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AN INVESTIGATION ABOUT BIOCHEMICAL COMPONENTS OF MUSCLE AND SOME VISCERAL ORGANS IN *Labeo rohita* (Hamilton, 1822) CAUGHT FROM TWO DIFFERENT WETLANDS IN INDIA

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author NK designed the study, performed experimental work, data analysis, written the protocol and final draft of the manuscript. Review, corrections and checking before final submission were done by author OSB. Both authors read and approved the final manuscript.

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ABSTRACT

The present study, it was aimed to examine and compare the total lipids, total proteins, carbohydrates, moisture and ash level in muscle and visceral organs of fish, *Labeo rohita* collected from two different wetlands in India seasonally. Simultaneously, the effect of industrial pollution on the health of fish has been scrutinized during the current research. The fish was collected during different seasons: rainy (July - September), autumn (September - November), winter (December - February), spring (March - mid April) and summer (Mid April - end June). In muscle, the higher average value of lipid (7.58±0.57%), protein (0.54±0.08%), carbohydrate (8.45±1.14%) and ash (3.62±0.20%) during all the seasons was reported in the control fish sample than test fish sample. Similarly, the higher mean value of lipid, protein and ash was found 8.58±0.46%, 0.91±0.23% and 2.84±0.11%, respectively, in control fish sample as compared to test fish sample. Furthermore, the maximum average value of lipid (7.76±0.36%), protein (0.37±0.07%) and carbohydrate (3.16±0.68%) was observed in the intestine of control fish sample than test fish sample. Biochemical parameters of muscle and visceral organs were decline significantly ($P<0.05$) in test fish samples than control fish. The decline in nutritional quality of the fish was caused by accumulation of industrial runoff because Harike wetland receives polluted water from the river Sutlej (Polluted water of Ludhiana city is being carried by the rivulet called Buddha Nullah and drained into the river Sutlej from left side) and another drain called Kala Sanghian carried polluted water from the Jalandhar district, Punjab and discharged into the river from right side. Protein concentration increased in the liver, muscle and intestine (autumn) of test fish samples during the present investigations. Increased production of total protein contents observed due to the acceleration of protein synthesis under the influence of toxicants in

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response to combat the stress situations. The increase or decrease in the values of carbohydrates, moisture and ash (%) in the muscle and visceral organs of test fish sample in contrast to control fish sample, proved the adverse impact of aquatic effluence on the health of fish. In conclusion, it is recommended that, possible measures should be taken to minimize the level of pollutants in this wetland.

Keywords: Aquatic pollution; *Labeo rohita*; biochemical changes; muscle; liver; intestine; bio-indicator; wetlands.

1. INTRODUCTION

Harike wetland is one of the most polluted wetland in Punjab. It is placed at the convergence of the rivers Sutlej and Beas after construction of barrage in 1952, it was meant to store water which was further provided for irrigation as well as consumption purposes toward major areas of Southern Punjab and its neighboring province Rajasthan. This wetland is surrounded by agricultural land from all sides. This wetland is having significant role in various aspects particularly in economic, scientific, ecological, socio - cultural in addition to recreational point of view. Furthermore, it holds up various plants, fishes and other faunal species which comes under threatened categories of red list of IUCN. The wetlands also attract huge population of avifauna during the winter season every year from places as far away as Siberia [1]. The Ludhiana city of Punjab is situated on the ridge of Buddah Nullah. With the increase in population pressure and rapid growth of industrialization has brought various environmental problems. The Buddha Nullah carried polluted water of the Ludhiana city and drains its polluted water into the Sutlej. Nearby this river, more than 1100 different industries are present which discharge their effluents rich water into the Harike wetland. Harike wetland also receives polluted water of industries from Jalandhar district through Kala Sanghian drain [2]. Due to discharge of polluted water in Harike wetland, it became highly polluted which posing a serious threats to the invaluable flora and fauna existing there [3]. On the other hand, Nangal wetland is enriched with diverse flora, fauna and hydrology indicating that it is free from pollutants. Shannon weaver index value was observed above two at all the studied sites of Nangal wetland represented that impact of pollution at this wetland was not recorded [4]. Shannon-weaver index is a combination of the number of species and the evenness of distribution of individuals among taxa. It may function as a sensitive indicator for pollution [5].

