Pakistan J. Zool., vol. 42(5), pp. 631-638, 2010.

Accumulation of Toxic and Essential Trace Metals in Fish and Prawns from Keti Bunder Thatta District, Sindh

A. B. Tabinda*, M. Hussain, I. Ahmed and A. Yasar
Sustainable Development Study Center, GC University, Lahore, 54000, Pakistan (ABT, AY), and Departments of Chemistry (MH) and Zoology (IA), University of Karachi, Karachi, 75270, Pakistan.

Abstract.- Toxic and essential trace element, contents of six fish and two prawn species from Keti Bunder were determined by atomic absorption spectrometry. The mean concentrations (μg g^{-1} wet weight) of toxic and trace elements in the muscle of fish and prawn were Cd: 0.024-0.035 and 0.025-0.026, As: nd-0.014 and 0.003-0.01, Ni: 0.149-1.420 and 0.155-0.157, Pb: 0.001-3.600 and 0.098-0.100, Zn: 0.460-1.490 and 0.250-1.454, Cu: 0.001-2.800 and 0.003-0.006, Fe: 0.081-7.350 and 0.085-0.133, Cr: 0.209-2.309 and 1.706-1.921. Level of Pb and Cu in *Pampus argenteus* and *Tenualosa ilisha* and Cr in most of the fish and prawn species exceeded the recommended limits.

Keywords: Toxic/trace metals, heavy metals, atomic absorption spectrometry, Keti Bunder

INTRODUCTION

Recent years have witnessed significant attention to the problems of heavy metals contamination which have been broadly studied (Tüzen, 2003; Canli and Atli, 2003; Karadede *et al*., 2004; Ansari *et al*., 2005; Tuzen and Soylak, 2007; Turkmen *et al*., 2009).

Heavy metals discharged into the marine environment can damage both marine species diversity and ecosystem due to their toxicity (Turkmen *et al*., 2009). Fish are often at the top of the aquatic food chain and may concentrate large amounts of some metals from the water (Mansour and Sidky, 2002). Furthermore, fish is one of the most sensitive indicators of trace metals pollution and risk potential of human consumption (Cid *et al*., 2001; Papagiannis *et al*., 2004; Ashraf, 2005; Lavilla *et al*., 2008). In natural life, some trace metals are essential at low levels but toxic at higher concentrations. They enter in the human body through food chain causing different diseases and damages to the humans (Tüzen, 2003; Tuzen and Soylak, 2007; Yilmaz *et al*., 2007).

Keti Bunder is located at a distance of about 200 km (125 miles) south-east of Karachi in Thatta district of Sindh province, Pakistan. River Indus joins Arabian Sea at Keti Bunder. Therefore Keti Bunder is recipient of heavy/trace metals load from five rivers of Punjab through river Indus as well as from Karachi City (110 million gallon of domestic wastewater per day and 37000 tons of industrial waste/annum from 30000 industrial units). Prawns, shrimp and fin fish from Keti Bunder are supplied within the country as well as exported to European countries and Middle-east. Present study was carried out to assess metal contamination in some important species of fish and prawn from Keti Bunder and evaluate the health risk for local population as well as for the population of other countries importing fish and prawn from the study area.

MATERIALS AND METHODS

Six species of fish *Pampus argenteus, Sardinella sindensis*, *Labeo rohita,*, *Platycephalus indicus, Kowala coval*, *Tenualosa ilisha* (10 specimen each) and two species of prawn *Penaeus indicus* and *Penaeus indicus, Pencillantus* (10 specimen each) were collected from Keti Bunder from random commercial catches (Fig. 1). The samples of fish muscle (on the dorsal surface) and prawn were washed with distilled water, dried in filter paper, homogenized, packed in polyethylene bags and stored below −20°C until analysis (Tüzen, 2003; Karadede *et al*., 2004; Papagiannis *et al*., 2004; Yilmaz *et al*., 2007).

Double de-ionized water was used for all dilutions. HNO_3, HCl were super pure from E.

* Corresponding author: amtulbaritabinda64@yahoo.com
0030-9923/2010/0005-0631 $ 8.00/0

Merck Darmstadt Germany. All glassware were cleaned by soaking in dilute HNO_3 (1/9, v/v) and were rinsed with de-ionized water before use (Uluozlu *et al*., 2007). Standard solutions of each metal were prepared by diluting a stock solution of 1g/l of each metal (E. Merck).

