Arch. Pol. Fish. (2013) 21: 19-27
DOI 10.2478/aopf-2013-0003

RESEARCH ARTICLE

Heavy metal bioaccumulation in edible fish species from an industrially polluted river and human health risk assessment

Usha Damodharan, M. Vikram Reddy

Received – 28 August 2012/Accepted – 25 November 2012. Published online: 31 March 2013; ©Inland Fisheries Institute in Olsztyn, Poland
Citation: Damodharan U., Vikram Reddy M. 2013 – Heavy metal bioaccumulation in edible fish species from an industrially polluted river and human health risk assessment – Arch. Pol. Fish. 21: 19-27.

Abstract. Trace metals, such as cadmium and lead, and micronutrients, such as copper, zinc, and manganese, were analyzed in water and muscle tissues of four edible fishes – barramundi, *Lates calcarifer* (Bloch), flat head gray mullet, *Mugil cephalus* L., giant catfish, *Netuma thalassina* (Rüppell) and Mozambique tilapia, *Oreochromis mossambicus* (Peters) sampled at upstream and downstream sites in the polluted Uppanar River during the dry (December – June) and wet (July – November) seasons from December 2009 to November 2010. The content of heavy metals in the fish species differed depending on the size, sampling site, or, in some cases, season. The analysis of the transfer factor (TF) and positive correlations ($P < 0.001$) between concentrations of selected metals in fish muscle tissues and water indicated the direct accumulation of metals from the water to the fish. Seasonal variations of metal concentrations in the water and fish muscle tissues indicated elevated concentrations during the dry season. The present study suggests that various metals were present in the fish muscle tissues at different levels, but these were within the maximum residual levels permitted by Indian standards and the WHO/FAO; thus, the fish from these areas are generally safe for human consumption.

Keywords: bioconcentration factor, downstream, dry and wet seasons, fish muscle tissues, trace metals and micronutrients, upstream

U. Damodharan [✉] M. Vikram Reddy
Department of Ecology and Environmental Sciences
Pondicherry Central University
Pondicherry, 605 014, India
e-mail: ushadamodhar@gmail.com

Introduction

Industrial wastes are a potential source of heavy metal pollution in aquatic environments (Lee and Stuebing 1990, Gumgum et al. 1994). Under certain environmental conditions, heavy metals can accumulate to toxic concentrations and cause ecological damage (Guven et al. 1999). Thus, heavy metals acquired through the food chain as a result of pollution are potential chemical hazards that can threaten consumers. Metals, such as copper, zinc, and manganese, are essential metals since they play important roles in biological systems, whereas lead and cadmium are toxic, even in trace amounts. The essential metals can also produce toxic effects at high concentrations. Only a few metals of proven hazardous nature are to be completely excluded in food for human consumption. Heavy metals like Cu, Zn, and Mn are essential for the metabolism of organisms, including fish, while Cd and Pb have no known role in biological systems and are toxic in elevated concentrations. Essential metals are taken up from the water or sediment for normal fish metabolism; however, non-essential metals, similarly to the route of essential ones, are also taken up by fish and accumulate in their tissues (Canli and Atli 2003). Heavy metal contamination is particularly significant in ecotoxicology since these metals are highly persistent and can bioaccumulate and biomagnify in the food chain,

Table 1
Total length (cm) and body weight (g) of the fish from the Uppanar River during the dry and wet seasons

		Length		Weight	
Species	N	mean ± SD	range	mean ± SD	range
L. calcarifer	58	7.929±4.100	4.19-18.5	55.035±28.570	14.43-120
M. cephalus	56	13.693±6.635	5.89-22.7	54.642±40.432	11.21-110.02
N. thalassina	63	10.702±3.566	5.6-18.21	36.363±15.936	10.23-64.12
O. mossambicus	60	10.594±2.804	5.8-15.6	33.334±12.281	14.87-53.24

thus becoming toxic to living organisms at higher trophic levels (Storelli et al. 2005). Heavy metal contamination, particularly of non-essential elements, can disadvantageously impact the ecological balance of the recipient aquatic environment and a diverse range of organisms, including fish (Farombi et al. 2007). Various studies have been conducted on heavy metal bioaccumulation in the muscle tissues of fish collected from different freshwater aquatic systems in relation to their concentrations in water in India (Batvari et al. 2008, Ebrahimpour and Mushrifah 2008, Raja et al. 2009, Biswas et al. 2011). However, studies on heavy metal bioaccumulation in fish collected from polluted water bodies in India are very few. Therefore, the aim of the present investigation was to assess the concentrations of heavy metals in the waters of the industrially polluted Uppanar River in relation to metal concentrations in the edible tissues of four fish species (*Oreochromis mossambicus*, *Netuma thalassina*, *Mugil cephalus*, *Lates calcarifer*) from the river, and to estimate the transfer factor (TF) of heavy metals (Cu, Pb, Cd, Zn, Mn) from the water to fish muscle tissues.

