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RESEARCH IN ENVIRONMENTAL PLANNING AND MANAGEMENT

Biochemical and histopathological effects in muscular tissue of carp fish (*Labeo rohita,* Hamilton 1822) following exposure to untreated and treated sewage water

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Abstract

Present work describes a laboratory study aiming at assessing the impact of sewage treatment plant (STP) effluents on fish health by means of biochemical and histopathological biomarkers in muscular tissue of fish. *Labeo rohita* (7.62 ±0.25 cm, 8.25±0.32 g) was exposed to sub-lethal concentrations of untreated (UT) and treated (T) sewage water obtained from STP, Ludhiana, India. Following the determination of the 96h lethal concentration (LC_{50}), the fish were exposed to control (de-chlorinated tap water), treated sewage water, $1/10^{th}$ of LC_{50} UT and $1/20^{th}$ of LC_{50} UT sewage water. The experiment was conducted for the period of 60 days. Experimental results depicted significant reduction ($p<0.05$) in muscle-somatic index of $1/10^{th}$ LC_{50} UT and $1/20^{th}$ LC_{50} UT groups compared to control and treated groups. Fish toxicity induced by untreated sewage water was evident from the significant decrease ($p<0.05$) in the levels of proteins and significant increase ($p<0.05$) in content of total lipids in muscular tissue of exposed group fingerlings. Drastic changes in the fatty acids profile and severe histological abnormalities viz. shortening of muscle bundles, edema, hyper-vacuolization, elongation of muscle bundles, gap formation in myofibrils, degenerated myotomes, hemorrhage, inter-myofibrillar space, necrosis, were also recorded in muscular tissue of exposed fingerlings. The intensity of muscular damage in *L.rohita* was found to increase with increase in duration of exposure. Results demonstrated that untreated sewage water could potentially induce physiological stress and somatic cell toxicity in fish *L.rohita.* Genotoxicity studies on germ cells of *L.rohita* fingerlings are further suggested to examine the genotoxic potential of untreated sewage water at high concentrations; this is especially of interest given that many effluents are genotoxic to fish.

Keywords Biochemistry, · Histology, · Muscles, · Sewage water, · *Labeo rohita*

Introduction

Untreated sewage water is the wastewater obtained from the domestic and the commercial establishments. The unregulated discharge of agricultural, industrial, and municipal pollutants has threatened the aquatic ecosystems throughout the world (Sachar and Raina 2014). A wide range of contaminants such as petroleum hydrocarbons, pesticide residues, heavy metals, acids, and dyes when released into the aquatic bodies not only leads to deterioration of surface water but also poses threat to natural ecosystems (Ashraf 2005; Kaur and Dua 2016).

Several studies have reported that the untreated wastewater consisted of several biocides, chemical dyes, acidic drugs, analgesics, and lipid regulators, which necessitates the treatment of untreated wastewater before its discharge into water bodies (Wang 2002; Dautremepuits et al. 2004; Noorjahan and Jamuna 2015). The effluents when discharged into receiving water bodies without adequate treatment can induce mild to severe toxic effects in the aquatic fauna (Koopaei and Abdollahi 2017). According to United Nations (UN), presently 80% of the wastewater is discharged into water bodies without any adequate treatment (WWAP 2017). Furthermore, inadequate sanitation infrastructure not only pollutes the environment but also permeates through all societal functions increasing the burden on human health, leading to the loss of economic activity and thus the overall development potential (Schellenberg et al. 2020). However, the availability

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of financially viable and feasible facilities for the treatment of wastewater still represents a significant challenge around the world, especially within a rapidly changing urban environment which fails to address current sustainable development goals (SDG) designated by UN of the wastewater sector, which could be further increased by global warming.

Another challenge for the aquatic environment is the increasing concentration of plastics in marine and freshwater ecosystems (UNEP 2011) which could become a major issue of concern in the coming times as weathering and degradation processes of plastics will liberate tremendous amounts of nanoparticles in the environment (Lambert and Wagner 2016). Nowadays, the release of nanoparticles in the water bodies and their adverse effect on aquatic environment and organisms has drawn much attention (Zhao et al. 2011). The potential sources for the release of microplastics and nanoparticles in aquatic environment are solid waste disposal sites and municipal effluents (Hernandez et al. 2019). The other sources for the release of nanoparticles in the various segments of environment are chemical industries, biomedicine, electronics, semiconductors, food additives, etc. (Klaine et al. 2011).

The uptake and effects of nanoparticles in the aquatic biota may be a major concern which demands extensive toxicological studies (Griffitt et al. 2008). Particles at the nanoscale have the potential to permeate cells of an organism and reach the cytoplasm where many biochemical processes are at play (Auclair and Gagné 2020). Thirty days exposure to sub-lethal concentrations of copper oxide nanoparticles (nCuO) to fish *Cyprinus carpio* has been found to cause reduction in fish growth and cholinesterase activity and accumulation of copper in various tissues/organs of fish viz. the intestine, gill, muscle, skin and scales, liver, and brain (Zhao et al. 2011). Besides fish, nanoparticles present in the municipal effluent may induce uneven effects in other aquatic organisms for example, 3-month exposure of mussels to primary treated municipal effluents of Saint Lawrence River which is a suspected source of microplastics and nanoplastics (polystyrene nanoparticles) was found to cause significant induction in lipid content, anisotropic changes in the subcellular fraction of digestive gland and to disrupt the spatial organization of enzyme clusters such as the fumarase-malate dehydrogenase (MDH)-citrate synthase (CS) system (Gagné et al. 2019; Auclair and Gagné 2020).

Since biological communities can integrate the effect of changes in chemical, physical, and biological factors of environment and hence, are good indicators of ecosystem health (Harrison et al. 2000). Fishes are sensitive to the changes in their surrounding environment making them good indicators of the status of a specific aquatic ecosystem (Nikalje et al. 2012). Any physical or chemical alteration in aquatic environment induces stress in fish by causing alterations at cellular or organ level including enzymatic, genetic, innate, and acquired immunotoxic effects which has negative impact on survival, growth, development and reproduction in fish (Sinha et al. 2018). Early toxic effects of pollution may be evident at cellular or tissue level before significant changes can be observed in fish behaviour or external appearance (Nikalje et al. 2012).

Fish muscle is an important valuable and recommended human nutrition possessing cardio protective effect due to the low content of fat and high content of proteins, minerals and optimal unsaturated fatty acids (Sumi and Chitra 2017). Muscle is not only an edible part of fish but it also act as a bioindicator of aquatic pollution (El-Serafy and Ibrahim 2005; Sitohy et al. 2006; Shakir et al. 2014; Sumi and Chitra 2017)). Physiological state of an organism is a key factor in determining species sustainability, survival, and availability because this factor is susceptible to the effects of pollutants at all stages of an organism life cycle (Adeogun and Chukwuka 2012). Biochemical responses and histomorphology of organs can be affected by physical and chemical abiotic factors, such as water pollution, temperature, age, disease, nutritional status, and seasonal changes (Lohner et al. 2001; Francis and Nagarajan 2013).

Several epidemiological studies have shown that untreated water can alter the level of total proteins, total lipids, fatty acids, cholesterol, and carbohydrates in muscles of fish (Kaur and Saxena 2002; Shakir et al. 2014; Giang et al. 2018). Untreated sewage water (UT) has already been shown to induce acute toxic effects (Workagegn 2013), altered behavioural responses (Kaur and Dua 2015) and morphometric defects (Sinha et al. 2018) in fish, but studies are lacking regarding the effect of UT at sub-lethal concentrations on biochemical and histopathological parameters of muscular tissue of fish *Labeo rohita*. So, the present research work was carried out to interpret the aforementioned defects, hypothesizing that the exposure to UT at sub-lethal concentrations can induce severe toxic health effects in *Labeo rohita* with the increase in duration of exposure.

Materials and methods

Test species

The present research work was carried out in the Department of Zoology, Punjab Agricultural University, Ludhiana, Punjab, India. Three-month-old healthy fingerlings of *Labeo rohita* (Hamilton 1822) with average length of 7.62 cm and weight of 8.25 g were obtained from College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) Ludhiana, Punjab, India. *Labeo rohita* is widely consumed by the people in South Asia especially for its muscle/flesh part (being delicious in taste and high nutritional value). Being a most preferable edible fish, it is widely cultivated for commercial purpose and being the prime cultured species in poly-culture practices in South Asia,

it occupies a prominent position in the aquatic ecosystem. Furthermore, there are several evidences in the literature which have proved that muscular tissue of this fish can accumulate toxicants/contaminants present in water which could be transferred to the consumers of this fish (Aditya and Chattopadhyaya 2000; Rauf et al. 2009; Malik et al. 2010; Mastan 2014). Keeping in view the nutritional, economical, and biological significance of this fish, it was selected as a model animal for the present study. The others reasons for choosing this fish are its wide geographic distribution, ease of maintenance under laboratory conditions, ready availability throughout the year, convenience of testing, and relative sensitivity to the pollutants.