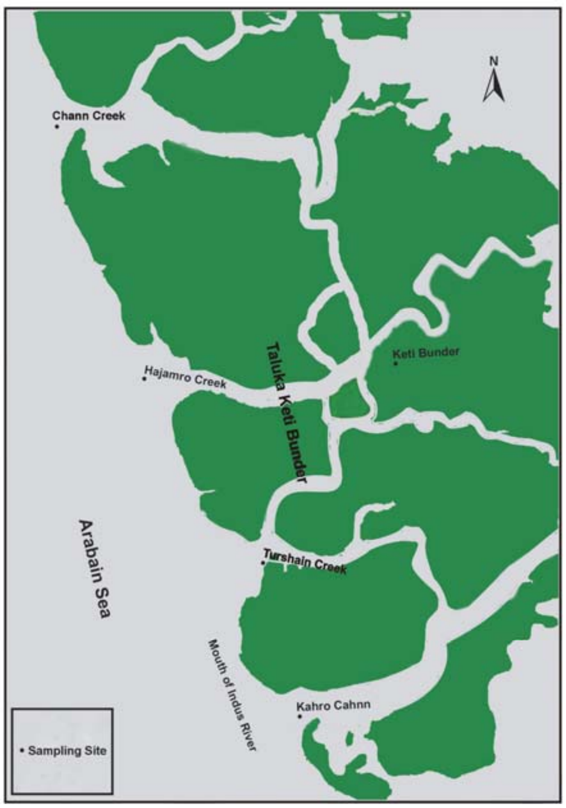

Fig. 1. Sampling sites in Keti Bunder.

Fish and prawn samples (5 gram each) were digested in concentrated HNO_3 and HCl (Analar grade, BDH 69%) (1:1) mixture (Zachariadis *et al*., 2005). They were placed in a hot-block digester first at low temperature for 1 h and then they were fully digested at high temperature (140 °C) for at least 3 h. The digested samples were then diluted to 50 ml with de-ionized water. Samples were filtered through 0.45 µm membrane filter.

After filtration, the prepared fish and prawn samples were analyzed for Cd, Ni, Pb, Zn, Fe, Cr using Unicam AAS 969 but As and Cu were analyzed using MHS 10 Hydride Generator Assembly. All elements were determined against aqueous standards (APHA, 1998; Karadede *et al*., 2004). The data were presented in µg $g^{-1}$of sample wet weight (ww). Procedural blanks were analyzed in every five samples. Quality control samples, made from standard solutions of Cd, As, Ni, Pb, Zn, Cu, Fe, Cr were analyzed in every five samples to check for the metal recoveries as described by Yap *et al*. (2004).

All the data were subjected to statistical analysis and correlation matrices were produced to examine the inter-relationships between the investigated metal concentrations of the fish samples. t test was performed to determine the significant difference between mean values.

RESULTS AND DISCUSSION

Sex and fork length of fish samples is given in Table I.

Table I.- Sex and length of fish samples.

Fish species	Male/female (n)	Fork length (cm)
Pampus argenteus	5♂/5♀	24± 03 (19-28)
Sardinella sindensis	6♂/4♀	18± 04 (17-22)
Labeo rohita	5♂/5♀	41± 05 (40-45)
Platycephalus indicus	4♂/6♀	37± 05 (30-42)
Kowala coval	7♂/3♀	07± 01 (06-08)
Tenualosa ilisha	4♂/6♀	32± 03 (30-35)

n – Number of samples; mean value ± standard deviation; Values within parenthesis are minimum and maximum levels

Recoveries (%) for different metals were 99, 98, 102, 97, 101, 95, 97, 96 for Cd, As, Ni, Pb, Zn, Cu, Fe and Cr respectively. Detection limits were 0.001 mg/L for Cd, Ni, Pb, Zn, Fe and Cr but 0.0004 mg/L for As and Cu.

The concentrations of toxic and trace metals (µg g^{-1}) in muscle of fish and prawn are summarized in Table II and Table III respectively.

During present studies mean values of Cd, As, Ni, Pb, Zn, Cu, Fe and Cr (µg g^{-1}) were 0.024, 0.014, 0.180, 3.600, 1.425, 0.002, 0.081, 0.240 for *Pampus argenteus,* 0.035, 0.001, 0.149, 0.133, 1.490, 0.001, 0.247 and 0.209 for *Labeo rohita*, 0.032 “nd”, 0.266, 0.001, 1.029, 0.001, 0.113 and 2.232 for *Platycephalus indicus* and 0.030, 0.001, 1.420, 0.690, 0.460, 2.800, 7.350 and 0.234 for *Tenualosa ilisha*, respectively.

