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Seasonal variations in the biochemical composition and bio accumulation of metals in selected fishes of Chirackal, Ernakulam district, Kerala

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Abstract

Aims: Quantification of metabolites and heavy metals in fish samples obtained in the coastal region of Chirackal on seasonal basis.

Methods: The proximate chemical composition was assessed using Spectrophotometer and the quantification of heavy metals using ICP-AES.

Key Findings: Proximate composition was found to change with the seasons, with the best body composition being recorded in pre monsoon and post monsoon seasons Highest levels of proteins (70.42 mg/g in *P. indicus*), carbohydrates (71.06 mg/g, *M. seenghala*) and fat (51.17 mg/g, *G. giuris*) were noted. The concentration of heavy metals in fish muscles varied significantly with the seasons. The highest content of Fe and Cu (73.13µg/g, 51.04 µg/g) in *P. indicus* during post and pre monsoon seasons, Zn and Co (24.06 µg/g, 27.39 µg/g) was recorded in *A. testudineus* during post monsoon and highest Ni (28.43 µg/g) and Cr (7.17 µg/g) were observed in *P. indicus* and *M. seenghala* during pre and post monsoon respectively.

Significance: Fish muscle is a valuable food of high quality protein and well balanced carbohydrate and fat for promoting health, prevention and healing of diseases in human body. The samples proved resistant to heavy metals and showed a high bioaccumulation rate. Thus, they can be used as a bio indicator of heavy metals.

Keywords: Heavy metals, spectrophotometer, bioaccumulation, bio indicators

Introduction

Heavy metals are natural components in the earth's crust that cannot be degraded or destroyed. They are dangerous substances because of their bioaccumulation and toxicity can threaten aquatic living organisms (Thiyagarajan *et al*., 2012) [54]. Fishes are widely used to monitor the variations in marine environment of anthropogenic pollutants (Zou *et al*., 2008) [59]. Fishes, crabs and shrimps form an important link in transferring the media to humans. Information on the level of heavy metal pollution in coastal origin is important because they cause serious environmental health hazards (Martin *et al*., 2012) [34]. The estimation of heavy metals in the food-chain will be used to know the heavy metal transfer to the human body through sea-food (Vijayakumar *et al*., 2011) [57].

The pollution of freshwater sources by sewage, industrial waste, oil and agricultural fertilizers and pesticides, endangers the existence of both flora and fauna. Fish are often considered as an important bio indicator for aquatic ecosystems, because they obtain a high trophic level, and because they have the inherent potential to accumulate heavy metals in their muscles (Rahman *et al*., 2012) [43]. Therefore and because fish are an important source of balanced protein in the human diet, the present study was carried out to determine the chemical composition of fish samples and to evaluate the bioaccumulation of selected heavy metals in this fish species collected from Chirackal mangrove area of Ernakulam District, Kerala.

Materials and Methods

Sample collection and preservation

Five species of fishes namely, *Tilapia mossambica, Glossogobius giuris, Anabas testudineus, Mystus seenghala*, and *Penaeus indicus* were collected from Chirackal (situated between 9.927658º N and 76.255159º N) mangrove creeks, Ernakulam District, Kerala. The period of observation and collection extended from February 2015 to January 2017. In order to present the data, the period from February 2015 to January 2016 has been considered as the first year and the period from February 2016 to January 2017 has been treated as the second year of study. The monthly data obtained has been pooled for three seasons *ie*, pre monsoon (February to May), Monsoon (June to September) and post monsoon (October to January). The samples were collected and transported to the laboratory within 5–6 h in ice packed condition in a storage box. The specimens were thawed at room temperature, washed with deionized distilled water. The samples were dried at 105 °C until they reach a constant weight. The dried samples were homogenized to fine powder using ceramic mortar and pestle and stored in -20 °C for heavy metal analysis.

Determination of Proximate composition of Samples

The proximate chemical composition of the fish muscles (moisture, protein, carbohydrates and lipids) was determined according to the standard analytical procedures of AOAC (2005).

Digestion of samples for Heavy metal determination

One gram of each of the ground fish tissues were transferred to a porcelain basin and put into a muffle furnace at a temperature of 550 °C for 5 h. Samples were digested with 10 ml tri-acid mixture (HNO_3: $HClO_4$:H_2SO_4) in a ratio of 6.5:6:2. The samples were heated at 105 °C until a clear colourless solution was obtained. The digested samples were allowed to cool and then diluted to 100 ml with deionized distilled water and then filtered through Whatman filter paper No. 42 and the filtrate was diluted to 100 ml with deionized water for the determination of heavy metal (Fe, Cu, Zn, Mn, Cd, Co, Ni, Pb, Cr) concentration by ICP-AES. Heavy metals concentrations were expressed as µg/g dry weight (AOAC, 2005).

Statistical analysis

Two way analysis of variance (ANOVA) was carried out to check the significant differences in heavy metal analysis between samples for two years of study. Correlation analysis was carried out to determine the positive and negative correlation between metals and samples during the course of study. The data was standardized to produce a normal distribution of all variables. All the statistical analyses were carried out using Statistical Package for Social Sciences (SPSS 20.0, 2014) statistical software packages.

Results

Proximate composition of Samples

The chemical composition of the muscles of fishes (as a percentage based on the fresh weight) is shown in Table 1. Overall, significant differences (p<0.05) were observed in the chemical composition of fish muscles between the three seasons. The highest moisture content (71.72%) was observed in *A. testudineus* during pre-monsoon followed by post monsoon (68.6%) in *T. mossambica* without a significant difference. The lowest average moisture content was observed in *G. giuris* muscles during monsoon (22.11%). Analysis of fish muscles showed that the highest protein value was observed in *P. indicus* samples collected during post monsoon (70.42 mg/g), and the lowest was shown during monsoon in *A. testudineus* (28.48 mg/g). There was a significant decrease in the protein content of fish muscles during monsoon season. *M. seenghala* showed the highest value of carbohydrates (71.06 mg/g) during pre-monsoon. The lipid content of fish muscles also differed significantly (p<0.05) between the various fish samples. The highest value (51.17%) was observed in *G. giuris* collected during monsoon, whereas the lowest value (18.44 mg/g) was found during pre-monsoon in *T. mossambica* samples.

