

Contamination of Metals in Different Tissues of Rohu (*Labeo rohita*, Cyprinidae) Collected from the Indian River Ganga

Huma Vaseem · T. K. Banerjee

Received: 17 August 2012 / Accepted: 17 April 2013 / Published online: 11 May 2013
© Springer Science+Business Media New York 2013

Abstract In the present paper, accumulation of zinc (Zn), iron (Fe), copper (Cu), chromium (Cr), nickel (Ni), manganese (Mn) and lead (Pb) was determined in different tissues (skin, muscles, liver, gills, kidney and brain) of rohu (*Labeo rohita*) collected from the River Ganga in Varanasi, India. Concentrations of Cu (except gills), Fe and Cr (except brain for Cr) in most of the tissues were above the permissible safe limits for human consumption suggested by the Food and Agricultural Organization (FAO 1983). Concentrations of all metals were higher in River Ganga fish than those from the University fish farm. With the exception of Zn in skin, muscle and brain tissue, the studied metals were bioaccumulated in all tissues. The total metal accumulation or metal pollution index was highest in liver (20.8 ± 0.50) followed by kidney (16.8 ± 0.44), gills (15.2 ± 0.15), muscles (12.1 ± 0.08), skin (10.5 ± 0.53) and brain (7.0 ± 0.02).

Keywords River Ganga · Contamination · Bioaccumulation of metals · *Labeo rohita*

The water of the River Ganga is used by Hindus for worship and is utilised and consumed by inhabitants along its banks from its origin in the Himalayas to its discharge at the Bay of Bengal. Industrial development and other anthropogenic activities have polluted the river water to such an extent that it has become a health concern due to

contamination with metals and other xenobiotics. A recent investigation by Vaseem and Banerjee (2013) illustrated the highly polluted condition of the Ganga water at Varanasi due to contamination of a large number of toxic metals. The concentrations of these metals (mg L^{-1}) reported by these authors were Zn: 12.553 ± 2.96 , Fe: 4.363 ± 2.18 , Mn: 1.967 ± 0.907 , Ni: 0.918 ± 0.247 , Cu: 0.290 ± 0.016 , Cr: 0.279 ± 0.19 , Pb: 0.150 ± 0.115 . These concentrations in water were greatly above the allowable levels suggested by the Bureau of Indian Standards (1991) for drinking water.

Toxic effluents, including those from leather and textile industries, are the main pollution sources of the river before its entry at Varanasi. Domestic waste is also a major polluting agent (Maceda-Veiga et al. 2012) because it contains metals from household products, such as insecticides, toothpaste, cleaning materials and cosmetics.

Metal pollutants are of concern due to their toxicity, persistence and bioaccumulation in the food chain (Storelli 2008; Groth 2010; Mendil et al. 2010). Aquatic organisms have been reported to accumulate heavy metals in their tissues several times higher than their ambient levels in water by absorption through the gills and by consumption of contaminated food and sediments (Canbek et al. 2007; Ayandiran et al. 2009). As fishes are constantly exposed to pollutants in contaminated water, they are excellent bio-indicators of heavy metals in aquatic ecosystems (Nsikak et al. 2007).

Labeo rohita (rohu), an endemic fish species found in large numbers in the Ganga River, and is one of the preferred fishes of the people. The bioaccumulation of the metals zinc (Zn), iron (Fe), copper (Cu), chromium (Cr), nickel (Ni), manganese (Mn) and lead (Pb) in various tissues of the rohu were analysed. The concentrations of metals in edible tissue were compared to guidelines of

H. Vaseem · T. K. Banerjee (✉)
Department of Zoology, Centre of Advanced Study,
Banaras Hindu University, Varanasi 221005, India
e-mail: tkbzool@yahoo.co.in

H. Vaseem
e-mail: vaseem18huma@gmail.com

maximum permissible concentrations to determine the relative safety for human consumers.