Alteration in fish biochemical parameters in reaction to various toxicants have been reported by investigators [6,7]. Research conducted on biochemical parameters of *Tor putitora* and observed that sufficient amount of total cholesterol, glucose, free amino acids and enzymatic activities have been decreased due to the effect of pollution [7]. Further study concluded that oil content and PUFAs (polyunsaturated fatty acids) has been decreased due to heavy metals effect [8]. The amount of EPA (eicosapentaenoic acid), DPA (docosapentaenoic acid), DHA (docosahexaenoic acid) and ALA (alpha-linolenic acid) has been drastically decreased in the fish, Nile tilapia samples collected from of Lake Victoria [9]. Biochemical profile can be advantageous to recognizing specific toxicity in organs in addition to physical condition of animals since it has been proved for depicting early alarms of probably destructive changes in strained life forms. Fish is an important food for millions of people having essential protein, minerals and micronutrients [10]. Thus, it is obligatory to study the pollution impact on fish health. Fish is rapid becoming the foremost resource of animal protein because of the strength along with lower price than meat, over the last decade. The demand for fish is also increasing to cater the food problems of large population as well as its high growth rate.

The goal of this research work was to monitor fish muscle, liver as well as intestine as bio-indicator of water pollution and its impact on the biochemical profile of *Labeo rohita* collected from Harike wetland (Polluted site) compared with Nangal wetland (Control site).

2. MATERIALS AND METHODS

Specimens of *Labeo rohita* (Hamilton, 1822) more than 500g was collected from the Nangal (n = 46) and Harike (n = 44) wetlands during different seasons i.e. rainy, autumn, winter, spring and summer (June, 2018 to June, 2019). Each fish was individually wrapped in a labeled and clean air-tight zip lock polythene bag and kept in the ice box. The samples were transported to the Lab and they will be stored in a freezer at -20^{0}C. The biometric measurements like, total length (cm), standard length (cm) and weight (g) were taken on the spot at collection site. Total length and standard length was measured using a measuring scale. Each fish sample was cleaned with tap water. Muscle and visceral organs (liver and intestine) removed from the fish and their weights were recorded. The muscles, livers and the intestine so collected from fish were then separately pooled together to form a composite samples of muscle, liver and intestine during every season. The entire process was preceded on ice and took approximate 10 minutes. The prepared composite samples of different organs were afterward preserved at -20°C in the freezer until analyzed.

The total lipid content (TLC) was done by Soxhlet lipid extraction/solvent extraction method [11]. Total soluble proteins in muscle, liver and intestine was estimated by method of Lowry method, 0.5 gram of frozen liver, muscle and intestine were processed as described before but homogenized in 4 ml PBS (Phosphate buffer saline) having pH = 7.4 [12]. For the estimation of moisture, accurately weighed samples (5g) were desiccated in an hot air oven at 100±2ºC, till constant weight was attained. The loss in weight was expressed in per cent moisture as recommended by A.O.A.C. [13]. Ash content was determined by placing the weighed samples in silica crucibles in muffle furnace at 525ºC for 4 hours as recommended by A.O.A.C. [13]. Carbohydrate content was determined by difference : Carbohydrate (%) = 100- [moisture(%) + proteins (%) + total lipids (%) + ash (%)] [14].

2.1 Statistical Analysis

All values were given as mean ± Standard error of mean. Statistical difference among the mean of biochemical parameters in different organs (muscle, liver and intestine) of control and test fish sample particularly were determined using one way and multifactor ANNOVA. The analysis was performed using Microsoft Excel and STATGRAPHICS statistical packages.

3. RESULTS AND DISCUSSION

Mean values of biometric measurements such as total length, standard length, body width with body weight of fish sampled from Nangal wetland (Control site) and Harike wetland (Heavily polluted) sites during different seasons are presented in Table 1. Maximum total length (cm) (41.8), standard length (cm) (34.5), body width (cm) (9.6) and highest body weight (kg) (1306.8) was observed in spring season at Nangal wetland, while during autumn season at Harike wetland (Table 1). The results depicted in Tables 2, 3 and 4 clearly reveal habitat and organ variation in terms of nutritional quality the fish samples collected from different wetlands.