Ashraf (1991) reported almost similar values of Cd and Fe, significantly high values of As and Cu ($P<0.0001$) but significantly low values of Ni, Pb, Zn and Cr ($P<0.001$); Sung and Lee (1993) reported significantly high values of Cd and As ($P<0.01$) but significantly low values of Pb ($P<0.0001$), whereas, Mitra *et al*. (2000) reported significantly high values of Cu and Pb ($P<0.0001$) but almost similar values of zinc in *Pampus argenteus* as compared to the values of these metals detected during present studies (Table II). Significantly high values of Pb and Cu detected by Mitra *et al*. (2000) are due to the analysis of these metals on dry weight basis.

Ashraf (1991) reported significantly high values of Cd, As, Cu ($P<0.0001$), Pb, Zn, Fe and Cr ($P<0.05$) but almost similar values of Ni. Rauf *et al*. (2009) reported significantly high values of Cd and Cr ($P<0.01$); Ashraf *et al*. (1992) reported significantly high values ($P<0.05$) of Zn but significantly low values of Cd ($P<0.01$) in *Labeo rohita* as compared to values of these metals determined during present studies (Table II).

In *Platycephalus indicus,* Abdel-Moati and Nasir ((1997) reported significantly high values of Cd, Pb ($P<0.001$) but non-significantly high value of Ni ($P<0.05$); Nahida *et al*. (1998) reported significantly high values of Cd ($P<0.05$), Pb, Zn and Fe ($P<0.001$) but significantly low value of Ni ($P<0.0001$) as compared to the values of these metals determined during present studies (Table II). High values of Cd, Pb and Ni reported by Nahida *et al*. (1998) seem to be due to their determination of metals on dry weight basis.

Ashraf (1991) reported significantly high values of Cd ($P<0.01$), As ($P<0.0001$) and Zn ($P<0.05$), significantly low values of Ni Fe ($P<0.01$), Pb ($P<0.0001$) and Cu ($P<0.05$) but non-significantly high value of Cr ($P<0.05$) in *Tenualosa ilisha* as compared with metals detected during present studies (Table II).

When metal contents detected in present studies were compared with the permissible limits, it was found that Cd concentrations in all samples of fish and prawn analysed were below the Turkish Food Code (TFC, 2002) and European Union (EU, 2001) limit of 0.05 mg kg^{-1}. The limit set by the Malaysian Food Regulation (1985) is 1.00 (μg g^{-1} wet wt.). According to Hong Kong Environmental Protection Department (HKEPD, 1987) the recommended limit is 2.00, United States Food and Drug Administration (USFDA, 1993) 3.70, United States Environmental Protection Agency (USEPA, 2000) 0.491, Food Standards Australia New Zealand (FSANZ, 2002) 0.200 and EUROPA (2004) 0.100.

Concentrations of arsenic in all fish and prawn samples were much below the permissible limit of USFDA (1993b) which is 76 mg kg^{-1}.

Ni concentrations in all the fish (except *Tenualosa ilisha*) and prawn samples were well within the toxic limit of 70-80 mg kg^{-1} set by USFDA (1993c) and the limit set by USEPA (2000) 1.00 μg g^{-1}.

Turkish acceptable limit and EU limit for Pb (μg g^{-1}) is 0.40, the range of international standard in fish is 0.5-10 (EU, 2001; TFC, 2002), permissible value by Malaysian Food Regulation (1985) is 2.00, by HKEPD (1987) it is 6.00, by USEPA (2000) it is 0.491, by FSANZ (2002) is 0.200, by EUROPA (2004) it is 0.100 and its toxic limit set by Food and Agriculture Organization (FAO, 1983) is 0.5 mg kg^{-1}. Pb contents in all prawn samples were within the different permissible limits but *Pampus argenteus* and *Tenualosa ilisha* exceeded international limits of EU, TCF, USEPA and toxic limit of FAO. *Pampus argenteus* exceeded Malaysian Food Regulation, *Pampus argenteus, Labeo rohita* and *Tenualosa ilisha* samples exceeded FSANZ guidelines and *Pampus argenteus, Sardinella sindensis, Labeo rohita* and *Tenualosa ilisha* samples exceeded EUROPA guidelines for human consumption.