Biology and ecology of test species

Labeo rohita is a native species of the carp family and is widely present in freshwater ecosystems of South Asia (Pakistan, India, Bangladesh, Myanmar, Nepal, Vietnam). This fish is found in tropical and temperate regions. It is a bony fish and its body is elongated, laterally compressed and spindle shaped tapering at either end. It has cycloid scales on body, head is without scales, and the snout is fairly depressed and projects beyond mouth. Lips are thick and fringed above and below the mouth, which is folded inwards. The color of the body is dark greyish on the back, but sides and belly are pale yellow or white in colour. Sexes are separate but there is no sexual dimorphism. This fish is oviparous, and breeds only in rivers but not in confined waters. The embroyonic development in *L. rohita* starts after 4 h of fertilization. The average diameter of fully swollen fertilized eggs is 4.5 mm. Post fertilization, the sequence of events during embroyonic development are as follows: first cleavage (after 45 min of fertilization), second cleavage (within next 5–6 min), third cell division occurs (within next 15–20 min), yolk invasion (within 2 h of fertilization), formation of yolk plug (within next 1 h), formation of myotome (within the next 1 h), formation of 17 myotomes and capfer vesicles (within 8 h), and the fetus begins to move. Egg hatching occurs after 14–18 h of fertilization. During normal breeding season, the embryonic development takes place within 78 h and during late breeding season, it takes 86 h. The length of this fish varies from 50 to 200 cm and it can attain maximum weight of 45 kg (Frimodt 1995). It has an average life span of 10 years and attains maturity at 2–5 years of age (Khan and Jhingran 1975).

Labeo rohita is an omnivore; however, it has specific food preferences at different life stages. It feeds on zooplankton (*Daphnia*, insect larvae) during the early stages of its lifecycle, but as it grows, it eats more, and more phytoplankton, and as a juvenile or adult is a herbivorous column feeder, eating mainly submerged vegetation and phytoplankton (Bakhtiyar et al. 2017). It has modified, thin hair-like gill rakers, suggesting that it feeds by sieving the water. Under culture/laboratory conditions, this fish can feed on supplementary fish food consisting of rice bran, wheat bran, oil cake, or any nutritionally balanced complete artificial fish diet (Mookerji and Rao 1995; Rahman 2005). It is a non-predatory fish and is usually cultivated in association with two other carps, *Catla catla* and *Cirrhina mrigala*.

Acclimatization of test species

For acclimatization, the fingerlings (total fingerlings acclimatized; n=200, total tanks; n=22, fingerlings per tank; n=9) were kept in tanks (42×53×42, 35 L capacity) containing dechlorinated tap water (temperature: 24±0.43°C, DO (dissolved oxygen): 6.47±0.35 mg/L, pH: 7.01±0.01) for the period of 10 days prior to the experiment. Normal photoperiod (12-h light/dark cycle) was maintained both for acclimatization period and experimentation. Commercial fish food TOYA fish feed (Trade name), marketed by Vasant Kunj, New Delhi (India), was used to fed the fingerlings during the experimental period. The experimental tanks were continuously aerated with electrically operated aerators (n=2 aerators per tank) and filters (n=2 filters per tank). The whole experiment was conducted according to the guidelines provided by Organisation for Economic Co-operation and Development (OECD 1992).

Municipal sewage water collection

The untreated and treated sewage water was obtained from Sewage Water Treatment (STP) plant located at village Bhattian (30° 57' 57 N and 75° 49' 54 E), Ludhiana, Punjab, India. The capacity of this STP to treat wastewater is 50 million L per day (MLD). This STP utilizes sequencing batch reactor (SBR) technology to treat untreated sewage water (Bhanot and Hundal 2019). As per the information obtained from the organization/authorities dealing with the maintenance of this STP, the sewage water consists of a mixture of large number of pollutants viz. heavy metals, insecticides, detergents, dyes, pharmaceutical agents, as it receives wastewater from multiple sources such as urban sewage discharges, textile industrial effluents, paper mill discharges, dyeing industrial effluents, agricultural runoffs, spinning mills waste, dairy farms waste and domestic discharges.

Standardized methods given by American Public Health Association American Water Works Association, and Water Environment (APHA 2005) were followed to analyze the physico-chemical parameters viz. pH, temperature, biochemical oxygen demand (BOD), dissolved oxygen (DO), free carbon-dioxide (CO_2), alkalinity and conductivity of control, untreated sewage water (UT), and treated sewage water.

It has been observed that the STP, using SBR technology, has significantly improved the quality of untreated sewage water with removal of objectionable values of physico-chemical measures with an overall removal efficiency of 90%.

Acute toxicity test

For calculating the value of lethal concentration (LC_{50}) of untreated sewage water (UT), an acute toxicity test was performed (Bhanot and Hundal 2019). In this test, fingerlings (two replicates per each group, n=7 fingerlings per each replicate, total n=14 fingerlings per group) were exposed to dechlorinated tap water (marked as control group), treated sewage water and different concentrations viz. 10%, 25%, 50%, 75%, and 100% of UT for the period of 24, 48, 72, and 96 h. No mortality was observed in the fingerlings exposed to control group, treated sewage water and 10% concentration of UT after 24, 48, 72, and 96 h of exposure. However, at 25% concentration of UT, two fingerlings were found dead after 96 h of exposure and at 50% concentration of UT, four fingerlings were found dead after 24 h of exposure, six fingerlings died after 48 h and none of the fingerling was found dead after 72 h of exposure. At 75% concentration of UT, six fingerlings were observed dead after 24 h and eight fingerlings showed mortality after 48 h of exposure. At 100% concentration of UT, all the fingerlings were found dead within 24 h of exposure. After analyzing the mortality rate in fingerlings, LC_{50} of UT was calculated using POLO software (Robertson et al. 2007) and the value of LC_{50} of UT was obtained as 51.70 % (v/v) (Bhanot and Hundal 2019) which revealed that UT did not meet the discharge limits of effluents into inland surface waters. Therefore, for conducting chronic bioassay, two sub-lethal concentrations, i.e., $1/10^{th}$ of LC_{50} and $1/20^{th}$ of LC_{50} which corresponds to 5.20 % (v/v) and 2.60 % (v/v) respectively, were taken.

Chronic bioassay

Two sub-lethal concentrations of the calculated LC_{50}, i.e., 1/10th LC_{50} and 1/20th LC_{50} were taken for the chronic bioassay test. Fingerlings were divided into four groups: control group, treated group, 1/10th LC_{50} UT and 1/20th LC_{50} group of UT (four replicates per each group were taken with each replicate consisting of nine healthy fingerlings (n=9); total n=36 fingerlings per each group). The fingerlings were exposed for the duration of 60 days and six fingerlings (n=6) from each group were dissected at an interval of 15, 30, 45, and 60 days.

Morphometric analysis

Muscle-somatic index (MSI)

Muscles were removed carefully and weighed in an electronic weighing machine, after removing moisture by blotting paper. The following formula was used to calculate the Muscle-somatic index (MSI):

$$\text{Muscle}-\text{somatic index (MSI)} = \frac{\text{Muscle weight}}{\text{Body weight}} \times 100$$

Biochemical studies

Muscle tissue (0.5 g) was homogenized in 2 ml of 0.1 molar of phosphate buffer saline (137 mM NaCl, 2.7 mM KCl, 10mM Na_2HPO_4, 1.8 mM KH_2PO_4), pH <7.4) and centrifuged at 755×g for 10 min to obtain supernatant for various biochemical estimations.

Estimation of proteins The total proteins were estimated in the supernatant of muscular tissue according to the method given by Lowry et al. (1951). After mixing all reagents, absorbance was recorded at 520 nm in a spectrophotometer against reagent blank and a standard curve was prepared by taking bovine serum albumin (BSA) solution in range of 20–200 μg/ml. Units were expressed as mg/g sample.

Estimation of total lipid content The total lipid content (TLC) was estimated in muscles of fingerlings by using Soxhlet lipid extraction/solvent extraction method (AACC 1976). The stored samples of muscle tissue were thawed for 3–4 h at 5°C and were soaked on filter paper before weighing. Thimble was prepared by folding 22 × 25 cm sheet of filter paper and then ten grams of sample was added. Cotton was placed at lower closed end of the extraction tube of Soxhlet apparatus. The sample was placed on the top of the cotton. The lipids were extracted with 125 ml petroleum ether in 250 ml flask of Soxhlet apparatus for 8 h at 60–80°C. The contents of the 250-ml flask were transferred into a previously weighed crucible. The excess ether was evaporated at 80°C in a hot air oven. The crucible was cooled and weighed. The process of heating and weighing after cooling was repeated till constant weight of the crucible was obtained.