The biochemical profiles of fish muscle, liver and intestine can be used as a stress indicator for aquatic life. The industrial runoff usually encloses abundant amount of dissolved and suspended solids, heavy metals, inorganic and organic chemicals, high biological oxygen demand and chemical oxygen demand, grease and oils, moreover lethal metals consequently, which pose injurious effects on the fish when released in to aquatic bodies. Lipids, proteins and carbohydrates are three main nutrients which are regarded as fish health biomarkers. Alteration in these parameters has been expressed as indications of long-lasting exposure to stressor [15]. In the present investigations, the analyzed biochemical parameters in muscle, liver and intestine were showed significant seasonal and habitat variation in the fish samples.

3.1 Muscle

Table 2 indicated Biochemical components (%) of muscle tissues of *Labeo rohita*. The mean total lipid and protein (except autumn) level in muscle tissue was observed to be reduced significantly ($P<0.05$) in test fish sample (Harike wetland) than control fish sample (Nangal wetland). The mean carbohydrate level decreased significantly ($P<0.05$) in polluted site sample as in contrast to control site sample in all seasons (except summer). Similar trend was observed in three fish species sampled from different habitat by other researchers [16]. At control site, season wise consequences of the present course of study concluded that the minimum level of lipid, protein and carbohydrates was observed during autumn (5.33±1.33%), autumn (0.09±0.03%) and summer (4.16±0.00%) season, respectively. However, maximum level of lipid, protein and carbohydrate was reported during summer (10.26±0.08%), summer (0.78±0.03%) and autumn (10.64±0.03%). At test sample site, the minimum level of lipid (3.53±0.73%), protein (0.11±0.05%) and carbohydrate (5.71±0.01%) was observed during rainy, winter and winter season, respectively. While, maximum level of lipid, protein and carbohydrate was recorded 7.06±0.06%, 0.57±0.05%, 9.24±0.00% in autumn, winter and winter season, respectively. The recorded maximum average value of lipid, protein and carbohydrate in the present research work was found to be elevated than those reported by other researchers [17]. While, highest average value of lipid content was found to be lower than those concluded by other ichthyologists in different seasons [18].

The mean moisture content in the test fish sample has increased significantly ($P<0.05$) in all the seasons (except autumn) as compared to control fish samples. The maximum value of moisture content was observed 72.2±1.1% and 72.0±0.05% in rainy season in presently studied control and test fish sample, respectively. In the present course of work, maximum value of moisture content at both the studied wetlands were found to be lower than those reported by other workers at upstream and downstream site [19] and close than those reported in *L. Rohita* and *C. mrigala* collected from Indus River during different seasons by other workers [20]. In another report, lowest value of moisture was recorded in *Mugil cephalus* in winter and maximum in summer season [21]. The moisture content of *Anabas testudineus* was maximum during summer and minimum in winter seasons [22]. No seasonal change in moisture content of catfish was observed [23]. The ash content varied between 2.90±0.42% (summer) to 4.91±0.11% (winter) in case of control fish sample and from 2.03±0.06% (summer) to 2.68±0.35% (winter) in test fish sample. These recorded values in the present research were found to be close than those reported in another research [24,19] and lower than those reported by other workers [25,20]. In another study, ash content found to be higher in small indigeneous fish species of Bangladesh, *Osteo brama cotio cotio* (3.06%), *Gadusia chapra* (4.54%) and *Amblypharyngodon mola* (3.40%) [26]. These values found to be lowest as compare to control and test fish samples during the course of present studies.

