ORIGINAL RESEARCH

# Examining muscles of *Cirrhinus mrigala* for biochemical parameters as a bio-indicator of water pollution by municipal and industrial effluents into River Ravi, Pakistan

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**Abstract**   We examined the muscles from replicated samples of *Cirrhinus mrigala* as a bio-indicator of water pollution following sewage and industrial discharges from the Lahore city into Ravi River of Pakistan. For this, *C. mrigala* was netted from three variably polluted downstream sites (B, C and D) and one less polluted upstream site (A). The fish was sampled during low (winter) and high (post monsoon) river flow seasons. The fish muscles from site D showed 28 % less total carbohydrates than the fish caught from site A. The muscle carbohydrate contents were further decreased at sites B and C by 58 and 59 % and 77 and 74 %, during low and high flows, respectively. Moreover, total lipids, cholesterol and RNA contents for the muscle of the fish from the downstream locations were also decreased up to 29, 68 and 58 %, respectively. Conversely, total protein, soluble protein and DNA contents were increased in fish muscles progressively downstream during both the low- and high-flow seasons. It appeared that the fish muscle components can change with the level of water pollution which may affect the fish attributes as a safe food.

## Introduction

Ravi River is perceived to be one of the most polluted rivers in Pakistan. There are over seven municipal sewage outlets and two drains which discharge their untreated effluents when the river passes through Lahore, the capital of the Punjab province of Pakistan (Saeed and Bahzad 2006; Shakir et al. 2013). Freshwaters such as the Ravi River are highly vulnerable to different sorts of pollutions, since they act as immediate sinks for the outputs of anthropogenic activities. Fishes are one of the richest sources of an essentially healthy diet. Therefore, it is important to know the impacts of water pollution on the health and growth of these animals. Indeed, different changes in aquatic medium can cause several physiological and compositional changes in fish. Industrial and municipal effluents are the main culprits for undesirable

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changes in the quality of water and its inhabiting fish (Wilson and Taylor 1993; Shakir et al. 2013). Alterations in biochemical composition of muscles in response to exposure to numerous pollutants have been reported by researchers (Bhathar et al. 2004; Yousafzai and Shakoori 2009). Biochemical profiles are commonly used as stress indicators because most biochemical defenses respond to cellular injury through self-regulating signal transduction mechanisms (Safahieh et al. 2010). Investigation of biochemical parameters can be helpful in identifying target organs of toxicity as well as general health status of animals because it has been advocated for presenting early warning of potentially damaging changes in stressed organisms (Jacobson-Kram and Keller 2001).

The aim of this study was to examine the fish muscles as a bio-indicator of potential impact of water pollution on the biochemical features of *C. mrigala* representing one upstream (less polluted as a reference or A) and three downstream (polluted as B, C and D) sites of the Ravi River. The fish species such as *C. mrigala* is one of the major fresh water carps native to Pakistan and other Asian countries. This fish is known for their high nutritive value, good taste, great price and huge demand in the market.