Materials and Methods

This study was conducted from January to April, 2011. Twenty-five fish were caught from each of three sites in the city of Varanasi (Fig. 1) (Vaseem and Banerjee 2013). The live fish (30 ± 10 g, 11 ± 2 cm) were brought to the laboratory in river water. Cold anaesthetised fish were sacrificed, and tissue samples were pooled before washing with double-distilled water. They were then placed into petri dishes to dry at 120°C until there was no weight loss. One gram of each dried tissue sample (in three replicates) was then digested with diacid [HNO_3 and HClO_4 in 2:1

ratio (Canli and Kalay 1998)] on a hot plate maintained at 130°C until all materials were dissolved. Metals were analysed using a flame atomic absorption spectrophotometer (AAS) (Perkin-Elmer Model 2380, Inc., Norwalk, CT, USA). Quality control measures were applied to detect contamination as well as the reliability of data. To calibrate the instrument, blanks and a drift standard were run after every ten readings. Detection limits for metals in mg kg^{-1} were 0.1 for Fe, Mn and Zn; 0.01 for Cu, Pb and Ni; and 0.02 for Cr. The results were expressed as mg kg^{-1} dry weight for the fish tissues. Heavy metal concentrations of the tissue samples were analysed in the laboratory following the standard methods of APHA (1998). The metal accumulation data of the Ganga River fish were compared with those observed in the same fish raised/maintained under controlled condition at a fish farm at the Institute of

Fig. 1 Map showing study area (Vaseem and Banerjee 2013)

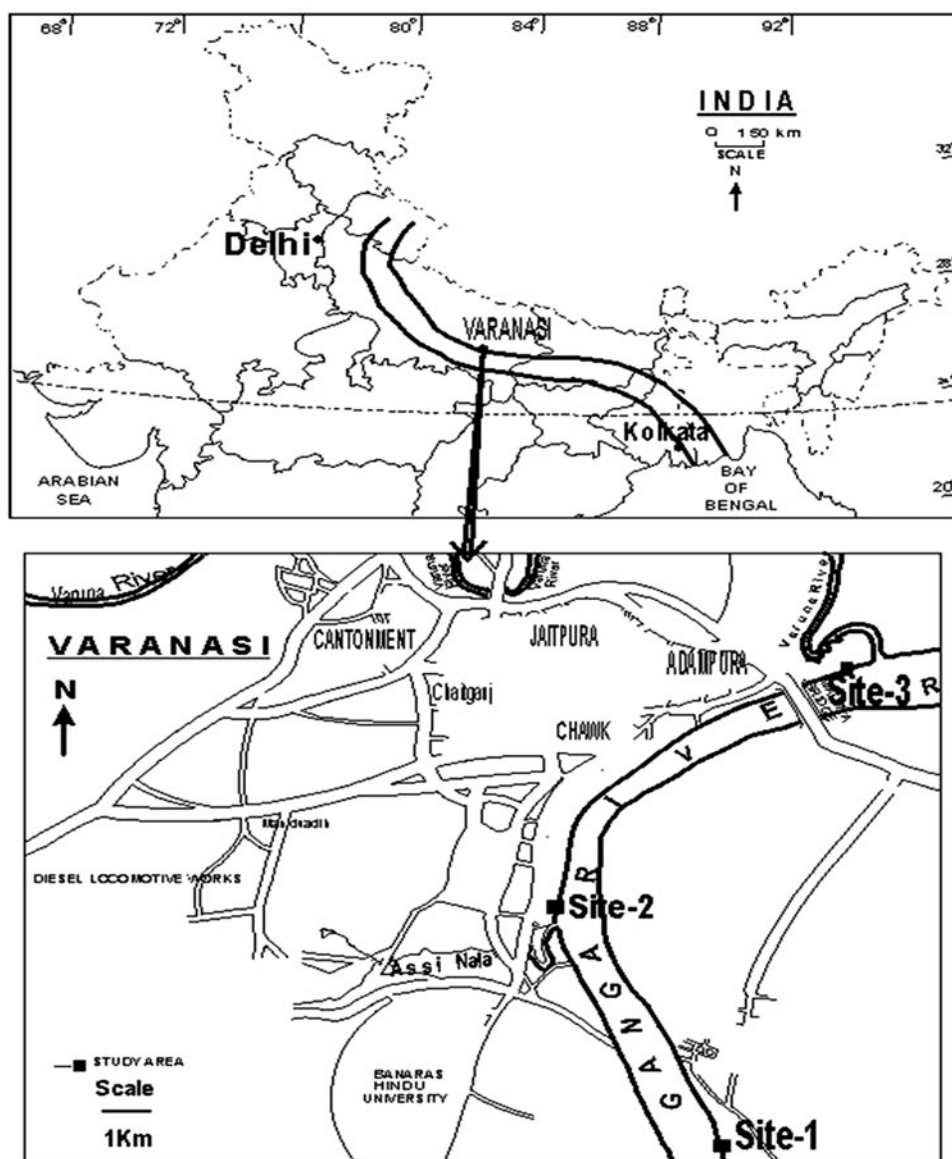


Table 1 Concentrations (mg kg⁻¹) of metals in various tissues of fish collected from a fish-rearing pond (control) and the River Ganga, compared to published values for maximum safe limits (FAO)

