INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 4, No. 12, p. 306-313, 2014

RESEARCH PAPER OPEN ACCESS

Lead and zinc levels in silver carp (*Hypophthalmichthys molitrix* Valenciennes, 1844), grass carp (*Ctenopharyngodon idella,* Cuvier and Valenciennes, 1844) and bighead carp (*Aristichthys nobilis,* Richardson, 1845) from Iran

Abolfazl Askary Sary[1*], Mohammad Velayatzadeh[2]

[1]Department of Fishery, College of Agriculture and Natural resource, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran

[2]Young Researchers Club, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran

Key words: Heavy metal, fishes, Zinc, Lead, Iran.

http://dx.doi.org/10.12692/ijb/4.12.306-313 Article published on June 28, 2014

Abstract

The present study was carried out to investigate contamination of heavy metals Pb and Zn in liver and muscle in *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella* and *Aristichthys nobilis*, Iran, in 2011. Heavy metal levels in fish samples were analyzed by Perkin Elmer 4100 zl atomic absorption. The results show that the highest concentration of Pb and Zn in the liver of *Ctenopharyngodon idella* 0.607±0.02 and 8.26±0.33 mgKg^{-1}dw and the determined lowest concentration of Pb and Zn in the muscle of *Hypophthalmichthys molitrix* 0.404±0.02 and 4.71±0.17 mgKg^{-1}dw. This study concentration of heavy metals Zn and Pb in the liver and muscle of *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella* and *Aristichthys nobilis* significant difference between ($P<0.05$). The mean estimated concentrations for Zn in the present study were lowest than International Standards for these metals as declare by the Ministry of Agriculture, Fisheries and Food (UK) and National Health & Medical Research Council (Australia), but concentrations of Pb in this study were lowest than International Standards World Health Organization (WHO), Ministry of Agriculture, Fisheries and Food (UK) and National Health & Medical Research Council (Australia)

* **Corresponding Author:** Abolfazl Askary Sary ✉ askary_sary@yahoo.com

Introduction

Fish are a major part of the human diet and it is therefore not surprising that numerous studies have been carried out on metal pollution in different species of edible fish. In recent years, fish lipids have also assumed great nutritional significance, because of their high polyunsaturated fatty acid levels and good source of digestible protein, vitamins, minerals (Prudente *et al.,* 1997; Puwastien *et al.,* 1999; Kucuksezgin *et al.,* 2001; Lewis *et al.,* 2002; Ikem and Egiebor, 2005).

Heavy metal pollution of aquatic environment has become a great concern in recent years. HMs can have toxic effects on organs (Macfarlane and Burchettt, 2000). Heavy metals have the tendency to accumulate in various organs of marine organisms, especially fish, which in turn may enter into the human metabolism through consumption causing serious health hazards. Iron, copper, zinc and manganese are essential metals while, mercury, lead and cadmium are toxic metals (Canli and Alti, 2003). Heavy metals still play an important role as pollutants affecting aquatic systems (Merian, 1991). 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 common heavy metals that are found in fish include copper, iron, copper, zinc and manganese, mercury, lead and cadmium (Rashed, 2001; Munoz-Olivas and Camara, 2001; Canli and Alti, 2003; Fernandes *et al.,* 2008). Toxic elements can be very harmful even at low concentration when ingested over a long time period. The essential metals can also produce toxic effects when the metal intake is excessively elevated (Celik and Oehlenschlager, 2007).

Lead finds its way in waters through the discharge of industrial waste waters, such as from painting, dyeing, battery manufacturing units and oil refineries etc. Pb also enters the rivers both from terrestrial sources and atmosphere and the atmospheric input of Pb aerosols can be substantial (Mitra *et al.,* 2010). Lead enters into the body with gill cells and especially is accumulated in gills and the later aim organs are liver and muscle (Sadeghi-Rad, 1997). Although, Zinc usually is accumulated in bone, skin, liver, gill and kidney are accumulated the great amount of them (Celik and Oehlenshlager, 2004).