For zinc (μg g^{-1}) the permissible limit by Canadian Food Standard is 100, by Hungarian Standard 80, by Australian Standard 10 (Papagiannis *et al*., 2004) and by Turkish Standards (TFC, 2002) it is 50 mg kg^{-1} . Zn concentrations in all fish and prawn samples analyzed were below these limits.

For Cu (μg g^{-1}) Canadian Food Standard is 100, Hungarian standard 60, the range of international standard 10-100, Turkish acceptable

Table II.- Concentrations of metals in fish species from Keti Bunder and their comparison with other study areas [a].

Fish species	Area	Cd	As	Ni	Pb	Zn	Cu	Fe	Cr
Pampus argenteus	Present study	0.024± 0.04[b] (0.012-0.035)	0.014± 0.001 (0.011-0.016)	0.180± 0.11 (0.140-0.190)	3.600± 0.314 (3.112–3.950)	1.425± 0.186 (1.143–1.715)	0.002± 0.001 (0.001-0.003)	0.081± 0.004 (0.066-0.090)	0.240 ± 0.030 (0.210 - 0.761)
	Pakistan[1]	0.031	2.06	0.036	0.211	0.383	0.680	0.086	0.073
	Korea[2]	0.061± 0.021	1.621± 1.521		0.335 ±0.134	-	-	-	-
	India[3]	-	-	-	98.5	1.50	5.50-	-	-
Sardinella sindensis	Present study	0.031± 0.003 (0.027-0.036)	nd	0.244± 0.003 (0.226-0.248)	0.200± 0.002 (0.192-0.206)	1.215± 0.136 (0.956–1.352)	0.007± 0.001 (0.006-0.01)	0.104± 0.001 (0.098–0.106)	1.813± 0.160 (1.432–1.921)
Labeo rohita	Present study	0.035± 0.04 (0.030-0.042)	0.001± 0.001 (0.000-0.004)	0.149± 0.002 (0.138-0.152)	0.133± 0.002 (0.124–0.143)	1.490± 0.109 (1..345-1.612)	0.001± 0.001 (0.000-0.003)	0.247± 0.002 (0.223-0.249)	0.209± 0.003 (0.191-0.213)
	Pakistan[1]	3.915	1.155	0.155	0.680	2.670	0.589	0.620	0.930
	River Ravi[4]	2.50± 1.32	-	-	-	-	-	-	3.40± 1.53
	Rawal Lake[5]	0.003	-	-					
Platycephalus indicus	Present study	0.032± 0.030 (0.012-0.045)	nd	0.266± 0.003 (0.261-0.275)	0.001± 0.001 (0.000-0.005)	1.029± 0.060 (0.515-1.041)			
	Qatar[6]	0.19± 0.03	-	0.31± 0.17	0.06± 0.02	-	-	-	-
	ROPME[7] Sea area	0.06	-	0.00	0.98	15.29	-	29.20	-
Kowala coval	Present study	0.033± 0.040 (0.011-0.073)	nd	0.286± 0.003 (0.244-0.298)	0.002± 0.001 (0.000-0.004)	1.184± 0.070 (0.654-1.942)	0.028± 0.003 (0.020-0.031)	0.189± 0.002 (0.186-0.194)	2.309± 0.120 (1.849-2.415)
Tenualosa ilisha	Present study	0.030± 0.030 (0.029-0.082)	0.001± 0.001 (0.000-0.005)	1.420± 0.152 (1.218-1.632)	0.690± 0.060 (0.56-0.711)	0.460± 0.100 (0.378-0.614)	2.800± 0.310 (1.969-2.965)	7.350± 0.760 (5.349-7.695)	0.234± 0.030 (0.193-0.256)
	Pakistan[1]	0.220	0.080	0.095	0.004	0.850	1.650	1.023	0.265

nd, not detectable.
[a] Results are expressed as μg g^{-1} wet weight; 3 and 7 – Results are expressed as μg g^{-1} dry weight
[b] Mean values and ± standard deviation.
Results within parenthesis indicate minimum and maximum values
1, Ashraf (1991); 2, Sung and Lee (1993); 3, Mitra *et al*. (2000); 4, Rauf *et al*. (2009); 5, Ashraf *et al*. (1992); 6, Moati *et al*. (1997); 7, Nahida *et al*. (1998).

limit 20 (Papagiannis *et al*., 2004), the permissible limits set by the Malaysian Food Regulation (1985) 30, USEPA (2000) Limit 120, and toxic limit for fish by FAO (1983) 30 mg kg^{-1}. Cu concentrations in all fish and prawn samples analyzed were below the corresponding authorized limits.