Tissue	Mn	Cu	Zn	Fe	Cr	Ni
Skin						
Control	0.52 ± 0.22	7.53 ± 3.2	0.85 ± 0.10	21.73 ± 4.38	bdl ^a	bdl
Ganga	5.70 ± 0.11*	20.09 ± 0.61*	5.79 ± 1.26*	35.41 ± 0.76*	5.8 ± 0.07	bdl
Muscles						
Control	0.56 ± 0.01	1.8 ± 0.02	5.65 ± 0.09	38.45 ± 0.83	bdl	bdl
Ganga	4.97 ± 0.13*	13.17 ± 1.13*	10.69 ± 1.75*	47.81 ± 1.05*	8.14 ± 0.17	bdl
Gills						
Control	0.61 ± 0.2	3.87 ± 0.1	5.69 ± 2.52	14.87 ± 2.79	bdl	bdl
Ganga	7.31 ± 0.15*	8.35 ± 0.18*	30.94 ± 1.32*	74.96 ± 2.16*	13.14 ± 0.15	7.48 ± 1.46
Liver						
Control	0.33 ± 0.22	14.13 ± 3.35	10.82 ± 0.63	49.88 ± 5.96	bdl	bdl
Ganga	10.59 ± 0.59*	28.19 ± 2.24*	38.08 ± 1.03*	131.05 ± 4.34*	7.92 ± 0.29	8.12 ± 2.05
Kidney						
Control	2.28 ± 0.04	14.95 ± 0.76	22.69 ± 0.49	36.23 ± 0.60	bdl	bdl
Ganga	7.79 ± 0.15*	22.51 ± 0.96*	35.28 ± 1.42*	55.21 ± 1.10*	12.25 ± 0.14	6.12 ± 0.93
Brain						
Control	0.92 ± 0.02	3.5 ± 0.159	4.38 ± 0.22	11.24 ± 1.89	bdl	bdl
Ganga	2.65 ± 0.07*	12.68 ± 3.36*	16.04 ± 1.27*	48.92 ± 2.17*	0.64 ± 0.02	bdl
FAO ^b (1983)	nl ^c	10	50	5.6	1	nl

* Denotes significant differences at $p < 0.05$

^a Below detectable limit

^b FAO Food and Agricultural Organisation (1983)

^c No limit has been suggested by FAO (1983)

Agriculture Sciences, Banaras Hindu University (BHU), Varanasi. To check if the metal contamination levels in fish from the Ganga River were safe for human consumption, the data were also compared with the permissible limits recommended by the Food and Agricultural Organization (FAO 1983) for fish and fishery products.

The bioaccumulation factor (BAF) denotes the ratio between the accumulated concentration of a given pollutant in any organ and its dissolved concentration in water (Authman and Abbas 2007). It was calculated using the following equation:

$$\text{BAF} = \frac{X(\text{mg/kg})}{Y(\text{mg/liter})}$$

where X denotes metal concentration in fish organ and Y denotes metal concentration in water (Vaseem and Banerjee 2013).

The metal pollution index (MPI) was calculated to determine total accumulation of the metals in the various tissues. The MPI values were calculated using the equation (Usero et al. 1997):

$$\text{MPI} = (\text{Cf}_1 * \text{Cf}_2 * \text{Cf}_3 * \dots * \text{Cf}_n)^{1/n}$$

where, Cf_n is the concentration for the metal n in the sample.

Statistical analyses of data were carried out using student's *t* test to compare the differences ($p < 0.05$) in metal accumulation in the Ganga River fish and BHU cultured fish. One way ANOVA followed by Duncan's Multiple Range Test ($p < 0.05$) were used to compare the calculated MPI values of different tissues of fish collected from Ganga River. The data in Tables 1, 2 and 3 are given as mean ± SD.

Result and Discussion

Table 1 provides metal concentrations (mg kg⁻¹) in different tissues (liver, kidney, gills, muscles, skin and brain) of riverine fish compared to concentrations in farm-raised fish and permissible limits for fish and fishery products (FAO 1983). Zinc concentrations in all tissues of the riverine fish were below the maximum permitted limit (50 mg kg⁻¹) suggested by FAO (1983), but were higher ($p < 0.05$) than those reported in the fish collected from the university pond. Zinc is an essential element involved in many metabolic activities, but higher concentrations in fish may result in the destruction of gill epithelium, causing hypoxia, growth retardation and mortality (Jones et al. 2001; Plachy 2003).

