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Bioaccumulation of Heavy Metals in Organs of Fresh Water Fish *Oreochromis mossambicus* Collected from two Sites of Ujani Backwaters

S.P. Chordiya[1]* and N.E. Chandanshive[2]

[1]Department of Zoology, Tuljaram Chaturchand College of Arts, Science & Commerce, Baramati 413 102, M.S., India
[2]Department of Zoology, Fergusson College, Pune, M.S., India

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ABSTRACT

In the present study, an attempt has been made to compare the bioaccumulation of some heavy metals in the different tissues of the most common fish *Oreochromis mossambicus* collected from two sites of Ujani backwaters during three seasons of the year. The backwater of Ujani dam is a major resource of freshwater fishes to local population, where fishery is practiced on large scale. It is essential to conduct a survey of the bioaccumulation pattern of toxic heavy metals such as Hg, Pb, Cu, and Zn in the fish species commonly consumed by people in this region. The survey was carried out during three seasons of the year. The accumulated toxic heavy metals were found in the order Zn>Cu>Pb> Hg. The seasonal order was Monsoon > Pre-monsoon > Post-monsoon. The seasonal variation might be the effect of monsoonal runoff from the highly urbanized and industrial city of Pune that convey huge quantum of effluents in the water of tributaries of Bhima. Such study has immense importance as fishes are consumed by a large percentage of the people in the area of backwaters. This study would also provide a roadmap for researchers and policymakers to identify and implement effective and sustainable measures to counteract the increasing trends of pollution levels in natural ecosystems.

***Key words**: Heavy metals, Water pollution, Tilapia, Aquatic ecosystem.*

Introduction

Pollution of fresh water due to a wide range of pollutants has become an issue of serious concern since the industrialization (MPCB, 2018; Canli *et al*., 1998; Dighavkar, 2016; Dirilgen, 2001; Vutukuru, 2005; Voegborlo *et al*., 1999). Surface water and ground water of natural aquatic systems may be contaminated with heavy metals released from domestic, industrial and other human activities (MPCB, 2018; Dighavkar, 2016; Conacher *et al*., 1993; Velez and Montoro, 1998). Heavy metal contamination may cause serious harmful effects on the ecosystem and a diversity of aquatic organisms (Ashraj, 2005; Farombi, *et al*., 2007; Vosyliene and Jankaite, 2006). Fishes and other living organisms cannot escape from the adverse effects of the pollution of aquatic bodies. (Clarkson, 1998; Olaifa *et al*., 2004; Dickman and Leung, 1998). Fish are very common organisms used to assess the health of aquatic ecosystems as pollutants build up in the food chain and finally rich the fish bodies in the aquatic ecosystem (Farkas *et*

([1]Assistant Prof., [2]Prof.)

al., 2002; Yousuf and El-Shahawi, 1999).

River Bhima is one of the main sources of irrigation in western Maharashtra and it confluences with the River Krishna in Andhra Pradesh. Origin of Bhima lies in the Ambegaon tehsil of Pune district; nearby the shrine of Bhimashankar. It flows through the states like Maharashtra, Karnataka, and Telangana in South-East direction for approximately 850 kilometres. Main tributaries like Mula-Mutha, Kukadi, Meena, Chandani, etc. contribute to its waters at different places of confluence (MPCB, 2018-19). Increasing water pollution in Pune and the cities upstream of the Ujani dam have serious environmental impacts at Ujani backwaters as its tributaries like Mula-Mutha, Indrayani, Ghod, Pavana flow through the metropolitan cities like Pune, Pimpri-Chinchwad. Pune, Chakan and Pimpri-Chinchwad have industrial areas nearby the course of the Bhima and its tributaries (Shinde *et al.*, 2020; Kalekar *et al.*, 2022). Domestic and industrial sewage from municipal areas of Pune, Pimpri-Chinchwad,Chakan, Cantonment settlements like Dehu, Pune & Khadki and agricultural run-off are major source of water pollution in Bhima River. Sewage disposal system of municipal and cantonment boards are not as per the standards of MPCB (Dighavkar, 2016). Heavy metal pollution due to anthropogenic activities since the last few decades, has led to new pattern of metal distribution as compared to natural distribution. Many heavy metals are used in large scale in automobiles, mining industries, pesticides, house-holds appliances, dental amalgams, paints, photographic papers, photo chemicals, etc. (WHO 1998; Lohani *et al*., 2008; Kumar *et al.*, 2012).