Cr contents in all the fish and prawn samples were well within the toxic limit of USFDA (1993a). But all the fish and prawn samples had higher Cr concentrations as compared to the limits of 0.200 set by FSANZ (2002) and 0.100 by EUROPA (2004). *Sardinella sindensis*, *Platycephalus indicus* and *Kowala coval* samples exceeded the Cr guidelines set by USEPA (2000) 0.491 and NEPA (1997) 500 μg g^{-1} for human consumption.

The potential hazards of metals transferred

Table III.- Toxic and essential element contents (mean, minimum and maximum) in prawn species from Keti Bunder, Pakistan[a].

Prawn species	Cd	As	Ni	Pb	Zn	Cu	Fe	Cr
Penaeus indicus	0.024± 0.04[b] (0.021-0.032)	0.01± 0.001 (0.009-0.014)	0.157± 0.08 (0.142-0.240)	0.101± 0.01 (0.089-0.114)	1.454± 0.08 (1.032 – 1.542)	0.006± 0.01 (0.002-0.171)	0.133± 0.40 (0.009–0.542)	1.921± 0.14 (1.626–1.983)
Penaeus indicus pencillantus	0.025± 0.03 (0.007-0.058)	0.003± 0.001 (0.000-0.006)	0.155± 0.05 (0.116-0.207)	0.098± 0.08 (0.052-0.181)	0.250± 0.050 (0.191-0.282)	0.003± 0.05 (0.000-0.058)	0.085± 0.32 (0.061-0.396)	1.706± 0.16 (1.212–1.824)

[a] Results are expressed as μg g^{-1} wet weight.
[b] Mean values ± standard deviation.

Table IV.- Correlations between metal concentrations in fish species.

	Zn	Cd	Cu	As	Fe	Ni	Pb	Cr
Cd	0.494							
Cu	−0.892	−0.106						
As	0.385	−0.884	−0.202					
Fe	−0.889	−0.094	1.000	−0.210				
Ni	−0.930	−0.089	0.995	−0.244	0.994			
Pb	0.248	−0.927	−0.031	0.984	0.038	0.077		
Cr	0.008	0.352	−0.434	−0.436	0.439	0.343	-0.542	

to humans are probably dependent on the amount of fish/prawn consumed by an individual. Average intake of fish in Pakistan is plus 2 kg per capita per annum (National policy and strategy for fisheries and aquaculture development in Pakistan, 2007). If an adult consumes 5.5 g day^{-1} of *Pampus argenteus, Sardinella sindensis, Labeo rohita*, *Platycephalus indicus, Kowala coval, Tenualosa ilisha* from Keti Bunder he would take in approximately 19.8 μg day^{-1} (138.6 μg week^{-1}), 1.342 μg day^{-1} (9.394 μg week^{-1}), 0.820 μg day^{-1} (5.74 μg week^{-1}), 1.463 μg day^{-1} (10.241 μg week^{-1}), 1.573 μg day^{-1} (11.011 μg week^{-1}), 7.81 μg day^{-1} (54.67 μg week^{-1}) of lead, respectively. If the consumer has to take *Penaeus indicus* and *Penaeus indicus pencillantus* for 7 days, then he would consume 0.55 μg day^{-1} (3.850 μg week^{-1}), 0.548 μg day^{-1} (3.836 μg week^{-1}) of Pb. This is lower than the provisional tolerable daily (weekly) intakes suggested by the WHO (1993) for Pb which is 245 μg day^{-1} (1715 μg week^{-1}) for a 70 kg person. The estimates of JEFCA (2000) regarding Provisional Daily Intake (PTDI) for Pb are 25 μg kg^{-1} and JEFCA (2003) recommended Provisional Tolerable Weekly Intake (PTWI) for lead 1.5 mg week^{-1} which is equivalent to 0.21 mg day^{-}1. The dietary intake of fish and prawn estimated from the present studies is well below these dietary intakes and the tested fish and prawn samples not represent any known risk to health for local population regarding Pb intake.