This matter that, importance of the heavy metals measuring relate to two important subjects which are aquatics ecosystem management and human health, the present study was carried out to determine the level of Lead and zinc in liver and muscle samples of *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella* and *Aristichthys nobilis* from Iran. The fish and fish products for the people in those ports are generally catch and carried by local vehicles from the Ahvaz. It should be noted that fish species are considered to be a heavy metals part of the diet in the region. No data exist on Zinc and Lead levels in this fish from mentioned areas.

The main objective of this study was to determinate the contents of zinc and lead in the muscle and liver of *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella* and *Aristichthys nobilis* from Iran, in order to assess fish quality and to assess the health risk for humans.

Material and methods

Sampling

The *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella* and *Aristichthys nobilis* in this study were collected 72 samples of farmed fishes from the Ahvaz, took place in 2011. After capture, fishes were placed in plastic bags and transported to the laboratory in freezer bags with ice. Samples were cut into pieces and labeled, and then all sampling procedures were carried out according to internationally recognized guidelines (UNEP, 1991). Total fish weight and length were measured to the centimeter and gram.

Apparatus

A Perkin-Elmer, model 4100 ZL atomic absorption spectrophotometer, equipped with a GTA Graphite furnace, was used. Pyrolytic-coated graphite tubes with a platform were used and signals were measured as peak areas. The instrument setting and furnace programmes for analysis of Zinc and Lead metals are described in table 2.

Reagents

All reagents were of analytical reagent grade unless otherwise stated. Double distilled water was used for the preparation of solution. All the plastic and glass ware were soaked in nitric acid for 15 min and rinsed with deionized water before use. The stock solutions of metals (1000 mgl^{-1}) were obtained by dissolving appropriate salts of the corresponding metals (E. merk) and further diluted prior to use. High purity Argon was used as inert gasted prior to use.

Chemical analyses (Wet-ashing)

The samples were solubilized using high-pressure decomposition vessels, commonly known as a digestion bomb. A sample (1gr) was placed in to Teflon container and 5 ml of concentrated HNO_3 was added. The system was heated to 130^o C for 90 min and finally diluted to 25 ml with deionized water. The sample solution was clear. A blank digest was carried out in the same way. Zinc and Lead metals were determined against aqueous standards.

Statistical analysis

Analysis of variance (ANOVA) was run for all the collected data for fish samples different using SPSS (16 version) computer programs. Mean values of each parameter were compared using Fisher's protected least tests with significance levels of 5% were conducted on each metal to test for significant differences between sites (Table 3 and 4). All statistical analyses were conducted using the Office Excel 2003 software package.

Results and discussion

Determination metals

Concentration levels of metals Zn and Pb in muscle and liver of *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella* and *Aristichthys nobilis* were measured and presented in table 3. Concentrations of metals are presented in mg Kg^{-1} dry weight unless otherwise mentioned. The highest concentration of Pb and Zn in tissues was done in liver of *Ctenopharyngodon idella*. The lowest concentration of Pb and Zn in tissues was done in muscle of *Hypophthalmichthys molitrix* (table 3). This study concentration of heavy metals Zn and Pb in the liver and muscle of *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella* and *Aristichthys nobilis* significant difference between ($P<0.05$). The distribution patterns of Zn in tissues of *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella* and *Aristichthys nobilis* follows the order: liver> muscle. Heavy metal concentrations were higher in the gill and liver, when compared with muscle. livers were chosen as target organs for assessing metal accumulation (Romeo *et al.*, 1999; Rodriguez *et al.*, 2003; Tuzen, 2009).

Table 1. Mean length and weight of the species examined in present study.

species	The number of samples	Length±SD (cm)	Weight±SD (g)
Hypophthalmichthys molitrix	24	53.38±2.51	1113.17±63.51
Ctenopharyngodon idella Aristichthys	24	38.83±1.63	626.61±25.62
nobilis	24	47.83±1.25	816±32.18

Estimation of the levels of various elements in different fish species as a measure of environmental pollution has been of great concern over decades. A variable range of different metal concentrations has been observed by various researchers worldwide (Ashraf *et al.*, 2006). The absorption of metals on to the gill surface, as the first target for pollutants in water, could also be an important influence in the total metal levels of the liver (Heath, 1987).