Table 2 Bioaccumulation factors for metals in various tissues of *L. rohita* collected from the River Ganga

Metals	Skin	Muscles	Gills	Liver	Kidney	Brain
Mn	2.68 ± 2.26	3.00 ± 1.30	4.46 ± 2.04	6.34 ± 2.60	4.70 ± 2.1	1.62 ± 0.77
Cu	30.19 ± 2.14	21.94 ± 9.49	28.80 ± 0.71	97.18 ± 7.20	77.74 ± 5.24	43.90 ± 11.96
Zn	0.46 ± 0.01	0.91 ± 0.34	2.62 ± 0.81	3.14 ± 0.56	2.92 ± 0.60	0.44 ± 0.19
Fe	9.38 ± 3.11	12.53 ± 3.90	19.89 ± 6.70	34.31 ± 10.50	14.49 ± 4.53	12.78 ± 3.92
Cr	30.26 ± 19.35	42.29 ± 27.11	68.48 ± 43.78	39.70 ± 25.77	63.69 ± 40.78	3.41 ± 2.17
Ni	–	–	8.58 ± 2.38	9.21 ± 2.16	6.85 ± 0.94	–

BAFs were calculated from the tissue concentrations observed in this study and the concentrations of metals in river water (Vaseem and Banerjee 2013) that was collected at the same time

Table 3 Metal pollution index (MPI) of different tissues in *L. rohita* collected from the River Ganga

Tissue	Skin	Muscles	Gills	Liver	Kidney	Brain
MPI	10.54 ± 0.53 ^b	12.09 ± 0.085 ^c	15.23 ± 0.15 ^d	20.83 ± 0.50 ^f	16.84 ± 0.44 ^e	6.99 ± 0.02 ^a

Different letters denote significant differences ($p < 0.05$)

The concentrations of Cu (Table 1) in skin, muscles, liver, kidney and brain of the fish from the river were above the permitted limit (10 mg kg^{-1}) recommended by FAO (1983). When compared to the BHU farm fish, all organs in the River Ganga fish contained significantly higher concentrations of Cu ($p < 0.05$). Copper is also an important element for good health, but the elevated intake may cause adverse health problems such as decreased reproductivity and oxygen consumption (Beyer et al. 2000).

Like Zn and Cu, the amounts of Mn, Fe and Cr were also higher in all the analyzed tissues of fish collected from the River Ganga compared to fish from the BHU pond. Concentrations of Fe and Cr in River Ganga fish exceeded the respective permissible FAO limits of 5.6 and 1.0 mg kg^{-1} , respectively, with the exception of brain tissue for Cr. Chromium plays an important role in glucose metabolism, but its higher concentration may lead to reduced growth, altered metabolism and chromosomal aberrations. Iron is an important component of the respiratory systems, but its higher concentration may cause gill damage, disrupt metabolism and osmoregulation (Dalzell and Macfarlane 1999).

No limit was set by FAO (1983) for Mn. It is an essential trace element that serves as a structural component of some enzymes and activates the functions of many enzymes. But it is also toxic when concentrations in the body are too high. It may cause hallucinations, forgetfulness and nerve damage in human subjects (www.lenntech.com/periodic/elements/mn.htm). The amounts of Ni and Cr in fish collected from the BHU pond were below detectable levels. Ni is required for normal growth and reproduction in animals and human beings, but has a carcinogenic effect when consumed in high amount. Ni was, however, present in the kidney, liver and gills of fish obtained from the River

Ganga. Lead was never detected in any tissue collected either from BHU or the River Ganga.

In aquatic ecological risk assessment, BAFs are used to quantify chemical accumulation in tissue relative to concentration in water or sediment (Fairbrother et al. 2007; Thomann et al. 1995). With the exception of Zn in skin, muscle and brain tissue, all metals had tissue concentrations that were higher than their respective concentrations in water (Table 2). Aquatic organisms have been reported to accumulate metals to concentrations that are many times higher than those present in water (Olaifa et al. 2004). While studying the trace metal concentration in the water, sediment and fish tissues from the Lake Tanganyika, Chale (2002) noted that concentrations of metals in fish tissues were always higher than in water.