The recommended daily amount indicated for Cr by the US National Research Council is about 60 μg day^{-1} (420 μg week^{-1}) for a 70 kg person (NRC, 1989). An adult who consumes 5.5 g day^{-1} of *Pampus argenteus, Sardinella sindensis*, *Labeo rohita*, *Platycephalus indicus, Kowala coval, Tenualosa ilisha,* from Keti Bunder he would take Cr approximately 1.320 μg day^{-1} (9.240 μg week^{-1}), 9.972 μg day^{-1} (69.801 μg week^{-1}), 1.150 μg day^{-1} (8.850 μg week^{-1}), 12.276 μg day^{-1} (85.9320 μg week^{-1}), 12.810 μg day^{-1} (89.670 μg week^{-1}), 1.287 μg day^{-1} (9.007 μg week^{-1}), respectively. If the consumer were to take *Penaeus indicus* and *Penaeus indicus* *pencillantus* for 7 days, then he would consume 10.566 µg day^{-1} (73.962 µg week^{-1}), 9.383 µg day^{-1} (65.681 µg week^{-1}) of Cr, respectively. This is lower than the recommended daily (weekly) amount indicated by NRC (1989) for all fish and prawn species.

If an adult consumes approximately 5.5 g of fish or prawn per day, then a person who consumes prawn or fish collected from Keti Bunder would consume Cd approximately 0.171 µg (0.031 µg g^{-1}×5.5 g) from fish or 0.143 µg (0.026 µg g^{-1}×5.5 g) from prawn each day. If the consumer takes the prawn or fish for 7 consecutive days, then he will consume 1.197 µg Cd (0.171×7 days) from fish or 1.029 µg Cd (0.147×7 days) from prawn. Expected Cd intake from Keti Bunder is lower than the recommended limit for the provisional tolerable weekly intake of Cd (6.70-8.30 µg adult^{-1}) recommended by FAO/WHO (1984), JEFCA (2003) which is 490 µg and Provisional Daily Intake (PTDI) of JEFCA (1993) which is 0.06 mg day^{-1}.

JEFCA (1989) established 7000 µg week^{-1} kg^{-1} as provisional tolerable weekly intake (PTWI) for zinc. The Provisional Maximum Tolerable Daily Intake (PMTDIs) set by JECFA (2003) for zinc is 1.0 mg kg^{-1} (equivalent to 70 mg day^{-1} for a 70 kg adult). PTDI for arsenic is 0.12 mg day^{-1} (JEFCA, 1993). The dietary intakes of fish and prawn estimated from the present studies is well below these dietary intakes and the tested fish and prawn samples do not represent any known risk to health regarding zinc and arsenic intake.

A linear regression correlation test was performed to investigate correlations between metal concentrations in fish. The values of correlation coefficients between metal concentrations in fish are given in Table IV. There is a very strong correlation between iron and copper ($r = 1.000$), correlations for copper and nickel, arsenic and lead, iron and nickel, and iron and manganese are strong, with corresponding r values of 0.995, 0.984, 0.994, respectively. A positive correlation exists between Cd and Zn, As and Zn, Pb and Zn, Cr and Fe, Cr and Ni with corresponding r values of 0.494, 0.385, 0.248, 0.439, 0.343, respectively. Correlations between Cr and Zn, Fe and Pb, Ni and Pb (r=0.008, 0.038, 0.077 respectively) are very weak. Correlations between other metals are negative.

CONCLUSIONS

This study was carried out to provide information on toxic and trace metal concentrations in six fish and two prawn species from Keti Bunder, Pakistan and potential health risk for local population due to their consumption. The majority of toxic and trace metal concentrations in the fish and prawn samples analyzed were well within the permitted limits set by various authorities and do not pose any health risk for the local population due to low intake of fish and prawn. But the contamination of Pb and Cu in *Pampus argenteus*, *Tenualosa ilisha* and Cr in most of the fish and prawn samples may pose threat for the importers due to high per capita consumption of fish.

ACKNOWLEDGEMENTS

Authors are grateful to Dr. Quddusi B. Kazmi, Director (Rtd.) MRCC, University of Karachi for her valuable help.

REFERENCES

ABDEL-MOATI, M.A.D. AND NASIR, N.A., 1997. Bioaccumulation of chromium, nickel, lead, and vanadium in some commercial fish and prawn from Qatri Waters. *Qatar Univ. Sci. J.*, **17**: 195-203.

