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Accumulation of Heavy Metals in Tissues of Long Tail Tuna from Karachi Fish Harbour, Pakistan

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Abstract

The present study was conducted to investigate the contamination of Fe, Mn, Cu, Zn, Ni, Pb, Cd, and Cr in the tissues of commercially important fish Thunnus tonggol and to evaluate risks to human health associated with seafood consumption. The results from this study showed that the concentrations of all metals measured except Cr were relatively high in the liver compared to the muscle, gills and gonads. High concentrations of Cr were present in the kidneys. Fe had the highest concentration compared with other metals tested in all tissues of followed by Cu and Zn. The highest mean concentration (µg/g) of Fe 36.43±11.41, Mn 1.29±1.08, Cu 23.35±11.47, Zn 3.89±2.23, Ni 0.35±0.12, Pb 0.27±0.12, Cd 0.71±0.13 and Cr 0.35±0.20 were recorded in the edible muscle tissues of the fish. Overall, the findings from the present study revealed that all metals except Cu and Cd concentrations in the muscle tissues were lower than the maximum permissible limit as recommended by the international regulations. However, the estimated the Provisional Tolerable Weekly Intake (PTWI) of Cu is below the established PTWI. The data indicate that the examined fish were polluted with Cd. It was also found that levels of the metals in other tissues in some samples were higher than the recommended concentrations. However, consumers do not consume the liver, kidney, gills and gonads.

Keywords: *Thunnus tonggol*, Heavy metals, Karachi Fish Harbour, Pakistan

1. Introduction

Fish have an important place in the diet of Pakistan and are a good source of digestible protein vitamins, minerals and polyunsaturated fatty acids (PUFA). However, fish are also source of metals. 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 term of heavy metals has been replaced in years by a classification scheme that considers their chemistry rather than relative density (Nieboer & Richardson, 1980). Separation of some essential and non-essential metal ions of importance as pollutants into class A (oxygen-seeking) including Ca, Mg, Mn, K, Sr, Na, class B (sulphur or nitrogen-seeking) including Zn, Pb, Fe, Cr, Co, Ni, As, V and borderline elements including Cd, Cu, Hg, Ag based on the classification scheme of Nieboer and Richardson (1980). As a results fish may accumulate large amounts of some metals from the water and from their food and then deposit them in the tissue. Therefore it is important to determine the concentration of heavy metals in commercial fish in order to evaluate the possible risk of fish consumption to human health (Pérez Cid et al., 2001). The common heavy metals that are found in fish include Cu, Fe, Zn, Mn, Hg, Pb and Cd (Connell, 1984). Evidence from the literature strongly indicates heavy metal toxicity in the marine environment as a majors concern to human health. Industrial and agricultural activities were reported as the likely source of the accumulation of pollutants in the aquatic environment including the sea (Tarra-Wahlberg et al., 2001; Akif et al., 2002).

Tuna, as a predator, is able to concentrate large amount of heavy metals via food chain. Some of them are used for biomonitoring of environmental contamination (Enomoto & Uchida, 1973; Schmitt & Brumbaugh, 1990). Numerous human health concerns have been linked to the injection of heavy metals. For example, the most common toxic effects of cadmium in humans is renal failure, accumulation in bone matter resulting in calcium loss and also malfunctioning of peripheral and central nervous system (Schroeder et al.,1965). Large fish such as Swordfish (*Xiphias gladius*), Yellowfin Tunas (*Tunnus al-bacares*), Skipjacks (*Katsuwonus pelamis*) and Common Dolfinfish (*Coryphaena hippurus*), which are at the top of marine food webs, are particularly exposed to high levels of trace elements through their food (Bryan, 1979). These pelagic organisms are high performance fish with very high metabolic rates, and consequently high food intake rates, a property that accentuates the exposure to heavy metals (Kojadinovic et al., 2007). Accumulations of heavy metals were generally found to be species specific and may be related to their feeding habits and the bio-concentration capacity of each species (Bat et al., 2013). Topping (1973) suggested that mainly plankton feeding fish contain much higher concentrations of some heavy metals than bottom feeding fish. Consequently, fish are widely used as biomonitor for assessing heavy metal contamination level of aquatic environment and the health state of marine ecosystems.