## Materials and methods

### Study sites and fish sampling

Fish specimen *C. mrigala* (mori) was collected from four sampling sites (A siphon, B Shahdara, C Sunder, D Balloki) of the Ravi River (Fig. 1) during low (winter months of Nov–Dec 2009) and high (post monsoon months of Sep–Oct 2010) flow seasons. Details of fish sampling and sites have been described previously (Shakir et al. 2013; Shakir and Qazi 2013). Nine fish specimens representing each sampling site of similar sizes were collected from three nets per site. The fish was immediately killed, washed with water, drained, and transferred to individually labeled polythene bags which were immediately transported in ice boxes to the laboratory. Biometric measurements of these fishes were recorded as described by Shakir and Qazi (2013). Each fish sample was then dissected to collect muscles which were stored in individually labeled sterilized polythene bags at −20 °C until further analysis.
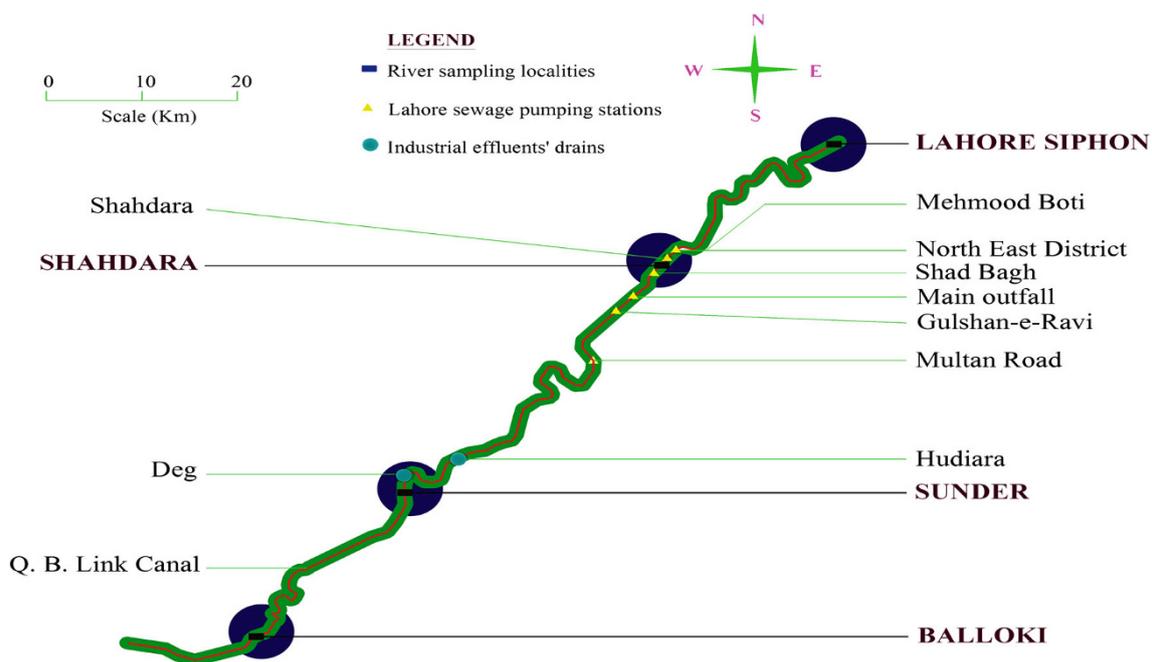


**Fig. 1** Map of the Ravi River Lahore showing the four sampling sites around major urban pollution inlets of Lahore (Shakir et al. 2013)

Biochemical analysis of the fish muscles

The frozen fish muscles were processed to obtain their extracts in ice-cold saline as described by Anwar et al. (2004). In brief, the fish muscles were cut into small pieces with a clean razor, thawed with distilled water and blotted with adsorbent paper. Then, 1 g muscle of a fish was homogenized in 4 ml ice-cold saline (0.89 % NaCl) with the help of a motor-driven homogenizer. The homogenate was centrifuged at 4,900 rpm for 45 min at 5 °C in a refrigerated centrifuge and the supernatant was separated for the determination of total carbohydrates by the method of Du Bios et al. (1956) and soluble protein as described by Lowry et al. (1951). For the estimation of total protein by the method of Lowry et al. (1951), 0.5 g of a frozen muscle was processed as described before but homogenized in 4 ml ice-cold 0.5 N NaOH.

For the estimation of nucleic acid contents, 0.5 g of a fish muscle was boiled in 3 ml ethanol in caped test tubes immersed in a boiling water bath at 90 °C for 1h. The contents in the test tubes were then incubated overnight at 37 °C. The homogenates were later centrifuged at 2,000 rpm for 10 min to get a clear ethanol supernatant. The pellets were used for nucleic acid extraction, while the ethanol supernatant was decanted into glass vials and evaporated at 70 °C in oven. The yellowish dried residue containing lipid and cholesterol components was then dissolved in 0.5 ml of chloroform and used for the analysis of total lipids as described by Henry and Henry (1974) and cholesterol as described by Henry (1964). Pellets were processed for the extraction of nucleic acids as adopted by Shakoori and Ahmed (1973). DNA and RNA contents were estimated according to Schneider (1957).