The following formula was used for the calculation of TLC:

$$\text{TLC (mg/g)} = \frac{(W_2 - W_1)}{A} \times 100$$

where,
A = weight of the sample taken
W_1 = weight of the empty crucible

W_2 = weight of crucible with extracted lipids

Estimation of fatty acids The lipids extracted from the muscles were dissolved and stored at 5°C in petroleum ether and then the fatty acid composition was determined in muscles by gas liquid chromatography (GLC) following the method given by Applequist et al. (1968). Homogenate (1.5 ml) was taken into a test tube to which 1.5 ml of sodium ethylate was added and allowed to stand for 30 min. After this, 1.5ml of 8% sodium chloride solution was added and contents were mixed well. It was kept undisturbed for another 30 min. As soon as two layers got separated, the upper layer (petroleum ether layer) was transferred to another test tube with a dropper. Two microliters of this upper layer was then injected into the oven using microsyringe (Hamilton) on M/s Nucon Engineers AIMIL Gas Chromatograph (solid state) model Nucon series 5700/5765 equipped with flame ionization detector fitted with SS column 1/8" outer diameter × 2M length, packed with 15% D.E.G.S on CHROMOSORB W.H.P, 80–100 mesh size. The conditions for the separation were oven temperature 200°C, injector temperature 230°C, detector temperature 240°C, hydrogen flow 30 ml/min, air flow 300 ml/min, and nitrogen flow 40 ml/min. Identification of peaks was done by comparison of their retention time with those of standard fatty acyl ester (M P Biomedical Inc. USA).

Relative concentration of fatty acid was calculated by use of automatic integrator-Windows based AIMIL Ltd., DASTA 710 Gas Chromatograph Data station software, version WinAcds 7.1.

Histomorphological examination

Six (n=6) fingerlings from each group were sacrificed and dissected to remove muscular tissue at an interval of 15, 30, 45, and 60 days of exposure. The tissues were then cleaned, weighed, and processed for histological studies after fixation in alcoholic bouin's solution and embedded in wax. Serial sections at 5–7μm were obtained and standard histological procedure as per Humason (1975), were followed for haematoxylin-eosin staining. After staining, the slides (n=30 slides per group per interval; n=5 slides per fingerling) were viewed under light microscope (Olympus, Tokyo, Japan) and photographed by digital camera at different magnifications (4×, 10×, 40×).

Statistical analysis

Data was expressed as Mean±Standard Error (S.E.). Statistical significance between the data of physico-chemical parameters of control, untreated and treated sewage water was determined using one-way analysis of variance (ANOVA) followed by Student's t-test (5% level of significance) using CPCS I software. Significant differences between groups and duration of exposure were analyzed using two-way analysis of variance (ANOVA), followed by Tukey's post hoc test at 5% level of significance for multicomparison between groups using SPSS software (version 20.0). Pearson correlation analysis was performed using SPSS software (version 20.0) to determine the relationship among various fatty acids of exposure groups. Statistical analysis was done in consultation with the Department of Mathematics, Statistics and Physics, Punjab Agricultural University, Ludhiana.

Results

Physico-chemical analysis of water

In the present study, the physico-chemical analysis of control, untreated sewage water (UT), and treated sewage water revealed that the pH value of UT (8.50±0.29) was non-significantly (p>0.05) higher as compared to control (7.10±0.18) and treated sewage water (7.50± 0.18) (Table 1). However, significantly (p<0.05) higher values of temperature (34.95±1.49), BOD (492.95±1.96), DO (10.70±0.79), conductivity (980.50±2.82), and free CO_2 (42.50±0.84) were observed in UT as compared to control (temperature: 26±1.78, BOD: 137.50±0.94, DO: 5.00 ±0.42, conductivity: 325.00±2.97 and free CO_2: 8.50 ±0.42) and treated sewage water (temperature: 22.50 ±1.73, BOD: 128.65±0.60, DO: 5.50±0.72, conductivity: 385.00±0.94 and free CO_2: 11.50±0.72) (Table 1). The levels of alkalinity were found to be significantly (p<0.05) lower in UT (77.50±1.62) in comparison to control (312.50±2.10) and treated sewage water (255.00 ±2.97). All the physico-chemical parameters have shown non-significant (p>0.05) difference between control and treated sewage water (Table 1). The physico-chemical parameters of untreated sewage water viz. BOD, DO, conductivity, and free CO_2 were observed to be more than the permissible limits and the value of alkalinity was found to be less than the permissible limits given by World Health Organization (WHO 2011) and Bureau of Indian Standards (BIS 2012).

Muscle-somatic index (MSI)

It has been observed that MSI of $1/10^{th}$ LC_{50}UT and $1/20^{th}$ LC_{50} UT groups decreased significantly (p<0.05) in comparison to control and treated groups (Table 2). Significant decrease (p<0.05) was observed in MSI of $1/10^{th}$ LC_{50} UT group in comparison to $1/20^{th}$ LC_{50} UT group. However, significant increase occurred (p<0.05) in MSI of $1/10^{th}$ LC_{50} UT and $1/20^{th}$ LC_{50} UT groups after completion of 30 days of exposure.

Table 1 Physico-chemical parameters of control, untreated (UT), and treated sewage water

S. No.	Parameters	Control water	Untreated sewage water (UT)	Treated sewage water	Permissible effluent discharge limits
1.	pH	7.10±0.18	8.50±0.29	7.50±0.18	6.5-8.5 (WHO 2011)
2.	Temperature (°C)	26±1.78	34.95±1.49*	22.50±1.73	< 40 (°C) (BIS 2012)
3.	BOD (mg/lt)	137.50±0.94	492.95±1.96*	128.65±0.60	100-300 (BIS 2012)
4.	DO (mg/lt)	5.00±0.42	10.70±0.79*	5.50±0.72	<5(WHO 2011)
5.	Conductivity (S/m)	325±2.97	980.50±2.82*	385±0.94	<400 (BIS 2012)
6.	Alkalinity (mg/lt)	312.50±2.10	77.50±1.62*	255±2.97	200-600 (BIS 2012)
7.	Free CO_2 (mg/lt)	8.50±0.42	42.50±0.84*	11.50±0.72	<10 (WHO 2011)

Values are Mean±S.E.

WHO World Health Organization, *BIS* Bureau of Indian Standards

*Significant difference at $p<0.05$ among control, untreated and treated group

Biochemical outcomes

Proteins

In the present study, it has been examined that the level of protein decreased significantly ($p<0.05$) in $1/10^{th}$ LC_{50} UT and $1/20^{th}$ LC_{50} UT groups in comparison to control and treated groups (Table 3). The level of protein was found to decrease more significantly ($p<0.05$) in $1/10^{th}$ LC_{50} UT group in comparison to $1/20^{th}$ LC_{50} UT group. Furthermore, with the increase in the days of experiment, the levels of protein showed significant decrease ($p<0.05$) in $1/10^{th}$ LC_{50} UT and $1/20^{th}$ LC_{50} UT groups (Table 3).

Total lipid content (TLC)

Contrary to the results of proteins, TLC increased significantly ($p<0.05$) in muscles of $1/10^{th}$ LC_{50} UT (the values of mean ±standard error are 2.46±0.70, 2.70±0.56, 3.16±0.20, and 3.37±0.09 at 15, 30, 45, and 60 days of exposure, respectively) and $1/20^{th}$ LC_{50} UT groups as 2.93±0.67, 3.20±0.70, 3.46 ±1.07, and 3.66±0.63 at 15, 30, 45, and 60 days of exposure, respectively in comparison to control (0.88±0.24, 0.86±0.56, 1.46±0.98, and 1.16±0.49 at 15, 30, 45, and 60 days of exposure, respectively) and treated groups (0.85± 0.17, 0.87± 0.32, 1.40± 0.50, and 1.53± 0.73 at 15, 30, 45, and 60 days of exposure, respectively) (Table 4). However, significant decrease ($p<0.05$) was observed in TLC of $1/10^{th}$ LC_{50} UT group in comparison to $1/20^{th}$ LC_{50} UT group. Non-significant increase ($p>0.05$) was observed in TLC in $1/10^{th}$ LC_{50} UT and $1/20^{th}$ LC_{50} UT groups with the increase in duration of exposure (Table 4).

Fatty acid composition

Polyunsaturated fatty acids (PUFAs)

In comparison to control and treated groups, the percent of linolenic acid (C18:3) was found to decrease significantly ($p<0.05$) in 1/10th LC_{50} UT (the values of mean±standard error are 7.35±0.04, 7.54±0.13, 7.85±0.41, and 8.10±0.43 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th

Table 2 Muscle-somatic index (MSI) of *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	$1/10^{th}$ LC_{50} UT	$1/20^{th}$ LC_{50} UT
15 days	$76.80±4.01^{a1}$	$78.14±2.29^{a1}$	$36.50±0.89^{b1}$	$50.02±5.53^{c1}$
30 days	$78.52±5.16^{a1}$	$80.39±1.49^{a1}$	$38.48±2.53^{b1}$	$51.46±5.98^{c1}$
45 days	$81.64±5.08^{a1}$	$82.63±3.73^{a1}$	$49.63±1.17^{b2}$	$58.20±0.13^{c2}$
60 days	$83.67±4.89^{a1}$	$84.26±2.60^{a1}$	$59.19±0.65^{b2}$	$68.09±3.76^{c2}$

Values are Mean±S.E

Values with different numeric superscript (1–2) in columns differ significantly ($p<0.05$)

Values with different alphabetic superscript (a-c) in rows differ significantly ($p<0.05$)