Table 1: Proximate Chemical composition of fish samples collected from Chirackal

Fish Species	Seasons of Collection	Moisture %	Protein (mg/g)	Carbohydrates (mg/g)	Fat (mg/g)
T. mossambica	Pre Monsoon	41.47±0.002	62.19±0.02	51.07±0.04	18.44±0.02
	Monsoon	53.69±0.04	46.44±0.04	29.62±0.02	37.03±0.04
	Post Monsoon	68.36±0.04	55.93±0.04	34.16±0.04	25.79±0.02
G. giuris	Pre Monsoon	50.69±0.02	66.27±0.002	37.27±0.04	24.77±0.02
	Monsoon	22.11±0.04	39.75±0.02	48.36±0.002	51.17±0.002
	Post Monsoon	33.06±0.02	51.94±0.04	39.81±0.02	29.73±0.02
A. testudineus	Pre Monsoon	71.72±0.002	65.13±0.04	54.28±0.04	23.51±0.02
	Monsoon	29.41±0.02	29.48±0.005	31.37±0.013	28.37±0.004
	Post Monsoon	36.47±0.02	51.27±0.03	39.66±0.002	34.83±0.05
M. seenghala	Pre Monsoon	29.36±0.001	61.37±0.04	71.06±0.04	18.94±0.04
	Monsoon	42.55±0.04	29.77±0.003	48.33±0.012	27.11±0.001
	Post Monsoon	26.32±0.02	47.42±0.04	52.94±0.04	37.41±0.04
P. indicus	Pre Monsoon	30.14±0.04	62.66±0.04	63.07±0.04	19.63±0.001
	Monsoon	38.26±0.02	49.31±0.02	29.62±0.02	24.51±0.002
	Post Monsoon	39.08±0.002	70.42±0.04	59.77±0.002	37.44±0.04

(Each data represents Mean±SD, Mean value is the average of two years. Percentage value based on the fresh weight).

Heavy metals in fish Samples

The concentrations of heavy metals Fe, Cu, Zn, Mn, Cd, Co, Ni, Pb and Cr in various fish samples were showed in Figures and Tables.

Iron (Fe) Concentration (µg/g dry weight)

The mean Fe concentration recorded high in the soft tissues of *T. mossambica* was 71.37 µg/g and 68.87 µg/g during post monsoon and pre monsoon for the first and second years of study. The values reported are 25.82 µg/g and 68.87 µg/g in pre monsoon, 62.48 µg/g and 36.82 µg/g in monsoon and 71.37 µg/g and 49.85 in post monsoon seasons. The mean concentration of Fe in *G. giuris* recorded as 17.45 µg/g and 38.87 µg/g, 27.07 µg/g and 41.77 µg/g, 36.31 µg/g and 53.28 µg/g during pre-monsoon, monsoon and post monsoon respectively. In *A. testudineus* at Chirackal, Fe showed its maximum concentration during post monsoon (51.73µ g/g) for the first year. The various levels of Fe content recorded in *A. testudineus* was 26.75 µg/g and 46.34 µg/g, 29.18 and 51.73 µg/g, 35.98 µg/g and 49.62 µg/g for pre monsoon, monsoon and post monsoon respectively. The Fe concentration in *M. seenghala* reported high (63.71 µg/g) in the first year of post monsoon. First year of monsoon showed a slight variation in Fe content (31.44 µg/g and 34 µg/g).

During monsoon the values reported was 30.06 µg/g and 34 µg/g and 57.42 µg/g and 63.7 µg/g for post monsoon. In the soft tissues of *P. indicus*, the concentration of Fe was comparatively high (73.13 µg/g) during post monsoon in the first year. The values recorded are 50.83 µg/g and 61.07 µg/g, 23.13 µg/g to 43.55 µg/g and 28.54 and 73.13 µg/g during pre-monsoon, monsoon and post monsoon respectively.

Copper (Cu) Concentration (µg/g dry weight)

In Chirackal, the mean Cu levels recorded maximum for the soft tissues of *T. mossambica* was 10.21 µg/g to 15.12 µg/g during post monsoon for two years of study. The mean values reported are 1.37 µg/g and 3.06 µg/g in pre monsoon and 3.03 µg/g and 9.36 µg/g in monsoon. The mean Cu value recorded in *G. giuris* as 3.36 µg/g and 5.11 µg/g, 1.93 and 2.68 µg/g, 6.86 and 13.24 µg/g in pre monsoon, monsoon and post monsoon respectively. In *A. testudineus* the values for Cu showed its maximum during post monsoon (11.08 µg/g) during the second year. The various levels of Cu content recorded as 2.96 µg/g and 4.34 µg/g, 1.98 µg/g and 5.03 µg/g for pre monsoon and monsoon respectively. Cu concentration in *M. seenghala* reported high (14.26 µg/g) in the second year of post monsoon. First year of monsoon also reported high Cu content (7.06 µg/g). The values observed as 3.78 µg/g and 5.36 µg/g, 2.85 µg/g and 7.06 µg/g, 3.74 µg/g and 14.26 µg/g during pre-monsoon, monsoon and post monsoon respectively. The concentration of Cu in the soft tissues of *P. indicus* was comparatively high during pre-monsoon in the first year of study. The mean values reported as 2.17 µg/g and 51.04 µg/g, 2.03 and 3.21 µg/g, 6.37 µg/g and 9.5 µg/g during pre-monsoon, monsoon and post monsoon respectively.

Zinc (Zn) Concentration (µg/g dry weight)

The mean Zn concentration in *T. mossambica* reported as 3.65 µg/g and 7.31 µg/g during pre-monsoon, 3.03 µg/g and 9.36 µg/g during monsoon, 13.45 µg/g and 15.08 µg/g during post monsoon respectively. In *G. giuris* the mean Zn concentration recorded was 2.32 and 9.25 µg/g, 1.28 and 6.35 µg/g, 8.68 µg/g and 9.84 µg/g during pre-monsoon, monsoon and post monsoon respectively. The concentration of Zn in *A. testudineus* was 3.16 µg/g and 4.03 µg/g during pre-monsoon, 3.58 µg/g and 7.24 µg/g for monsoon, 11.29 µg/g and 24.06 µg/g for post monsoon. Maximum Zn concentration in *M. seenghala* was 12.25 µg/g in the first year during post monsoon. In pre monsoon and monsoon maximum Zn content was 10.35 µg/g and 11.1 µg/g during the second and first year of the study. In *P. indicus* post monsoon showed maximum Zn concentration (9.72 µg/g and 19.07 µg/g) and minimum was during pre-monsoon (4.22 µg/g and 6.13 µg/g). In monsoon, the concentration reported was 2.84 µg/g to 7.47 µg/g.