3.2 Liver

Table 3 indicated Biochemical components (%) in liver tissue of *Labeo rohita*. The mean total lipid and protein level in liver tissue was reported to be reduced significantly ($P<0.05$) at Harike wetland than Nangal wetland in all the seasons. The mean value of total protein decreased significantly ($P<0.05$) at polluted site in contrast to control site during all the seasons (except autumn). The mean carbohydrate level increased significantly ($p<0.05$) at polluted site than control site in all the studied seasons (except winter). Season wise results of the study conducted at control site revealed that the minimum level of lipid (7.33±0.03%), protein (0.06±0.02%) and carbohydrate (3.08±0.00%) was observed in rainy, autumn and summer season, respectively. However, maximum level of lipid (11.33±0.33%), protein (1.96±0.56%), and carbohydrate (7.71±0.00%) was recorded during summer, rainy and rainy season, respectively. At test sample site, the minimum level of lipid, protein and carbohydrate was observed 5.6±0.58%, 0.14±0.00% and 4.19±0.00% in rainy, winter and winter season, respectively. On the other hand, maximum value of lipid (7.73±0.49%), protein (1.38±0.51%) and carbohydrate (7.83±0.00%) was observed in summer, rainy and rainy season, respectively in the present findings. Maximum mean value of total lipid in the present course of study was observed to be lower than those reported by other scientists [24,27].

The mean moisture content in test fish sample has increased significantly ($P<0.05$) in all the seasons (except rainy) than the control fish sample. The maximum values of moisture content were observed 77.7±1.16% (rainy) and 77.53±1.29% (spring) in control and test fish sample, respectively. Highest value of moisture content during present investigation was observed to be higher than those reported in the liver of *Catla catla* collected from local fish pond Bhubaneswar by other ichthyologists [24]. The ash content varied between 2.60±0.02% (autumn) to 2.96±0.14% (winter) in control fish sample, while from 2.09±0.05% (rainy) to 3.05±0.05% (winter) in test fish sample which was found to be higher than those reported by other researchers [27].

3.3 Intestine

Table 4 indicated Biochemical components (%) in intestine tissues of *Labeo rohita*. The mean total lipid and protein (except autumn) level in intestine tissues was observed to be decline significantly ($P<0.05$) in Harike wetland fish sample than Nangal wetland fish sample. The mean carbohydrate level enhanced significantly ($P<0.05$) in spring and summer season in polluted site as compared to control site (except autumn). In control fish sample presently recorded season wise results revealed that the minimum level of lipid (6.96±0.49%), protein (0.16±0.00%) and carbohydrate (1.73±0.02%) was recorded in rainy, autumn and spring season, respectively. On the other hand, maximum level of lipid (8.5±0.05%), protein (0.85±0.00%) and carbohydrate (5.65±0.00%) was reported in summer, rainy and rainy season, respectively. In test sample fish, the minimum level of lipid (5.2±0.69%), protein (0.20±0.00%) and carbohydrate (0.89±0.00%) was found in rainy, winter and winter season, respectively. While, the highest value of lipid (7.1±1.15%), protein (0.48±0.04%) and carbohydrate (3.87±0.00%) was observed in autumn, rainy and summer season, respectively. The maximum mean value of total lipid content values detected in the present conducted research work were recorded less than results reported by other reporters in different weight groups of different fish species [28,29,27].

The mean moisture content in test fish sample has increased significantly ($P<0.05$) in all the seasons (except rainy) than control site. The maximum value of moisture content was recorded 76.66±0.14% (rainy) and 76.63±1.21% (spring) in control and test fish samples, respectively. The ash content varied between 1.15±0.08% (spring) to 2.10±0.06% (winter) in control fish sample, while from 0.16±0.04% (rainy) to 1.43±0.03% (winter) in test fish sample. These values were found to be lower than those reported by other scientists in [meagre and gilthead sea bream] intestine [30].

Table 1. Mean biometric data (cm and Kg) of *Labeo rohita* collected from Nangal Wetland (Control site) and Harike wetland (Polluted site) during different seasons (June, 2018 to June, 2019)

Sites	Nangal wetland (Control site)					Harike wetland (Polluted site)				
Seasons	**Rainy**	**Autumn**	**Winter**	**Spring**	**Summer**	**Rainy**	**Autumn**	**Winter**	**Spring**	**Summer**
Total length (cm)	37.5	38.8	41.4	41.8	40.0	40.1	42.3	40.4	40.2	40.1
Standard length (cm)	30.95	31.7	34.4	34.5	34.5	34.6	36.8	34.8	34.5	34.5
Body width (cm)	9.6	8.7	9.5	9.6	8.9	9.0	9.8	9.2	9.0	9.0
Body weight (Kg)	872.1	892.9	1306.6	1306.8	1148	1150.2	1401.1	1153.9	1150	1146