Distribution patterns of metal concentrations in liver and muscle of *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella* and *Aristichthys nobilis* follows the sequence: Zn>Pb. There are various studies on the heavy metal levels in fish from different waters. Oymak *et al*., (2009) studied the heavy metal levels in kidney, liver, gill and muscle of *Tor grypus* and Maaboodi *et al*., (2011) studied the concentration of Zn and Pb in liver of *Carrassius* sp., *Cyprinus carpio*, *C. aculeate* and *C. damasciana* which concentration of Zn were higher than Pb. Also, Turkmen *et al*., (2010) studied the heavy metal levels in muscle, liver, gonad, and gill of gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), and keeled mullet (*Liza carinata*) which concentration of Zn were higher than Pb. The levels of Zn in all tissues were higher than the Pb levels, as Zn is present in many enzymes throughout the fishes body (Oymak *et al*., 2009).

Table 2. The instrument setting and furnace programmes for analysis of Zn and Pb by Perkin-Elmer, model 4100 ZL (AAS).

Working conditions	Pb	Zn
Wavelenght (nm)	283.3	307.5
Slit width	0.5	0.7
Lamp current (MA)	8	15
Ar Flow (ml/min)	250	250
Injection Volume (µl)	25	20
Heating programme temprture^{O} C [ramp time (5), hold time (5)]		
Drying 1	125(1.20)	115(1.20)
Drying 2	150(5.30)	150(5.30)
Pyrolysis	900(15.10)	1250(15.10)
Atomization	2150(0.5)	1900(0.5)
Cleaning	2400(1.2)	2400(1.2)

Table 3. The concentrations and comparison of Pb and Zn in muscle and liver *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella* and *Aristichthys nobilis* (mgKg^{-1})

Sample	Location	Lead	Zinc
Hypophthalmichthys molitrix	muscle	0.404±0.02^{a}	4.71±0.17^{a}
	liver	0.44±0.03^{a}	5.27±0.23^{a}
Ctenopharyngodon idella	muscle	0.541±0.03^{a}	7.9±0.21^{a}
	liver	0.607±0.02^{b}	8.26±0.33^{b}
Aristichthys nobilis	muscle	0.45±0.01^{a}	6.57±0.28^{a}
	liver	0.518±0.04^{b}	7.11±0.31^{b}

a: non-significant differences at p<0.05, b: significant differences at p<0.05 Comparison Zn and Pb.

Lead

In this study minimum Pb levels 0.404 mgKg^{-1}dw and maximum concentrations of this metals 0.607 mgKg^{-1}dw. Agah *et al*., (2009) that among Pb in five fishes from Persian Gulf, which concentration of Grunt, Flathead, Greasy grouper, Tiger-tooth and Silver pomfret were 2-25, 0.2-17, 2-9, 1-9 and 3-33 ngg^{-1}. In other study concentration of Pb in muscle Indo-Pacific king mackerel and Tigertooth croaker were 0.625 and 0.3 mgKg^{-1}. The Pb values in fish species were found to be in range of 0.068–0.874 mgg-1. These values were lower than those reported earlier in fish species of different lakes (Aucoin *et al*., 1999; Mendil *et al*., 2005). Turkish acceptable limits and

EU limits were 0.4 mgg^{-1}. The range of international standards for Pb in fish is 0.5–10 mgg^{-1} (EU, 2001; TFC, 2002). The concentrations of Pb in liver higher than muscle. Muscle tissue is the main edible fish part and can directly influence human health. Lead enters into the body with gill cells and especially is accumulated in gills and the later aim organs are liver and muscle (Sadeghi-Rad, 1997).

Table 4. The tolerable values of some heavy metals in the fish ($mgkg^{-1}$).

Standards	Pb	Zn	References
WHO[1]	2	-	WHO, 1996
FDA[2]	0.5	-	Chen and Chen, 2001
UK(MAFF)[3]	2	50	MAFF, 1995
NHMRC[4]	1.5	150	Chen and Chen, 2001
FAO[5]	0.5	30	FAO, 1983
This study	0.40-0.64	4.66-8.39	

1- World Health Organization

2- U.S. Food and Drug Administration

3- Ministry of Agriculture, Fisheries & Food (UK)

4- National Health & Medical Research Council (Australia)

5- Food and Agriculture Organization.