When one or more metal contaminants are present in the tissue(s), it becomes mandatory to understand the total metal load/stress on the different organ of the fish. This load is reflected in the MPI value. The MPI values (Table 3) in this study were calculated to compare the total metal uptake in different tissues of the fish. The order of MPI values was liver > kidneys > gills > muscles > skin > brain.

A possible reason that liver tissue has the highest MPI value may be due to its role as the main site for synthesis of various proteins and other molecules which have high affinities for metals (Fernandes et al. 2008), as well as being the main site of detoxification of various contaminants. Also, metals are transported to the liver from different sites of absorption (Kargin and Erdem 1991). The kidney was the second highest organ for metal accumulation, likely due to its excretory function. Gill tissue also exhibited high level of metal accumulation. Gills have a large surface area, and are in continuous contact with the aquatic environment. Hence chances for bioaccumulation

of metals are very high. Further, the barrier distance between the blood in the gills and ambient toxicants is very thin, causing easy transfer of the metals into the gills. The other reason for increased metal accumulation in the gills is perhaps due to the increased density of chloride cells whose number increases in contaminated waters because of their role in picking up of cations, including metal ions (Mazon et al. 1999, 2002; Costa and Fernandez 2002).

The concentrations of different metals in muscle tissue were relatively low compared to the liver, kidney and gills. This may be due to less extensive blood circulation in muscles than other vital tissues like liver, kidney and gills. Also, muscle is a less metabolically active tissue (Adhikari et al. 2009; Wagner and Boman 2003; Radhakrishnan 2010). Even though the skin is a boundary tissue, the accumulation of metals is not as high as that observed in the gills, perhaps due to continuous production and sloughing of slime from the body surface (Singh and Banerjee 2008) resulting in the loss of metals bound with—SH groups. Metal accumulation in the brain tissue was minimal. This might be due to the presence of a blood brain barrier (Takeda 2004).

In conclusion, the concentrations of three metals (Cu, Fe and Cr) in Ganga River fish were higher than the permissible levels suggested by an international organisation, the FAO. Hence, it is not presently safe to consume *L. rohita* collected from the River Ganga in the Varanasi region. If preventive measures are not taken to curb the inputs of metals pollutants, the condition may worsen in the future.

Acknowledgments Ms. Huma Vaseem is highly thankful to the University Grant Commission, Government of India, New Delhi for providing a Senior Research Fellowship.

