INT. J. BIOL. BIOTECH., 15 (2): 277-284, 2018.

ASSESSMENT OF HEAVY METALS IN SOFT TISSUES OF FEMALE RED TILAPIA REARED IN CONTAMINATED SEA WATER

Rahat Rukhsana[1,*], Samina Bano[2], Madiha Shakir[2] and Muhammad Hanif Soomro[1]

[1]*Seed Production Unit, Hawks Bay. Directorate of Fisheries Sindh, Research and Development, Livestock and Fisheries Department, Government of Sindh Karachi. Pakistan.*
E-mail: rahatbazmi@hotmail.com
[2]*Department of Biochemistry, University of Karachi, Karachi. Pakistan*
E-mail: samina_ku@hotmail.com
Corresponding Author*: rahatbazmi@hotmail.com;Cell#: 03333012243

ABSTRACT

Fish is consumed as food by many species, including humans. Water pollution and fish contamination remain significant problems. In the present work the concentration of heavy metals (Lead, Copper, Chromium and Iron) in contaminated sea water of Red tilapia (hybrid) rearing tank and their bioaccumulation in different tissues of Female Red tilapia were investigated and compared with the values obtained from the tissues of fish reared in a tank contained hatchery managed filtered sea water. The results showed a direct relationship between the heavy metal levels in contaminated sea water and their accumulation in soft tissues of red tilapia. Concentration of lead and chromium in muscles were found higher than the maximum permissible limit. This situation may pose health risk to fish population and an ultimate threat to humans.

Key words: Contamination, Heavy metals, Bio accumulation, Red tilapia, fish rearing, soft tissues.

INTRODUCTION

Consumption of fish is increasing day by day due to its nutritional and therapeutic importance. It is not only a good source of protein but also rich in essential amino acids, minerals, vitamins and above all omega-3-fatty acids. (Medeiros *et al.*, 2012).Water quality is considered to be the key factor in fish production, health and reproduction. Pollution of the aquatic environment due to the anthropogenic activities poses serious threats to fish population and other aquatic organisms. The major sources of pollutants in sea water are industrial effluents, contaminated petroleum and raw sewage disposal (Santos *et al.*, 2005). Their potential toxic effect is revealed not only by decreasing the aquatic population but by exposing human beings to such toxic substances directly through food chain (Andersen *et al.*, 1996). Metals from discharges can contaminate water and can be accumulated in tissues of living organisms. However fish can normally accumulate heavy metals from contaminated water and sediments and subsequently become the part of food chain (Yilmaz *et al.*, 2007; Zhao *et al.*, 2012).

Olojo *et al.* (2005) reported that long term exposure to heavy metals raised morbidity of juvenile fish and decreased breading of adults. It can pose a health risk to fish consumers, which may include decreased fertility, dysfunction of variety of organs leading to death (Al-Busaidi *et al.*, 2011; Rahman *et al.*, 2012).

In the last few decades, the concentrations of heavy metals were studied mostly in the edible part (muscles) (Elnabris *et al.*, 2013). However other studies reported the distribution of metals in different organs like liver, kidney, heart, gonads, bone, digestive tract and brain.

In previous studies fish samples were collected from different habitat/stations along with water samples and were investigated for the accumulation of heavy metals in their organs and muscles. A correlation was also developed between heavy metal content in their organs and water contamination found at their site of collection. Although fish are mostly migratory and seldom settle in one place, metals accumulation in fish organs provides evidences of exposure to contaminated aquatic environment (Qadir and Malik, 2011) and could be used to assess the health condition of the area from which they are collected. In recent years various fish farms have been established in order to meet the growing demands. Cultured fish may accumulate significant amount of heavy metals in different tissues from its feeding diets and surrounding water (McCarthy and Shugart, 1990).In the present study fingerlings of Red tilapia (A hybrid fish of *O.mossambicus* and *O.niloticus*) were reared (stocked) in a contaminated aquatic system for six months. After six months these grown up fishes were investigated for the accumulation of heavy metals like Lead, Copper, Chromium and Iron in their tissues. Also metals content in muscles were compared against permissible limit provided by WHO/FAO to assess the quality of fish for human consumption.