Materials and Methods

Study area

The Uppanar River flows by the State Industries Promotion Corporation of Tamil Nadu area of the coastal town of Cuddalore. The river is located on the south-east coast of India along with the Gadilam River to the north, and both drain into the Bay of Bengal. Industrial development on the banks of the Uppanar River has increased three-fold over the past two decades with the establishment of many small- and large-scale industries including fertilizer, pharmaceutical, dye, and chemical production, mineral processing plants, and metal-based industries. Currently, the effluents from these industries find their way into the Uppanar River through small channels and pipelines, and this could account for the severe pollution of these river waters that are contaminated with heavy metals, and consequently, the likely contamination of fish with toxic heavy metals (Ayyamperumal et al. 2006).

Sample Collection

Fresh fish samples were collected every month during the dry and wet seasons from December 2009 to November 2010 on the Uppanar River. Study sites were located upstream at Semmankuppam (uncontaminated site; 11.685985 N, 79.763739 E) and downstream at Kudikadu (contaminated site; 11649378 N, 79.742929 E). These two areas are major fishing sites for the local people. A total of 150 fish specimens belonging to four fish species were collected. The fish samples were delivered to the laboratory on the same day, and the total length (cm) and weight (g) of each of the fish was measured (Table 1). Water samples were collected from the same sites on the river at a depth of 25 cm, stored in clean polyethylene bottles, and delivered to the laboratory for heavy metal analyses.

Sample preparation

The edible parts, or muscle tissues, of the fish were separated and washed with tap water followed by double-distilled water, weighed, and then oven-dried to constant weight at 101°C for 3 h. The dried fish samples were crushed and powdered in an agate mortar, and then stored in polyethylene bottles at 30°C until analysis. The dried muscle tissue samples of 2.5 ± 0.5 g from each fish were digested using a microwave digestion system. The advantages of microwave digestion over the classical method include shorter time, lowered acid consumption, and the retention of volatile compounds in the solution (Krushevska et al. 1993). After digestion, the residues were diluted to 25 ml with 2.5% of HNO_3. Approximately 1 ml of concentrated HNO_3 was added to the water samples to prevent the microbial utilization of heavy metals. Suspended particulate matter in the water samples was separated by filtering through 0.45-mm Whatman GF/C filters. River water samples were acidified to 0.5% (v/v) separately using concentrated nitric acid for sample precipitation. The digested water and fish samples were assessed with atomic absorption spectrometry (AAS) (GBC-AVANTA, Victoria, Australia).

Analytical Procedure

All the reagents used were of analytical grade. Working standards of zinc, copper, cadmium, lead, manganese, and zinc were prepared by diluting concentrated stock solutions (Merck, Germany) of 1000 mg l^{-1} in ultra-pure water (MilliQ, Millipore – USA). Reagent grade chemicals and distilled deionized water were used throughout the analysis. Glassware was soaked with aqua regia for 2 h and rinsed with distilled and deionized water. Standard stock solutions (1000 mg l^{-1}) of Cd, Cu, Pb, Mn, and Zn (Reagecon, Ireland) were prepared into working standards of various concentrations. Reagents' blank determinations were used to correct the instrument readings, and the analyses were replicated three times.

Contamination Control

Accurate analysis of heavy metals is dependent upon the presentation of element contamination. All laboratory materials used were made of pyrex and high-density polyethylene washed with 30% HNO_3 (Ross 1986), and then rinsed three times with double-distilled water, and allowed to dry in an oven at 105°C for 2 h.