Zinc

In this study minimum and maximum of Zn 4.71 and 8.26 $mgKg^{-1}$dw. Yilmaz *et al.*, (2007) reported that Zn concentration was 6.350–28.550 $mgkg^{-1}$ in tissues of *Leucis cephalus* and 6.540–16.064 $mgkg^{-1}$ in tissues of *Lepomis gibbosus*. Levels of Zn in muscle and liver of *Sciaena umbra* were 11.6 and 28.3 $mgkg^{-1}$ (Turkmen *et al.*, 2008). Also Abu Hilal and Ismail (2008) reported that concentrations of Zn were 1.9-35 $mgkg^{-1}$ in muscles, livers, gills, gonads, and stomachs of eleven common fish species collected from three sites in the northern Gulf of Aqaba.

The concentrations of Zn in liver was higher than muscle of *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella* and *Aristichthys nobilis*. In other study such as *Sciaena umbra* (Turkmen *et al.*, 2008), *Sparus auratus, Trigla cuculus, Sardina pilchardus*, *Mugil cephalus*, *Atherina hepsetus*, *Scomberesox saurus* (Canli and Alti, 2003) concentrations of Zn in liver were higher than muscle. The observed variability of heavy metal levels such as Zn and Pb in different species depends on feeding habits ecological needs, metabolism age, size and length of the fish (Linde *et al.*, 1998) and their habitats (Canli and Atli, 2003; Tuzen and Soylak, 2007).

Comparison of International Standards

The mean estimated concentrations for Zn in the present study were higher than international standards for these metals as declare by the Ministry of Agriculture, Fisheries and Food (UK), Food and Agriculture Organization (FAO) and National Health & Medical Research Council (Australia). Concentrations of Pb in this study were lower than international standards World Health Organization (WHO), Ministry of Agriculture, Fisheries and Food (UK) and National Health & Medical Research Council (Australia), but the Pb concentrations were higher than Food and Agriculture Organization (FAO) and U.S. Food and Drug Administration (FDA).

Conclusions

The concentrations of Zn in liver was higher than muscle of *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella* and *Aristichthys nobilis* ($P>0.05$). Distribution patterns of metal concentrations in liver and muscle of *Hypophthalmichthys molitrix*, *Ctenopharyngodon* *idella* and *Aristichthys nobilis* from Iran follows the sequence: Zn>Pb. The mean estimated concentrations for Zn in the present study were higher than International Standards for these metals as declare by the Ministry of Agriculture, Fisheries and Food (UK) and National Health & Medical Research Council (Australia), but concentrations of Pb in this study were lowest than International Standards World Health Organization (WHO), Ministry of Agriculture, Fisheries and Food (UK) and National Health & Medical Research Council (Australia).

References

Abu Hilal AH, Ismail NS. 2008. Heavy Metals in Eleven Common Species of Fish from the Gulf of Aqaba, Red Sea. Jordan Journal of Biological Sciences **1,** 13-18.

Agah H, Leermakers M, Elskens M, Fatemi SMR, Baeyens W. 2009. Accumulation of trace metals in the muscle and liver tissues of five species from the Persian Gulf. Environment Monitoring and Assessment **157,** 499-514. http://dx.doi.org/10.1007/s10661-008-0551-8.

Ashraf W, Seddigi Z, Abulkibash A, Khalid M. 2006. Levels of selected metals in canned fish consumed in Kingdom of Saudi Arabia. Environmental Monitoring and Assessment **117(1),** 271-279. http://dx.doi.org/10.1007/s10661-006-0989-5.

Aucoin J, Blanchard R, Billiot C, Partridge C, Schultz D, Mandhare K, Beck MJ, Beck JN. 1999. Trace metals in fish and sediments from Lake Boeuf, Southeastern Louisiana. Microchem Journal **62,** 299-307. http://dx.doi.org/10.1006/mchj.1999.1735.