UT untreated sewage water

Table 3 Protein content (mg/g tissue) in muscles of *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	1/10th LC_{50} UT	1/20th LC_{50} UT
15 days	3.84 ± 0.03^{a1}	3.67 ± 0.11^{a1}	0.92 ± 0.04^{b1}	1.38 ± 0.03^{c1}
30 days	5.17 ± 0.01^{a1}	3.71 ± 0.12^{a1}	0.68 ± 0.05^{b2}	1.29 ± 0.01^{c2}
45 days	6.45 ± 0.72^{a1}	5.70 ± 0.69^{a1}	0.42 ± 0.17^{b3}	1.13 ± 0.18^{c2}
60 days	6.85 ± 0.05^{a1}	6.41 ± 0.01^{a1}	0.23 ± 0.02^{b4}	0.87 ± 0.01^{c3}

Values are Mean±S.E

Values with different numeric superscript (1–4) in columns differ significantly ($p<0.05$)

Values with different alphabetic superscript (a–c) in rows differ significantly ($p<0.05$)

LC_{50} UT groups (8.43±0.45, 9.15±1.03, 9.46±0.60, and 10.15 ±0.61 at 15, 30, 45, and 60 days of exposure, respectively). However, non-significant ($p>0.05$) decrease was observed in 1/10th LC_{50} UT group in comparison to 1/20th LC_{50} UT group. With the increase in duration of exposure also non-significant ($p>0.05$) increase was observed in exposed groups (Table 5). In the present study, the percent of eicosapentanoic acid (C20:5) was observed to decrease significantly ($p<0.05$) in 1/10th LC_{50} UT (1.15±0.12) and 1/20th LC_{50} UT groups (1.23±0.11) after 15 days of exposure, in comparison to control (1.99±0.11) and treated groups (1.82±0.04). Non-significant ($p>0.05$) decrease was observed in 1/10th LC_{50} UT group in comparison to 1/20th LC_{50} UT group. Significant ($p>0.05$) decrease was examined in 1/10th LC_{50} UT group (0.71±0.04) after 30 days of exposure and 1/20th LC_{50} UT group as 0.92±0.05 and 0.83±0.02 at 45 and 60 days of exposure, respectively (Table 6). The percent of docosahexanoic acid (C22:6) was observed to decrease significantly ($p<0.05$) in 1/10th LC_{50} UT (0.48±0.06, 0.47±0.01, 0.40±0.01, and 0.36±0.02 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT groups (0.53±0.01, 0.50±0.02, 0.42±0.01, and 0.41 at 15. 30, 45, and 60 days of exposure, respectively) on comparison with control (0.73 ±0.05, 0.76±0.02, 0.81±0.02, and 0.83±0.01 at 15, 30, 45, and 60 days of exposure, respectively) and treated groups (0.75±0.03, 0.77±0.03, 0.79±0.04, 0.80±0.02 at 15, 30, 45, and 60 days of exposure, respectively). However, the percent was found to be decreased non-significantly ($p>0.05$) in 1/10th LC_{50} UT group in comparison to 1/20th LC_{50} UT group. Significant ($p>0.05$) decrease was examined in 1/10th LC_{50} UT and 1/20th LC_{50} UT groups after 30 days of exposure for 60 days (Table 7). The results of the present study depicted that total PUFAs (n-3) decrease significantly ($p<0.05$) in 1/10th LC_{50} UT (2.06±2.85, 2.56±2.32, 2.62±2.10, and 2.79 ±1.88 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT groups (2.64±3.19, 2.89±2.78, 2.97 ±2.49, and 3.01±2.17 at 15, 30, 45, and 60 days of exposure, respectively) as compared to control (5.34±3.99, 5.31±3.74, 5.25±3.65, and 5.68±3.89 at 15, 30, 45, and 60 days of exposure, respectively) and treated groups (4.72±3.45, 5.12±3.67, 5.31±0.71, and 5.61±3.76 at 15, 30, 45, and 60 days of exposure, respectively). Non-significant ($p>0.05$) difference was observed between 1/10th LC_{50} UT and 1/20th LC_{50} UT groups. Significant increase ($p>0.05$) was demonstrated in 1/10th LC_{50} UT and 1/20th LC_{50} UT groups at 60 days of exposure period (Table 8). The percent of linoleic acid (C18:2) was observed to decrease significantly ($p<0.05$) in 1/10th LC_{50} UT (8.50±0.20, 8.62±0.17, 9.22±0.47, and 9.56 ±0.61 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT groups (8.61±0.05, 9.00±0.44, 9.84±0.06, and 10.21±0.53 at 15, 30, 45, and 60 days of exposure, respectively) in comparison to control (11.74±0.05, 11.84±0.06, 13.18±1.07, and 13.71±0.44 at 15, 30, 45, and 60 days of exposure, respectively) and treated groups (12.11±0.25, 12.63±0.22, 13.28±0.39, and 13.49±0.01 at 15, 30, 45, and 60 days of exposure, respectively). Non-significant decrease ($p>0.05$) was observed in 1/10th LC_{50} UT group in comparison to 1/20th LC_{50} UT group. The percent was found to be

Table 4 Total lipid content (mg/g tissue) in muscles of *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	1/10th LC_{50} UT	1/20th LC_{50} UT
15 days	0.88 ± 0.24^{a1}	0.85 ± 0.17^{a1}	2.46 ± 0.70^{b1}	2.93 ± 0.67^{c1}
30 days	0.86 ± 0.56^{a1}	0.87 ± 0.32^{a1}	2.70 ± 0.56^{b1}	3.20 ± 0.70^{c1}
45 days	1.46 ± 0.98^{a1}	1.40 ± 0.50^{a1}	3.16 ± 0.20^{b1}	3.46 ± 1.07^{c1}
60 days	1.61 ± 0.49^{a1}	1.53 ± 0.73^{a1}	3.37 ± 0.09^{b1}	3.66 ± 0.63^{c1}

Values are Mean±S.E

Values with same numeric superscript (1) in columns differ non-significantly ($p>0.05$)

Values with different alphabetic superscript (a-c) in rows differ significantly ($p<0.05$)

Table 5 Percent (%) of linolenic acid (C18:3) in *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	1/10th LC_{50} UT	1/20th LC_{50} UT
15 days	13.31±0.58^{a1}	12.60±0.19^{a1}	7.35±0.04^{b1}	8.43±0.45^{b1}
30 days	13.66±0.21^{a1}	13.43±0.02^{a1}	7.54±0.13^{b1}	9.15±1.03^{b1}
45 days	14.70±0.16^{a1}	13.67±0.11^{a1}	7.85±0.41^{b1}	9.46±0.60^{b1}
60 days	14.39±0.15^{a1}	14.04±0.20^{a1}	8.10±0.43^{b1}	10.15±0.61^{b1}

Values are Mean±S.E

Values with same numeric superscript (1) in columns differ non-significantly ($p>0.05$)

Values with different alphabetic superscript (a–b) in rows differ significantly ($p<0.05$)

increased non-significantly ($p>0.05$) in 1/10th LC_{50} UT group and 1/20th LC_{50} UT groups with increase in the days of exposure (Table 9). Arachidonic acid (C20:4) percent was found to decrease non-significantly ($p>0.05$) in 1/10th LC_{50} UT (8.60±0.15, 8.64±0.49, 9.35±0.07, and 9.43±0.44 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT groups (8.39±0.05, 9.39±0.04, 9.46±0.47, and 9.70 ±0.05 at 15, 30, 45, and 60 days of exposure, respectively) as compared to control and treated groups. Non-significant decrease ($p>0.05$) was observed in 1/10th LC_{50} UT group in comparison to 1/20th LC_{50} UT group. Also, non-significant increase ($p>0.05$) was found to occur in the percent of arachidonic acid in 1/10th LC_{50} UT and 1/20th LC_{50} UT groups with the increase in the duration of exposure (Table 10). In comparison to control and treated groups, the percent of total PUFAs (n-6) was significantly decreased ($p<0.05$) in 1/10th LC_{50} UT (3.01±2.68, 3.44±2.44, 3.99±2.35, and 4.51±2.18 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT groups (4.60± 2.96, 4.70± 2.53, 5.89± 2.76, and 7.05 ± 2.43 at 15, 30, 45, and 60 days of exposure, respectively) (Table 11). Non-significant ($p>0.05$) decrease was observed in PUFAs (n-6) in 1/10th LC_{50} UT group in comparison to 1/20th LC_{50} UT group; however, after 60 days of treatment, significant increase was observed in 1/20th LC_{50} UT group in comparison to 1/10th LC_{50} UT groups. Non-significant ($p>0.05$) increase was observed in the percent level of PUFAs (n-6) in 1/10th LC_{50} UT and 1/20th LC_{50} UT groups with the increase in duration of exposure (Table 11). The results of the present study indicate that PUFAs (n-9), i.e., eicosadienoic acid (C20:2) decreased significantly ($p<0.05$) in 1/10th LC_{50} UT (0.32±0.01, 0.25±0.04, 0.18±0.01, and 0.11±0.02 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT groups (0.34±0.02, 0.26±0.01, 0.19 ±0.01, and 0.13±0.01 at 15, 30, 45, and 60 days of exposure, respectively) as compared to control (0.88±0.01, 0.86±0.03, 0.93±0.02, and 0.94±0.01 at 15, 30, 45, and 60 days of exposure, respectively) and treated groups (0.81± 0.02, 0.87± 0.02, 0.88± 0.02, and 0.90± 0.01 at 15, 30, 45, and 60 days of exposure, respectively) (Table 12). However, non-significant ($p>0.05$) decrease was observed in PUFAs (n-9) in 1/10th LC_{50} UT group in comparison to 1/20th LC_{50} UT group. Significant ($p<0.05$) decrease was observed in the percent levels of PUFAs (n-9) in 1/10th LC_{50} UT and 1/20th LC_{50} UT groups with the increase in duration of exposure (Table 12).