Analytical Measurement

AAS was performed with element hollow cathode lamps and an air-acetylene flame. The equipment was calibrated by analyzing five standard solutions and two reagent blank samples. For quality assurance, ten samples of each blank, standard reference material (APHA 1992), and muscle tissues of fish spiked with a known amount of analytical standard Cd, Cu, Mn, Pb, and Zn solutions were analyzed, and the recoveries were calculated. The mean recovery was 101.2-102.3% (blank), 100-101.8% (muscle), and 101.2-104% (water). Recoveries for the certified standard reference material were between 98 and 105%.

Health risk analysis

This study also evaluated the health risk of humans consuming the fish caught in the river. The transfer factor is an approach based on the water-fish transfer factor that provides a straightforward, constructive method for assessing heavy metal accumulation for the purposes of health risk assessment of humans consuming the fish. The water-fish transfer factor (TF) of the biological accumulation coefficient (BAC), which expresses the ratio of contaminant concentration in fish to the concentration in water, was used to characterize quantitatively the transfer of an element from the water to fish (Rodríguez et al. 2002, Vera Tome et al. 2003).

$$TF = Con_{fish}/Con_{water}$$

where Con_{fish} and Con_{water} are the fish muscle tissue and water concentrations, respectively. The parameter is zero if the element accumulates only from the water. A prerequisite of the water-fish transfer factor concept is the presence of a statistically significant relationship between the content of a given element in the water and soil (Bunzl et al. 2000). These values are needed for many assessment models to predict the concentration of an element for a given fish species at an anticipated contamination level in the water (Bunzl et al. 2000). TF describes the amount of an element expected to accumulate in a fish from the water when conditions are equilibrious. This theory assumes a linear relationship between the concentrations of a certain element in the fish with that in the water. This linearity does not hold for essential elements, the content of which is under strict metabolic control, but it does apply to nonessential elements and pollutants.

Statistical Analysis

All data for trace metals are presented as means ± SD. The significance of differences between the metal concentration means in the muscle tissues of the four species of fish from the two sampling sites during the wet and dry seasons were calculated with two-way analysis of variance (ANOVA), and the correlation of trace metals in the water and fish muscle tissues are presented in scatter plot diagrams. Statistical analyses were performed with the statistical software package SPSS 19.0 (SPSS Science, Chicago, IL, USA).

Results

Heavy metal concentration in river water

The results obtained for the river water samples from two sampling sites are presented in Table 2. The copper, lead, cadmium, zinc, and manganese values in the water samples from the upstream site were lower than those from the downstream site during the dry and wet seasons. In general, the heavy metal

Table 2

Heavy metal concentrations in water ($\mu g\ l^{-1}$) and muscle tissues of fish ($\mu g\ g^{-1}$ d.w.) during the dry and wet seasons at the upstream and downstream sites in the Uppanar River. Data are means ± SD

Heavy metals	Site	Water	*L. calcarifer*	*M. cephalus*	*N. thalassina*	*O. mossambicus*
Dry season						
Cu	Upstream	0.013±0.003	0.024±0.013	0.040±0.0115	0.041±0.021	0.052±0.011
Pb	Upstream	0.006±0.004	0.027±0.030	0.055±0.019	0.034±0.011	0.026±0.019
Cd	Upstream	0.007±0.001	0.005±0.001	0.005±0.001	0.004±0.001	0.004±0.001
Zn	Upstream	104.330*±21.240	3.114±1.156	14.091±3.145	12.238±1.240	11.539±0.714
Mn	Upstream	6.320*±1.150	4.564±0.913	8.110±0.787	4.078±1.246	7.262±0.408
Cu	Downstream	123.120*±13.540	0.914±0.465	0.181±0.010	0.099±0.208	0.252±0.003
Pb	Downstream	2.333*±1.130	0.160±0.033	0.189±0.007	0.119±0.041	0.144±0.071
Cd	Downstream	3.233*±1.110	0.008±0.001	0.010±0.001	0.01± 0.003	0.011±0.001
Zn	Downstream	233.320*±39.290	9.198±1.260	16.261±2.087	14.347±0.986	15.687±2.117
Mn	Downstream	39.000*±7.560	11.153±3.758	26.269±3.045	21.225±11.339	23.373±8.102
Wet season						
Cu	Upstream	0.010±0.001	0.012±0.003	0.023±0.007	0.013±0.003	0.022±0.008
Pb	Upstream	0.002±0.001	0.014±0.004	0.015±0.009	0.004±0.001	0.004±0.001
Cd	Upstream	0.002±0.001	0.003±0.001	0.002±0.001	0.004±0.002	0.005±0.002
Zn	Upstream	62.120*±12.360	1.147±0.548	3.108±1.729	6.584±2.398	3.529±1.280
Mn	Upstream	2.500*±0.932	2.085±0.936	10.121±3.296	5.323±1.213	12.257±4.210
Cu	Downstream	46.780*±0.11.230	0.623±0.187	0.127±0.040	0.072±0.008	0.124±0.090
Pb	Downstream	1.213*±0.342	0.044±0.003	0.098±0.004	0.070±0.004	0.091±0.007
Cd	Downstream	2.750*±0.681	0.006±0.002	0.007±0.002	0.005±0.001	0.008±0.003
Zn	Downstream	121.640*±45.290	4.288±1.497	8.317±2.530	9.436±2.309	9.315±3.459
Mn	Downstream	18.350*±4.320	9.221±2.340	8.349±1.750	16.210±5.426	18.130±5.420