Statistical analysis

The data were statistically analyzed using Minitab software to find the effect of sites, seasons and site × season on the biochemical parameters of fish muscles for either very high significance at $P < 0.001$ or high significance at $P < 0.01$ or significance at $P < 0.05$. Turkey test was used if there were more than two means to compare for significant difference at $P < 0.05$.

## Results

Mean values of biometric measurements (total length, wet weight) and various biochemical parameters of muscles of the fish sampled from four sites of Ravi River during both the flow seasons are presented in Table 1. The biometric data did not differ significantly ($P > 0.05$) for sites, seasons and sites × seasons interaction. However, all the biochemical parameters showed significant differences between seasons and among downstream sites, except the DNA content ($P < 0.05$). The entire biochemical parameters showed non-significant site × season interactions ($P > 0.05$).

The biochemical parameters appeared to be changing in response to the perceived pollution levels of the downstream sites. Here, total and soluble proteins and DNA contents of the muscles showed increases while carbohydrate, total lipids, cholesterol and RNA contents decreased for up to site C during both low- and high-flow seasons. These changes in the biochemical parameters tended to stabilize at site D and rather showed a recovery as compared to the values obtained for the site C (Table 1). The mean total protein contents of the fish muscles increased for up to 56, 105, 62 %, while soluble proteins showed elevations of 37, 106 and 78 % for the sites B, C and D, respectively, over the corresponding values for the site A when the data were averaged over the low- and high-flow seasons. The increases in protein contents of muscles sampled from the three downstream locations were comparable during both low- and high-flow seasons. While vivid differences appeared for the DNA content, which increased for up to 2.8, 6.2 and 3.5 % at sites B, C and D, respectively, during high flow but could show elevation of 2 % at site B but decrease of 2.7 % at site C during low-flow season compared to the relevant values at site A in respective seasons (Fig. 2).

Carbohydrates, total lipids, cholesterol and RNA showed variable decreases for the muscles sampled from the downstream sites as compared to their respective values obtained at the upstream site A. The decreases, in general, appeared to be intensified during low-flow season (Fig. 2). Reductions in muscle carbohydrate ranged from 28 to 77 % and from 29 to 74 %, total lipids from 10.8 to 28.9 % and from 12.9 to 27.4 %, cholesterol

**Table 1** Mean biometric (cm and g) and biochemical components (mg/g) of muscles of *Cirrhinus mrigala* sampled from upstream (Siphon as reference = A) and downstream heavily polluted (Shahdara = B; Sunder = C; and head Balloki = D) sites during low- and high-flow seasons

| Sites | A | | B | | C | | D | | SEM with significance | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Seasons | Low | High | Low | High | Low | high | Low | High | Site | Season | Site × season |
| **Biometric data** | | | | | | | | | | | |
| Total length (cm) | 40.31$^a$ | 40.16$^a$ | 39.94$^a$ | 39.84$^a$ | 39.49$^a$ | 39.46$^a$ | 40.19$^a$ | 39.89$^a$ | 0.881 | 0.623 | 1.246 |
| Wet weight (g) | 650$^a$ | 650$^a$ | 665$^a$ | 636$^a$ | 662$^a$ | 649$^a$ | 652$^a$ | 643$^a$ | 43.96 | 31.08 | 62.16 |
| **Biochemical parameters (mg/g)** | | | | | | | | | | | |
| Carbohydrate | 47.51$^b$ | 54.95$^a$ | 19.91$^d$ | 22.48$^d$ | 10.90$^e$ | 14.17$^e$ | 34.23$^c$ | 39.25$^c$ | 0.871*** | 0.616*** | 1.23 |
| Total protein | 139.27$^c$ | 128.35$^c$ | 218.40$^b$ | 199.67$^b$ | 274.28$^a$ | 272.93$^a$ | 220.86$^b$ | 212.52$^b$ | 3.741*** | 2.645** | 5.29 |
| Soluble protein | 54.79$^e$ | 51.47$^e$ | 77.19$^c$ | 68.53$^d$ | 111.65$^a$ | 106.73$^a$ | 96.43$^b$ | 92.40$^b$ | 0.952*** | 0.673*** | 1.35 |
| Total lipids | 25.66$^{ab}$ | 27.92$^a$ | 22.88$^{bc}$ | 24.33$^{ab}$ | 18.24$^d$ | 20.27$^{cd}$ | 22.24$^{bc}$ | 24.02$^{bc}$ | 0.612*** | 0.433** | 0.865 |
| Cholesterol | 1.64$^a$ | 1.68$^a$ | 1.35$^b$ | 1.38$^{ab}$ | 0.52$^e$ | 0.66$^{de}$ | 0.81$^{cd}$ | 0.91$^c$ | 0.030*** | 0.021** | 0.043 |
| DNA | 1.48$^a$ | 1.45$^a$ | 1.51$^a$ | 1.49$^a$ | 1.44$^a$ | 1.54$^a$ | 1.48$^a$ | 1.50$^a$ | 0.050 | 0.036 | 0.071 |
| RNA | 6.17$^{abc}$ | 6.37$^a$ | 6.13$^{abc}$ | 6.23$^{ab}$ | 5.81$^c$ | 6.01$^{abc}$ | 5.90$^{bc}$ | 6.08$^{abc}$ | 0.065*** | 0.046** | 0.092 |

