monsoon		6.34±1.60	3.24-8.24	0.46
South-west monsoon		10.41±3.19	6.24-16.63	0.75
Autumn inter- monsoon		0.50±0.21	0.26-1.08	0.06
North-east monsoon	Ni	0.52±0.12	0.23-0.76	0.42
Spring inter- monsoon		0.61±0.32	0.27-1.02	0.09
South-west monsoon		0.61±0.39	0.20-1.64	0.09
Autumn inter- monsoon		0.46±0.14	0.26-0.66	0.04
North-east monsoon	Pb	0.50±0.14	0.36-0.86	0.39
Spring inter- monsoon		0.49±0.14	0.23-0.66	0.41
South-west monsoon		0.42±0.09	0.23-0.56	0.02

Iron was the highest and lead was the lowest in both muscle and liver of analysed fish species in the present study. Similar findings were found by many researchers (Honda *et al*. 1983; Bat *et al.* 1996; Agusa *et al*. 2005 and 2007; Uluozlu *et al.* 2007; Bat *et al.* 2012), who suggest that the liver plays an important role in the metabolic processes of heavy metal in fishes. According to Kotze *et al*. (1999), among the different organs of fishes, the muscles are a primary part of metal intake and availability of the metals in muscles reflect the metal concentration in waters, whereas increased metal concentrations in liver may represent storage of sequestered products in this organ.

The considerably higher iron levels in fish liver comparing to the muscle tissue are expected due to the physiological role of this organ in blood synthesis. Fish is widely consumed in many parts of the world because it has high protein content, low saturated fat and also contains omega fatty acids known to support good health (Ikem and Egiebor 2005). They are constantly exposed to chemicals in polluted and contaminated waters. Heavy metal content in fishery products, therefore, needs to be well established. Top predator fish, like *E. affinis*, accumulate heavy metals through their position in the food web. The intake of these heavy metals by humans through the consumption of fish causes serious health hazards (Puel *et al.* 1987; Luoma and Rainbow 2008). Basically, the marine organisms accumulate contaminants such as metals from the environment (Connell 2005) and have been extensively used in marine pollution monitoring programmes (Linde *et al.* 1998; Mora *et al.* 2004).

Table 3. Heavy metal levels in the liver tissues of *E. affinis* collected at Karachi fish Harbour from October 2010 to September 2011(µg/g dry wt.)

Seasons	Metal	Mean±SD	Min-Max	SE
Autumn inter- monsoon		575.71± 159.92	318.96-752.61	44.35
North-east monsoon		506.65±186.33	326.83-841.63	53.79
Spring inter- monsoon	Fe	625.72±173.54	366.52-8223.41	42.09
South-west monsoon		660.24±141.24	463.21-866.33	23.54
Autumn inter- monsoon		36.97±11.97	21.94-63.21	3.32
North-east monsoon		33.03±8.07	16.53-46.32	2.33
Spring inter- monsoon	Mn	35.78±6.96	28.61-46.34	1.67
South-west monsoon		47.37±12.29	24.56-64.41	2.05
Autumn inter- monsoon		2.01±0.77	1.05-3.74	0.21
North-east monsoon		2.39±1.02	1.19-3.78	0.29
Spring inter- monsoon	Ni	2.10±0.60	1.23-2.84	0.14
South-west monsoon		2.80±0.0.82	1.48-4.46	0.14
Autumn inter- monsoon		1.29±0.14	1.06-1.56	0.39
North-east monsoon		1.41±0.15	1.21-1.68	0.45
Spring inter- monsoon	Pb	1.38±0.17	1.08-1.63	0.04
South-west monsoon		1.59±0.30	1.26-2.46	0.51

These metals accumulate in fish through water, food, sediment and some suspended particulate materials (Agusa *et al.* 2005). The increased accumulation of heavy metal levels in the aquatic environment is disastrous to aquatic organisms and humans alike (Uluturhan and Kucuksezgin 2007; Naji *et al.* 2010). In the present study the metal concentrations decrease in the order Fe>Mn>Ni>Pb (Table 3). Elements such as Fe and Mn are essential metals since they play an important role in biological systems whereas non-essential elements, such as Ni and Pb are toxic even in trace amounts. Nonessential elements are well known to be toxic. However, the essential metals can also produce toxic effects at high concentrations (Ray 1994; Oehlenschlager 2002). In the present study the highest concentration of Fe 46.9644±12.54456 µg/g and Mn 10.4089±3.19444 µg/g were measured in muscles during South-west monsoon (Table 2). The maximum concentrations of Fe 660.2367±141.23909 µg/g, Mn 47.3711±12.29087 µg/g, Ni 2.7983±0.0.81944 µg/g were also recorded in liver during South-west monsoon season (Table 3). *E. affinis* is a migratory fish and able to concentrate large amounts of heavy metals (Anonymous 2005). Ni and Pb showed low level of concentration during all seasons of the year. Only a few metals, of proven hazardous nature are to be completely excluded in food for human consumption. For example, Pb has been included in the regulations of the European Union for hazardous metals (Anonymous 2006). The maximum Pb concentration in muscle and liver of *E. affinis* were 0.4958±0.13641 µg/g and 1.5950±0.3045 µg/g, respectively in the present study was considerably higher than the maximum level (0.30 mg/kg wet weight) set by EC (Commission Regulation) and TFC (Turkish Food Codex) (Anonymous 2006 and 2008). It should be noted that Pb levels in *E. affinis* were expressed in µg metal g^{-1} dry weight in the present study. This makes it very difficult to compare. Moreover, the maximum Pb level reported by The Food Safety (MAFF 1995) for fish is 2.0 mg/kg wet weight.

Furthermore, the tolerable daily and weekly intakes were estimated by means of references for *E. affinis* consumed by human over a lifetime without appreciable risk. The average daily fish consumption in Pakistan is 5 g per person (Anonymous 2010). This is also equivalent to 35 g/week. Maximum Pb levels in dorsal muscles and liver were 0.4958±0.13641 and 1.5950±0.3045 µg/g dry wt., respectively. An internationally accepted safe level of Pb is 25 µg per kg body weight per week for PTWI (FAO/WHO 2010). This is equivalent to 1750 µg/week/70 kg body weight and 250 µg/day/70 kg body weight. Estimated Weekly Intake (EWI) and Estimated Daily Intake (EDI) for a 70 kg body weight of an adult person according to the results of present study were calculated as 0.017±0.0048 µg for muscle and 0.056±0.011 µg for liver and 0.002±0.0007 µg for muscle and 0.008±0.0016 µg for liver, respectively. The present study showed that different metals were present in *E. affinis* samples at different levels but within the maximum residual levels prescribed by the EU and FAO/WHO and the fish from the area, in general, is safe for human consumption. The liver had higher concentrations of all metals than the muscle, but the livers of *E. affinis* are not consumed for human consumption. However, it is recommended that the liver of *E. affinis* from Karachi fish Harbour should be removed and well washed before consumption. Moreover if the liver of *E. affinis* used in pet food for dogs and cats, this point should be considered. These results indicated the need for further monitoring of heavy metals in that local area.

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