*indicates values exceeding permissible limits of IS (Indian Standard) specification for drinking water concentrations in the water in the wet season were lower than during the dry season. The total concentration of Cu, Pb, Cd, Zn, and Mn in the river water at the downstream site and the concentration of Mn and Zn in the water at the upstream site during dry and wet seasons exceeded maximum permissible limits (Table 2).

Heavy metal concentration in fish muscle tissues

Results of heavy metal concentrations in the tissues of fish caught at the Uppanar River are summarized in Table 2. The metal concentrations exhibited high variation among the species. High Mn concentrations were noted in the muscles of all the studied species from the Uppanar River, but mainly in *M. cephalus*

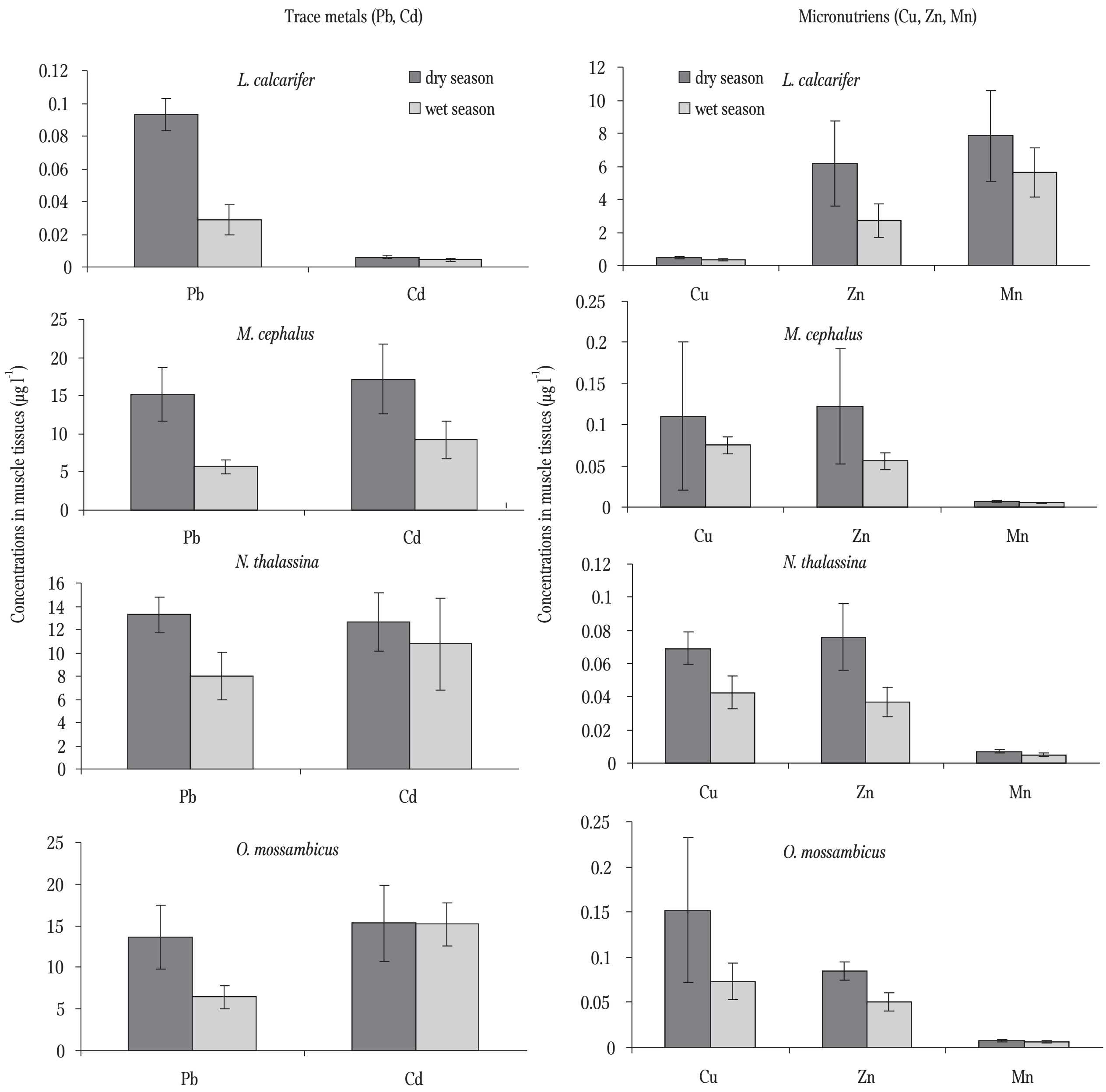

Figure 1. Total concentrations of trace metals and micronutrients in muscle tissues during dry and wet seasons.

and *O. mossambicus*, whereas Cd was lowest. The Cu concentration in muscle tissues of *O. mossambicus* was higher at the upstream site during the dry season, and its concentration was higher in *L. calcarifer* at the downstream during the dry and wet seasons. The Pb concentration in the muscle tissues of *M. cephalus* was higher at the upstream and downstream sites during the dry and wet seasons. The concentration of Zn in *M. cephalus* was several times higher at the upstream and downstream sites during the dry season in comparison to that noted during the wet season.

Hence, the ranking order of heavy metal concentrations in the muscle tissues of fish was Zn>Mn>Cu>Pb>Cd in all the fish species. The ranking order of heavy metal concentrations in the fish species was *M. cephalus* > *N. thalassina*> *O. mossambicus* > *L. calcarifer*. The mean metal concentrations in the muscles of fish were, on average, below the maximum tolerance levels for human consumption set forth by Indian Standards for fish. The total concentration of trace