Heavy metals are deposited, assimilated, or incorporated in water, sediment and aquatic animals as they are non-degradable (Linnik and Zubenko, 2000). Once they enter the aquatic ecosystem, they can be bioaccumulated and biomagnified via the food chain and finally reach the humans causing serious health risks (Agah *et al*., 2009). Fishes feed upon the zooplanktons and phytoplanktons and thereby occupy higher trophic levels in the aquatic ecosystem. Tilapia is one of the common and affordable food sources for the poor people. Hence, they are an important and very suitable specimen to assess the heavy metals pollution in the inland aquatic ecosystem (Blasco *et al*., 1998; Agah *et al.*, 2009). Determination of concentrations of heavy metals like Hg, Pb, Cu and Zn in water and different tissues of *Oreochromis mossambicus* was main purpose of the present study. Samples were collected from two sites at Ujani backwaters, Maharashtra, India.

Materials and Methods

Study Area

Study area selected for the present study is backwaters of Ujani dam constructed on River Bhima. Ujani dam is built on a comparatively flat land. Its submergence area stretches nearly 40 kilometres from the dam wall. The environmental impacts at Ujani backwater are an indication of the increasing water pollution in Pune and the cities upstream of the dam. (MPCB, 2018) Water samples & fish were collected from two sites as Site-I & Site-II.

Collection of water sample

Water samples and fish specimens were collected from the selected sites in three seasons as pre-monsoon (February – May), Monsoon (June – September) & Post-monsoon (October to January) seasons. For laboratory analysis, water samples were collected in pre-acid washed dry polyethylene bottles.

Fish were collected with the help of local fishermen and brought to the laboratory in icebox. Immediately, they were dissected to collect the gills, muscles & liver. Collected tissues were weighed and kept for drying in hot air oven at 60 °C till the constant dry weight. Dried tissues were powdered in porcelain mortar. Powdered tissues were stored at -18°c till the further analysis (Gorakhe and Chandanshive, 2020).

Heavy metal: Concentrations of Heavy metals were determined by Atomic Absorption Spectrophotometer; LABINDIA AA8000 as per the protocols recommended by APHA (2005).

Ethical statement: We declare that, the fish under study is not protected under wildlife conservation act and is routinely caught by professional fisherman and sold as a food fish in local markets. No specific permit is required for obtaining this fish in India, and no experimentation was conducted on live specimens in the laboratory.

Results and Discussion

Mercury: Average concentration of mercury in the gills, muscles and liver of the fish collected at site-I & II was reported to be 0.24, 0.25, 0.32 and 0.43, 0.34, 0.42 mg kg^{-1} respectively. Concentration of mercury in these tissues of the fish are within the limit prescribed by FSSAI and WHO. Tissue-wise trend of concentration seems to be like liver > gill > muscles. Similar trend has been reported by Gupta *et al*. (2009); Malik *et al*. (2010); Ayejuyo *et al*. (2009); Mastan (2014); and Lippy *et al*.(2021). Season-wise trend of concentration in the tissues was found to be maximum in the pre-monsoon (summer), followed by monsoon (rainy) and the least concentration was reported during post-monsoon (winter) season. Similar trend has been reported by Kumar *et al.* (2014) in waters of River Yamuna, Allahabad.

Average concentration of mercury in water samples was found to be 0.0025 mgl^{-1} and it was slightly higher than the standard limit recommended by EPA, USA.

Lead: Average concentration of the lead in the tissues like gills, muscles and liver were reported as 0.3, 0.33, 0.27 and 0.34, 0.35, 0.33 mg kg^{-1} of the fish from site-I & II respectively. These values indicate that the concentration of lead is slightly higher than the limits recommended by FSSAI and WHO. Thus, tissue-wise accumulation trend is observed as muscles > gills > liver. Similar observations are recorded for the fish *Mystus cavasius* by Gorakhe and Chandanshive (2020). However, Gorakhe and Chandanshive (2020) have reported maximum concentration of lead in liver; followed by gills and muscles in *Oreochromis mossambicus*. However, Arbind Kumar *et al.* (2020) have reported somewhat different trend in carps. Dural *et al.* (2007) has reported mixed trends for lead in case of different fish species. Season-wise variation of lead in fish tissues is reported as monsoon > pre-monsoon > post-monsoon. Pandey *et al*. (2020) have reported similar trends for fishes from Mahi estuary, Gujarat, India.