The objective of the present study is to provide information on the Fe, Mn, Cu, Zn, Ni, Pb, Cd and Cr levels in the muscle, liver, kidney, gills and gonads tissues of *Thunnus tonggol*. The fish for the metal determinations were collected from Karachi Fish Harbour, Pakistan between August 2006 and December 2011. It is also attempted to compare the measured values with international standards for food and human health.

2. Material and Method

Fish samples (*T. tonggol*) were collected in August, 2006 to December, 2011 from the fish landing Centre, at Fish Harbour West Wharf Karachi, Pakistan, located in the north eastern border of the Arabia Sea, lying between 24°50′54.71′′N and 66°58′38.68′′E (Figure 1). Collected samples were immediately transported to the laboratory in ice boxes and allowed to thaw and rinsed in distilled water. Total lengths (cm) and weights (g) of fish were determined (Table 1; Figure 2). The fishes were dissected. To avoid contamination problems the glassware and sample containers were pre-cleaned by soaking in a 10 % HNO3 solution more than 24 hours, and then rinsed at least three times with bi-distilled water before use.

Figure 1. Fish Harbour of Karachi, Pakistan

Table 1. Mean length and weight of fish during years 2006-2011

Years	n	Length (cm) Mean ±SD (Min-Max)	Weight (g) Mean ±SD (Min-Max)
2006-2007	52	62.0±2.23 (59-64.8)	2500±222.92 (2200-2900)
2007-2008	63	62.0±2.11 (58-66)	2200±248.84 (2200-3100)
2008-2009	65	60.0±4.44 (48-68)	2400±495.95 (1200-3200)
2009-2010	62	61.0±1.70 (58-66)	2400±317.08 (1800-3100)
2010-2011	58	61.0±3.61 (52-72)	2600±586.64 (1400-3900)

Figure 2. Collection of *Thunnus tonggol* at Fish Harbour

The muscle, liver, kidney, gills and gonads tissues of the fish were prepared for analysis according to the method described by Bernhard (1976). Approximately 5 g dorsal muscles, entire liver, 2 rakers of gills, entire kidney, and entire gonads were prepared separately from individual fish which were then cut into small pieces and homogenized. The samples were then placed in a muffle furnace and the temperature was gradually increased to 500oC over a period of 3 hours to avoid a loss of material due to sudden combustion. The samples remained in the muffle furnace for 72 hours until they had been reduced to a grey to white ash. The ashes were dissolved with 10 millilitres of 0.1 M HCl according to the method of Gutierrez et al. (1978).

As a means of comparison, three working standards (2 ppm, 4 ppm and 6 ppm) were prepared from 1000 ppm stock solution. The samples were analyzed for heavy metals using the equipment Analyst 700 with programme win lab 32 software.

The results of the research were processed using statistical methods (ANOVA). The data transformations allowed for adjusting of all the zero values in the analytical results prior to the ANOVA test (Zar, 1984).

3. Results and Discussion

The seasonal variations of heavy metals in T. tonggol collected from Fish Harbour Karachi were studied. The mean concentration of heavy metals (Fe, Mn, Cu, Zn, Ni, Pb, Cd and Cr) determined in the muscle, liver, kidney, gills and gonads tissues of T. tonggol are given Table 2. The levels of metals ranged from 6.96±3.67 µg/g in kidney to 553.92±169.45 µg/g in liver for Fe, 0.40±0.23 µg/g in kidney to 6.47±2.47 µg/g in liver for Mn, 3.16±1.37 µg/g in gonads to 63.36±19.51 µg/g in liver for Cu, 1.27±20.65 µg/g in gills to 19.72±9.15 µg/g in liver for Zn, 0.14±0.10 µg/g in kidney to 1.46±0.56 µg/g in liver for Ni, 0.17±0.08 µg/g in gonads to 2.63±0.62 µg/g in liver for Pb, 0.19±0.12 µg/g in gills to 2.7±1.05 µg/g in liver for Cd and 0.16±0.14 µg/g in gonads to 1.5±0.78 µg/g in kidney for Cr at Fish Harbour Karachi in T. tonggol. Liver concentrations of all metals except Cr, were higher than other tissues tested. The statistical results presented in Table 3 indicate that there is a considerable greater accumulation of the metals in liver than in muscle tissues (P<0.05) and that there was a statistically significant difference between the concentrations of the metals among years.