metals (Pb, Cd) and micronutrients (Cu, Mn, Zn) and their seasonal variations during the dry and wet seasons are presented in Fig. 1. Trace metal and micronutrient concentrations in the muscle tissues of the four fish species examined varied significantly during both the seasons. Pb, Zn, and Mn concentrations in *L. calcarifer* were several times higher during the dry season, while Cu and Cd showed little variation. However, the trace metal and micronutrient concentrations in *O. mossambicus*, *N. thalassina*, and *M. cephalus* varied highly significantly seasonally. The correlation between the heavy metals Cu, Cd, Zn, and Mn and total length and body weight of *L. calcarifer*, *N. thalassina*, *M. cephalus*, and *O. mossambicus* were negatively highly significant ($P < 0.001$), except for Pb in *L. calcarifer*, for which there was no correlation (Table 3).

Heavy metal transfer from water to fish

The transfer factor of the heavy metals in the muscle tissues of fish from the river water are presented in Fig. 2. The TF value of the four fish species for five heavy metals, which shows the transfer factor of the different heavy metals from the water to the fish, is one of the key components of human exposure to metals through the food chain. The highest TF values for Pb varied greatly among the fishes, and they were generally higher than those for the other four metals, followed by those for Mn, for which the TF came from water. One of the reasons for these results is that Pb occurs with Mn in nature, while Cu is retained less so by water than other toxic cations. The highest TF in *O. mossambicus* was followed by *N. thalassina* and *M. cephalus*. The highest TF values were found for Pb and Mn because these metals are more mobile in nature. TF values for Pb and Mn from the water to the fish indicate the potent accumulation of Pb and Mn by the fishes. The ranking order trends for BCF for the heavy metals in the four fish species were Pb > Mn > Cu> Zn> Cd.