MATERIALS AND METHODS

A six months study program was conducted at Seed Production Unit Hawks bay, Department of Fisheries Sindh, in which 22 fingerlings of red tilapia (hybrid of *O. mossambicus and O. niloticus*) of average weight 20 grams were reared in a tank of capacity two tons labeled as T(Test) for six months. Source of water was a mixture of sea water contaminated with heavy metals and municipal water. In another tank labeled as C (Control) 22 fingerlings of similar size were reared where source of water was hatchery managed filtered sea water mixed with municipal water. Water was replenished every week for six months. Water quality parameters like Temperature, Dissolved oxygen, Ammonia nitrogen, Free Carbon dioxide, pH, Salinity, Nitrate/Nitrite nitrogen, Hardness, Alkalinity, etc. were monitored and maintained at safe/optimum level throughout the study course in both the tanks (Table 1).

Sampling: Water samples from red tilapia rearing tanks were taken at depth 15 cm below the water surface in acid washed, polyethylene bottles on weekly basis for heavy metal analysis.

After six months of rearing period, 15 female red tilapia of quite a similar size and weight from each tank were selected, washed with sea water then with ultrapure deionized water, immediately preserved in an ice box and transferred to the laboratory where they were kept frozen at -20°C till further analysis.

Sample preparation: Water samples were filtered through nitrocellulose membrane filter of pore size 0.45 µm. The filtrate was boiled gently with 1M nitric acid for heavy metal analysis. Frozen fish were partially thawed, and each fish was dissected using stainless steel instruments. Liver, gills, ovary and muscles were taken out from the sampled fish. Tissue samples were allowed to dry in an electric oven at 105°C till constant weight then cooled and exposed to ambient temperature. In this study wet digestion technique using a digestion block was considered (Holak, 1980). 1.0 gram of each dried solid tissue samples were digested in 10 ml mixture of ultrapure nitric acid and perchloric acid (4:1) for two hours at 120°C. The clear solution so obtained was diluted to 100 ml with deionized water and filtered through nitrocellulose membrane filter of pore size 0.45µm. Similarly a blank was also prepared. All the reagents and reference standards of 1000 mg /L were supplied by Merck and deionized water was used throughout the work. Composite samples from each organ/tissues were used for subsequent analysis.

Analytical methods: Water samples were analyzed immediately for lead, copper, chromium and iron by SPECTROQUANT PHOTOMETER, NOVA 400. The measurement technique used was based upon the complex formation with reagents such as pyridyl azo resorcinol, cuprizone, diphenylecarbazide and phenanthroline respectively, following the methods developed by Merck (KGaA, Damstadt, Germany) and analogous to APHA, 1992 .The results obtained were expressed in mg/L.

All the tissue samples, blank and standards were analyzed for the detection of heavy metals viz., lead, copper, chromium and iron following the standard methods of APHA (1992) by using an automated PC controlled Atomic Absorption Spectrometer (Hitachi, A-1800, Japan). The wavelengths of cathode lamp selected were 283.3nm, 324.8nm, 357.9nm and 248.3nm for lead, copper, chromium and iron respectively. An air: acetylene flame composition was applied throughout the measurement. Each measurement was repeated. The results obtained were expressed in µg / g dry weight.

RESULT

Table 2 presents the heavy metal concentrations (mg/L) in water of rearing tanks C and T. The measured mean values of Pb, Cu, Cr and Fe in filtered sea water of control tank C were recorded as 0.16 ± 0.03 mg/L, 0.15 ± 0.03 mg/L, 0.09 ± 0.02 mg/L and 0.88 ± 0.14 mg/L respectively, while in contaminated water of test tank T it is found as 1.18 ± 0.34 mg/L, 1.85 ± 0.44 mg/L, 0.27 ± 0.05 mg/L and 2.15 ± 0.53 mg/L, respectively.

Data on the accumulation of lead, copper, chromium and iron in fish organs viz., Gills, liver, muscles and ovary is presented in Table 3.

Significant amount of heavy metals were found in organ tissues of red tilapia reared in tank T as compared to control tank C.