Table 2. Mean concentration of metals with standard deviation (μg/g dry wt.) in different organs of fish collected from Karachi fish harbour during years (2006-2011)

Metals	Years	n	Muscles (Mean ±SD)	Liver (Mean ±SD)	Kidney (Mean ±SD)	Gills (Mean ±SD)	Gonads (Mean ±SD)
Fe	2006-2007	52	26.11±10.48	553.92±169.45	6.96±3.67	15.06±7.91	12.34±3.51
	2007-2008	63	24.7±11.51	437.65±178.80	8.73±5.02	16.41±8.19	14.52±3.81
	2008-2009	65	25.65±11.20	433.14±134.56	12.74±9.52	19.87±8.33	14.56±3.92
	2009-2010	62	35.72±12.44	427.65±113.78	14.58±5.84	20.10±9.74	16.52±5.45
	2010-2011	58	36.43±11.41	464.36±154.98	16.32±8.68	25.51±12.37	24.14±8.40
Mn	2006-2007	52	0.50±0.30	3.87±2.59	0.40±0.23	1.46±1.12	0.46±0.25
	2007-2008	63	0.52±0.32	4.64±2.37	0.51±0.25	1.84±1.05	0.41±0.33
	2008-2009	65	1.29±1.08	5.54±2.74	0.49±0.24	1.98±0.93	0.58±0.39
	2009-2010	62	0.63±0.55	6.47±2.47	0.59±0.42	2.23±1.45	0.63±0.44
	2010-2011	58	0.44±0.23	6.35±3.11	0.50±0.32	2.16±1.75	0.46±0.28
Cu	2006-2007	52	16.21±6.18	63.36±19.51	6.81±3.40	3.63±1.81	3.16±1.37
	2007-2008	63	14.25±7.41	56.21±18.10	5.86±3.21	3.50±2.80	4.12±1.38
	2008-2009	65	20.44±8.23	51.59±17.03	10.21±3.69	6.43±2.66	4.41±1.26
	2009-2010	62	23.35±11.47	41.46±24.53	5.58±2.87	5.52±2.40	5.08±1.50
	2010-2011	58	23.22±11.07	53.25±14.54	5.36±3.57	6.55±2.38	4.31±1.32
Zn	2006-2007	52	2.73±1.83	10.01±4.20	3.46±2.03	1.27±0.65	1.49±1.29
	2007-2008	63	3.01±1.94	15.63±11.98	3.69±1.06	1.62±1.23	1.75 ±1.09
	2008-2009	65	2.65±1.91	17.73±8.89	4.09±1.79	3.37±2.10	3.26±1.66
	2009-2010	62	1.93±1.21	16.78±9.80	3.46±2.45	2.63±1.93	4.45±1.32
	2010-2011	58	3.89±2.23	19.72±9.15	4.13±2.38	2.20±1.43	4.98±1.89
Ni	2006-2007	52	0.18±0.12	0.54±0.12	0.14±0.10	0.19±0.06	0.16±0.12
	2007-2008	63	0.22±0.16	0.63±0.43	0.33±0.17	0.26±0.10	0.33 ±0.12
	2008-2009	65	0.21±0.15	1.26±0.38	0.51±0.19	0.42±0.22	0.33±0.14
	2009-2010	62	0.31±0.17	1.46±0.56	0.41±0.14	0.62±0.48	0.44±0.21
	2010-2011	58	0.35±0.12	0.93±0.53	0.79±0.19	1.26±0.35	0.74±0.29
Pb	2006-2007	52	0.18±0.10	0.58±0.21	0.18±0.04	0.19±0.12	0.18±0.06
	2007-2008	63	0.23±0.14	0.52±0.18	0.35±0.17	0.32±0.18	0.17 ±0.08
	2008-2009	65	0.19±0.11	0.46±0.21	0.40±0.21	0.26±0.16	0.36±0.10
	2009-2010	62	0.27±0.12	1.52±0.24	0.45±0.22	0.68±0.22	0.72±0.23
	2010-2011	58	0.22±0.18	2.63±0.62	1.23±0.42	1.88±0.68	0.83±0.21
Cd	2006-2007	52	0.23±0.11	0.75±0.40	0.36±0.26	0.19±0.12	0.21±0.15
	2007-2008	63	0.61±0.19	1.61±0.40	0.51±0.25	0.31±0.19	0.34±0.19
	2008-2009	65	0.42±0.23	1.64±0.48	0.44±0.23	0.26±0.16	0.26±0.19
	2009-2010	62	0.28±0.15	2.72±1.05	0.45±0.22	0.32±0.17	0.36±0.15
	2010-2011	58	0.71±0.13	2.47±0.94	1.44±0.62	1.20±0.55	0.41±0.19
Cr	2006-2007	52	0.21±0.12	0.68±0.41	0.49±0.26	0.18±0.13	0.16±0.14
	2007-2008	63	0.24±0.12	1.21±0.44	0.36±0.19	0.31±0.20	0.21±0.14
	2008-2009	65	0.26±0.19	1.38±0.51	0.52±0.17	0.38±0.23	0.36±0.18
	2009-2010	62	0.35±0.20	1.49±0.57	1.51±0.78	0.51±0.18	0.31±0.14
	2010-2011	58	0.24±0.11	1.14±0.60	0.41±0.16	0.42±0.16	0.41±0.19