Table 3
Correlation between heavy metal concentration in muscle tissues (μg g^{-1} d.w.) and total length (cm) or body weight (g) of the fish

Species	Parameter	Length	Weight
L. calcarifer	Cu	-0.847**	-0.851**
	Pb	-0.194ns	-0.185ns
	Cd	-0.744**	-0.731**
	Zn	-0.886**	-0.882**
	Mn	-0.910**	-0.908**
M. cephalus	Cu	-0.804**	-0.792**
	Pb	-0.714**	-0.681**
	Cd	-0.736**	-0.710**
	Zn	-0.773**	-0.778**
	Mn	-0.767**	-0.753**
N. thalassina	Cu	-0.865**	-0.821**
	Pb	-0.895**	-0.882**
	Cd	-0.631*	-0.584*
	Zn	-0.524*	-0.695*
	Mn	-0.644*	-0.618*
O. mossambicus	Cu	-0.947**	-0.952**
	Pb	-0.925**	-0.931**
	Cd	-0.967**	-0.969**
	Zn	-0.957**	-0.962**
	Mn	-0.743**	-0.755**

*P value significant at α=0.05; **P value significant at α=0.01; ns – not significant

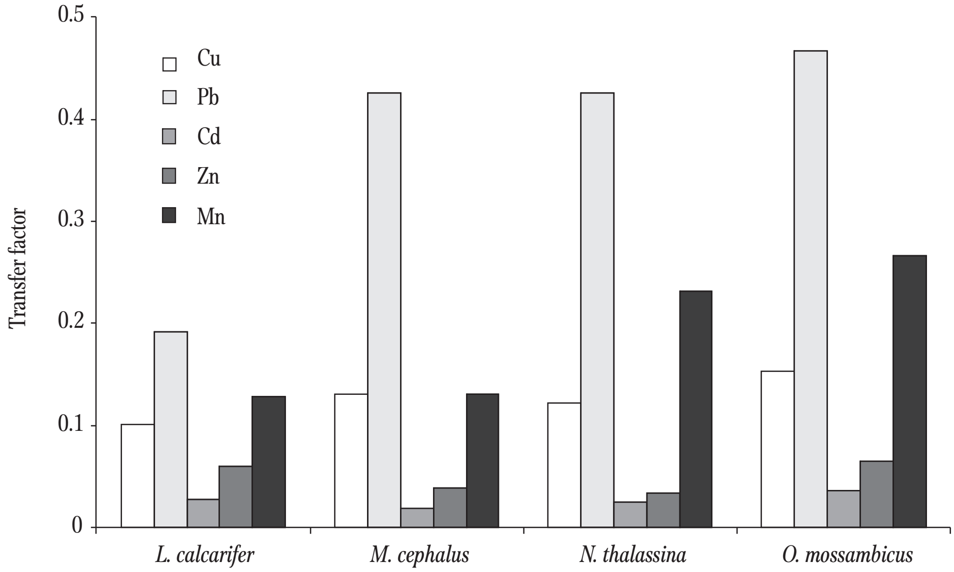

Figure 2. Transfer factor of five metals from water to fish.

Discussion

The concentration of metal in fish tissues is generally related to the age of the fish, and, consequently, on size and length (Scerbo et al. 2005), but the concentrations of metals in the current samples did not correlate strictly with fish length. The results of the present study showed significant negative correlation between fish size (length and weight) and heavy metal concentration in muscle tissues, which is supported by the literature (Lakshmanan 1988, Douben 1989, Roesijadi and Robinson 1994, Canli and Atli 2003). Nevertheless, the present results showed significant decline in almost all heavy metals with increasing fish size, and metal accumulation was higher in younger individuals, which discharged far less compared to that of older individuals. This is probably due to the difference in metabolic activities between younger and older fish. Many previous investigators reported similar results (Ebrahimpour and Mushrifah 2008), which corroborate the present findings. However, fish growth and its relationship with metal concentration in the aquatic environment should be monitored occasionally in the field to foster a better understanding of the effects metals have on fish development.

The accumulation of metals in fish tissues depends on numerous factors, such as environmental concentrations, environmental conditions (pH, water temperature, hardness), exposure time, and species-specific living and feeding habits (Moore and Ramamoorthy 1984, Deram et al. 2006, Lalonde et al. 2011). All the fish species in the present study are of commercial value. Based on the samples collected, the metal concentrations noted in the edible tissues of these species are not heavily contaminated with metals, and concentrations are below the limits for fish proposed by Nauen (1983). The concentrations Mn and Zn in the present study were the highest, while that of Cd was the lowest of all the metals in the tissues of the analyzed fishes; this is consistent with the findings of earlier workers (Petkovšek et al. 2012).