Means within the same row with the same letters did not differ significantly ($P > 0.05$)

*, ** and *** represent significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively
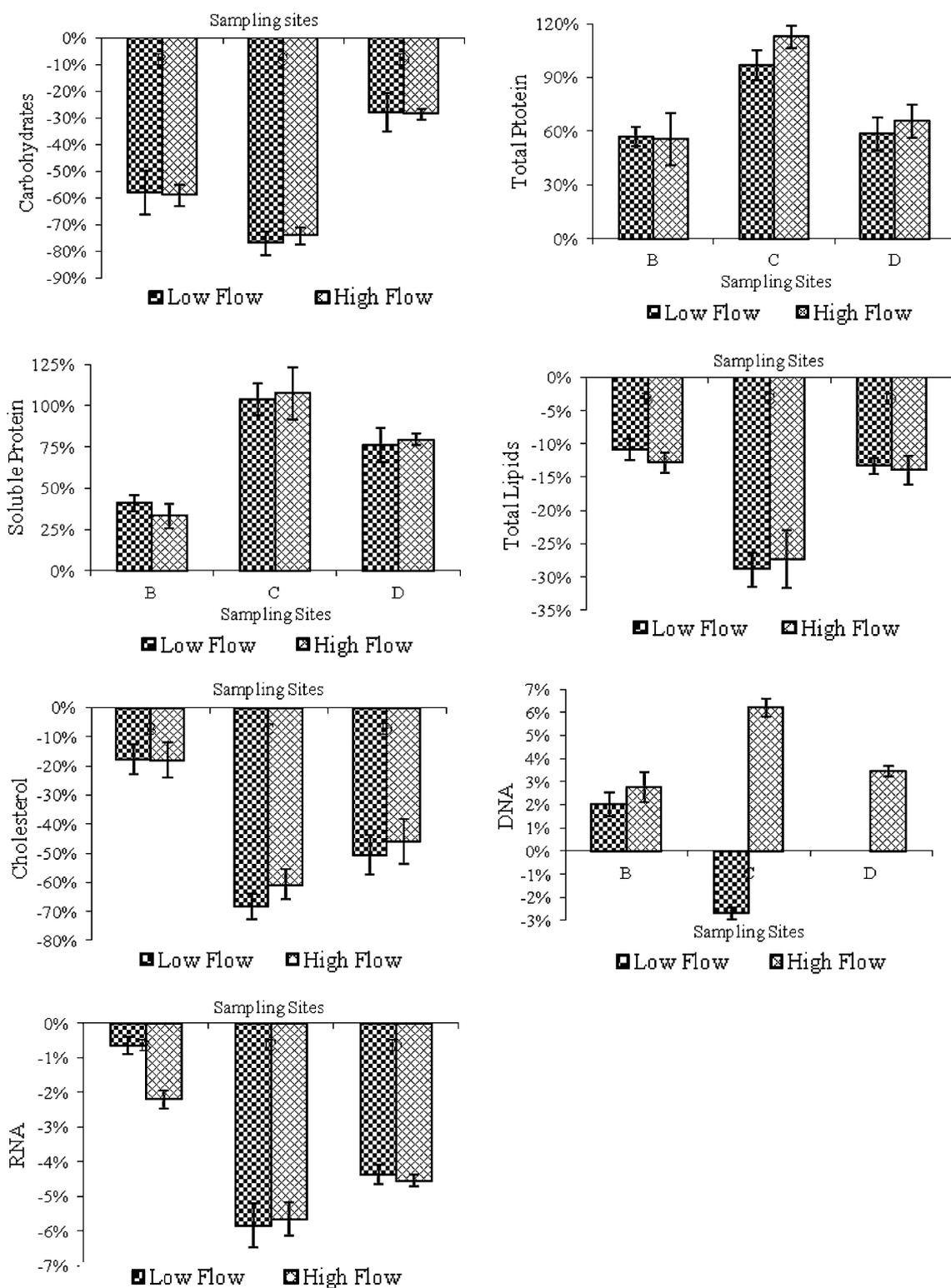
**Fig. 2** Percent increase (+) or decrease (−) with standard deviation in biochemical profiles of muscles of *Cirrhinus mrigala* representing different downstream polluted sites; **B** (Shahdara); **C** (Sunder); and **D** (Balloki) as compared with less polluted reference *A* (Siphon) during the low- and high-flow seasons