Manganese (Mn) Concentration (µg/g dry weight)

Average Mn concentration in *T. mossambica* was higher than all other fish samples with average value of 35.37 µg/g followed by *P.indicas* (31.87 µg/g), *A. testudineus* (26.95 µg/g) and *M. seenghala* (25.53 µg/g) during post monsoon in the second year of study. In *T. mossambica* Mn content reported was 4.3 µg/g and 7.15 µg/g, 14.42 µg/g and 19.06 µg/g, 19.88 µg/g and 35.37 µg/g during pre-monsoon, monsoon and post monsoon respectively. The highest concentration of Mn in *G. giuris* reported as 20.54 µg/g and 7.31 µg/g as lowest concentration during post monsoon and pre-monsoon in the second year of study. The values during pre-monsoon was 7.31 µg/g and 14.25 µg/g, 7.38 µg/g and 9.65 µg/g for monsoon, 12.53 µg/g and 20.54 µg/g for post monsoon. Mean Mn concentration reported in *A. testudineus* was between 5.52 µg/g and 26.95 µg/g with maximum during post monsoon and minimum during pre-monsoon in the second year of study. During pre-monsoon, mean concentration was 5.52 µg/g and 19.42 µg/g. During monsoon, mean concentration was 8.04 µg/g and 11.62 µg/g and in post monsoon the concentration was 18.64 µg/g and 26.95 µg/g.

The Mn concentration in the tissues of fish samples in Chirackal are in the decreasing order were *T. mossambica* > *P. indicus* > *A. testudineus*> *M. seenghala* > *G. giuris* with average values of 4.3 µg/g and 35.37 µg/g, 4.06 µg/g and 31.87 µg/g, 5.52 µg/g and 26.95 µg/g, 5.38 µg/g and 25.53 µg/g, 7.31 µg/g and 20.54 µg/g respectively.

Cadmium (Cd) Concentration (µg/g dry weight)

The distribution of Cd in *T. mossambica* varied with seasons. Effect of season on the distribution pattern of Cd was very much pronounced. Mean Cd content in whole *T. mossambica* was 0.22 µg/g and 0.87 µg/g during pre-monsoon, 0.37 µg/g and 0.83 µg/g during monsoon, 0.97 µg/g and 1.42 µg/g during post monsoon. Elevated levels of Cd was found during post monsoon than pre monsoon and monsoon in the soft tissues of *T. mossambica* for two years. It was pre monsoon < monsoon< post monsoon in the first year and monsoon < pre monsoon < post monsoon in the second year. In *G. giuris*, high Cd concentration was reported during post monsoon (1.87 µg/g) in the second year. The difference in the range of Cd concentration between pre monsoon and monsoon season was 1.03 to 1.12 µg/g and 0.51 to 0.62 µg/g respectively. Cd content of *A. testudineus* reported as 0.62 µg/g and 0.63 µg/g during pre-monsoon, 0.25 µg/g and 1.94 µg/g during monsoon, 0.83 µg/g and 1.05 µg/g during post monsoon. In *M. seenghala* the value recorded as 0.67 µg/g and 1.07 µg/g during pre-monsoon, followed by 0.42 µg/g and 2.04 µg/g in monsoon, 1.07 µg/g and 1.73 µg/g in post monsoon. Cd content in *P. indicus* was 0.92 µg/g and 1.34 µg/g during pre-monsoon followed by 0.46 µg/g and 1.48 µg/g, 0.83 µg/g and 0.98 µg/g during monsoon and post monsoon respectively.

Cobalt (Co) Concentration (µg/g dry weight)

The lowest and highest values of Co in *T. mossambica* were 2.73 µg/g and 25.11 µg/g during monsoon and post monsoon respectively. During post monsoon, the values reported as 12.78 µg/g to 25.11 µg/g. In *G. giuris* the highest value 1.07 µg/g to 20.06 µg/g, 3.64 µg/g to 27.39 µg/g in *A. testudineus*, 3.78 µg/g to 24.33 in M. *seenghala* and 5.36 µg/g to 20.39 µg/g in *P. indicus.* The average concentration of Co in *A. testudineus* was higher than other species with value of 27.39 µg/g followed by *T. mossambica* with 25.11 µg/g and *M. seenghala* with 24.33 µg/g during post monsoon season in the second year of study.

Nickel (Ni) Concentration (µg/g dry weight)

The mean Ni levels recorded for the soft tissues of *T. mossambica* were 1.07 µg/g and 19.72 µg/g in pre monsoon, 4.04 µg/g and 11.09 µg/g in monsoon. The monthly Ni levels varied moderately in different months and the highest value (28.43 µg/g) was recorded in the second year of study. The Ni content in *G. giuris* reported as 1.31 27 µg/g and 27.07 µg/g, 2.07 µg/g and 8.27 µg/g, 18.19 µg/g and 23.98 µg/g during pre-monsoon, monsoon and post monsoon respectively. In *A. testudineus* maximum Ni concentration reported during post monsoon (15.08 µg/g) in the second year. The various levels of Ni content recorded was 5.35 µg/g and 13.34 µg/g, 2.86 µg/g and 5.25 µg/g for pre monsoon and monsoon respectively. Ni concentration in *M. seenghala* reported high (24.22 µg/g) in the second year of pre monsoon. Second year of monsoon also reported high Ni content (18.28 µg/g). In the soft tissues, the mean concentration reported as 2.43 µg/g and 24.22 µg/g, 14.96 g/g and 18.28 µg/g, 6.43 µg/g and 9.88 µg/g during pre-monsoon, monsoon and post monsoon respectively. The concentration of Ni in the soft tissues of *P. indicus* was comparatively high (28.43 µg/g) during post monsoon. The mean concentration reported as 4.82 µg/g and 16.34 µg/g 9.42 µg/g and 10.27 µg/g, 9.55 µg/g and 28.43 µg/g during pre-monsoon, monsoon and post monsoon seasons (Table 6).

Lead (Pb) Concentration (µg/g dry weight)

The average concentration of Pb in the tissues of *T. mossambica* during pre-monsoon is 2.88 µg/g to 3.21 µg/g. 2.14 to 4.26 µg/g and 1.96 µg/g to 3.67 µg/g for monsoon and post monsoon respectively. Here the Pb concentration of Tilapia during post monsoon is higher than monsoon and pre monsoon. This may be due to difference of feeding habits of the species, Tilapia fish feeds mainly on phytoplankton. It was also found that Pb varied significantly between the sampling stations ($P<0.05$). The concentration of Pb in *G. giuris* reported as 1.08 µg/g and 5.62 µg/g, 0.97 µg/g and 1.87 µg/g and 0.87 µg/g and 4.68 µg/g during pre-monsoon, monsoon and post monsoon for two years of study respectively. These levels therefore constitute an immediate hazard to both aquatic fauna and human consumers. In the soft tissues of *A. testudineus,* the Pb content varied from 1.97 µg/g to 3.03 µg/g during pre-monsoon followed by 2.06 µg/g to 3.52 µg/g during monsoon and 0.57 µg/g to 3.03 µg/g during post monsoon.