Table 2. Biochemical components (%) of muscles of Labeo rohita collected from Nangal wetland (Control site) and Harike wetland (Polluted site) during different seasons (June, 2018 to June, 2019)

Sites	Nangal wetland (Control site)					Harike wetland (Polluted site)				
Seasons	**Rainy**	**Autumn**	**Winter**	**Spring**	**Summer**	**Rainy**	**Autumn**	**Winter**	**Spring**	**Summer**
Total lipids	6.6 ± 0.11^{b}	5.33 ± 1.33^{a}	6.63 ± 0.38^{b}	9.06 ± 0.03^{c}	10.26 ± 0.08^{d}	3.53 ± 0.73^{a}	7.06 ± 0.06^{c}	5.0 ± 1.05^{b}	6.56 ± 0.20^{c}	7.03 ± 1.33^{c}
Total proteins	0.64 ± 0.06^{b}	0.09 ± 0.03^{a}	0.66 ± 0.00^{b}	0.56 ± 0.06^{b}	0.78 ± 0.03^{c}	0.28 ± 0.06^{c}	0.19 ± 0.00^{b}	0.11 ± 0.05^{a}	0.25 ± 0.05^{c}	0.57 ± 0.00^{d}
Carbohydrates	10.11 ± 0.02^{c}	10.64 ± 0.03^{c}	8.67 ± 0.00^{b}	8.67 ± 0.00^{b}	4.16 ± 0.00^{a}	8.12 ± 0.01^{c}	8.08 ± 0.06^{b}	5.71 ± 0.01^{a}	8.24 ± 0.21^{c}	9.24 ± 0.00^{d}
Moisture	72.2 ± 1.1^{b}	67.23 ± 3.61^{a}	66.66 ± 3.38^{a}	68.23 ± 0.56^{a}	69.0 ± 2.64^{a}	72.0 ± 0.5^{c}	65.4 ± 0.46^{a}	69.8 ± 0.05^{b}	70.0 ± 0.11^{b}	70.66 ± 0.52^{b}
Ash	3.60 ± 0.06^{c}	3.15 ± 0.04^{b}	4.91 ± 0.11^{d}	3.53 ± 0.09^{c}	2.90 ± 0.42^{a}	2.13 ± 0.07^{a}	2.66 ± 0.02^{b}	2.68 ± 0.35^{b}	2.53 ± 0.39^{b}	2.03 ± 0.06^{a}

Values are mean ±S.E values in a row at individual site during different seasons with the different superscript differ significantly ($P<0.05$)

Table 3. Biochemical components (%) in liver of *Labeo rohita* collected from Nangal wetland (Control site) and Harike wetland (Polluted site) during different seasons (June, 2018 to June, 2019)

Sites	**Nangal wetland (Control site)**					**Harike wetland (Polluted site)**				
Seasons	**Rainy**	**Autumn**	**Winter**	**Spring**	**Summer**	**Rainy**	**Autumn**	**Winter**	**Spring**	**Summer**
Total lipids	7.33±0.03^{a}	7.36±0.82^{a}	7.40±0.55^{a}	9.50±0.00^{b}	11.33±0.33^{c}	5.6±0.58^{a}	6.9±1.80^{b}	6.96±1.33^{b}	7.03±1.28^{b}	7.73±0.49^{b}
Total proteins	1.96±0.56^{d}	0.06±0.02^{a}	0.43±0.03^{a}	1.02±0.00^{b}	1.12±0.02^{c}	1.38±0.51^{c}	1.04±0.02^{b}	0.14±0.00^{a}	0.70±0.02^{a}	0.37±0.00^{a}
Carbohydrates	7.71±0.00^{d}	5.55±0.05^{c}	4.21±0.00^{b}	3.08±0.05^{a}	3.08±0.00^{a}	7.83±0.00^{d}	5.63±0.00^{c}	4.19±0.00^{a}	4.8±0.00^{b}	4.73±0.00^{b}
Moisture	77.7±1.16^{d}	65.4±1.8^{a}	72.4±0.25^{b}	74.4±1.6^{c}	73.0±0.17^{c}	74.4±2.2^{b}	67.1±0.05^{a}	77.46±1.26^{d}	77.53±1.29^{d}	76.63±2.43^{c}
Ash	2.90±0.12^{b}	2.60±0.02^{a}	2.96±0.14^{b}	2.90±0.43^{b}	2.82±0.41^{b}	2.09±0.05^{a}	2.11±0.04^{b}	3.05±0.05^{d}	2.09±0.05^{a}	2.23±0.14^{c}