Copper: Average concentration of copper in the gills

Fig. 1. Site-I: Mercury in water & fish tissues

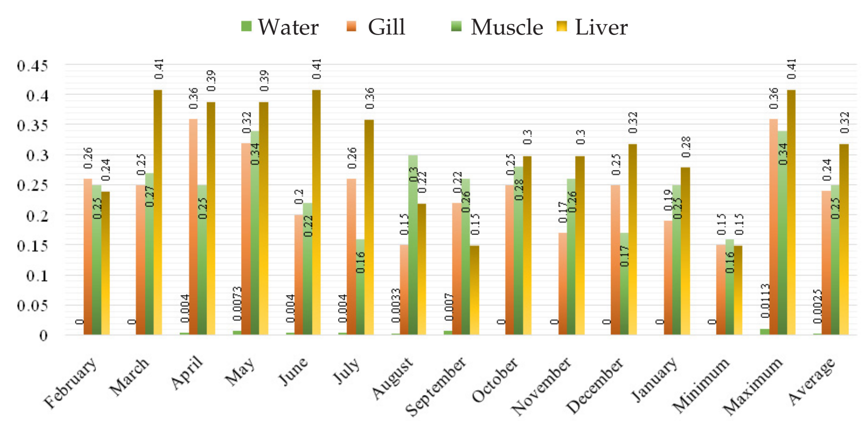

Fig. 2. Site-I: Lead in water & fish tissues

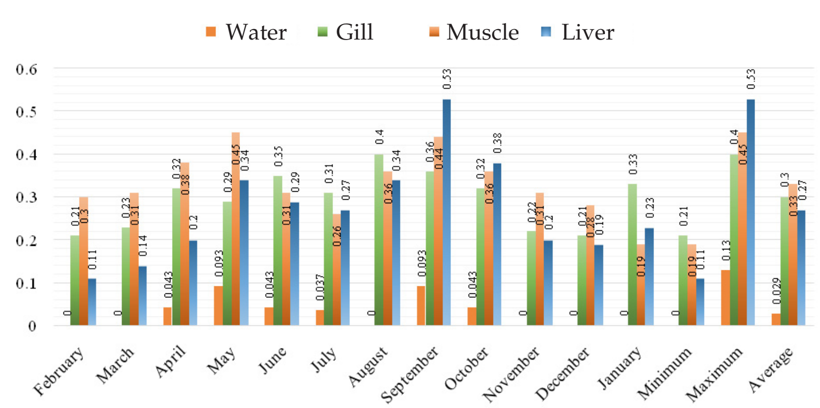

Table 1. Concentrations of Hg & Pb in Surface Water (mgl^{-1}) and Tissues of Fish ($mgkg^{-1}$) at Site-I. Values are Average ± S.D. (n=3), BDL: Below Detectible Limit, EPA: Environmental Protection Agency (US), FSSAI: Food Standards & Safety Authority of India, WHO: World Health Organization, EU: European Union. All standard values are in mgl^{-1} or $mgkg^{-1}$ as applicable

Period	Month	Hg				Pb			
		Water	Fish			Water	Fish		
			Gill	Muscle	Liver		Gill	Muscle	Liver
Pre-monsoon	February	BDL	0.26±0.06	0.25±0.14	0.24±0.1	BDL	0.21±0.06	0.30±0.05	0.11±0.1
	March	BDL	0.25±0.11	0.27±0.05	0.41±0.21	BDL	0.23± 0.07	0.31±0.05	0.14±0.03
	April	0.004±0.007	0.36±0.04	0.25±0.17	0.39±0.12	0.043±0.075	0.32±0.04	0.38±0.06	0.20±0.04
	May	0.0073±0.006	0.32±0.05	0.34±0.11	0.39±0.25	0.093±0.083	0.29±0.12	0.45±0.07	0.34±0.03
Monsoon	June	0.004±0.007	0.20±0.07	0.22±0.11	0.41±.07	0.043±0.075	0.35±0.09	0.31±0.04	0.29±0.08
	July	0.004±0.007	0.26±0.05	0.16±0.08	0.36±0.11	0.037±0.064	0.31±0.09	0.26±0.04	0.27±0.06
	August	0.0033±0.006	0.15±0.04	0.30±0.11	0.22±0.11	BDL	0.40±0.03	0.36±0.05	0.34±0.05
	September	0.007±0.006	0.22±0.08	0.26±0.14	0.15±0.04	0.093±0.083	0.36±0.08	0.44±0.06	0.53±0.06
Post-monsoon	October	BDL	0.25±0.1	0.28±0.1	0.30±0.17	0.043±0.075	0.32±0.08	0.36±0.03	0.38±0.05
	November	BDL	0.17±0.06	0.26±0.05	0.30±0.16	BDL	0.22±0.11	0.31±0.08	0.20±0.08
	December	BDL	0.25±0.14	0.17±0.11	0.32±0.18	BDL	0.21±0.08	0.28±0.09	0.19±0.08
	January	BDL	0.19±0.09	0.25±.09	0.28±0.04	BDL	0.33±0.06	0.19±0.08	0.23±0.06
	Minimum	BDL	0.15	0.16	0.15	BDL	0.21	0.19	0.11
	Maximum	0.0113	0.36	0.34	0.41	0.130	0.40	0.45	0.53
	Average	0.0025±0.005	0.24±0.09	0.25±0.1	0.32±0.14	0.029±0.056	0.30±0.09	0.33±0.09	0.27±0.12
EPA Standards (mgl^{-1})		0.002				0.015			
FSSAI				0.5				0.3	
WHO, EU				0.5				0.3	