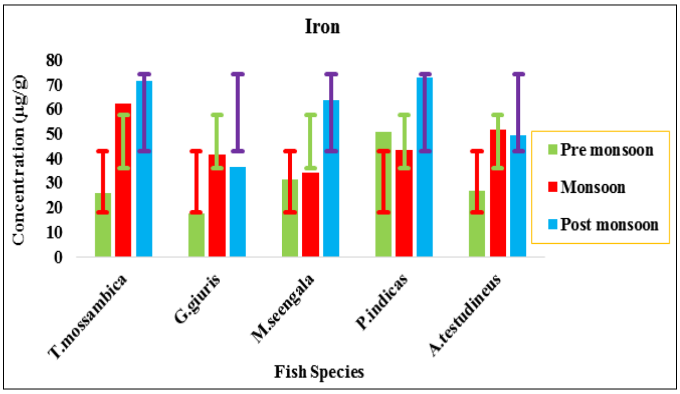
The concentration of Pb in *M. seenghala* recorded as 2.32 µg/g and 5.39 µg/g, 2.75 µg/g and 3.27 µg/g, 3.26 µg/g and 5.16 µg/g during pre-monsoon, monsoon and post monsoon for two years of study respectively. In *P. indicus* the mean concentration of Pb recorded was 2.07 µg/g and 3.95 µg/g during pre-monsoon season. In monsoon and post monsoon the mean Pb values reported were 2.37 µg/g and 3.58 µg/g, 1.36 µg/g and 4.15 µg/g.

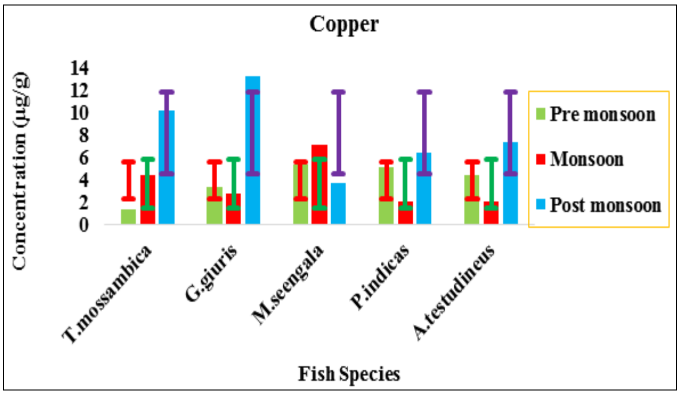
Chromium (Cr) Concentration (µg/g dry weight)

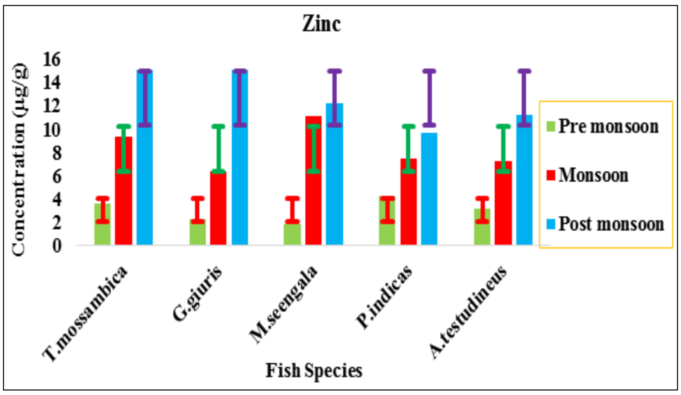
In Chirackal mangrove region, the maximum Cr levels recorded for the soft tissues of *T. mossambica* was 4.26 µg/g during post monsoon for the second year. The range of values reported are 0.64 µg/g and 1.877 µg/g in pre monsoon and 1.14 µg/g and 3.85 µg/g in monsoon.

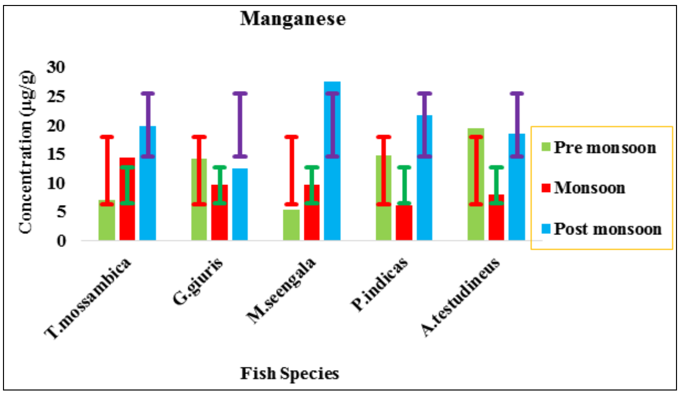
The highest concentration of Cr in *G. giuris* reported as 3.53 µg/g and 0.42 µg/g as lowest concentration during post monsoon and pre monsoon in the second and first years of study. The range of values during pre-monsoon was 0.42 µg/g and 0.97 µg/g, 0.48 µg/g and 2.32 µg/g for monsoon, 1.86 µg/g and 3.53 µg/g for post monsoon. Mean Cr concentration reported in *A. testudineus* was 0.74 µg/g and 4.97 µg/g with maximum during post monsoon and minimum during pre-monsoon in the first and second years of study. During pre-monsoon, mean concentration recorded as 0.74 µg/g and 2.05 µg/g. During monsoon, mean concentration was 1.58 µg/g and 2.73 µg/g and in post monsoon the concentration was 2.11 µg/g and 4.97 µg/g.

In Chirackal, mean Cr concentration reported in *M. seenghala* was between 1.05 µg/g and 7.17 µg/g with maximum during post monsoon and minimum during pre-monsoon in the first and second years of study. During pre-monsoon, mean concentration recorded as 1.05 µg/g and 1.63 µg/g. During monsoon, mean concentration was 2.12 µg/g and 2.52 µg/g and in post monsoon the concentration was 4.83 µg/g and 7.17 µg/g. The mean Cr concentration reported in *P. indicus* was 0.58 µg/g and 3.95 µg/g with maximum during post monsoon and minimum during pre-monsoon in the first years of study. During pre-monsoon, mean concentration recorded as 0.58 µg/g and 0.94 µg/g. During monsoon, mean concentration was 2.91 µg/g and 3.05 µg/g and in post monsoon the concentration was 3.05 µg/g and 3.96 µg/g.