Table 3. Analysis of variance (ANOVA) in *Thunnus tonggol* fish from the coast of Karachi

Metals	Effect	Sum of square	degree of freedom	Mean of square	F	p
Fe	Organs	6.5567	4	1.6397	3633.574	.000
	Year	63967.280	5	12793.456	2.836	.015
	Organs * Year	277665.736	16	17354.109	3.847	.000
	Error	8317667.114	1400	4510.665	1.983	
	Total	1.0288	1500			
	Corrected Total	7.7257	1499			
Mn	Organs	6031.759	4	1507.940	1413.356	.000
	Year	92.881	5	23.220	21.764	.000
	Organs * Year	150.582	16	9.411	8.821	.000
	Error	1493.690	1400	1.067		
	Total	15456.946	1500			
	Corrected Total	8723.561	1499			
Cu	Organs	510604.561	4	127651.140	1427.575	.000
	Year	156.329	5	39.082	.437	.782
	Organs * Year	10243.006	16	640.188	7.159	.000
	Error	125185.425	1400	89.418		
	Total	1192517.869	1500			
	Corrected Total	676540.922	1499			
Zn	Organs	49983.109	4	12495.777	658.913	.000
	Year	1766.351	5	441.588	23.285	.000
	Organs * Year	3508.929	16	219.308	11.564	.000
	Error	26834.375	1400	18.964		
	Total	152925.645	1500			
	Corrected Total	91269.733	1499			
Ni	Organs	58.576	4	14.644	218.844	.000
	Year	45.287	5	11.322	169.195	.000
	Organs * Year	32.723	16	2.045	30.564	.000
	Error	93.682	1400	.067		
	Total	681.637	1500			
	Corrected Total	255.914	1499			
Pb	Organs	15.552	4	3.888	117.965	.000
	Year	4.482	5	1.120	33.996	.000
	Organs * Year	4.943	16	.309	9.373	.000
	Error	46.142	1400	.033		
	Total	261.296				
	Corrected Total	77.918				
Cd	Organs	62.603	4	15.651	132.796	000
	Year	3.266	5	.817	6.928	.000
	Organs * Year	6.791	16	.424	3.601	.000
	Error	164.999	1400	.118		
	Total	567.067	1500			
	Corrected Total	275.872	1499			
Cr	Organs	242.802	4	60.700	746.879	.000
	Year	10.940	5	2.735	33.651	.000
	Organs * Year	8.179	16	.511	6.290	.000
	Error	113.781	1400	.081		
	Total	889.759	1500			
	Corrected Total	412.699	1499			

Note: * = significant at $P < 0.05$.