Saturated fatty acids (SFAs)

The percent of lauric acid (C12:0) was found to increase significantly ($p<0.05$) in 1/10th LC_{50} UT (0.82±0.01, 0.83±0.02, 0.87±0.05, and 0.89±0.01 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT groups (0.79±0.03, 0.81±0.02, 0.83±0.01, and 0.84±0.02 at 15, 30, 45, and 60 days of exposure, respectively) in comparison to control (0.54±0.02, 0.52±0.01, 0.51±0.01, and 0.49±0.03 at 15, 30, 45, and 60 days of exposure, respectively) and treated groups (0.57±0.03, 0.55±0.02, 0.49±0.01, and 0.47±0.05 at 15, 30, 45, and 60 days of exposure, respectively). Non-significant

Table 6 Percent (%) of eicosapentanoic acid (C20:5) in *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	1/10th LC_{50} UT	1/20th LC_{50} UT
15 days	1.99±0.11^{a1}	1.82±0.04^{a1}	1.15±0.12^{a1}	1.23±0.11^{a1}
30 days	2.22±0.24^{a1}	2.16±0.17^{a1}	0.71±0.04^{b2}	1.11±0.09^{b1}
45 days	2.38±0.07^{a1}	2.48±0.08^{a1}	0.60±0.01^{b3}	0.92±0.05^{b2}
60 days	2.84±0.36^{a1}	2.96±0.02^{a1}	0.57±0.06^{b3}	0.83±0.02^{b3}

Values are Mean±S.E

Values with same numeric superscript (1–3) in columns differ non-significantly ($p>0.05$)

Values with different alphabetic superscript (a–b) in rows differ significantly ($p<0.05$)

Table 7 Percent (%) of docosahexanoic acid (C22:6) in *Labeo rohita* fingerlings after rearing in control, treated and different concentrations of UT

Groups/Duration	Control	Treated	$1/10^{th}$ LC_{50} UT	$1/20^{th}$ LC_{50} UT
15 days	0.73 ± 0.05^{a1}	0.75 ± 0.03^{a1}	0.48 ± 0.06^{b1}	0.53 ± 0.01^{b1}
30 days	0.76 ± 0.02^{a1}	0.77 ± 0.03^{a1}	0.47 ± 0.01^{b1}	0.50 ± 0.02^{b1}
45 days	0.81 ± 0.02^{a1}	0.79 ± 0.04^{a1}	0.40 ± 0.01^{b2}	0.42 ± 0.01^{b2}
60 days	0.83 ± 0.01^{a1}	0.80 ± 0.02^{a1}	0.36 ± 0.02^{b23}	0.41 ± 0.01^{b23}

Values are Mean±S.E

Values with different numeric superscript (1–3) in columns differ significantly ($p<0.05$)

Values with different alphabetic superscript (a–b) in rows differ significantly ($p<0.05$)

increase ($p>0.05$) was observed in 1/10th LC_{50} UT in comparison to 1/20th LC_{50} UT group. The percent was found to be increased non-significantly ($p>0.05$) in 1/10th LC_{50} UT and 1/20th LC_{50} UT groups with the increase in days of experiment (Table 13). Percent of myristic acid (C14:0) was observed to be increased significantly ($p<0.05$) in 1/10th LC_{50} UT (6.40 ±0.17, 8.32±0.64, 10.60±0.16, and 10.80±0.50 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT (5.72±0.12, 6.43±1.15, 9.41±0.04, and 10.52±0.05 at 15, 30, 45, and 60 days of exposure, respectively) in comparison to control (2.78±0.01, 2.62±0.04, 2.04±0.06, and 1.65±0.20 at 15, 30, 45 and 60 days of exposure, respectively) and treated groups (2.51±0.05, 1.67±0.32, 1.11±0.11, and 0.70±0.06 at 15, 30, 45, and 60 days of exposure, respectively). Non-significant increase ($p>0.05$) was observed in 1/10th LC_{50} UT group in comparison to 1/20th LC_{50} UT group. Significant increase ($p<0.05$) was found to occur in 1/10th LC_{50} UT and 1/20th LC_{50} UT groups at 45 and 60 days of exposure (Table 14). Percent of pentadecanoic acid (C15:0) was found to decrease non-significantly ($p>0.05$) in 1/10th LC_{50} UT (1.16±0.02, 0.98±0.01, 0.65±0.02, and 0.25 ±0.02 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT groups (2.10±0.01, 1.92±0.01, 1.29 ±0.05, and 1.09±0.10 at 15, 30, 45, and 60 days of exposure, respectively) in comparison to control (2.62±0.06, 2.79±0.10, 2.52±0.15, and 3.39±0.40 at 15, 30, 45, and 60 days of exposure, respectively) and treated groups (2.72±0.03, 2.88±0.01, 2.94±0.05, and 3.22±0.42 at 15, 30, 45, and 60 days of exposure, respectively). Non-significant difference ($p>0.05$) was observed in 1/10th LC_{50} UT in comparison to 1/20th LC_{50} UT group. Non-significant ($p>0.05$) decrease occurred in 1/10th LC_{50} UT and 1/20th LC_{50} UT groups as the duration of exposure period increased (Table 15). The percent of palmitic acid (C16:0) was observed to increase significantly ($p<0.05$) in 1/10th LC_{50} UT (53.91±0.56, 53.86±0.15, 56.97±0.60, and 57.03±0.45 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT groups (50.47±0.01, 51.96±1.50, 54.52±0.05, and 55.92±0.56 at 15, 30, 45, and 60 days of exposure, respectively) as compared to control (26.74±0.15, 22.02±0.44, 20.96±0.49, and 20.02±0.45 at 15, 30, 45, and 60 days of exposure, respectively) and treated groups (27.68 ±0.01, 27.46±0.98, 24.57±0.99, and 22.96±0.49 at 15, 30, 45, and 60 days of exposure, respectively). Non-significant increase ($p>0.05$) was observed in 1/10th LC_{50} UT in comparison to 1/20th LC_{50} UT group. With the increase in period of exposure, the percent was found to increase non-significantly ($p>0.05$) in 1/10th LC_{50} UT and 1/20th LC_{50} UT groups (Table 16). The results of the present study revealed that the percent of steric acid (C18:0) increased significantly ($p<0.05$) in 1/10th LC_{50} UT (5.91±0.44, 6.42±0.07, 6.47±0.01, and 12.24±0.06 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT groups (5.41±0.06, 6.23±0.06, 6.58±0.01, and 11.41±0.06 at 15, 30, 45, and 60 days of exposure, respectively) in comparison to control (2.91 ±0.43, 1.52±0.06, 0.98±0.02, and 0.35±0.11 at 15, 30, 45, and 60 days of exposure, respectively) and treated groups (2.35 ±0.22, 1.97±0.02, 1.27±0.07, and 0.95±0.01 at 15, 30, 45, and 60 days of exposure, respectively). However, non-significant

Table 8 Percent (%) of total polyunsaturated fatty acids (PUFAs) (n-3) in *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	$1/10^{th}$ LC_{50} UT	$1/20^{th}$ LC_{50} UT
15 days	5.34 ± 3.99^{a1}	4.72 ± 3.45^{a1}	2.06 ± 2.85^{b1}	2.64 ± 3.19^{b1}
30 days	5.31 ± 3.74^{a1}	5.12 ± 3.67^{a1}	2.56 ± 2.32^{b1}	2.89 ± 2.78^{b1}
45 days	5.25 ± 3.65^{a1}	5.31 ± 0.71^{a1}	2.62 ± 2.10^{b1}	2.97 ± 2.49^{b1}
60 days	5.68 ± 3.89^{a1}	5.61 ± 3.76^{a1}	2.79 ± 1.88^{b2}	3.01 ± 2.17^{b2}

Values are Mean±S.E

Values with same numeric superscript (1–2) in columns differ non-significantly ($p>0.05$)

Values with different alphabetic superscript (a–b) in rows differ significantly ($p<0.05$)

Table 9 Percent (%) of linoleic acid (C18:2) in *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	1/10th LC_{50} UT	1/20th LC_{50} UT
15 days	11.74±0.05^{a1}	12.11±0.25^{a1}	8.50±0.20^{b1}	8.61±0.05^{b1}
30 days	11.84±0.06^{a1}	12.63±0.22^{a1}	8.62±0.17^{b1}	9.00±0.44^{b1}
45 days	13.18±1.07^{a1}	13.28±0.39^{a1}	9.22±0.47^{b1}	9.84±0.06^{b1}
60 days	13.71±0.44^{a1}	13.49±0.01^{a1}	9.56±0.61^{b1}	10.21±0.53^{b1}