References

- Adhikari S, Ghosh L, Giri BS, Ayyappan S (2009) Distribution of metals in the food web of fishponds of Kolleru Lake India. *Ecotoxicol Environ Safe* 72:1242–1248
- APHA-AWWA-WPCF (1998) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association American Water Works Association and Water Pollution Control Federation, Washington
- Authman M, Abbas H (2007) Accumulation and distribution of copper and zinc in both water and some vital tissues of two fish species (*Tilapia zilli* and *Mugil cephalus*) of Lake Qaran, Fayoum Province, Egypt. *Pak J Biol Sci* 10:2106–2122
- Ayandiran TA, Fawole OO, Adewoye SO, Ogundiran MA (2009) Bioconcentration of metals in the body muscle and gut of *Clarias gariepinus* exposed to sublethal concentrations of soap and detergent effluent. *J Cell Anim Biol* 3:113–118
- Beyer WN, Day D, Melancon MJ, Sileo L (2000) Toxicity of Anacostia River, Washington DC, USA, sediment fed to mute swans (*Cygnus olor*). *Environ Toxicol Chem* 19:731–735
- Bureau of Indian Standard (BIS) (1991) Drinking water specification IS: 10500: 1991. New Delhi
- Canbek M, Demir TA, Uyanoglu M, Bayramoglu G, Emiroglu O, Arslan N (2007) Preliminary assessment of heavy metals in water and some cyprinidae species from the Porsuk River, Turkey. *J Appl Biol Sci* 1:91–95
- Canli M, Kalay OAM (1998) Levels of heavy metals (Cd, Pb, Cu, Cr and Ni) in tissue of *Cyprinus carpio*, *Barbus capito* and *Chondrostoma regium* from the Seyhan River, Turkey. *Turk J Zool* 22:149–157
- Chale FM (2002) Trace metal concentrations in water, sediments and fish tissue, Lake Tanganyika. *Sci Total Environ* 299:115–121
- Costa OTF, Fernandez MN (2002) Chloride cell changes induced by nitrite exposure in an Amazonian fish species. In: Kennedy C, Kolok A, MacKinlay D (eds) *Aquatic toxicology: mechanism and consequences*. International Congress of Fish Biology, Canada, pp 51–61
- Dalzell DJB, Macfarlane NAA (1999) The toxicity of iron to brown trout and effects on the gills: a comparison of two grades of iron sulphate. *J Fish Biol* 55:301–315
- Fairbrother A, Wenstel R, Sappington S, Wood W (2007) Framework for metals risk assessment. *Ecotoxicol Environ Safe* 68:145–227
- FAO (1983) Compilation of legal limits for hazardous substances in fish and fishery products. *Food Agric Organ Fish Circ No.* 464
- Fernandes D, Bebianno MJ, Porte C (2008) Hepatic levels of metal and metallothioneins in two commercial fish species of the Northern Iberian shelf. *Sci Total Environ* 39:159–167
- Groth E (2010) Ranking the contributions of commercial fish and shellfish varieties to mercury exposure in the United States: implication for risk communication. *Environ Res* 110:226–236
- Jones I, Kille P, Sweeney G (2001) Cadmium delays growth hormone expression during rainbow trout development. *J Fish Biol* 59:1015–1022
- Kargin F, Erdem C (1991) Accumulation of copper in liver, spleen, stomach, intestine, gill and muscle of *Cyprinus carpio*. *Doga Turk J Zool* 15:306–314
- Maceda-Veiga A, Monroy M, De Sostoa A (2012) Metal bioaccumulation in the Mediterranean barbell (*Barbus meridionalis*) in a Mediterranean river receiving effluents from urban and industrial wastewater treatment plants. *Ecotoxicol Environ Safe* 76:93–101
- Mazon AF, Cerqueria CCC, Monteiro EAS, Fernandez MN (1999) Acute copper in fresh water fish: morphological and physiological effects. In: Val AL, Almeida-val VMF (eds) *Biology of tropical fishes*. INPA, Manaus, pp 261–275
- Mazon AF, Monteiro EAS, Pinheiro GHD, Fernandez MN (2002) Hematological and physiological changes induced by short-term exposure to copper in the freshwater fish, *Prochilodus scrofa*. *Braz J Biol* 62:621–631
- Mendil D, Demirci Z, Tuzen M, Soylak M (2010) Seasonal investigation of trace element contents in commercially valuable fish species from the Black Sea, Turkey. *Food Chem Toxicol* 48:865–870
- Nsikak UB, Joseph PE, Akan BW, David EB (2007) Mercury accumulation in fishes from tropical aquatic ecosystems in the Niger Delta, Nigeria. *Curr Sci* 92:781–785
- Olaifa FE, Olaifa AK, Adelaja AA, Owolabi AG (2004) Heavy metal contamination of *Clarias gariepinus* from a lake and fish farm in Ibadan, Nigeria. *Afr J Biomed Research* 7:145–148
- Plachy J (2003) Cadmium. In: USGS. *US geological survey minerals yearbook—2003*, Reston, pp 15.1–15.5
- Radhakrishnan MV (2010) Accumulation of trace metals in tissues of *Heteropneustes fossilis* collected from Chaliyar River, Kerala, India. *World J Fish Mar Sci* 2:303–306
- Singh AK, Banerjee TK (2008) Toxic effects of sodium arsenate on the skin epidermis of air breathing catfish, *Clarius batrachus* (L.). *Vet Arhiv* 78:73–88
- Storelli MM (2008) Potential human health risk from metals (Hg, Cd and Pb) and polychlorinated biphenyls (PCBs) via seafood

- consumption: estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). *Food Chem Toxicol* 46:2782–2788
- Takeda A (2004) Analysis of brain function and prevention of brain diseases: the action of trace metals. *J Health Sci* 50:429–442
- Thomann RV, Mahony JD, Mueller R (1995) Steady state model of biota—sediment accumulation factor for metals in two marine bivalves. *Environ Toxicol Chem* 4:989–998
- Usero J, Gonzalez-regalada E, Gracia I (1997) Trace metal in the bivalve molluscs *Ruditapes decussates* and *Ruditapes philippinarium* from the Atlantic coast of southern Spain (J). *Environ Int* 23:291–298
- Vaseem H, Banerjee TKB (2013) Contamination of the River Ganga and its toxic implication in the blood parameters of the major carp *Labeo rohita* (Ham). *Environ Sci Pollut Res*. doi: [10.1007/s11356-013-570-8](https://doi.org/10.1007/s11356-013-570-8)
- Wagner A, Boman J (2003) Biomonitoring of trace elements in muscle and liver tissue of freshwater fish. *Spectrochim Acta B* 58:2215–2226