Table 2. Concentrations of Cu & Zn in Surface Water (mgl^{-1}) and Tissues of Fish ($mgkg^{-1}$) at Site-I. Values are Average±S.D. (n=3), BDL: Below Detectible Limit, EPA: Environmental Protection Agency (US), FSSAI: Food Standards & Safety Authority of India, WHO: World Health Organization, EU: European Union. All standard values are in mgl^{-1}or $mgkg^{-1}$ as applicable

Period	Month	Cu				Zn			
		Water	Fish			Water	Fish		
			Gill	Muscle	Liver		Gill	Muscle	Liver
Pre-monsoon	February	1.55±0.4	22.18±1.2	21.29±0.53	20.64±0.06	4.41±0.81	41.70±7.44	43.40±6.16	39.32±10.33
	March	1.39±0.077	22.90±1.26	20.68±0.63	21.71±1.28	4.08±1.80	47.47±3.35	42.61±11.17	33.67±11.83
	April	1.37±0.11	22.55±0.08	22.09±0.36	21.80±1.10	4.61±1.27	41.18±11.10	38.36±8.04	33.68±1.10
	May	1.98±0.34	23.98±1.13	22.89±0.79	22.61±0.96	5.58±0.39	45.47±10.09	48.20±4.36	34.07±11.25
Monsoon	June	1.52±0.16	23.84±0.61	22.32±0.49	22.61±0.83	4.28±1.63	42.11±7.35	48.45±3.77	43.15±9.54
	July	1.41±0.18	23.20±0.81	21.24±0.54	23.06±1.06	3.99±0.35	50.24±6.81	45.23±9.98	37.77±5.07
	August	1.51±0.15	23.56±1.06	23.29±0.77	22.97±0.52	4.17±0.85	46.71±7.96	37.50±5.12	35.13±3.40
	September	2.15±0.49	24.48±0.78	23.75±1.06	23.03±1.51	5.90±0.88	47.43±7.80	46.41±2.69	47.20±2.88

Table 2. *Continued ...*

Period	Month	Cu				Zn			
		Water	Fish			Water	Fish		
			Gill	Muscle	Liver		Gill	Muscle	Liver
Post-monsoon	October	1.67±0.25	23.65±0.09	19.61±0.96	21.61±0.67	4.16±1.19	46.89±7.93	44.86±2.31	34.32±7.25
	November	1.51±0.07	22.35±0.21	20.16±0.84	21.10±1.51	4.08±1.87	40.80±11.40	35.25±9.76	34.46±9.03
	December	1.52±0.17	21.89±0.65	20.98±0.89	20.87±0.86	3.69±1.13	37.87±1.60	40.47±9.75	40.26±10.48
	January	1.4±0.043	21.36±0.33	22.47±0.57	20.39±0.31	3.76±0.43	45.34±6.24	45.02±3.45	35.93±7.31
	Minimum	1.3260	21.36	19.61	20.39	3.69	37.87	35.25	33.67
	Maximum	2.1523	24.48	23.75	23.06	5.92	50.24	48.45	47.20
	Average	1.58±0.313	23.00±1.13	21.73±1.4	21.87±1.25	4.39±1.17	44.43±7.44	42.98±7.17	37.41±8.00
EPA Standards (mg/l)		1.3				5			
FSSAI				30				Not specified	
EU				Not Specified				Not specified	
WHO				Not specified				Not specified	

Table 3. Concentrations of Hg & Pb in Surface Water (mgl^{-1}) and Tissues of Fish ($mgkg^{-1}$) at Site-II. Values are Average ± S.D. (n=3), BDL: Below Detectible Limit, EPA: Environmental Protection Agency (US), FSSAI: Food Standards & Safety Authority of India, WHO: World Health Organization, EU: European Union. All standard values are in mgl^{-1} or $mgkg^{-1}$ as applicable.