Values are Mean±S.E

Values with same numeric superscript (1) in columns differ non-significantly (p>0.05)

Values with different alphabetic superscript (a–b) in rows differ significantly (p<0.05)

increase (p>0.05) was observed in 1/10th LC_{50} UT in comparison to 1/20th LC_{50} UT group. Percent was found to increase significantly (p<0.05) in 1/10th LC_{50} UT and 1/20th LC_{50} UT groups at 60 days of exposure (Table 17). The percent of undecyclic acid (C11:0) was found to increase significantly (p<0.05) in 1/10th LC_{50} UT (15.02±0.44, 15.97±0.49, 16.46±0.01, and 16.62±0.84 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT groups (14.07 ±0.38, 15.50±0.95, 15.68±1.03, and 16.51±1.06 at 15, 30, 45, and 60 days of exposure, respectively) as compared to control (10.52±0.05, 8.52±0.06, 8.47±0.01, and 7.41±0.06 at 15, 30, 45, and 60 days of exposure, respectively) and treated groups (12.45±0.13, 11.57±0.10, 11.45±0.12, and 10.52 ±0.06 at 15, 30, 45, and 60 days of exposure, respectively). However, non-significant increase (p>0.05) was observed in 1/10th LC_{50} UT in comparison to 1/20th LC_{50} UT group. Percent was found to show non-significant (p>0.05) increase in 1/10th LC_{50} UT and 1/20th LC_{50} UT groups with the increase in duration of exposure (Table 18). It has been observed that the percent of arachidic acid (C20:0) decreased non-significantly (p>0.05) in 1/10th LC_{50} UT (0.67±0.04, 0.62 ±0.01, 0.57±0.01, and 0.53±0.01 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT groups (0.69 ±0.03, 0.65±0.02, 0.62±0.01, and 0.59±0.05 at 15, 30, 45, and 60 days of exposure, respectively) in comparison to control and treated groups. Non-significant decrease (p>0.05) was also observed in 1/10th LC_{50} UT in comparison to 1/20th LC_{50} UT group. Percent was found to show non-significant (p>0.05) decrease in 1/10th LC_{50} UT and 1/20th LC_{50} UT groups as the duration of the experiment increased (Table 19). In the present study, total SFAs were found to increase significantly (p<0.05) in 1/10th LC_{50} UT (8.90 ±1.52, 12.95±4.89, 13.73±0.12, and 14.49±0.17 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT groups (9.93±1.66, 13.06±4.93, 14.55±0.90, and 16.48 ±0.14 at 15, 30, 45, and 60 days of exposure, respectively) as compared to control (5.45±0.93, 6.63±2.59, 6.87±0.41, and 7.22±0.43 at 15, 30, 45, and 60 days of exposure, respectively) and treated groups (5.37± 0.60, 6.01± 2.19, 6.11± 0.40, and 7.30± 0.77 at 15, 30, 45, and 60 days of exposure, respectively) (Table 20). However, non-significant (p>0.05) decrease was observed in 1/10th LC_{50} UT group in comparison to 1/20th LC_{50} UT group. Significant increase (p<0.05) was observed in 1/10th LC_{50} UT and 1/20th LC_{50} UT groups after 15 days of exposure as the duration of exposure period increased (Table 20).

Monounsaturated fatty acids (MUFAs)

The percent of palmitoleic acid (C16:1) was found to decrease significantly (p<0.05) in 1/10th LC_{50} UT (0.23±0.02, 0.17 ±0.01, 0.11±0.01, and 0.07±0.01 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT groups (0.32 ±0.01, 0.26±0.02, 0.19±0.01, and 0.11±0.02 at 15, 30, 45, and 60 days of exposure, respectively) in comparison to control (0.57±0.01, 0.62±0.01, 0.65±0.05, and 0.71±0.02 at 15, 30, 45, and 60 days of exposure, respectively) and treated groups (0.58±0.03, 0.64±0.02, 0.68±0.01, and 0.70±0.01 at 15, 30, 45, and 60 days of exposure, respectively). Significant decrease (p<0.05) was also observed in 1/10th LC_{50} UT group in comparison to 1/20th LC_{50} UT group. Percent level of palmitoleic acid (C16:1) was found to indicate significant

Table 10 Percent (%) of arachidonic acid (C20:4) in *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	1/10th LC_{50} UT	1/20th LC_{50} UT
15 days	9.56±0.11	10.15±0.26	8.60±0.15	8.39±0.05
30 days	9.69±0.28	10.22±0.33	8.64±0.49	9.39±0.04
45 days	10.66±0.21	10.60±0.17	9.35±0.07	9.46±0.47
60 days	11.23±0.25	11.17±0.72	9.43±0.44	9.70±0.05

Values are Mean±S.E

Table 11 Percent (%) of total PUFAs (n-6) in *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	1/10th LC_{50} UT	1/20th LC_{50} UT
15 days	7.84 ± 2.93^{a1}	8.60 ± 3.31^{a1}	3.01 ± 2.68^{b1}	4.60 ± 2.96^{b1}
30 days	8.61 ± 3.21^{a1}	8.82 ± 3.39^{a1}	3.44 ± 2.44^{b1}	4.70 ± 2.53^{b1}
45 days	9.48 ± 3.62^{a1}	9.37 ± 3.64^{a1}	3.99 ± 2.35^{b1}	5.89 ± 2.76^{b1}
60 days	9.61 ± 3.59^{a1}	9.87 ± 3.73^{a1}	4.51 ± 2.18^{b1}	7.05 ± 2.43^{c1}

Values are Mean±S.E

Values with same numeric superscript (1) in columns differ non-significantly ($p>0.05$)

Values with different alphabetic superscript (a-c) in rows differ significantly ($p<0.05$)

decrease ($p<0.05$) in 1/10th LC_{50} UT and 1/20th LC_{50} UT groups with increase in days of exposure (Table 21). It has been observed that the percent of oleic acid (C18:1) decreased significantly ($p<0.05$) in 1/10th LC_{50} UT (24.57±0.19, 23.66 ±1.10, 18.16±0.45, and 16.99±0.67 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT groups (28.72 ±0.16, 28.13±1.88, 22.11±1.24, and 20.67±0.22 at 15, 30, 45, and 60 days of exposure, respectively) as compared to control (36.98±0.44, 39.21±0.65, 39.60±0.81, and 42.24±1.01 at 15, 30, 45, and 60 days of exposure, respectively) and treated groups (37.57±0.29, 37.77±1.20, 39.50±0.71 and 40.82 ±0.95 at 15, 30, 45, and 60 days of exposure, respectively). Furthermore, significant decrease ($p<0.05$) was also observed in 1/10th LC_{50} UT group in comparison to 1/20th LC_{50} UT group. Percent was found to indicate significant decrease ($p<0.05$) in 1/10th LC_{50} UT and 1/20th LC_{50} UT groups at 45 and 60 days of exposure (Table 22). The results of the present study demonstrated significant decrease ($p<0.05$) in percent levels of total MUFAs in 1/10th LC_{50} UT (13.40 ±0.12, 10.91±0.11, 8.12±0.10, and 6.53±0.74 at 15, 30, 45, and 60 days of exposure, respectively) and 1/20th LC_{50} UT groups (15.52±0.14, 13.19±0.13, 11.60±0.12, and 9.39 ±0.10 at 15, 30, 45, and 60 days of exposure, respectively) in comparison to control (18.77±0.18, 19.91±0.19, 20.12 ±0.19, and 21.47±0.20 at 15, 30, 45, and 60 days of exposure, respectively) and treated groups (19.07± 0.18, 19.20± 0.18, 20.09± 0.19, and 20.76± 0.21 at 15, 30, 45, and 60 days of exposure, respectively) (Table 23). Significant decrease ($p<0.05$) was observed in 1/10th LC_{50} UT group in comparison to 1/20th LC_{50} UT group for 60 days. Significant decrease ($p<0.05$) was observed in 1/10th LC_{50} UT group and 1/20th LC_{50} UT group with the increase in duration of exposure period (Table 23).

Relative percent (%) PUFAs (n-3) to total % of fatty acids in fingerlings The relative contribution of PUFAs (n-3) to the total percent of fatty acids in fingerlings for 15, 30, 45, and 60 days was 36%, 34%, 33%, and 33% , respectively in control group, 32%, 32%, 33%, and 33%, respectively in treated group, 14%, 16%, 16%, and 16% , respectively in 1/10th LC_{50} UT group and 18%, 18%, 18%, and 18%, respectively in 1/ 20th LC_{50} UT group (Fig. 1). It clearly indicates that in 1/10th LC_{50} UT and 1/10th LC_{50} UT groups, the relative contribution of PUFAs (n-3) to the total percent of fatty acids was significantly less ($p<0.05$) as compared to control and treated groups.