Canli M, Atli G. 2003. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environment Pollution **121(1),** 129-36. http://dx.doi.org/10.1016/S0269-7491(02)00194-X

Celik U, Oehlenschlager J. 2004. Determination of zinc and copper in fish samples collected from Northeast Atlantic By DPSAV. Food Chemistry **87,** 343-347. http://dx.doi.org/10.1016/j.foodchem.2003.11.018.

Celik U, Oehlenschlager J. 2007. High contents of cadmium, lead, zinc and copper in popular fishery products sold in Turkish supermarkets. Food Control **18,** 258-261. http://dx.doi.org/10.1016/j.foodcont. 2005.10.004.

Chen YC, Chen MH. 2001. Heavy metal concentrations in nine species of fishes caught in coastal waters off Ann-Ping, S.W. Taiwan. Journal of Food and Drug Analysis **9,** 107-114.

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

Food Agriculture Organization (FAO). 1983. Compilation of legal limits for hazardous substances in fish and fishery products," *FAO Fishery Circular*, no. 464, 5–10 p.

Fernandes C, Fontaínhas-Fernandes A, Cabral D, Salgado MA. 2008. Heavy metals in water, sediment and tissues of *Liza saliens* from Esmoriz–Paramos lagoon, Portugal. Environment Monitoring and Assessment **136,** 267–275. http://dx.doi.org/10.1007/s10661-007-9682-6.

Heath AG. 1987. Water Pollution and Fish Physiology. CRC Press, Florida, USA, 245 p.

Ikem A, Egiebor NO. 2005. Assessment of trace elements in canned fishes (mackerel, tuna, salmon, sardines and herrings) marketed in Georgia and Alabama (United States of America). Journal of Food Composition Analytic **18,** 771-787. http://dx.doi.org/10.1016/j.jfca.2004.11.002.

Kucuksezgin F, Altayl O, Uluturhan E, Kontas A. 2001. Trace metal and organochlorine residue levels in red mullet (*Mullus barbatus*) from the

Eastern Aegean, Turkey. Water Research **35(9)**, 2327-2332.
DOI: org/10.1016/S0043-1354(00)00504-2.

Lewis MA, Scott GI, Bearden DW, Quarles RL, James M. 2002. Fish tissue quality in near- coastal areas of the Gulf of Mexico receiving point source discharges. Sciences of The Total Environment **284,** 249-261.
http://dx.doi.org/10.1016/S0048-9697(01)00891-9.

Linde AR, Sanchez-Galan S, Izquierdo JI, Arribas P, Maranon E, Garcya-Vazquez E. 1998. Brown Trout as biomonitor of heavy metal pollution: effect of age on the reliability of the assessment. Ecotoxicological and Environmental Safety **40,** 120-125.
http://dx.doi.org/org/10.1006/eesa.1998.1652.

Macfarlane GB, Burchettt MD. 2000. Cellular distribution of Cu, Pb and Zn in the Grey Mangrove *Avicennia marina* (Forsk). Aquatic Botany **68,** 45-49.
http://dx.doi.org/10.1016/S0304-3770(00)00105-4.

Maaboodi H, Jamili S, Maddani H. 2011. Accumulation of heavy metals (Lead and Zinc) in the Liver of some edible fishes in Zayandeh-rood. Research Journal of Environmental Sciences **5 (3)**, 295-301.
http://dx.doi.org/10.3923/rjes.2011.295.301.

MAFF. 1995. Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of wastes at sea, 1993. Aquatic Environment Monitoring Report No. 44. Direcorate of Fisheries Research, Lowestoft.

Mendil D, Uluozlu OD, Hasdemir E, Tuzen M, Sari H, Suicmez M. 2005. Determination of trace metal levels in seven fish species in lakes in Tokat, Turkey. Food Chemistry **90,** 175-179.
http://dx.doi.org/10.1016/j.foodchem.2004.03.039.

Merian E. 1991. Metals and their Compounds in Environment and Life. Occurrence, Analysis and Biological Relevance. New York, Verlag Chemie Weinheim.

Mitra A, Mondal K, Banerjee K. 2010. Concentration of Heavy Metals in Fish Juveniles of Gangetic Delta of West Bengal, India. Journal of Fisheries and Hydrobiology **5(1)**, 21-26.