ANSARI, T. M., MAHBOOB, S., HANIF, T., ARIF, M. AND SALAM. A., 2005. Dry ashing or wet digestion? A comparative study for estimation of zinc and calcium in freshwater fish samples by atomic absorption spectrometry. *J. chem. Soc. Pakistan*, **27**: 90–94.

APHA, 1998. *Standard method for water and wastewater*. 20th edition, American Public Health Association.

ASHRAF, M., 1991. *Studies on heavy trace metals distribution in local fish and relevant waters*. Ph. D dissertation, Quaid-e-Azam University, Islamabad, Pakistan.

ASHRAF, M., JAFFAR, M. AND TARIQ, J., 1992. Annual variation of selected trace metals in freshwater lake fish, *Labeo rohita* as an index of environmental pollution. *Toxicol. Environ. Chem.,* **35**: 1-7.

ASHRAF, W., 2005. Accumulation of heavy metals in kidney and heart tissues of *Epinephelus Microdon* fish from the Arabian Gulf. *Environ. Monitor. Assess.*, **101**: 311–316.

CANLI, M. AND ATIL, G., 2003. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environ. Pollut.,* **121**: 129–136.

CID, B. P., BOIA, C., POMBO, L. AND REBELO, E., 2001. Determination of trace metals in fish species of the Ria de Aveiro (Portugal) by electro thermal atomic absorption spectrometry. *Fd. Chem.,* **75**: 93–100.

EU, 2001. *Commission Regulation as regards heavy metals*, Directive 2001/22/EC, No: 466/2001.

EUROPA, 2004. *Assessment of the dietary exposure to arsenic, cadmium, lead and mercury of the population of the EU Member States*. Directorate-General Health and Consumer Protection: European Union.

FAO, 1983. Compilation of legal limits for hazardous substance in fish and fishery products (Food and agricultural organization). *FAO Fish. Circ.*, **464**: 5-100.

FAO/WHO, 1984. *Lists of contaminants and their maximum levels in foods.* CAC/Vol XVII (1st ed.).

FSANZ, 2002. *Food Standards Code*. Food Standards Australia New Zealand, Anstat Pty Ltd, Australia.

JECFA, 1989. *Joint FAO/WHO Expert Committee on Food Additives*. *Toxicological evaluation of certain food additives and contaminants*. Cambridge University Press, 1989; 155-162.

JECFA, 1993. *Evaluation of certain food additives and contaminants:* Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives. (WHO Technical Report Series No. 837). Geneva, World Health Organization.

JEFCA, 2000. *Joint FAO/WHO Expert Committee on Food Additives*. Fifty-fifth Meeting. Summary and Conclusions , World Health Organization, Geneva.

JEFCA, 2003. *Joint FAO/WHO Expert Committee on Food Additives*. Sixty-first Meeting. Summary and Conclusions. , World Health Organization, Geneva.

KARADEDE, H., OYMAK, S.A. AND ÜNLÜ**,** E., 2004. Heavy metals in mullet, *Liza abu* and Cat fish, *Silurus triostegus*, from the Atatürk Dam Lake (Euphrates), Turkey. *Environ. Int.,* **30**: 183–188.

HKEPD, 1987. *Marine water quality in Hong Kong in 1997*. Government Printer, Hong Kong.

LAVILLA, I., VILAS, P. AND BENDICHO, C., 2008. Fast determination of arsenic, selenium, nickel and vanadium in fish and shellfish by electrothermal atomic absorption spectrometry following ultrasound-assisted extraction. *Fd. Chem.,* **106**: 403-409.

MALAYSIAN FOOD REGULATION, 1985. *Malaysian law on food and drugs*. Malaysian Law Publishers.

MANSOUR, S.A. AND SIDKY, M.M., 2002. Ecotoxicological studies.3. Heavy metals contaminating water and fish from Fayoum Governorate, Egypt. *Fd. Chem.*, **78:** 15–22.

MITRA, A., MITRA, S., HAZRA, S. AND CHAUDRI, A., 2000. Heavy metal concentrations in Indian Coastal fishes. *Res. J. Chem. Environ.*, **4:** 35–37.