from 17.7 to 68.3 % and from 17.9 to 60.7 % and RNA from 0.65 to 5.83 % and from 2.2 to 5.7 % during low- and high-flow seasons, respectively, as compared to the respective values at the upstream site A.

## Discussion

Fish muscle biochemical profiles can be used as a stress indicator for biological systems such as aquatic life. Vivid differences in the muscle's biochemical parameters appeared in fish specimens collected from downstream sites receiving heavy insults of sewage and industrial effluents than the fish from the upstream site of the Ravi River before its entry through the Lahore city. Evaluation of three nutrients: carbohydrates, proteins and lipids, is generally considered as indicators of fish health. Variations in these nutrients have been described as indicative of long-term exposure to stressors (Mayer et al. 1992). Biochemical responses can be affected by environmental factors, such as physico-chemical profiles of aquatic medium, season, fish nutritional status, age and health (Lohner et al. 2001).

Carbohydrate contents of the fish muscles decreased retrogressively from the site B to C. The nutrient decrease was reached at 77.1 and 74.2 % for the site C during low and high flows than the corresponding values at site A. This clearly demonstrates that during high-flow season, dilution of the urban pollutants was able to mitigate their negative effects. Carbohydrates are considered to be the first degraded molecules under stress condition of different animals. Chemical stress causes depletion of stored carbohydrates (Vijayavel and Balasubramanian 2006). Pollutants' stresses might have induced metabolic changes resulting in the increased utility of stored carbohydrates as an energy source. The present results are in accordance with the findings of Garg et al. (2009), who reported significant reduction in carbohydrate contents in the muscles of *Labeo rohita*, *C. mrigala* and *Catla catla* after their separate exposures to heavy metals. Toxicant stress may induce glycogenolysis possibly by accelerating the activity of glycogen phosphorylase to meet the enhanced energy demands or the toxicant may have an effect on glycogenesis by inhibiting the activity of carbohydrate metabolism (Valarmathi and Azariah 2002). Dhavale and Masurekar (1986) suggested that reduction in the tissues' carbohydrate content may be due to the prevalence of hypoxic condition as in oxygen limitation carbohydrate consumption is enhanced. Reduction in dissolved oxygen at the same sampling sites and flow seasons reported by Shakir et al. (2013) also supports this conclusion that decreases in the carbohydrate contents in fish muscles are reflective of less availability of dissolved oxygen and direct effects of heavy metals and other pollutants on the fish.