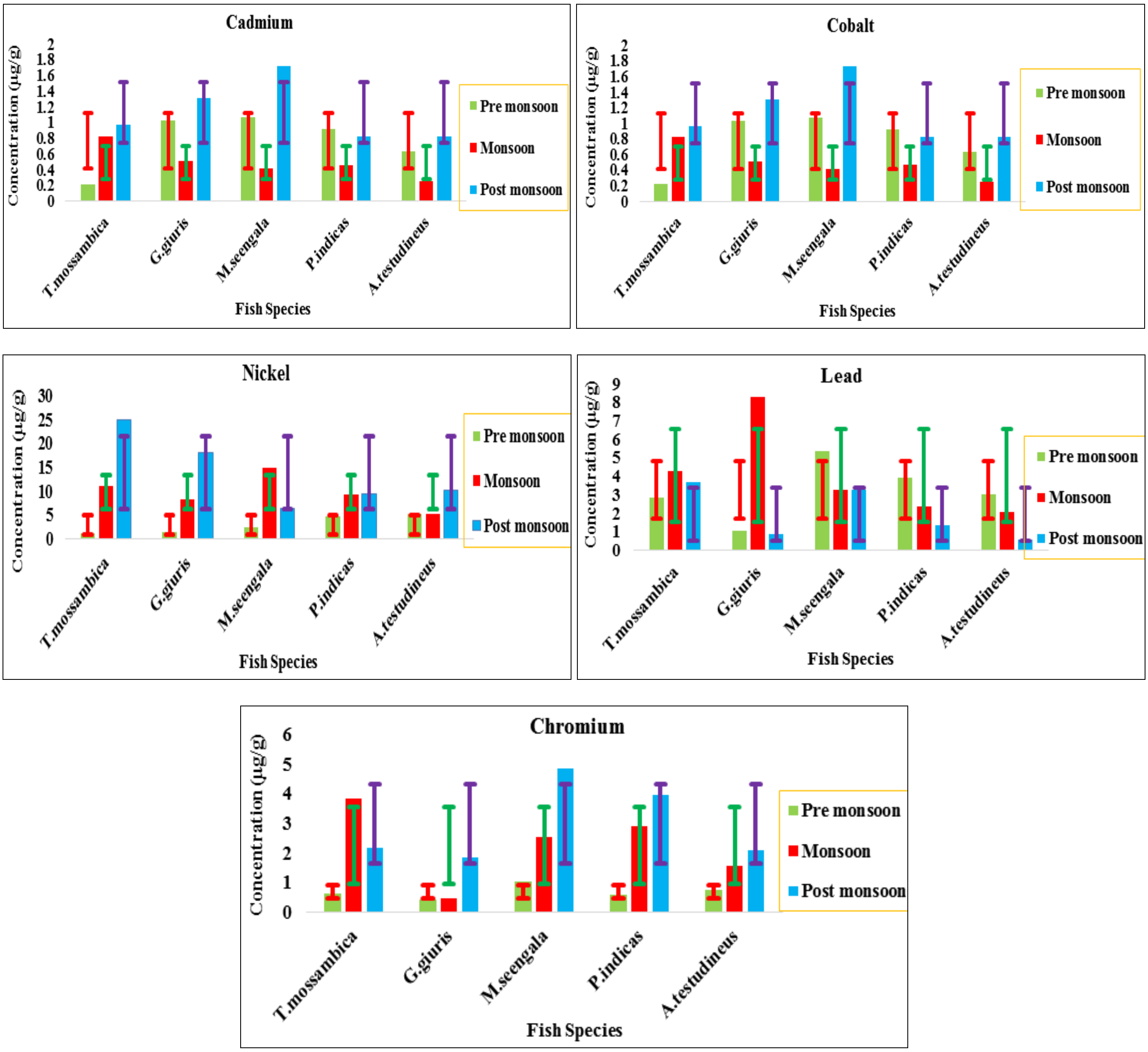


Fig 1-9: Seasonal Variations of heavy metal concentrations in the fish samples at Chirackal

Table 2: Seasonal Variations of heavy metals in *T. mossambica*

T. mossambica							
S. No.	**Metals**	**2015-16**			**2016-17**		
		Pre monsoon	**Monsoon**	**Post monsoon**	**Pre monsoon**	**Monsoon**	**Post monsoon**
1	Fe	25.82±0.01	62.48±0.531	71.37±0.006	68.87±0.476	36.82±0.006	49.85±0.026
2	Cu	1.37±0.01	4.36±0.01	10.21±0.185	3.06±0.012	3.86±0.006	15.32±0.01
3	Zn	3.65±0.01	9.36±0.006	15.08±0.093	7.31±0.006	3.03±0.02	13.45±0.01
4	Mn	7.15±0.01	14.42±0	19.88±0.01	4.29±0.041	19.06±0.015	35.37±0.015
5	Cd	0.22±0.01	0.83±0	0.97±0.01	0.87±0.01	0.37±0.01	1.42±0.01
6	Co	3.04±0.021	4.17±0	12.78±0.015	3.12±0.006	2.73±0.01	25.11±0.01
7	Ni	1.07±0.061	11.09±0.006	25.04±0.015	19.72±0.006	4.04±0.02	16.68±0.01
8	Pb	2.88±0.01	4.26±0	3.67±0.015	3.21±0.032	2.14±0.006	1.96±0.023
9	Cr	0.64±0.006	3.85±0.006	2.19±0.006	1.88±0.012	1.14±0.006	4.26±0.01

Table 3: Seasonal Variations of heavy metals in *G. giuris*

G. giuris							
S. No.	**Metals**	**2015-16**			**2016-17**		
		Pre monsoon	**Monsoon**	**Post monsoon**	**Pre monsoon**	**Monsoon**	**Post monsoon**
1	Fe	17.45±0.01	41.77±0.015	36.31±0.01	38.87±0.087	27.07±0.056	53.28±0.01
2	Cu	3.36±0.01	2.68±0	13.24±0.015	5.11±0.006	1.93±0.006	6.86±0.012
3	Zn	2.32±0.006	6.35±0	9.84±0.006	9.25±0.01	1.28±0.006	8.68±0.01
4	Mn	14.25±0.015	9.65±5.764	12.53±0.015	7.31±0.051	7.38±0.01	20.54±0.472
5	Cd	1.03±0.01	0.51±0	1.31±0.006	1.12±0.006	0.62±0.01	1.87±0.015
6	Co	4.57±0.01	3.02±0.006	16.32±0.01	8.52±0.01	1.07±0.064	20.06±0.049
7	Ni	1.31±0.006	8.27±0.01	18.19±0.01	27.07±0.017	2.077±0.068	23.98±0.01
8	Pb	1.08±0.01	0.97±0.006	0.87±0.015	5.62±0.015	1.87±0.02	4.68±0.01
9	Cr	0.42±0.006	0.48±0.007	1.86±0.006	0.97±0.015	2.32±0.017	3.53±0.012