NAHIDA, B., MAJED, A., SAFAR, A., RAJAB, W. A., FARHAN, M.S. AND IQBAL, A. R., 1998. Levels of trace metals and hydrocarbons in fish from ROPME Sea Area Offshore. In: *Environment of the ROPME Sea Area after the war-Related oil spills*. (eds. A. Otuski *et al*.), Terra Scientific Publishing Company, Tokyo, pp. 257-279.

NATIONAL POLICY AND STRATEGY FOR FISHERIES AND AQUACULTURE DEVELOPMENT IN PAKISTAN, 2007. Ministry of Food, Agriculture and Livestock, Government of Pakistan, Islamabad.

NEPA, 1997. China environmental state bulletin: 1996. *Environ. Protect.*, **236**: 2-5.

NRC, 1989. *National Research Council recommended dietary allowances*. 10th ed. Washington: National Academy Press.

PAPAGIANNIS, I., KAGALOU, I., LEONARDOS, J., PETRIDIS, D. AND KALFAKAKOU, V., 2004. Copper and zinc in four freshwater fish species from Lake Pamvotis (Greece). *Environ. Int.,* **30**: 357-362.

RAUF, A., JAVED, M. AND UBAIDULLAH, M., 2009. Heavy metal levels in three major carps (*Catla catla*, *Cirrhina mrigala* and *Labeo rohita*) from the river Ravi, Pakistan.. *Pakistan Vet. J.,* **29**: 24-26.

SUNG, D.W. AND LEE, Y. K., 1993. Study on contents of heavy metals of marine fish in Korean Coastal Waters. *Korean J. Fd. Hyg.,* **8**: 231-240.

TFC, 2002. *Turkish food codes, Official Gazette*, 23 September 2002, No: 24885.

TURKMEN, M., TURKMEN, A., TEPE, Y., TÖRE, Y. AND ATES, A., 2009. Determination of metals in fish species from Aegean and Mediterranean Seas. *Fd. Chem.,* **113**: 233–237.

TUZEN, M., 2003. Determination of heavy metals in fish samples of the middle Black Sea (Turkey) by graphite furnace atomic absorption spectrometry. *Fd. Chem.,* **80:** 119–123.

TUZEN, M. AND SOYLAK, M., 2007. Determination of trace metals in canned fish marketed in Turkey**.** *Fd. Chem.*, **101**: 1378–1382.

ULUOZLU, O.D., TUZEN, M., MENDIL, D. AND SOYLAK, M., 2007. Trace metal content in nine species of fish from the Black and Aegean Seas, Turkey. *Fd. Chem.*, **104**: 835–840.

USEPA, 2000. *Guidance for assessing chemical contaminant, Data for use in fish advisories*, Vol. 1.

Fish sampling and Analysis. 3rd ed. EPA 823-R-95-007. Office of Water: Washington, DC.

USFDA., 1993. *Guidance documents for trace elements in seafood*. Washington DC: US Food and Drug Administration.

USFDA, 1993a. *Food and drug administration. Guidance document for chromium in shellfish*. DHHS/PHS/FDA/CFSAN/Office of Seafood, Washington, DC.

USFDA, 1993b. *Food and drug administration. Guidance document for arsenic in shellfish*. DHHS/PHS/FDA/CFSAN/Office of Seafood, Washington, D.C.

USFDA, 1993c. *Food and drug administration. Guidance document for nickel in shellfish.* DHHS/PHS/FDA/CFSAN/Office of Seafood, Washington, D.C.

WHO, 1993. *Guidelines for drinking water quality*. 2nd ed. Vol.1. Geneva, pp. 114-121.

YAP, C. K., ISMAIL, A. AND TAN, S.G., 2004. Heavy metal (Cd, Cu, Pb and Zn) concentrations in the green-lipped mussel *Perna viridis* (Linnaeus) collected from some wild and aquacultural sites in the west coast of Peninsular Malaysia. *Fd. Chem.,* **84**: 569-575.

YILMAZ, F., OZDEMIR, N., DEMIRAK, A. AND TUNA, A.L., 2007. Heavy metal levels in two fish species *Leuciscus cephalus* and *Lepomis gibbosus*. *Fd. Chem.,* **100**: 830-835.

ZACHARIADIS, J. A., SRATIS, J. A., KANIOU, A. AND KALLIGAS, G., 2005. Critical comparison of wet and dry digestion procedures of trace metal analysis of meat and fish tissues. *Microchim. Acta* DOI. **10**. 1007/BF 01243998.

(Received 1 December 2009, revised 1 February 2010)