Contamination with heavy metals on local, regional and global scales, have been intensively studied in recent years. Due to the fact that metals are persistent and toxic, they tend to accumulate and pose a risk to humans and ecosystems health (Szefer, 2002; Rainbow, 2002). The main reason for this is the increasing metal input to the coastal zone from both rivers and non-point sources (NPS) whichcomes from many diffuse sources. NPS pollution is caused by rainfall or snowmelt moving over and through the ground. As the runoff moves, it picks up and carries away pollutants, finally depositing them into coastal waters, especially in developing countries. Fish have been considered good indicators for heavy metal contamination in aquatic systems because they occupy different trophic levels with different sizes and ages (Burger et al*.,* 2002). Meanwhile, fish are widely consumed in many parts of the world by humans, and polluted fish may endanger human health. The levels of toxic elements in fish are related to age, sex, season and habitat (Kagi et al*.,* 1988). It is also reported that cooking reduces the amount of some metals (Atta et al*.,* 1997). Cd may accumulate in the human body and may induce kidney dysfunction, skeletal damage and reproductive deficiencies. The amount of Cd in tuna was 0.32, 0.35, µg/g in each sample respectively which were higher than those in our samples in southern Iran (Voegborlo et al*.,* 1999; Amundsen et al*.,* 1997). Kojadinovic et al. (2007) suggested that that their muscle impregnation is the result of the accumulation of heavy metals from preys living in other environments than where the fish were caught and which contained high metal levels.

In the present study, Fe was the highest in all tissues of analysed species in the present study, followed by Cu and Zn. Similar results were found by many researchers (Tepe et al., 2007; Türkmen et al., 2008 and 2009). Metals, such as Fe, Cu and Zn, are essential metals since they play important roles in biological systems (Türkmen et al., 2009) and the contaminant uptake rate is positively linked to the metabolic rate in marine organisms, it can be supposed that metal accumulation would be high (Kojadinovic et al., 2007) .

Furthermore, the tolerable weekly intakes were estimated by means of references for muscles tissues *T. tonggol* consumed by people. The average daily fish consumption in Pakistan is 5 g per person (FAO, 2010). The Joint FAO/WHO Expert Committee on Food Additives established a PTWI for Fe of 5.6 mg/kg body weight/week which was equivalent to 392 mg/week for a 70 kg adult (FAO/WHO, 2010). By using the means of weekly fish consumption in Pakistan of 35 g per person and the maximum iron levels in muscle tissues of *T. tonggol*, weekly intake calculated as 1.28±0.40 mg (35 g x 36.43±11.41 mg/1000 g) per person for Fe in muscles of fish. The Joint FAO/WHO Expert Committee on Food Additives established a PTWI for Cu of 3.5 mg/kg body weight/week which was equivalent to 245 mg/week for a 70 kg adult (WHO, 1996; Council of Europe, 2001). By using the means of weekly fish consumption in Pakistan of 35 g per person and the maximum copper concentrations in muscle tissues of *T. tonggol*, weekly intake calculated as 0.82±0.40 mg (35 g x 23.35±11.47 mg/1000 g) per person for Cu in muscles of fish. The Joint FAO/WHO Expert Committee on Food Additives established a PTWI for Zn of 7 mg/kg body weight/week which was equivalent to 490 mg/week for a 70 kg adult (WHO, 1996; Council of Europe, 2001). By using the means of weekly fish consumption in Pakistan of 35 g per person and the maximum Zn levels in muscle tissues of *T. tonggol*, weekly intake calculated as 0.136±0.078 mg (35 g x 3.89±2.23 mg/1000 g) per person for zinc in muscles of fish. Fe, Cu and Zn are important components of the human body. They are also toxic to most forms of organisms when present in amounts exceeding certain limits Bryan, 1979). Legal thresholds are not available for essential elements in Commission Regulation. However, in the edible muscle tissues of fish the average Zn concentrations were well below the maximum tolerance levels for human consumption established by compared with the Turkish Food Codex (TGK, 2002) and the Food Safety of Fish Product (MAFF, 1995) (50 µg/g wet wt.); Georgian Food Safety Rules (2001) and Russian Federation (GAIN, 2002) (40 µg/g wet wt.). Cu levels (23.35±11.47 µg/g) in the edible muscle tissues exceeded the critical limits set by the Turkish Food Codex (TGK, 2002) and the Food Safety of Fish Product (MAFF, 1995) (20 µg/g wet wt.); Georgian Food Safety Rules (2001) and Russian Federation (GAIN, 2002) (10 µg/g wet wt.). Cu is often association with sewage sludge, where it is most likely complexes with a variety of organic compounds. However, the estimated PTWI of Fe, Cu and Zn in the present study are below the established PTWI. The results of the present study from Table 2 were given as dry wt. and this should be taken into account. On the contrary Mn, Ni and Cr metals were generally low. Mn is essential element and an important enzyme cofactor for the activation of a number of enzymes. Mn accumulation was higher in liver followed in gills. It is indicated that fish take Mn from the surrounding water through their gills and accumulated it. Ni is also essential for normal growth and reproduction in human beings, but shows carcinogenic effect when consumed in high amount. Cr accumulation was higher in kidney of *T. tonggol*. Cr is essential element and an important enzyme cofactor which may become toxic when accumulating at a certain level as other essential elements (Underwood, 1977).