The highest concentration of different metals in different soft tissue of female red tilapia reared in tank T were found as lead (18.05 ± 0.43 µg/g) in gills, Iron (286.71 ± 6.5µg/g) in liver, copper (35.64 ± 0.28 µg/g) in liver and chromium (4.84 ± 0.02 µg/g) in liver. Heavy metal accumulation ordered as Fe>Pb>Cu>Cr except in case of liver where copper is significantly higher than lead. In general all metals showed considerable variation among tissues but iron was found significant. Results showed that all soft tissues of fish contained the lowest concentration of chromium and highest concentration of iron. Liver and ovary showed remarkable concentrations of chromium. Copper concentration in ovary was found 4.78 ± 0.07 µg/g which is slightly greater than in gills. Similarly chromium concentration in ovary is measured as 2.94 ± 0.07 µg/g which is also greater than that found in gills. Similar pattern of heavy metals accumulation in muscles and organ tissues of red tilapia was observed in control tank C except in case of lead which is found higher in muscles (0.26 ± 0.02 µg/g) than in ovary (0.15 ± 0.04 µg/g) unlike in tank T where more amount of lead accumulated in ovary (11.86 ± 0.27 µg/g) than in muscles (8.03 ± 0.05 µg/g).

Table 4 shows maximum permissible limit in µ/g dry weight of heavy metals in edible part (muscles) of fish according to international standard and amount of heavy metals accumulated in edible part of red tilapia. The concentration of lead and chromium was found higher than the permissible limit provided by FAO/WHO 1989 in tank T.

Table 1. Optimum range of water quality parameters.

Sr.No.	**Parameters**	**Values Obtained**
01.	Dissolved Oxygen	5.5-7.5 mg/L
02.	Temperature	25-31 °C
03.	pH	7.5-8.2
04.	Unionized Ammonia Nitrogen	<0.1 mg/L
05.	Salinity	5-18 ppt.
06.	Carbon dioxide	0-7 mg/L
07.	Alkalinity	2.5-2.8 mm/L
08.	Total Hardness	160-200 mg/L
09.	Turbidity	20-60 FAU
10.	Nitrate Nitrogen	5-18 mg/L
11.	Nitrite nitrogen	<0.3 mg/L

Table 1 shows the water quality parameters measured weekly to maintain optimum/safe level in both the tanks C and T for six months.

Table 2. Concentration of heavy metals in Red tilapia rearing tanks C and T.

Source of water	Lead (mg/L) Mean± SEM	Copper (mg/L) Mean± SEM	Chromium(mg/L) Mean± SEM	Iron (mg/L) Mean± SEM
Filtered sea water (C)	0.16± 0.03	0.15± 0.03	0.09± 0.02	0.88± 0.14
Contaminated sea water (T)	1.18± 0.34	1.85± 0.44	0.27± 0.05	2.15± 0.53

*Table 2 shows mean ± SEM concentration of heavy metals (mg/L) in control tank C and test tank T, measured on weekly basis, for a period of six months.

DISCUSSION

We can infer that the high level of heavy metals in rearing tank T having contaminated sea water is due to the discharge of agriculture and industrial waste through the coastal water. The high level of lead in water may be the result of leaded petrol spill from fishing boats and discharge of oil and bilge water from ships and industrial plants (McEldowney *et al.*, 1993; Mason, 2002). The present results corroborate with the previous studies that reported high metal levels in the sea water of Suez Bay (El-Moselhy, 2000) and in Red Sea (Hamed and El-Moselhy, 2000).

Table 3. Concentration of Heavy Metals (µg/g DW) in different tissues of Red Tilapia.

Organ	Control tank C				Test Tank T				2-way ANOVA df 1,112		
	Pb (µg/g)	Cu (µg/g)	Cr (µg/g)	Fe (µg/g)	Pb (µg/g)	Cu (µg/g)	Cr (µg/g)	Fe (µg/g)	Condition	Metal	Condition × Metal
Muscle	0.26± 0.02	0.31± 0.007	0.11± 0.005	25.97± 0.06	8.03± 0.05*	3.62± 0.04*	1.42± 0.09*	50.09± 0.53*	F=1013 P<0.01	F=3773 P<0.01	F=14799 P<0.01
Liver	2.08± 0.033	9.6± 0.15	2.4± 0.03	71.68 ± 0.34	12.58 ± 0.34*	35.64 ± 0.28*	4.84± 0.02 Ns	286.71± 6.5*	F=385 P<0.01	F=1758 P<0.01	F=3738 P<0.01
Gills	4.51±0.06	1.68± 0.007	0.15± 0.005	44.52 ± 0.29	18.05± 0.43*	4.61± 0.074*	2.1± 0.02*	249.8± 0.48*	F=12127 P<0.01	F=98888 P<0.01	F=226339 P<0.01
Ovary	0.15±0.04	1.98± 0.02	1.5± 0.005	29.23 ± 0.14	11.86 ± 0.27*	4.78± 0.07*	2.94± 0.07*	89.8± 0.51*	F=2643 P<0.01	F=15518 P<0.01	F=38915 P<0.01

*C=Control Tank contains filtered water. T=Test Tank contains contaminated/polluted water.