Table 4: Seasonal Variations of heavy metals in *A. testudineus*

A. testudineus							
S. No.	**Metals**	**2015-16**			**2016-17**		
		Pre monsoon	**Monsoon**	**Post monsoon**	**Pre monsoon**	**Monsoon**	**Post monsoon**
1	Fe	26.75±0.02	51.73±0.01	49.62±0.01	46.34±0.17	29.18±0.06	35.98±0.01
2	Cu	4.34±0.139	1.98±0.01	7.32±0.01	2.96±0.01	5.03±0.006	11.08±0.01
3	Zn	3.16±0.01	7.24±0.012	11.29±0.28	4.03±0.006	3.58±0.578	24.06±0.01
4	Mn	19.42±0.1	8.04±0.01	18.64±0.02	5.52±0.303	11.62±0.6	26.95±0.01
5	Cd	0.63±0.01	0.25±0.017	0.83±0.015	0.62±0.01	6.94±11.54	1.05±0.015
6	Co	7.82±0.060	6.74±0.038	14.35±0.02	5.98±0.015	3.64±0.02	27.39±0.04
7	Ni	5.35±0.006	5.25±0.006	10.21±0.18	13.34±0.06	2.86±0.01	15.08±0.02
8	Pb	3.03±0.012	2.06±0.006	0.57±0.01	1.97±0.02	3.52±0	3.03±0.01
9	Cr	0.74±0.059	1.58±0.006	2.11±0.02	2.05±0.01	2.73±0.01	4.97±0.02

Table 5: Seasonal Variations of heavy metals in *M. seenghala*

M. seenghala							
S. No.	**Metals**	**2015-16**			**2016-17**		
		Pre monsoon	**Monsoon**	**Post monsoon**	**Pre monsoon**	**Monsoon**	**Post monsoon**
1	Fe	31.44±0.17	34.01±0.103	63.71±0.02	28.13±0.01	30.06±0.05	57.42±0.012
2	Cu	5.36±0.01	7.06±0.05	3.74±0.02	3.78±0.02	2.85±0.01	14.26±0.01
3	Zn	1.88±0.012	11.1±0.10	12.25±0.02	10.35±0.03	2.66±0.012	7.43±0.02
4	Mn	5.38±0.01	9.81±0.01	27.53±0.02	6.33±0.01	8.37±0.01	25.53±1.73
5	Cd	1.07±0.012	0.42±0.01	1.73±0.02	0.67±0.02	2.04±0.01	1.07±0.061
6	Co	12.63±0.025	3.78±0.02	21.34±0.02	11.45±0.02	13.67±0.03	24.33±0.02
7	Ni	2.44±0.021	14.96±0.01	6.43±0.01	24.22±0.01	18.28±0.02	9.88±0.01
8	Pb	5.39±0.05	3.27±0.01	3.26±0	2.32±0.01	2.75±0.01	5.16±0.034
9	Cr	1.05±0.01	2.52±0.012	4.84±0.01	1.63±0.01	2.12±0.02	7.17±0.015

Table 6: Seasonal Variations of heavy metals in *P. indicus*

P. indicus							
S. No.	**Metals**	**2015-16**			**2016-17**		
		Pre monsoon	**Monsoon**	**Post monsoon**	**Pre monsoon**	**Monsoon**	**Post monsoon**
1	Fe	50.83±0.06	43.55±0.02	73.13±0.015	61.07±0.015	23.13±0.015	28.54±0.01
2	Cu	51.04±0.02	2.03±0.075	6.37±0.012	2.16±0	3.21±0.006	9.5±0.049
3	Zn	4.22±0.01	7.47±0.006	9.72±0.006	6.13±0.006	2.84±0	19.07±0.01
4	Mn	14.72±0.01	6.13±0.006	21.84±0.006	4.06±0.006	14.63±0.03	31.87±0.01
5	Cd	0.92±0.1	0.46±0.006	0.83±0.006+	1.34±0.006	1.48±0.006	0.98±0.015
6	Co	8.75±0.021	5.36±0.01	17.47±0.02	6.87±0.01	7.97±0.02	20.39±0.072
7	Ni	4.82±0.01	9.42±0.012	9.55±0.01	16.34±0.015	10.27±0.32	28.43±0.01
8	Pb	3.95±0.04	2.37±0.006	1.36±0.006	2.08±0.015	3.58±0	4.15±0.006
9	Cr	0.58±0.02	2.91±0.006	3.96±0.006	0.94±0.015	3.05±0.012	3.05±0.01

Statistical Analysis

Analysis of Variance (ANOVA)

The statistical significance of annual and seasonal differences in trace metal concentration in fish samples were tested using two-way ANOVA. Results indicate that variability in concentrations of all metals was statistically significant (p<0.01 and p<0.05; Table 7) in fish samples. During all seasons, the annual variations of in trace metal concentrations in fishes were quite significantly different among the samples.

The two-way ANOVA for Fe revealed a significant variation between years and between seasons (p<0.05). The interaction between seasons and years showed high significance (p<0.05). Statistical analysis by two-way ANOVA proved significant variations in the concentration of Cu, Zn and Mn between years and between seasons (p<0.05). Significant variation was observed in the interaction between years and seasons (p<0.05). Statistical analysis by two-way ANOVA proved significant variations in Cd and Co between seasons (p<0.05). Significant variation was negligible in the interaction between years and seasons. Two-way ANOVA for Ni showed high significant variations between seasons and between samples ($p<0.05$). Pb and Cr showed significant variations in the interactions between seasons and samples ($p<0.05$) and the significance was negligible in the interaction between years and seasons.