Pb and Cd belong to the group of non-essential and toxic metals and they have no known function in biochemical processes and are accumulated in multiple organs (Phillips, 1977). The maximum Pb concentrations in all tissues except edible muscle were considerably higher than the maximum level (0.30 µg/g wet wt.) set by EC (Commission Regulation) (Anonymous, 2006) and Turkish Food Codex (TGK, 2008). Pb is a heavy metal that occurs in nature mainly lead sulphide and a powerful neurotoxin even in trace quantities, it is currently used in batteries, alloys, shot, paints and gasoline (The Earth Report 3, 1992). This metal is extremely insoluble and is readily absorbed by organic matter, especially under reducing conditions and is highest in the immediate vicinity of industrial activity and river inputs (GESAMP, 1990). The Joint FAO/WHO Expert Committee on Food Additives established a PTWI for Pb of 0.025 mg/kg body weight/week which was equivalent to 1.725 mg/week for a 70 kg adult (WHO, 2000; Council of Europe, 2001; FAO/WHO, 2010). By using the means of weekly fish consumption in Pakistan of 35 g per person and the maximum Pb levels in muscle tissues of *T. tonggol*, weekly intake calculated ranged from 0.009±0.004 mg (35 g x 0.27±0.12 mg/1000 g) per person for Pb in muscles of fish. In terms of Pb concentrations in the edible muscle tissues of *T. tonggol* was lower than permissible limits and seems to be appropriate for human health. It is important to note that the liver and visceral of *T. tonggol* from Karachi Fish Harbour should be removed and well washed before consumption. Similarly, the maximum Cd concentrations in all tissues were considerably higher than the maximum level (0.05 µg/g wet wt.) set by EC (Commission Regulation) (Anonymous, 2006) and Turkish Food Codex (TGK, 2008). The Joint FAO/WHO Expert Committee on Food Additives established a PTWI for Cd of 0.007 mg/kg body weight/week which was equivalent to 0.49 mg/week for a 70 kg adult (WHO, 1989 and 2004; Council of Europe, 2001; FAO/WHO, 2010). By using the means of weekly fish consumption in Pakistan of 35 g per person and the maximum Cd levels in muscle tissues of *T. tonggol*, weekly intake calculated ranged from 0.025±0.005 mg (35 g x 0.71±0.13 mg/1000 g) per person for Cd in muscles of fish. As regards Cd concentrations in muscle tissues of *T. tonggol*, the estimated PTWI in the present study is above the established PTWI. Cd is naturally present in phosphate fertilizers and is discharged to the environment when they refined (The Earth Report 3, 1992). The available data suggest that Cd in the open ocean is derived, for the most part, from natural sources such as rock weathering or sea-bed eruptions (GESAMP, 1990). Cd is used in metal plating in certain plastics as pigment and in some rechargeable batteries and chronic Cd poisoning causes damage to the kidneys and heart (The Earth Report 3, 1992). Fish is one of the most important food sources, and thus, intake of metals when consuming fish, especially toxic metals if one of great concern for human health. As a consequence of heavy metal toxicity and of the serious contamination of food that occurs from time to time during commercial handling and processing, most countries monitor the levels of toxic elements in foods. However, the potential hazards of metals transferred to humans are probably dependent on amount of muscles consumed by an individual.

4. Conclusion

The values of all metals except Cd in muscles of analysed fish in the present study stayed below the established limit values. It is suggesting that the concentration of Cd in muscle tissues of *T. tonggol* from Karachi Fish Harbour pose to health hazards to the consumers. Therefore it is concluded that the regular monitoring of commercial fish of marine coastal areas is essentially required.

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