Experimental details are described in materials and methods section. Table shows accumulation of heavy metals (µg/g DW) in different soft tissues of female Red Tilapia (hybrid fish). Values are expressed as mean ± SEM for n=15. The results obtained for Control tank C were compared with Test tank T. The data was analyzed using 2-way ANOVA following Newman Keul's q test. Effects of condition, Metal and condition × metal interactions of condition were significant in all tissues assessed. The significance of difference is indicated by *P<0.01 whereas NS=non-significant.

Table 4. Maximum Permissible Limit of heavy metals in Fish Muscles.
.

Organization/ country/rearing tank	Heavy Metal Concentration in ug/g				Reference
	Pb	Cu	Cr	Fe	
European Community	0.2				EC , 2005
England	2.0	20			MAFF, 2000
FAO 1983	0.5	30			FAO, 1983
FAO/WHO 1989	0.5	30	0.15	100	WHO/FAO, 1989
WHO 1989	2.0	30		100	WHO, 1989
TANK C	0.26	0.31	0.11	25.97	
TANK T	8.03	3.62	1.42	50.09	

*Table 4 shows maximum permissible limit of heavy metals in µg/g dry weight in edible part (muscles) of fish according to international standards and concentration of heavy metals accumulated in muscles of studied fish Red Tilapia.
Abbreviations: EC= European Committee; MAFF= Ministry of Agriculture fisheries and food; FAO= Food and agriculture organization; WHO = world health organization

The present study reveals that the metal accumulation in different tissues of fish viz; Gills, liver, muscles and ovaries is closely related to the metal concentration in water. Each tissue has different capacity of accumulation. Among the four fish organs liver exhibited the highest tendency to accumulate heavy metals while muscles showed the lowest tendency to accumulate such metals. The concentration of toxic metals was highest in liver tissues of *O.niloticu*s followed by gills then muscles. (Jasim *et al*, 2016). Amundsen *et al.* (1997) reported that in white fish the heavy metal accumulation was usually lowest in muscles and highest in liver or gills. Previously it has been shown that the lowest concentration of metals accumulated in fish muscles. Bioaccumulation of the essential metals copper, zinc and iron was found mainly in the liver, while gills showed the highest accumulation of lead and manganese (El-Moselhy *et al.*, 2014). High level of copper in liver was observed in many field studies (Zhao, 2012 and Eisler, 2010). Essential metals copper, zinc and iron mainly accumulated in the liver which may be attributed to its role in normal metabolic processes (Zhao, 2012). Jobling (1995) reported that the high accumulation of heavy metals in gills and hepatic tissues is related to the natural binding proteins such as metallothionine which are synthesized in liver and gills tissues and detoxify them. Similarly accumulation of iron in liver is likely linked to its normal physiological role in blood cells and hemoglobin synthesis (Gorur *et al.*, 2012). Present study showed the highest concentrations of lead in gills. Similar results for high concentrations of lead in gills were recorded by Kargin (1998), Abu-Hilal and Ismail (2008) and Qadir and Malik (2011). Exchange of metal ions from water occurs mainly through the gills (Qadir and Malik, 2011). The large surface areas of the gills help the toxic metals to diffuse rapidly (Dhaneesh *et al.*, 2012). However Saleh (1982) reported that the high level of pollutants accumulation in fish liver may be attributed to the degree of pollution in the aquatic environment by heavy metals. This report was supported by the observations of other researchers with a variety of fish species (Guerrin *et al.*, 1990; Saeed and Sakr, 2008). The order of accumulation of heavy metals in muscles of *Thunnus tonggol* collected from Karachi Fish Harbour were Fe>Cu>Cr>Pb (Ahmed *et al.*,2015), whereas in present study Pb accumulation is greater than Cu and Cr in test tank T. The edible part of farmed fish species *O.niloticus* and *S.aurata* showed a considerable level of heavy metals accumulation (Elnabris *et al.*, 2013). Asgedom *et al.* (2012) reported the accumulation of heavy metals in the bone and muscle tissues of Nile Tilapia and Common Carp. Gills of *Oreochromis mosssambicus* showed greater concentration of Pb, liver was the target organ for Cu and Cr whereas lowest bioaccumulation of metals was observed in gonads.(Raheela *et al.*, 2014) Ovaries exhibited different patterns in metal accumulation in the present study. However, the fish samples collected from within tank C had non-significantly higher levels of metals in their bodies than those obtained from tank T. Among the four fish organs, fish liver showed the highest tendency of metal accumulation. The present findings revealed a higher concentration of heavy metals in fish organs in tank T as compared to those of tank C. This may be the result of their high concentrations in water.