Values are mean ±S.E values in a row at individual site during different seasons with the different superscript differ significantly (P<0.05)

Table 4. Biochemical components (%) of intestine of *Labeo rohita* collected from Nangal wetland (Control site) and Harike wetland (Polluted site) during different seasons (June, 2018 to June, 2019)

Sites	**Nangal wetland (Control site)**					**Harike wetland (Polluted site)**				
Seasons	**Rainy**	**Autumn**	**Winter**	**Spring**	**Summer**	**Rainy**	**Autumn**	**Winter**	**Spring**	**Summer**
Total lipids	6.96±0.49^{a}	7.2±1.68^{a}	7.73±0.46^{a}	8.4±0.05^{b}	8.5±0.5^{b}	5.2±0.69^{a}	7.1±1.15^{b}	6.56±0.20^{b}	6.03±0.08^{b}	6.56±0.20^{b}
Total proteins	0.85±0.00^{c}	0.16±0.00^{a}	0.21±0.00^{a}	0.37±0.02^{b}	0.34±0.01^{b}	0.48±0.04^{c}	0.38±0.07^{b}	0.20±0.00^{a}	0.25±0.02^{a}	0.32±0.00^{b}
Carbohydrates	5.65±0.00^{d}	2.44±0.06^{b}	3.49±0.00^{c}	1.73±0.02^{a}	2.49±0.00^{b}	2.12±0.00^{c}	1.77±0.01^{b}	0.89±0.00^{a}	2.07±0.03^{b}	3.87±0.00^{d}
Moisture	76.66±0.14^{c}	64.83±0.44^{b}	71.1±1.1^{a}	71.0±1.52^{a}	71.0±0.57^{a}	71.1±1.1^{a}	72.0±1.10^{a}	75.53±2.23^{b}	76.63±1.21^{b}	74.5±1.60^{b}
Ash	1.91±0.01^{c}	2.06±0.14^{d}	2.10±0.06^{d}	1.15±0.08^{a}	1.58±0.17^{b}	0.16±0.04^{a}	1.03±0.00^{b}	1.43±0.03^{c}	0.20±0.05^{a}	0.19±0.05^{a}

Values are mean ±S.E values in a row at individual site during different seasons with the different superscript differ significantly (P<0.05)

Table 5. Mean ± S.E of five seasons (June, 2018 to June, 2019) biochemical parameters (%) in various different organs of *Labeo rohita* collected from different wetlands

Sites	**Nangal wetland (Control site)**			**Harike wetland (Polluted site)**		
Body part / organ	**Muscle**	**Liver**	**Intestine**	**Muscle**	**Liver**	**Intestine**
Total lipids	7.58±0.57	8.58±0.46	7.76±0.36	5.84±0.48	6.84±0.49	6.29±0.28
Total proteins	0.54±0.08	0.91±0.23	0.37±0.07	0.28±0.05	0.73±0.16	0.27±0.05
Carbohydrates	8.45±1.14	4.72±0.87	3.16±0.68	7.87±0.58	5.43±0.64	2.14±0.48
Moisture	68.66±1.10	72.58±1.16	70.92±1.05	69.57±0.61	74.62±1.22	73.95±0.79
Ash	3.62±0.20	2.84±0.11	1.76±0.10	2.40±0.11	2.31±0.10	0.60±0.14