Period	Month	Hg				Pb			
		Water	Fish			Water	Fish		
			Gill	Muscle	Liver		Gill	Muscle	Liver
Pre-monsoon	February	BDL	0.42±0.21	0.27±0.14	0.51±0.21	BDL	0.25±0.07	0.31±0.02	0.24±0.12
	March	BDL	0.52±0.11	0.35±0.20	0.57±0.10	BDL	0.35±0.03	0.35±0.03	0.24±0.08
	April	0.0037±0.006	0.48±0.21	0.35±0.12	0.65±0.08	0.047±0.081	0.40±0.04	0.39±0.02	0.36±0.04
	May	0.0080±0.007	0.55±014	0.50±0.15	0.56±0.08	0.147±0.025	0.38±0.04	0.43±0.02	0.41±0.06
Monsoon	June	0.0040±0.007	0.49±0.25	0.39±0.20	0.45±0.09	0.050±0.087	0.28±0.06	0.35±0.03	0.30±0.04
	July	BDL	0.38±0.11	0.27±0.22	0.48±0.11	BDL	0.28±0.06	0.33±0.02	0.28±0.05
	August	0.0037±0.006	0.47±0.23	0.40±0.28	0.31±0.09	0.040±0.069	0.41±0.06	0.40±0.02	0.37±0.02
	September	0.0080±0.007	0.38±0.14	0.19±0.13	0.34±0.11	0.167±0.015	0.48±0.04	0.43±0.02	0.45±0.02
Post-monsoon	October	BDL	0.20±0.13	0.27±0.03	0.32±0.10	0.037±0.064	0.28±0.04	0.33±0.05	0.38±0.03
	November	BDL	0.37±0.17	0.36±0.13	0.34±0.16	BDL	0.35±0.02	0.27±0.02	0.33±0.03
	December	BDL	0.44±0.20	0.30±0.12	0.24±0.08	0.047±0.081	0.32±0.10	0.27±0.05	0.30±0.05
	January	BDL	0.42±0.18	0.42±0.04	0.25±0.20	BDL	0.26±0.04	0.27±0.05	0.29±0.05
	Minimum	BDL	0.20	0.19	0.24	BDL	0.25	0.27	0.24
	Maximum	0.0080	0.55	0.50	0.65	0.167	0.48	0.43	0.45
	Average	0.00240±0.005	0.43±0.17	0.34±0.16	0.42±0.17	0.047±0.067	0.34±0.081	0.35±0.064	0.33±0.08
EPA Standards (mg/l)		0.002				0.015			
FSSAI				0.5				0.3	
WHO, EU				0.5				0.3	

was recorded as 23.00 mgkg^{-1}; in the muscles as 21.73 mgkg^{-1}; in the liver as 21.87 mgkg^{-1} at site-I and 25.58 mgkg^{-1}, 22.64 mgkg^{-1} and 22.94 mgkg^{-1} respectively at site-II. Concentration of copper in surface water was observed to 1.88 mgl^{-1}. All values of copper concentration in fish tissues are well below the standards recommended by FSSAI. These reported values show the maximum accumulation in gillsfollowed by liver and muscles i.e. gills > liver > muscles at both sites. However, Nayak *et al.* (2015); Gorakhe & Chandanshive (2020); Abdel-Baki *et al.* (2011) have reported maximum concentration of copper in liver followed by gill and muscles. No significant seasonal variations are reported in copper concentration in case of water and all tissues.

Zinc: Average concentrations of zinc in gills was 44.43 mgkg^{-1}, in muscles, it was 42.98 mgkg^{-1}, and in liver, it was 37.41 mgkg^{-1} at site-I and same was 47.56 mgkg^{-1}, 49.67 mgkg^{-1} and 49.43mgkg^{-1} at site-II. Thus, maximum zinc accumulation was in muscles followed by liver and gills. However, Nayak *et al.* (2015); Gorakhe and Chandanshive (2020) have reported the zinc accumulation in the order of liver > gill > muscles. Seasonal trend in fish tissues was in the sequence of monsoon > pre-monsoon > post-monsoon. Similar trends are reported for heavy metals in general by Pandey *et al.* (2020). Concentration of Zinc in water was found to be within limit