Presently many researchers from different countries are studying the accumulation of heavy metals content in different fish organs and its effect on human health. Metals such as copper, zinc and iron are essentially required for the normal physiological functions but their higher level may exhibit toxicological effect on human health (Tuzen, 2003). Bioaccumulation of non-essential metals may lead to muscles dystrophy, infertility as well as have deleterious effect on the liver, kidney and respiratory organs (Fernandes, 2008).

It is clear from the Table 4 that the heavy metals accumulation in the edible part of red tilapia reared in tank C was found below the permissible limit, therefore it is safe for consumers. However the measured values of essential micronutrients copper (3.62 ± 0.04) and Iron (50.09 ± 0.53) in edible part of fish in tank T were found within the permissible limit while the concentration of chromium (1.42 ± 0.09) was higher than the permissible limit according to FAO/WHO Standards. Whereas the measured value of lead (8.03 ± 0.05) which is potentially a toxic element was found significantly higher than the MPL and may pose health risks to consumers. Normally fish muscles do not accumulate chromium. However young fish may have higher tendency to absorb chromium which declines with age causing reduction in chromium level in tissues. (Dara, 1995). High concentration of chromium in water was related to the waste water disposal from textile, photography and paint industries as well as discharge from agriculture field (Rahman *et al.*, 2012). Chromium has toxic effects on a variety of organs with hemorrhagic effect, it may cause ulceration of the tissues in organisms when exposed to high level of chromium. The major sources of lead are plumbing, ammunition, glass and paint industry. Lead is a non-essential element that causes neurotoxicity, nephrotoxicity and other effects on fish and other animals (Garcia- Leston *et al.*, 2010).

CONCLUSION

In this regard a constant and regular monitoring of the aquatic system is required during fish farming and management strategies should be framed and followed to control the waste water disposal in the area used for aquaculture activities. There is a great relationship between the metal content in aquatic system and their accumulation in soft tissues of cultured fish.

ACKNOWLEDGMENT

Authors wish to thank Mr. Ghulam Muhammad Mehr, Director General Fisheries and Mr. Athar Mian Ishaqui, Director Fisheries, Research and Development, for providing facilities.

REFERENCES

Abu-Hilal, A. H. and N. S. Ismail (2008). Heavy metals in eleven common species of fish from the Gulf of Aqaba, Red Sea, Jordan. *Journal of Biological Sciences*, 1: 13-18. ISSN 1995-6673.

Ahmed, Q.,L. Bat.,F. Yousuf. (2015). Accumulation of heavy metals in tissues of long tail tuna from Karachi Fish Harbour, Pakistan. *Aquatic Science and Technology*, 3(1), 103-115.

Al-Busaidi, M., P.Yesudhason., S. Al-Mughairi., W. A. K Al-Rahbi., K.S Al-Harthy and N.A. AlMazrooei (2011). Toxic metals in commercial marine fish in Oman with reference to national and international standards. *Chemosphere*, 85(1): 67

American Public Health Association (APHA) (1992). *Standard methods for the examination of water and waste water*. 18th ed., Academic press. Washington DC.

Amundsen, P. A., F. J. Staldvik., A. A. Lukin., N. A. Kashulin,.,O.A.Popova and Y. S. Reshetnikov (1997). Heavy metal contamination in freshwater fish from the border region between Norway and Russia. *Science of the Total Environment*, 201(3): 211-224.