Table 7: P values of heavy metals in Fish samples at Chirackal

	Sample Wise		Year Wise		Season Wise	
Metals	**F Value**	**P Value**	**F Value**	**P Value**	**F Value**	**P Value**
Fe	5.069	0.001	9.068	0.003	15.400	0.000
Cu	2.250	0.066	0.045	0.832	5.593	0.004
Zn	1.045	0.385	0.446	0.505	70.162	0.000
Mn	0.858	0.490	1.124	0.290	97.834	0.000
Cd	0.418	0.795	5.507	0.020	0.285	0.752
Co	4.825	0.001	8.182	0.005	159.24	0.000
Ni	2.016	0.094	50.812	0.000	15.156	0.000
Pb	0.828	0.509	8.356	0.004	5.276	0.006
Cr	2.202	0.071	0.185	0.668	79.69	0.000

Pearson Correlation Coefficient Matrix

Possible associations among the metals in the muscle tissue of fish samples during pre-monsoon, monsoon and post monsoon were assessed by means of Pearson's correlation coefficients and is represented in Table 8. Statistically significant correlations at 0.01 level of significance were observed in Fe with Zn and Cr (r = 0.298 and r = 0.366). Cu showed a statistically significant correlation at 0.01 and 0.05 level with Mn (r = 0.293) and Co (r = 0.272). The metals like Mn (r = 0.594), Co (r = 0.664), Ni (r = 0.586) and Cr (r = 0.490) were highly correlated with Zn. Co content in fishes showed significant correlations for Ni (r = 0.387, $p<0.01$), Cr (r = 0.687, $p<0.01$).

Table 8: Pearson Correlation Matrix of heavy metals in Fish samples at Chirackal

	Fe	**Cu**	**Zn**	**Mn**	**Cd**	**Co**	**Ni**	**Pb**	**Cr**	**Year**	**Season**
Fe	1										
Cu	0.163	1									
Zn	0.298**	0.132	1								
Mn	0.184	0.293**	0.594**	1							
Cd	-0.062	0.001	-0.047	0.039	1						
Co	0.243*	0.272**	0.664**	0.756**	0.030	1					
Ni	0.227*	0.003	0.586**	0.152	-0.030	0.387**	1				
Pb	0.061	0.193	0.066	0.087	0.100	0.183	0.245*	1			
Cr	0.366**	0.040	0.490**	0.618**	0.095	0.685**	0.157	0.264*	1		
Year	-0.142	-0.125	0.053	0.076	0.168	0.178	0.410**	0.228*	0.260*	1	
Season	0.042	0.216*	-0.020	-0.099	-0.107	0.112	0.148	0.146	-0.311**	0.000	1

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Discussion

Proximate body composition is the analysis of moisture, fat, protein, carbohydrate and ash content of the fish and is a good indicator of its physiological condition and health (Saliu *et al*., 2007) [49]. The present study has shown changes in the chemical composition of fish samples of Chirackal which are related to the seasons. It shows similar results with that of Javaid *et al.* (2018) [24] and Oliveira *et al*. (2003) [39]. Reports indicated that in different environmental conditions, the body composition of the same fish may change in relation to differences in water quality, feeding conditions, sex, state of maturity and the period which the fish was captured. Saeed (2018) [48] reported that the best condition and proximate body composition of fish (*Oreochromis aureus* and *Tilapia zillii*) were recorded in the spring and winter seasons with an increase in protein and fat and a decrease in water content.

The study of heavy metal concentrations in fishes were important with respect to human consumption of fish. Several studies showed that heavy metal concentrations in tissue of coastal fishes vary considerably among different species. This was possibly due to the differences in metabolism and feeding patterns of the fishes. Fish is among the dominant bio indicator species used for acute toxicity assay of pollutants such as heavy metals. The rapid development of industries and agricultures have promoted the increase of environmental pollution although heavy metals in aquatic system can be naturally produced by slow leaching from rocks and soil into water which occur at low levels. Cd and Pb are among the aquatic metal pollutants which usually present at significant level in water system which pose high toxicities on the aquatic organisms (Zhou *et al*., 2008; Jose *et al*., 2014) [59, 26]. Fe, Zn and Pb levels in the fishes collected from Cuddalore was higher than the levels reported by Thiyagarajan *et al.* (2018) [55]. This is due to the industrial activities and anthropogenic introduction of pollution resulted from local people. Increased levels of Cu, Zn, Fe and Mn in gills and alimentary canal compared to the muscle were reported from fishes of Cochin area (Maheswari *et al*., 2017) [33]. Mn is recognized as an essential trace element for humans and several of their metabolic roles have been well established (Mendil and Uluozlu, 2007) [35].

The tissue accumulation of metals in aquatic animals can reflect the past exposure (Dural *et al.*, 2007) [14]. Higher concentrations of metals found in gill tissue of fish species. Ahmed *et al*. (2016) [3] investigated the heavy metal concentration in fish and oyster from the Shitalakhya River, Bangladesh and found seasonal variation of Ni, Cr, Cd, Pb and Cu. Haque *et al*. (2006) [23] studied the seasonal variation of heavy metal concentrations in *Gudusia chapra* inhabiting the Sundarban mangrove forest and found the concentration of Cu, Zn, Fe, Pb, Cd, Cr and Ni seasonally. The increase of heavy metals concentration in fish tissues from aquatic environment is a good indicator of pollution status of the environment (Kalyoncu *et al*., 2012, Rejomon and Joseph, 2013; Ali and Abdel, 2015) [29, 45, 4]. The present study investigated the concentration of heavy metals (Fe, Cu, Zn, Mn, Cd, Co, Ni, Pb and Cr) in fishes from Chirackal mangrove area. The results of this study, demonstrated that all the heavy metal species investigated have been detected to some extent.

Fe is considered as essential metal because of its biochemical and physiological role in blood cells and haemoglobin synthesis and cofactor of many enzymes (Gorur *et al*., 2012; Edward *et al*., 2013) [21]. High amount of Fe above the physiological level in living organisms may result in iron overload (Stancheva *et al*., 2014) [52]. The source of this pollution could have resulted from discharge of industrial effluents and municipal wastes, geology of river bed and catchment area (Bijoy *et al*., 2013; Ciji and Bijoy, 2014; Dhaneesh *et al*., 2015) [10, 11, 13].

Cu is an essential element play vital role in enzymes activity and is necessary for the synthesis of haemoglobin (Dhaneesh *et al*., 2012) [12]. These higher accumulations of essential metals such as Cu in the tissues may be attributed to the binding proteins such as metallothionines and its role in storage, metabolism and detoxification which may increase its tendency to accumulate essential metals at higher concentrations (Kaladharan *et al*., 2015)[28]. When accumulated to higher amounts it cause health hazards to both animals and humans (Bashir *et al*., 2018) [8]. Thus, our data clearly demonstrated that concentrations of Cu in fish tissues were significantly higher than maximum permissible limits set by the Joint FAO/ WHO committee (FAO, 2007; WHO, 1989) [8].

Zn is the essential element for both human and aquatic organisms and they showed productive activity against Cd and Pb toxicity in biological organisms (Ardakani and Jafari, 2014) [6]. Zinc being an essential metal, is required in certain amount for normal metabolic functions and is involved in many cellular processes either as structural component of regulatory proteins or catalytic part of enzymes. When in excess amounts, Zn can be toxic to all living organisms including fish (Ardakani and Jafari, 2014) [6]. Our data showed quite variations in mean concentration of Zn in the studied fish tissues as well as sampling sites.