Fig. 3. Site-I: Copper in water & fish tissues

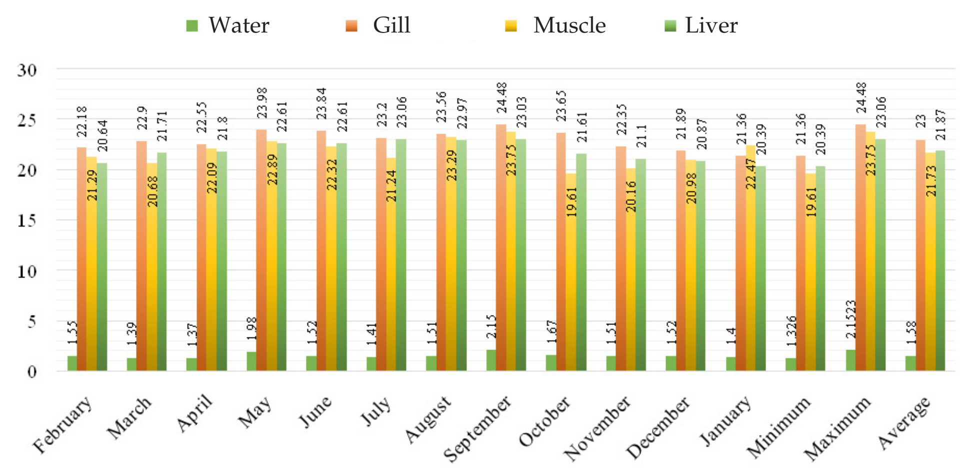

Fig. 4. Site-I: Zinc in water & fish tissues

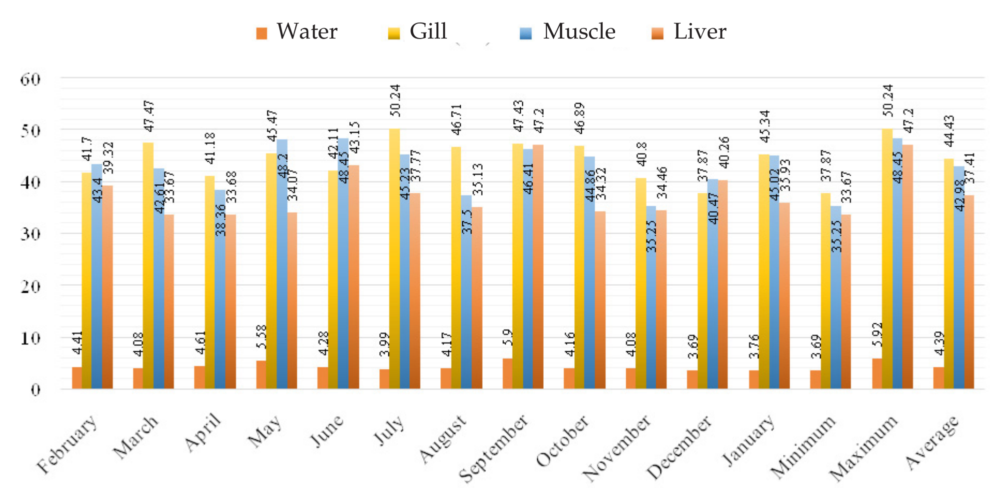

Fig. 5. Site-II: Mercury in water & fish tissues

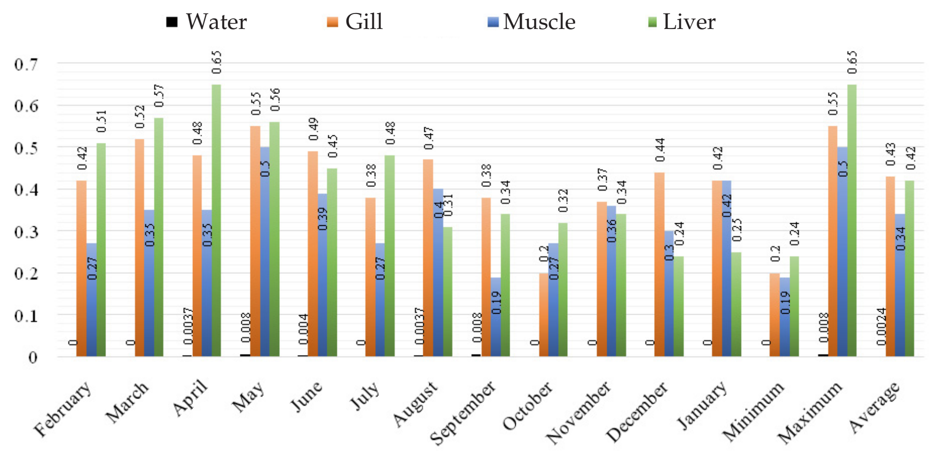

Fig. 6. Site-II: Lead in water & fish tissues

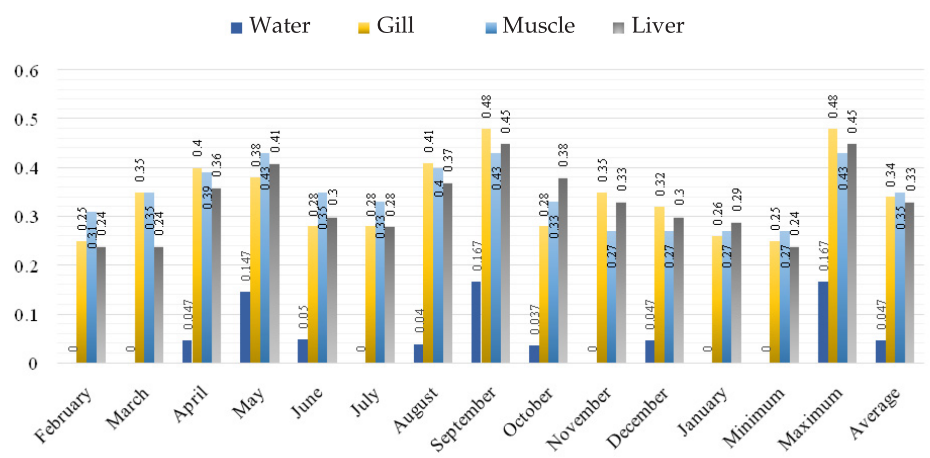

Fig. 7. Site-II: Copper in water & fish tissues

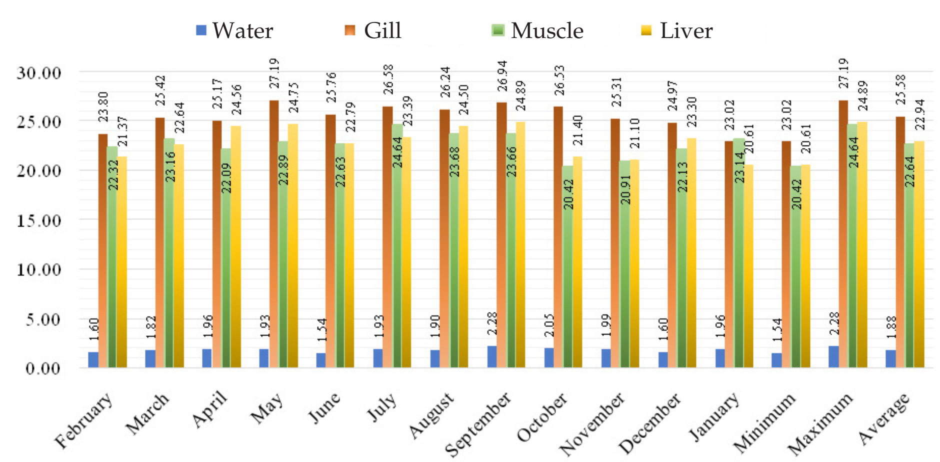