Relative % PUFAs (n-6) to total % of fatty acids in fingerlings Furthermore, the relative contribution of PUFAs (n-6) to the total percent of fatty acids in 1/10th LC_{50} UT and 1/ 10th LC_{50} UT groups was also significantly less ($p<0.05$) as compared to control and treated groups for 15, 30, 45, and 60 days as depicted from the obtained values as 33%, 32%, 33%, and 31%, respectively in control group, 37%, 35%, 33%, and 32% , respectively in treated group, 13%, 14%, 14%, and 14%, respectively in 1/10th LC_{50} UT group and 17%, 19%, 20%, and 23%, respectively in 1/ 20th LC_{50} UT group (Fig. 2).

Table 12 Percent (%) of eicosadienoic acid (C20:2) or total PUFAs (n-9) in *Labeo rohita* fingerlings after rearing in control, treated and different concentrations of UT

Groups/Duration	Control	Treated	1/10th LC_{50} UT	1/20th LC_{50} UT
15 days	0.88 ± 0.01^{a1}	0.81 ± 0.02^{a1}	0.32 ± 0.01^{b1}	0.34 ± 0.02^{b1}
30 days	0.86 ± 0.03^{a1}	0.87 ± 0.02^{a1}	0.25 ± 0.04^{b2}	0.26 ± 0.01^{b2}
45 days	0.93 ± 0.02^{a1}	0.88 ± 0.02^{a1}	0.18 ± 0.01^{b3}	0.19 ± 0.01^{b3}
60 days	0.94 ± 0.01^{a1}	0.90 ± 0.01^{a1}	0.11 ± 0.02^{b4}	0.13 ± 0.01^{b4}

Values are Mean±S.E

Values with different numeric superscript (1–4) in columns differ significantly ($p<0.05$)

Values with different alphabetic superscript (a–b) in rows differ significantly ($p<0.05$)

Table 13 Percent (%) of lauric acid in (C12:0) *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	1/10th LC_{50} UT	1/20th LC_{50} UT
15 days	0.54 ± 0.02^{a1}	0.57 ± 0.03^{a1}	0.82 ± 0.01^{b1}	0.79 ± 0.03^{b1}
30 days	0.52 ± 0.01^{a1}	0.55 ± 0.02^{a1}	0.83 ± 0.02^{b1}	0.81 ± 0.02^{b1}
45 days	0.51 ± 0.01^{a1}	0.49 ± 0.01^{a1}	0.87 ± 0.05^{b1}	0.83 ± 0.01^{b1}
60 days	0.49 ± 0.03^{a1}	0.47 ± 0.05^{a1}	0.89 ± 0.01^{b1}	0.84 ± 0.02^{b1}

Values are Mean±S.E

Values with same numeric superscript (1) in columns differ non-significantly ($p>0.05$)

Values with different alphabetic superscript (a-b) in rows differ significantly ($p<0.05$)

Relative % PUFAs (n-9) to total % of fatty acids in fingerlings The results of the present study revealed that the relative contribution of PUFAs (n-9) to the total percent of fatty acids in fingerlings for 15, 30, 45, and 60 days was 37%, 38%, 43%, and 45%, respectively in control group, 34%, 39%, 40%, and 43%, respectively in treated group, 14%, 11%, 8%, and 6%, respectively in 1/10th LC_{50} UT group and 15%, 12%, 9%, and 6%, respectively in 1/20th LC_{50} UT group (Fig. 3). It indicates that in 1/10th LC_{50} UT and 1/10th LC_{50} UT groups, the relative contribution of PUFAs (n-9) to the total percent of fatty acids was significantly less ($p<0.05$) in comparison to control and treated groups.

Relative % SFAs to total % of fatty acids in fingerlings In the present study, it has been observed that the relative contribution of SFAs to the total percent of fatty acids in fingerlings for 15, 30, 45, and 60 days was 18%, 17%, 17%, and 16%, respectively in control group, 18%, 16%, 15%, and 16%, respectively in treated group, 30%, 33%, 33%, and 32%, respectively in 1/10th LC_{50} UT group and 34%, 34%, 35%, and 36%, respectively in 1/20th LC_{50} UT group (Fig. 4). It indicates that in 1/10th LC_{50} UT and 1/10th LC_{50} UT groups, the relative contribution of SFAs to the total percent of fatty acids was significantly more ($p<0.05$) as compared to control and treated groups.

Relative % MUFAs to total % of fatty acids in fingerlings In the present study, it has been observed that the relative contribution of MUFAs to the total percent of fatty acids in fingerlings for 15, 30, 45, and 60 days was 28%, 32%, 33%, and 37%, respectively, in control group, 29%, 30%, 33%, and 36%, respectively, in treated group, 19%, 17%, 15%, and 11%, respectively, in 1/10th LC_{50} UT group and 24%, 21%, 19%, and 16%, respectively, in 1/20th LC_{50} UT group (Fig. 5). It indicates that in 1/10th LC_{50} UT and 1/10th LC_{50} UT groups, the relative contribution of MUFAs to the total percent of fatty acids was significantly less ($p<0.05$) as compared to control and treated groups.

Correlation analysis of fatty acids According to Pearson correlation analysis, non-significant positive correlation was observed among the fatty acids in muscles of control and treated group fingerlings at 0.01 and 0.05 level of significance (Tables 24 and 25). In case of 1/10th LC_{50} group, arachidonic acid was found to have significantly positive correlation with linoleic acid ($r=0.830$; r represents correlation value), lauric acid ($r=0.759$), linolenic acid ($r=0.707$), myristic acid ($r=0.878$), pentadecanoic acid ($r=0.761$), palmitic acid ($r=0.820$), undecyclic acid ($r=0.736$), arachidic acid ($r=0.812$), and palmitoleic acid ($r=0.798$) at 0.05 level and significantly positive correlation with steric acid ($r=0.839$) at 0.01 level of significance; however, arachidonic acid has non-significant positive correlation with eicosapentanoic acid ($r=0.651$) and docosahexanoic acid ($r=0.678$) (Table 26). In case of 1/20th LC_{50} group, arachidonic acid was found to have significantly positive correlation with docosahexanoic acid ($r=0.774$), linoleic acid ($r=0.812$), linolenic acid ($r=0.733$), pentadecanoic acid ($r=0.827$), steric acid ($r=0.822$),

Table 14 Percent (%) of myristic acid (C14:0) in *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	1/10th LC_{50} UT	1/20th LC_{50} UT
15 days	2.78 ± 0.01^{a1}	2.51 ± 0.05^{a1}	6.40 ± 0.17^{b1}	5.72 ± 0.12^{b1}
30 days	2.62 ± 0.04^{a1}	1.67 ± 0.32^{a1}	8.32 ± 0.64^{b1}	6.43 ± 1.15^{b1}
45 days	2.04 ± 0.06^{a1}	1.11 ± 0.11^{a1}	10.60 ± 0.16^{b2}	9.41 ± 0.04^{b2}
60 days	1.65 ± 0.20^{a1}	0.70 ± 0.06^{a1}	10.80 ± 0.50^{b2}	10.52 ± 0.05^{b2}

Values are Mean±S.E

Values with different numeric superscript (1-2) in columns differ significantly ($p<0.05$)

Values with different alphabetic superscript (a-b) in rows differ significantly ($p<0.05$)

Table 15 Percent (%) of pentadecanoic acid (C15:0) in *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	$1/10^{th}$ LC_{50} UT	$1/20^{th}$ LC_{50} UT
15 days	2.62±0.06	2.72±0.03	1.16±0.02	2.10±0.01
30 days	2.79±0.10	2.88±0.01	0.98±0.01	1.92±0.07
45 days	2.52±0.15	2.94±0.05	0.65±0.02	1.29±0.05
60 days	3.39±0.40	3.22±0.42	0.25±0.02	1.09±0.10

Values are Mean±S.E

palmitoleic acid (r=0.767) at 0.05 level of significance and significantly positive correlation with eicosadienoic acid (r=0.901), lauric acid (r=0.880), myristic acid (r=0.890), palmitic acid (r=0.884), undecyclic acid (r=0.867), and arachidic acid (r=0.902) at 0.01 level of significance; however, it has non-significant positive correlation with eicosapentanoic acid (r=0.691) and oleic acid (r=0.683) (Table 27). Similarly, the other fatty acids in 1/10th and 1/20th LC_{50} group were also found to have significant positive correlation with respect to each other at 0.01 and 0.05 level of significance as shown in Tables 26 and 27.

Histomorphological examination

The results of histological study in muscles of control fingerlings at 15 days of exposure period indicated intact muscular bundles, myocyte nuclei, polygonal muscle fibre and muscular bundles (Plate 1; Fig. A, B). Intact muscular bundles, connective tissue, and myocyte nuclei were also observed in the fingerlings belonging to treated group (Plate 1; C, D). In comparison to the control and treated groups, shortening of muscular bundles, elongation of muscular bundles, hypervacuolization, edema, necrotic cells, gap formation in myofibrils, and haemorrhage in muscular bundles were observed in the muscle cells of 1/10th LC_{50} UT and 1/20th LC_{50} UT groups (Plate 1; Fig. E–H).

Furthermore, at 30 days of exposure, control and treated group fingerlings showed normal muscular bundles, interstitial material, endomysium (Plate 2; Fig. A–D), on the other hand, in 1/10th LC_{50} UT and 1/20th LC_{50} UT groups, the alterations viz. gap formation in muscular bundles, hypervacuolization, shortening of muscular bundles, haemorrhage, ruptured muscular bundles, and inter-myofibrillar space were observed (Plate 2; Fig. E–H).