Values are mean ±S.E

In the present investigation, significantly ($P<0.05$) depletion in total lipid contents (average value of all the seasons) of the muscle (7.58 to 5.84%), liver (8.58 to 6.84%) and intestine (7.76 to 6.29%) was observed in the test fish samples (Harike wetland), when compare to control fish samples (Nangal wetland) (Table 5). In another study, glycogen with lipid profile declined significantly in the liver and muscles of *Channa punctatus* due to polluted water enclosing heavy metals induces strain, making fish feeble and susceptible to ailments [31]. Lipid serves as energy storage to meet the metabolic demand for more energy to diminish toxic stress. In the present study, there is alteration in lipid content of different body parts of test fish sample, *Labeo rohita* as compared to control fish. Similar results have been observed in the freshwater teleost fish, *Oreochromis mossabicus* collected from diverse habitat [32]. Under sub-lethal exposure to *Channa punctatus* to sugar mill effluent and diminish in the lipid content of muscle plus liver organs was observed [33]. Dwindle in total lipid content of muscle, liver as well as intestine from the test fish samples during the present course of study advocates that lipid may have been channeled for energy synthesis intended for other metabolic activities where these products play major role in stress condition.

Significant decline in the mean value of all the seasons of protein in the muscle (0.54 to 0.28%), liver (0.91 to 0.73%) and intestine (0.37 to 0.27%) was determined in test fish samples in the present investigation (Table 5). In another research decline in total proteins of *Channa punctatus* liver due to tannery effluent exposure was observed [34]. Under the stress of numerous trace metals, significant diminish in the level of protein in various organs of animals was reported. When fish was exposed to heavy metals, significant decline in the level of tissues protein of *Channa punctatus* was detected [35,36]. Increase in glycoprotein content of *Channa punctatus* collected from polluted canal is caused by increased synthesis of vital proteins for fighting the toxicants. In any polluted water bodies the fishes defend themselves by generation of defense mechanism [31]. During the present investigation the rise or fall in the value of protein in sample collected from polluted site as a result of pollution stress.

Carbohydrates are major source of energy for all life form and are available in huge quantities in muscle and liver body part of fish. The decrease in the carbohydrate content in the muscle (8.45 to 7.87%) and intestine (3.16 to 2.14%) in present studied test samples were observed (Table 5). In another finding, the decline in carbohydrate amount in brain plus muscles perhaps as a result of use of glucose to meet overload energy requirement imposed by severe anaerobic stress caused by mercury intoxication [37]. Similarly, the glucose level of muscle, liver along with kidney of *Clarias batrachus* was decreased under sodium arsenite exposure [38]. In present research, maximum value of carbohydrate in muscle (10.11%), liver (7.71%) as well as intestine (5.65%) was recorded in rainy season and declined during the autumn season which is similar with previously studies [39]. In another research, steady raise of muscle in addition to reproductive organs carbohydrate content of fish, *Cyprinus carpio* has been observed. The elevated level of carbohydrate cause damaging physiological impact, decline hormonal immune response with enhanced dietary toxicity in fish [40]. It can be summarized that carbohydrate was also the major energy fuel in the test fish sample during stress. In the present course of study period, a significantly ($P<0.05$) depletion in ash contents (average value of all the seasons) of the muscle (3.62 to 2.40%), liver (2.84 to 2.31%) and intestine (1.76 to 0.60%) was observed in the test fish samples (Harike wetland), when compare to control fish samples (Nangal wetland) (Table 5). However, moisture content in all the organs of fish sample increased significantly ($P<0.05$) in test fish sample as than control fish sample (Table 5).

4. CONCLUSION

The alteration in biochemical parameters in the muscle, liver and intestine of fish was due to stress induced by various pollutants in the samples collected from Harike wetland (test fish sample) when compared with the samples collected from Nangal wetland (control fish sample). This may be due to discharge of industrial effluents, municipality waste and domestic waste into Harike wetland, disturbing the growth, health of fish and more noticeably the population of fish at the wetland. From these investigations, it is concluded that biochemical parameters in of muscle, liver and intestine could serves as biomarkers for evaluating effect of xenobiotic in *Labeo rohita*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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