Andersen, V., A .Maage and P. J. Johannessen (1996). Heavy metals in blue mussels (Mytilusedulis) in the Bergen Harbor area, western Norway. *Bulletin of Environmental Contamination and Toxicology*, 57(4): 589-596.

Asgedom, A. G., M. B. Desta and Y. W. Gebremedh (2012). Bioaccumulation of heavy metals in fishes of Hashenge Lake, Tigray, Northern Highlands of Ethiopia. *American Journal of Chemistry*, 2(6): 326-334.

Dhaneesh, K. V., M. Gopi., R. Ganeshamurthy., T. T. A. Kumar and T Balasubramanian (2012). Bio-accumulation of metals on reef associated organisms of Lakshadweep Archipelago. *Food chemistry*, 131(3): 985-991.

Dara, S.S. (1995) *Environmental Chemistry and pollution control.* New Delhi, India. S. Chandand Company LTD. 191-1912 ISBN-13: 978-8121908832.

EC. (EUROPEAN COMMUNITY). (2005). Commission regulation No 78/2005 (pp. L16/43-L16/45). *Official journal of European Union*

Eisler, R. (2010). Compendium of Trace Metals and Marine Biota: Volume *2: Vertebrates*. St. Louis, MO: Elsevier Science, 522 pages, Amesterdam. ISBN:978-0-444-53439-2

El Moselhy, K. M. (2000). Accumulation of Copper, Zinc, Cadmium, and Lead in Some Fish Species from the Gulf of Suez. *Egyptian Journal of Aquatic Biology and Fisheries*, 4(3): 235-249.

El-Moselhy, K. M., A. I. Othman., H. A. El-Azem and M. E. A. El-Metwally (2014). Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt. *Egyptian Journal of Basic and Applied Sciences*, 1(2): 97-105.

Elnabris, K. J., S. K Muzyed and N. M. El-Ashgar (2013). Heavy metal concentrations in some commercially important fishes and their contribution to heavy metals exposure in Palestinian people of Gaza Strip (Palestine). *Journal of the Association of Arab Universities for Basic and Applied Sciences*, 13(1): 44-51.

FAO (1983). *Compilation of legal limits for hazardous substances in fish and fishery products*. FAO Fishery Circular No. 464. Food and Agriculture Organization, pp. 5-100.

Fernandes, C., A. Fontaínhas-Fernandes., D Cabral and M. A. Salgado (2008). Heavy metals in water, sediment and tissues of Liza Saliens from Esmoriz–Paramos Lagoon, Portugal. *Environmental Monitoring and Assessment*, *136*(1-3), 267-275. EPUB: 2007 April 20 PbMed.

García-Lestón, J., J. Méndez., E..Pásaro and B. Laffon (2010). Genotoxic effects of lead: an updated review. *Environment International*, 36(6): 623-636.

Görür, F. K., R. Keser.,N. Akçay and S. Dizman (2012). Radioactivity and heavy metal concentrations of some commercial fish species consumed in the Black Sea Region of Turkey. *Chemosphere*, 87(4): 356-361.

Guerrin, F., V. Burgat-Sacaze and P. de Saqui-Sames (1990). Levels of heavy metals and organochlorine pesticides of cyprinid fish reared four years in a wastewater treatment pond. *Bulletin of environmental contamination and toxicology*, 44(3): 461-467. PMID:2328354

Hamed, M. A. and K. M. El-Moselhy (2000). Levels of some metals in the coastal water and sediments of the Red Sea, Egypt. *Modell Meas Control C*, 61(1): 43-57.

Holak, W. (1980). Analysis of foods for lead, cadmium, copper, zinc, arsenic, and selenium, using closed system sample digestion: collaborative study. *Journal of the Association of Official Analytical Chemists*, 63(3): 485-495. PMID: 7430035

Jasim. M.A.,M. Motior Rahman., I. Yosoff and M.Sofian-Azirun (2016). Acute Toxicity and Bioaccumulation of Heavy metals in Red Tilapia fish. *Journal of Animal and Plant Sciences*. 26(2): 507-513

Jobling, M. (1995). Environmental Biology of Fishes. Xii, 455 pages. London. ISBN 10: 0412580802 / ISBN 13: 9780412580802 Published by Chapman & Hall, 1995

Kargin, F. (1998). Metal concentrations in tissues of the freshwater fish *Capoeta barroisi* from the Seyhan River (Turkey). *Bulletin of environmental contamination and toxicology*, 60(5): 822-828.