Mn is an essential metal, and low level is necessary for human health, however, excess amount can induced oxidative stress and toxic effects in aquatic organisms (Vieira *et al*., 2011) [56]. In this study, Mn concentration fluctuates between organs and the locations of sample collections. Cd exposure is regarded as being lethal and capable of producing chronic lung disease and testicular degeneration (stancheva *et al*., 2013) [51]. Our results revealed that Cd was detected only in the gills from both locations of sample collection. This abnormal high level of heavy metals in fish tissues could be attributed to the frequent crude oil spills as well as other industrial discharge around the region particularly, Finima river which contained highest concentrations of all the metals investigated. The concentrations of some of the heavy metals in fish organs analysed were above the recommended maximum permissible limits set by the joint FAO/WHO standards. Manganese can lead to a variety of psychiatric and motor disturbances, termed manganism which has occurred in people employed in the production and processing of manganese alloys (Ranjitha and Sujatha, 2011) [44]. Adoption of adequate measures to remove the heavy metal load from the industrial waste water and renovation of sewage treatment plants are suggested to avoid further deterioration of the aquatic ecosystem quality (Bashir *et al*., 2018) [8].

The highest concentration of Co is mainly due to increased boating activities, recurrent usage of antifouling paint, oil dropping from boats and commercial fishing activity in the study area. Co showed wild array of essential role in haemoglobin biosynthesis (Bebbington *et al*., 1997) [9] and also causes adverse effects of liver and kidney damage. Various studies showed the concentration of Co are as follows: Bighead carp (2.06 ppm), Mandarin fish (0.79 ppm) from Pearl River Delta (PRD), China (Leung *et al*., 2014) [22], *Channa striata* (0.24 ppm), Catla catla (6.45 ppm), *Oreochromis mossambicus* (3.69 ppm), *Etroplus suratensis* (3.55 ppm), *Mystus vittatus* (1.80 ppm) and *Cirrhinus mrigala* (0.46 ppm) from Cauvery delta region, India (Dhanakumar *et al*., 2015) [46].

Coetzee *et al*. (2012) [30] reported that kidney and gills of *Clarias gariepinus* and *Labeo umbratus* showed high degree of metal accumulating behaviour and the present study was disagreed. Hence, it was confirmed that the metal accumulation behaviour of fishes is depending not only the organs and species but also influenced by various environmental parameters.

Fish are known to accumulate Ni in tissues when exposed to elevated levels of environment (Papanikolaou, 2008) [38]. Excess Ni can cause variety of pulmonary adverse health effects, such as lung inflammation, fibrosis, emphysema and tumors (Palanichamy and Rajendran, 2010) [47]. The high levels of Ni is a result of waste from rivers, municipal and domestic wastes (Solai *et al*., 2010) [50]. As a result, the consumption of fish from these water bodies is risks contracting the Ni related illness where nickel sulphide fume and dust is believed to be carcinogenic with industrial Ni causing cancer of the respiratory tract and dermatitis (Jiya *et al*., 2017) [25]. These concentrations were above the WHO recommended limit which, constitute immediate health hazard to the people consuming fish and water. Based on Ni concentrations, it was recommended that control and monitoring of the heavy metal was necessary.

Pb is a nonessential element for living organism and also it possess various adverse effects such as neuro and nephro toxicity, rapid behavioural malfunction, and decreases the growth, metabolism, and survival rate, alteration of social behaviour in some mammals (Garcia *et al*., 2015) [20]. This study evidenced that the major accumulation of Pb was in kidney of the fishes (Abdel *et al*., 2011) [2]. Rashed (2001) [32] found that elevated Pb level in fishes obtained from fresh water ecosystem affected by extended agriculture, poultry forms, textile, industrial and other activities. So the sediments could be the major sources of Pb contamination and the bottom feeders may directly affects with this deposited element in consequence to their feeding habitat (Sarkar *et al*., 2016) [53]. From the literature survey of Papanikolaou *et al*. (2005) [38] the half-life of lead in blood and soft tissues have short period. It may use as a biomarker of resent lead contaminant on polluted environment and it cause longer chronic effect in children. Fishes have the ability to accumulate Cd in various parts of the body tissues, especially in the kidney and liver, adjacent to other tissues like muscle and skin (El-Nemr, 2008) [1]. Pb did not show any significant biological activity towards living organism, even when it was toxic at tracer level (Robert, 2001) [19] which damages the kidney, testicular and blood cells (Gupta and Mathur, 1998) [7].

The amount of Cr in tissues of various shrimp species is due to anthropogenic activities in the aquatic environment. The considerable variations in Cr bioaccumulation may be the due to the difference in their feeding habits. Even Cr concentrations have harmful effects on reproduction, fertilization in aquatic animals (Sarkar *et al*., 2016) [53] the study of Nurjanah *et al*. (2015) [37] reported that the banana puffer fish from West Java having higher concentration of Cr in internal organs than skin and muscle (Makedonski *et al*., 2015) [31].

The metals Cu, Pb and Cd exceeded the maximum limit recommended by international agencies because of the uncontrolled anthropogenic activities in the study area. There are numerous literatures reported that the sediment and water from south east coast has elevated level of heavy metals when compared to permissible limit (Ajeeshkumar *et al*., 2015) [27]. Developmental activities along the coastal area are the prime source for elevated level of heavy metals including large scale industries, thermal power plants, chemical and fertilizer industries, textile mills (Sankar *et al*., 2017) [42] and also attributed by municipal wastes, mining wastes, aquaculture and agricultural discharges (Dhinesh *et al*., 2016) [40]. The source for Zn is mainly influenced by effluents from the metal processing, paint and pigment industries, Cd from both point and non-point source (Malik *et al*., 2010; Dhanakumar *et al*., 2015) [36, 46], Cu from number of deteriorated boats in the boatyard (Rajaram *et al*., 2013) [41], copper smelter industry, petrochemicals and Tuticorin port trust are the major source of metals like Pb, Cu and Zn (Abukashim *et al*., 2014) [15].

Conclusion

The study proved that the body composition of the same fish species vary depending on changes in seasonal variations. The results indicated that the metal content in fish muscle varies depending on the area of study and the period of catching. Despite the bio concentration the samples are an efficient bio-indicator of heavy metals. In this study, there is health risk through an exposure of consumption of certain fish. Additionally the results obtained for the elements in analysed fish species were above the acceptable limits for human consumption.

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