Munoz-Olivas R, Camara C. 2001. Speciation related to human health. In: Ebdon, L., Pitts, L., Cornelis, R., Crews, H., Donard, O.F.X., Quevauviller, P. (Eds.), Trace Element Speciation for Environment, Food and Health. The Royal Society of Chemistry 331–353.
http://dx.doi.org/10.1039/9781847552204-00331.

Oymak SA, Karadede-Akin H, Dogan N. 2009. Heavy metal in tissues of *Tor grypus* from Ataturk Dam Lake, Euphrates River-Turkey. Biologia **64(1),** 151-155.

Prudente M, Kim EY, Tanabe S, Tatsukawa R. 1997. Metal levels in some commercial fish species from Manila Bay, the Philippines. Marine Pollution Bulltein **34(8),** 671-674.
http://dx.doi.org/org/10.1016/S0025326X(97)00035-0.

Puwastien P, Judprasong K, Kettwan E, Vasanachitt K, Nakngamanong Y, Bhattacharjee L. 1999. Proximate composition of raw and cooked Thai freshwater and marine fish. Journal of Food Composition and Analysis **12,** 9-16.
http://dx.doi.org/ org/10.1006/jfca.1998.0800.

Rashed MN. 2001. Monitoring of environmental heavy metals in fish from Nasser lake. Environmental International **27,** 27-33.

Rodriguez VM, Jimenez-Capdeville ME, Giordano M. 2003. The effects of arsenic exposure on the nervous system. Toxicology Letters **145(1),** 1-18.
http://dx.doi.org/10.1016/S0378-4274(03)00262-5.

Romeo M, Siau Y, Sidoumou Z, Gnassia-Barelli M. 1999. Heavy metal distribution in different fish species from the Mauritania coast. Science of The Total Environment **232,** 169-175. http://dx.doi.org/10.1016/S0048-9697(99)00099-6.

Sadeghi-Rad M. 1997. Studying and determination of heavy metals (Mercury, Cadmium, Lead, Zinc, Cobalt) in some species of Anzali swamp edible fish (Carp, Duck fish, Carassius, Phytophag). Science Magazine Iran Fish **4,** 1-16.

TFC. 2002. Turkish Food Codes, Official Gazette, 23 September 2002. No: 24885.

Turkmen M, Turkmen A, Tepe Y, Ates A, Gokku K. 2008. Determination of metal contaminations in sea foods from Marmara, Aegean and Mediterranean seas: Twelve fish species. Food Chemistry **108**, 794-800. http://dx.doi.org/10.1016/j.foodchem.2007.11.025.

Turkmen A, Turkmen M, Tepe Y, Cekic M. 2010. Metals in tissues of fish from Yelkoma Lagoon, northeastern Mediterranean. Environmental Monitoring and Assessment **168,** 223-230. http://dx.doi.org/10.1007/s10661-009-1106-3.

Tuzen M, Soylak M. 2007. Determination of trace metals in canned fish marketed in Turkey. Food Chemistry **101(4),** 1378-1382. http://dx.doi.org/org/10.1016/j.foodchem.2006.03.044.

Tuzen M. 2009. Toxic and essential trace elemental contents in fish species from the Black Sea, Turkey. Journal of Food and chemical Toxicology **47(9),** 2302-2307. http://dx.doi.org/org/10.1016/j.fct.2009.04.029.

UNEP. 1991. Sampling of selected marine organisms and sample preparation for the analysis of chlorinated hydrocarbons Reference Methods for Marine Pollution Studies No. 12. Rev. 2. UNEP. Nairobi. 17.

WHO. 1996. Health criteria other supporting information. In: Guidelines for Drinking Water Quality, vol. 2, 2nd ed. 31-388 p, Geneva.

Yilmaz F, Ozdemir N, Demirak A, Tuna AL. 2007. Heavy metal levels in two fish species *Leuciscus cephalus* and *Lepomis gibbosus*. Food Chemistry **100,** 830-835. http://dx.doi.org/org/10.1016/j.foodchem.2005.09.020