Total and soluble protein contents of the fish muscle significantly increased for the downstream polluted sites. Pollutants' stresses appeared to accelerate synthesis of body protein. The present results are in agreement with the study of Lohner et al. (2001) who attributed increase in protein contents in response to the exposure of increasing metal concentrations, to synthesis of proteins required to sequester the metals. Similarly, Susan et al. (2010) noticed an increase in protein content in the muscles of *C. mrigala* being exposed to fenvalerate (synthetic pyrethroids) and suggested that the toxicant stress might have stimulated protein synthesis for enzymic detoxification at the expense of glycogen to meet additional requirement in the synthetic activity of body tissues.

Total lipid contents of the fish muscle decreased downstream resembling the pattern of carbohydrate decline. Liver dysfunction or inhibition of oxidative phosphorylation or metabolization of glycerol for energy demand under stress condition due to the polluted environment might have been the possible causes. Vincent et al. (1996) reported variable decline in lipids contents in *Catla catla* after an exposure of 20, 25, 30 and 35 mg chromium/L. Similarly, reductions in lipids profile have been reported by Kaur and Saxena (2001) in fish flesh sampled from polluted waters of river Sutluj in Pakistan. Shukla et al. (2002) observed decrease in lipids content of *Channa punctatus* after 60 days' exposure of cadmium individually and in combination with other metals. Giridhar and Indira (1997) suggested that to overcome the stress, animal tends to mobilize lipids by stimulating the lipase which acts on lipids to break them down to free fatty acids. These fatty acids may undergo β-oxidation leading to the formation of Acetyl CoA (acetyl-coenzyme A) which enters TCA cycle to make the energy available for metabolic functions. Cholesterol contents also showed similar pattern of decreases in response to pollution at downstream sites. Results of the present study are in accordance with Al-Kahtani (2011) who reported decline in cholesterol content of muscle of *Oreochromis niloticus* (tilapia) following exposure to different insecticides. Inhibition of cholesterol biosynthesis in the liver might have

occured due to lack of cholesterol precursors (acetyl-coenzyme A) as suggested by Ali (1989). Reduced absorption of dietary cholesterol as reported by Kanagaraj et al. (1993) and utilization of fatty deposits instead of glucose for energy purpose as reported by Remia et al. (2008) might have also contributed to the observed deficits of cholesterol content.

The DNA contents of the muscle were not significantly different among the downstream sites and seasons. However, elevations for downstream sampling sites, especially during high-flow season, were apparent as compared to the value obtained for fish muscle sampled from the reference site A. The RNA content differed significantly in different seasons and it decreased for the downstream sites. Similar findings have been reported by Yousafzai and Shakoori (2009) who suggested that DNA seemed to be resistant to the ambient toxicants. Das and Mukherjee (2003) also reported elevated DNA and decreased RNA contents in the muscle of *Labeo rohita* after their exposure to sub-lethal concentration of cypermethrin.

The results indicated significant increases in soluble and total protein contents in fish muscles being exposed to the potential pollutants from the sewage and industrial effluents as compared to the respective values obtained for the fish sampled from the upstream reference site. Conversely, significant reduction in carbohydrates, total lipids, cholesterol and RNA contents of the pollution exposed fish meat as compared to the respective values of fish muscle from the upstream reference site was evident that the fish health was under strong negative pressure due to the pollutants loads. These results reflect that much attention is needed to regulate water pollution to minimize its impact on fish and ultimately human health.

## Conclusions

This study showed that the fish muscles can be used as a bio-indicator of river water pollution. In fact, the muscle biochemical parameters were able to show fluctuations in response to the changes in different study sites during different water flow periods. It appeared that the urban pollutant loads via untreated effluents of the Lahore city have been exerting drastic negative effects on fish health. However, some of the negative effects appeared to be partly mitigated by a higher river flow at some parts of the city. These observations clearly demonstrate that the fish life has been affected at the river's study segment. Therefore, prompt action is needed to strictly regulate the influx of urban effluents into the Ravi River to safeguard the aquatic and public health. Meanwhile, further studies should be conducted by covering a longer distance along the Ravi River around Lahore city to reveal the impact of other sources of pollutions on different fish species of river Ravi.

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