The present results indicated that industrial discharge and agricultural runoffs forms of human activities that are released into the river caused heavy metal pollution in the water seasonally, and that these persistent pollutants accumulated more readily in fish during the dry season (Cogun et al. 2006, Dural et al. 2006). Moreover, because of evaporation resulting from increased temperatures during the dry season, heavy metal concentrations in the water were generally higher compared to those in the wet season.

Lower heavy metal concentrations in the wet season are attributable to water dilution caused by moderate to heavy rainfall during the rainy season (Tekin-Özan 2008).

Based on the present findings, it was concluded that the heavy metals concentration in muscle tissues of fish were related to those of the surface waters of the polluted Uppanar River. Higher concentration of Cu, Zn, Mn, Cd, and Pb in the surface waters at the sampling sites downstream and upstream in the river could be related to industrialization at the SIPCOT area and the anthropogenic activities of the Cuddalore municipality. The heavy metal concentrations found in the four fish species examined correspond with the metal contamination levels of the river water. Moreover, these results can also be used to understand the heavy metal contamination of fish inhabiting polluted water bodies and to evaluate the possible risk associated with their consumption. Heavy metal concentrations noted in the edible parts of the four fish species examined are within WHO permissible limits for human consumption. Thus, there appears to be no immediate threat to the fisheries of the river from heavy metal contamination. However, since the results of the present investigation indicate that the heavy metal contamination of the river affects its aquatic life, including fish, a scientific method for detoxifying the river water is essential to improve the health of its fish, and, in turn, that of the humans who consume them.

Acknowledgment. This study, as part of Usha Damodharan's doctoral research work, was funded by Pondicherry Central University. Thus, the author is grateful to Pondicherry University for providing the necessary laboratory facilities and for providing a university fellowship.

Authors contribution. M.V.R. designed the experiment and supervised the research work. U. D. performed the experiment, collected and analyzed the data, and wrote the paper. M.V.R. revised the paper.

References

APHA 1992 – Standard Methods for the Examination of Water and Waste Water – American Public Health Association, 18th ed., Academic Press. Washington D.C.

Ayyamperumal T., Jonathan M.P., Srinivasalu S., Armstrong Altrinb J.S., Ram-Mohan V. 2006 – Assessment of acid leachable trace metals in sediment cores from River Uppanar, Cuddalore – Environ. Pollut. 143: 34-45.

Batvari B.P.D., Kamala Kannan S., Shanthi K., Krishnamoorthy R., Lee K.J. 2008 – Heavy metals in two fish species (Carangoidel malabaricus and Belonestronglurus) from Pulicat lake, north of Chennai, southeast coast of India-Environ Monit Assess. 145: 167-175.

Biswas S., Prabhu R.K., Hussain K.J., Selvanayagam M., Satpathy K.K. 2011 – Heavy metals concentration in edible fishes from coastal region of Kalpakkam, Southern part of India – Environ. Monit. Assess. 184: 5097-5104.

Bunzl K., Albers B.P., Schimmack W., Belli M., Ciuffo L., Menegon S. 2000 – Examination of a relationship between the concentrations in soils and plants from alpine pastures – J. Environ. Radioactiv. 48: 145-158.

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 – Environ Pollut. 129-136.

Cogun H., Yuzereroglu T., Firat O., Gok G., Kargin F. 2006 – Metal concentrations in fish species from the Northeast Mediterranean Sea – Environ. Monit. Assess. 121:429-436.

Deram A., Denayer F.O., Petit D., Van Haluwyn C. 2006 – Seasonal Variations of Cadmium and Zinc in Arrhena therumelatius, a perennial grass species from highly contaminated soils – Environ. Pollut. 140: 62-70.

Douben P.E. 1989 – Lead and cadmium in stone loach (*Noemacheilus barbatulus* L.) from three rivers in Derbyshire – Ecotox. Environ. Safe. 18: 35-58.

Dural M., Goksu L.Z.M., Ozak A.A., Derící B. 2006 – Bioaccumulation of some heavy metals in different tissues of *Dicentrachus labrax* L, 1758, *Sparus aurata* L, 1758 and *Mugil cephalus* L, 1758 from the Çamlik Lagoon of the eastern cost of Mediterranean (Turkey) – Environ. Monit. Assess. 18: 65-74.