Fig. 8. Site-II: Zinc in water & fish tissues

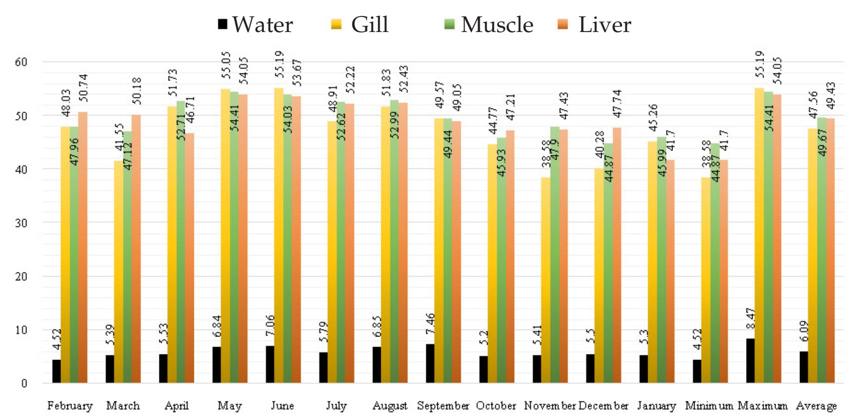

Table 4. Concentrations of Cu & Zn in Surface Water (mgl^{-1}) and Tissues of Fish ($mgkg^{-1}$) at Site-II. Values are Average±S.D. (n=3), BDL: Below Detectible Limit, EPA: Environmental Protection Agency (US), FSSAI: Food Standards & Safety Authority of India, WHO: World Health Organization, EU: European Union. All standard values are in mgl^{-1} or $mgkg^{-1}$ as applicable.