MAFF. (Ministry of Agriculture, Fisheries and Food) (2000). *Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of wastes at sea, 1997*. Aquatic environment monitoring report No 52, Centre for Environment, Fisheries and Aquaculture Science, Lowestoft, UK. 92 pages ISSN 0142-2499 J. Jones, A. Franklin.

Mason, C. F. (2002). *Biology of freshwater pollution*. 4th ed. Essex university, Harlow , England. 387 pages, ISBN-10:0130906395, Publisher; Benjamin Cummings.

McCarthy, J. F. and L. R. Shugart (1990). *Biomarkers of environmental contamination*, Lewis Publishers, Science, 457 pages. University of California.

McEldowney, S., D.J. Hardman. and S .Waite (1993). *Pollution, ecology and biotreatment*. Longman Scientific and Technical, England, 322 pages.

Medeiros, R. J., L. M. G. dos Santos., A. S. Freire., R. E. Santelli., A. M. C. Braga., T. M. Krauss and S. D. C. Jacob (2012). Determination of inorganic trace elements in edible marine fish from Rio de Janeiro State, Brazil. *Food Control*, 23(2): 535-541.

Olojo, E. A. A., K. B. Olurin, and A. D. Oluwemimo (2005). Histopathology of the gill and liver tissues of the African catfish *Clariasgariepinus* exposed to lead. *African Journal of Biotechnology*, 4(1): 117-122

Qadir, A., and R. N. Malik (2011). Heavy metals in eight edible fish species from two polluted tributaries (Aik and Palkhu) of the River Chenab, Pakistan. *Biological Trace Element Research*, 143(3): 1524-1540.

Rahman, M. S., A. H. Molla., N. Saha and A. Rahman (2012). Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Savar, Dhaka, Bangladesh. *Food Chemistry*, 134(4): 1847-1854.

Saeed, S. M and S. F. Sakr (2008). Impact of cage-fish culture in the river nile on physico-chemical characteristics of water, metals accumulation, histological and some biochemical parameters in fish. *Abbassa International Journal of Aquaculture.*, 1A: 179-202.

Saleh, H. H. (1982). Fish liver as indicator of aquatic environmental pollution. *Bull. Inst. Oceanogr. & Fish*, 8(1): 69-79.

Santos, I. R., E. V. Silva-Filho., C. E. Schaefer., M. R..Albuquerque-Filho and L. S. Campos (2005). Heavy metal contamination in coastal sediments and soils near the Brazilian Antarctic Station, King George Island. *Marine Pollution Bulletin*, 50(2): 185-194.

Sharmeen,R.,M. Zaheer Khan.,G. Yasmeen and S. A. Ghalib (2014). Levels of heavy metals (Cadmium, Chromium, Copper and Lead) in water and selected tissues of *Oreochromis mossambicus* from different locations of Malir River Karachi. *Canadian Journal of Pure and Applied Sciences*. (British Columbia), 8(3): 1920-3853

Tuzen, M. (2003). Determination of heavy metals in fish samples of the middle Black Sea (Turkey) by graphite furnace atomic absorption spectrometry. *Food chemistry*, 80(1): 119-123.
WHO (World Health Organization) (1989). *Heavy metals-environmental aspects*. Environment health criteria. No. 85. Geneva, Switzerland. Science and Education.
WHO/FAO. (1989). National Research Council Recommended Dietary 626 Allowances (10thed). National Academy Press. Washington, DC. USA.
Yilmaz, F., N. Özdemir., A. Demirak and A. L. Tuna (2007). Heavy metal levels in two fish species *Leuciscuscephalus* and *Lepomisgibbosus. Food Chemistry*, 100(2): 830-835.
Zhao, S., C. Feng., W. Quan., X.Chen., J. Niu and Z. Shen, (2012). Role of living environments in the accumulation characteristics of heavy metals in fishes and crabs in the Yangtze River Estuary, China. *Marine pollution bulletin*, 64(6): 1163-1171

(*Accepted for publication March 2018*)