Ebrahimpour M., Mushrifah I. 2008 – Heavy metal concentrations in water and sediments in Tasik Chini, a freshwater lake, Malaysia – Environ Monit Assess. 141: 297-307.

Farombi E.O., Adelowo O.A., Ajimoko Y.R. 2007 – Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African Cat fish (*Clarias gariepinus*) from Nigeria Ogun River – Int. J. Environ. Res. Public Health 4: 158-165.

Gumgum B., Unlu E., Tez Z., Gulsun Z. 1994 – Heavy metal pollution in water, sediment and fish from the Tigris River in Turkey – Chemosphere. 29:111-116.

Guven K., Ozbay C., Unlu E., Satar A. 1999 – Acute lethal toxicity and accumulation of copper in *Gamma ruspulex* (L.) (Amphipoda) – Turk. J. Biol. 23: 51-521.

Krushevska A., Barnes M.R., Chita A. 1993 – Decomposition of biological samples for inductively coupled plasma atomic emission spectrometry using an open focused microwave digestion system – Analyst 118: 1175-1181.

Lakshmanan P.T. 1988 – Levels of cadmium in seafood products – Fish. Technol. 25: 142-146.

Lalonde B.A., Ernst W., Comeau F. 2011 – Trace metal concentrations in sediments and fish in the vicinity of Ash Lagoon discharges from coal-combustion plants in New Brunswick and Nova Scotia, Canada – Arch. Environ. Contam. Toxicol. 61: 472-481.

Lee Y.H., Stuebing R.B. 1990 – Heavy metal contamination in the River Toad, *Bufo juxtasper* (Inger), near a copper mine in East Malaysia – Bull. Environ. Contam. Toxicol. 45: 272-279.

Moore J.W., Ramamoorthy E. 1984 – Heavy metals in the natural waters: Applied Monitoring and Impact assessment – Springer, New York, 268 p.

Nauen C.E. 1983 – Compilation of legal limits for hazardous substances in fish and fishery products – FAO Fisheries Circular, 764, 102 p.

Raja P., Verasingam S., Suresh G., Marichamy G., Venkatachalapathy R. 2009 – Heavy metal concentration in four commercially valuable marine edible fish species from Parangipettai coast, south east coast of India – Int. J. Anim. Vet. Adv. 1: 10-14.

Rodríguez P.B., Tomé F.V., Lozano J.C. 2002 – About the assumption of linearity in soil-to plant transfer factors for uranium and thorium isotopes and ^{226}Ra – Sci. Total. Environ. 284: 167-175.

Roesijadi G., Robinson W.E. 1994 – Metal regulation in aquatic animals: mechanism of uptake, accumulation and release – In: Aquatic Toxicology: Molecular, Biochemical and Cellular Perspectives (Eds) D.C Malins, G.K. Ostrander, Lewis Publishers, London: 387-420.

Ross H.B. 1986 – The importance of reducing sample contamination in routine monitoring of trace metals in atmospheric precipitation – Atmos. Environ. 20: 401-405.

Petkovšek S.A.S., Grudnik Z.M., Pokorny B. 2012 – Heavy metals and arsenic concentrations in ten fish species from the Salek lakes Slovenia: assessment of potential human health risks due to fish consumption – Environ Monit Assess. 184: 2647-2662.

Scerbo R., Ristori T., Stefanini B., De Ranieri S., Barghigiani C. 2005 – Mercury assessment and evaluation of its impact of fish in the Cecina river basin (Tuscany, Italy) – Environ. Pollut. 135: 179-186.

Storelli M.M., Storelli A., D'Addabbo R., Marano C., Bruno R., Marcotrigiano G.O. 2005 – Trace elements in loggerhead turtles (*Caretta caretta*) from the eastern Mediterranean Sea: overview and evaluation – Environ. Pollut. 135: 163-170.

Tekin-Özan S 2008 – Determination of heavy metal levels in water, sediment and tissues of tench (*Tinca tinca* L. 1758) from Beyşehir Lake (Turkey) – Environ. Monit. Assess. 145: 295-302.

Vera Tome F., Rodríguez M.B., Lozano J.C. 2003 – Soil-to-plant transfer factors for natural radionuclides and stable elements in a Mediterranean area – J. Environ. Radioact. 65: 161-75.