Period	Month	Cu				Zn			
		Water	Fish			Water	Fish		
			Gill	Muscle	Liver		Gill	Muscle	Liver
Pre-monsoon	February	1.60±0.32	23.80±2.08	22.32±1.36	21.37±0.62	4.52±0.44	48.03±1.58	47.96±3.56	50.74±4.12
	March	1.82±0.42	25.42±0.94	23.16±1.08	22.64±1.43	5.39±0.54	41.55±5.02	47.12±4.78	50.18±3.33
	April	1.96±0.28	25.17±0.49	22.09±0.36	24.56±1.14	5.53±1.01	51.73±2.48	52.71±2.53	46.71±6.28
	May	1.93±0.16	27.19±0.80	22.89±0.79	24.75±1.29	6.84±1.52	55.05±2.59	54.41±1.61	54.05±2.92
Monsoon	June	1.54±0.26	25.76±0.68	22.63±0.85	22.79±1.22	7.06±0.31	55.19±5.03	54.03±0.26	53.67±0.21
	July	1.93±0.26	26.58±1.38	24.64±1.12	23.39±1.35	5.79±1.07	48.91±3.18	52.62±2.08	52.22±2.09
	August	1.90±0.38	26.24±0.74	23.68±0.48	24.50±1.41	6.85±1.10	51.83±7.58	52.99±2.95	52.43±5.66
	September	2.28±0.27	26.94±0.96	23.66±1.05	24.89±0.74	7.46±0.07	49.57±3.13	49.44±1.70	49.05±3.97
Post-monsoon	October	2.05±0.10	26.53±0.92	20.42±0.52	21.40±0.97	5.20±0.59	44.77±5.13	45.93±1.14	47.21±7.06
	November	1.99±0.29	25.31±1.15	20.91±0.58	21.10±1.51	5.41±0.21	38.58±5.98	47.90±5.67	47.43±2.20
	December	1.60±0.31	24.97±3.47	22.13±1.92	23.30±0.30	5.50±1.95	40.28±6.45	44.87±8.68	47.74±0.87
	January	1.96±0.45	23.02±0.91	23.14±1.25	20.61±0.76	5.30±2.06	45.26±2.56	45.99±0.22	41.70±6.37
	Minimum	1.54	23.02	20.42	20.61	4.52	38.58	44.87	41.70
	Maximum	2.28	27.19	24.64	24.89	8.47	55.19	54.41	54.05
	Average	1.88±0.27	25.58±1.70	22.64±1.43	22.94±1.76	6.09±1.281	47.56±6.6	49.67±4.6	49.43±4.96
EPA Standards (mg/L)		1.3				5			
FSSAI				30			Not specified		
EU				Not Specified			Not specified		
WHO				Not specified			Not specified		

prescribed by EPA, US (2011). No agency has prescribed any such limit for Zinc in fish tissues.

Conclusion

In case of fish tissues, all the heavy metals under observation in this study are found to within prescribed limits except lead (Pb). Lead (Pb) has been found to be slightly above the prescribed limit. Possible source may be the industrial effluents, automobile exhaust precipitate, idol immersion, etc. as per reports of Dighavkar (2016); Gorakhe and Chandanshive (2020). Though the mercury has been reported within limits, its presence cannot be ignored. Possible source of mercury pollution needs to be ascertained and fixed. Copper and Zinc are found to be within limits; hence their presence is not the major concern.

The accumulated toxic heavy metals were found in the order of concentration, Zn > Cu > Pb > Hg.

The season-wise variation order was Monsoon > Pre-monsoon > Post-monsoon for almost all heavy metals. The seasonal variation might be the effect of monsoonal runoff from the highly urbanized and industrial city of Pune that convey huge quantum of effluents in the water of tributaries of Bhima.

No specific pattern of tissue-wise accumulation was observed in present study. Each heavy metals showed different affinity for different tissue.

In case of water samples, concentrations of Hg & Pb are found to be above the prescribed concentrations.

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Declarations

Conflict of interest: The authors have no conflicts of interest to declare. Both authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

Informed consent: The manuscript in part or in full has not been submitted or published anywhere.

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