The normal myotomes, connective tissue, and normal muscular bundles were observed on completion of 45 days of experimental period in control and treated group fingerlings (Plate 3; Fig. A–D); however, gap formation in myofibrils, necrotic cells, distortion in muscular bundles, edema, and hyper-vacuolization was observed in the myotomes of exposed group fingerlings (Plate 3; Fig. E–H).

After 60 days of exposure, normal muscular bundles, normal myotomes, and normal muscular fibers were observed in the control and treated group fingerlings (Plate 4; Fig. A–D), however, in case of 1/10th LC_{50} UT and 1/20th LC_{50} UT group fingerlings, prominent changes viz. shortening of muscular bundles, haemorrhage along with hyper-vacuolization in muscular bundles were observed (Plate 4 ; Fig. E–H).

Discussion

Pollution of water bodies affects the physiochemical parameters which further leads to the systematic destruction of the community level ecostructure, thus disturbing the delicate food web which in turn is hazardous to human health. The quality of water in any ecosystem provides significant information about the available resources for supporting life in that ecosystem (Tripathi et al. 2008). Similar to the findings of the present study, the analysis of physico-chemical parameters of effluents from inlet and outlet of the biological lagoons of Hawassa (Ethiopia) textile waste treatment plant indicated that

Table 16 Percent (%) of palmitic acid (C16:0) in *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	$1/10^{th}$ LC_{50} UT	$1/20^{th}$ LC_{50} UT
15 days	$26.74±0.15^{a1}$	$27.68±0.01^{a1}$	$53.91±0.56^{b1}$	$50.47±0.01^{b1}$
30 days	$22.02±0.44^{a1}$	$27.46±0.98^{a1}$	$53.86±0.15^{b1}$	$51.96±1.50^{b1}$
45 days	$20.96±0.49^{a1}$	$24.57±0.99^{a1}$	$56.97±0.60^{b1}$	$54.52±0.05^{b1}$
60 days	$20.02±0.45^{a1}$	$22.96±0.49^{a1}$	$57.03±0.45^{b1}$	$55.92±0.56^{b1}$

Values are Mean±S.E

Values with same numeric superscript (1) in columns differ non-significantly (p>0.05)

Values with different alphabetic superscript (a-b) in rows differ significantly (p<0.05)

Table 17 Percent (%) of steric acid (C18:0) in *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	$1/10^{th}$ LC_{50} UT	$1/20^{th}$ LC_{50} UT
15 days	2.91 ± 0.43^{a1}	2.35 ± 0.22^{a1}	5.91 ± 0.44^{b1}	5.41 ± 0.06^{b1}
30 days	1.52 ± 0.06^{a1}	1.97 ± 0.02^{a1}	6.42 ± 0.07^{b1}	6.23 ± 0.06^{b1}
45 days	0.98 ± 0.02^{a1}	1.27 ± 0.07^{a1}	6.47 ± 0.01^{b1}	6.58 ± 0.01^{b1}
60 days	0.35 ± 0.11^{a1}	0.95 ± 0.01^{a1}	12.24 ± 0.06^{b2}	11.41 ± 0.06^{b2}

Values are Mean±S.E

Values with different numeric superscript (1-2) in columns differ significantly ($p<0.05$)

Values with different alphabetic superscript (a-b) in rows differ significantly ($p<0.05$)

pH and conductivity were increased and had a direct relationship with effluent concentration whereas the level of DO decreased as effluent concentration increased (Workagegn 2013). Popa et al. (2012) have also studied physico-chemical characteristics of domestic and industrial wastewater collected by wastewater collectors and then discharged into Danube River and Siret River, Romania. Their study concluded that the domestic wastewater has a more negative impact on water quality as observed from high levels of pH, free CO_2, and extremely low values of DO as compared to wastewater collected from industrial areas.

In the present study, high levels of pH, temperature, BOD, and DO in untreated sewage water indicates that this untreated sewage water consists of highly alkaline pollutants which may induce lethal effects in fish (Wang et al. 2007; Lokhande et al. 2011). The higher values of conductivity depict the presence of large amount of ionized form of organic and inorganic toxicants/pollutants in wastewater (Benit and Roslin 2015). The observed decrease in the levels of alkalinity in untreated sewage water indicates lesser amount of salts viz. phosphates, carbonates, bi-carbonates, nitrates, and hydroxyl ions in free state (Bhutiani et al. 2016). Likewise the present study, the high levels of free CO_2 in urban wastewater indicate the oxidation of organic and reductive inorganic compounds at high rate (Lu et al. 2006).

Besides being an edible part, fish muscle may be the depositary for different contaminants such as dioxins, polychlorinated biphenyls (PCBs), heavy metals, pesticides, etc. which occur/discharge in the aquatic ecosystem and are global threat to food safety as fish muscle could lose its properties (energy components viz. proteins, lipids, fatty acids) due to environmental contamination (Tashla et al. 2018). Several researchers have shown that the presence of pollutants in aquatic ecosystem have a potential to induce degenerative changes in muscular tissue which could be a probable reason for decrease in the weight of muscles (Brraich and Kaur 2014; Kaur and Dua 2015), as indicated by decreased MSI in the present study after exposing fingerlings to 1/10th LC_{50} and 1/20th LC_{50} UT. Adult *Clarias gariepinus* revealed significant increase in organo-somatic index viz. hepato-somatic index, spleeno-somatic index and renato-somatic index on exposure to oilfield wastewater at the concentration of 0, 10, 20, 30, 40, 50, and 60% (Akani and Daka 2015); however, the studies having data on muscle-somatic index are still lacking.

Biochemical constituents are generally evaluated to assess the toxic stress, integrity of the immune system, and potential damage in tissues of an organism (Kavitha et al. 2010). Any alteration in biochemical parameters is simply quantifiable and provides an integrated measure of the entire physiological changes occurring in an organism (Remyla et al. 2008). In corroboration to the findings of the present study, *Cirrhinus mrigala*, *Cirrhina reha*, and *Mystus cavasius* collected from polluted water of Buddha Nullah (tributary of river Satluj, Punjab, India) indicated significantly decreased content of proteins and carbohydrates in muscular tissue (Kaur and Saxena 2002). However, *Cirrhinus mrigala* collected from the polluted sites of River Ravi indicated increased content of total proteins and decreased level of total lipids in muscles

Table 18 Percent (%) of undecyclic acid (C11:0) in *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	$1/10^{th}$ LC_{50} UT	$1/20^{th}$ LC_{50} UT
15 days	10.52 ± 0.05^{a1}	12.45 ± 0.13^{a1}	15.02 ± 0.44^{b1}	14.07 ± 0.38^{b1}
30 days	8.52 ± 0.06^{a1}	11.57 ± 0.10^{a1}	15.97 ± 0.49^{b1}	15.50 ± 0.95^{b1}
45 days	8.47 ± 0.01^{a1}	11.45 ± 0.12^{a1}	16.46 ± 0.01^{b1}	15.68 ± 1.03^{b1}
60 days	7.41 ± 0.06^{a1}	10.52 ± 0.06^{a1}	16.62 ± 0.84^{b1}	16.51 ± 1.06^{b1}

Values are Mean±S.E

Values with same numeric superscript (1) in columns differ non-significantly ($p>0.05$)

Values with different alphabetic superscript (a-b) in rows differ significantly ($p<0.05$)

Table 19 Percent (%) of arachidic acid (C20:0) in *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	$1/10^{th}$ LC_{50} UT	$1/20^{th}$ LC_{50} UT
15 days	0.73±0.05	0.75±0.02	0.67±0.04	0.69±0.03
30 days	0.70±0.10	0.72±0.06	0.62±0.01	0.65±0.02
45 days	0.67±0.19	0.69±0.10	0.57±0.01	0.62±0.01
60 days	0.62±0.24	0.65±0.10	0.53±0.01	0.59±0.05

Values are Mean±S.E

(Shakir et al. 2014). Chronic exposure of wastewater-borne nanoparticles of silver (AgNPs, 1.4–36.2 μgL^{-1}) and titanium dioxide (TiO_2NPs, 3.1–50.2 μgL^{-1}) was found to cause decrease in the levels of total proteins, lipids, and carbohydrates in the muscles of fish rainbow trout *Oncorhynchus mykiss* (Zeumer et al. 2020). In the muscles, the depletion of proteins occurs as a result of their transfer to the associated detoxification mechanisms due to the metabolic dysfunction that eventually occurred in another organs/tissues (Zeumer et al. 2020).

The common carp *Cyprinus carpio* exposed to untreated sewage water for 1 year indicated increased levels of total lipids and MUFAs, however, decreased level of SFAs and PUFAs (Sakalli et al. 2018). Variations in the lipid content and fatty acid composition of fish muscles could occur as an effect of chemicals present in the effluent discharge on the metabolism of total lipids (Giang et al. 2018). Lipids are the major energy reserves, essential components of protoplasm and hence are stored by an organism in large quantities (Hoar 1984). In the present study, exposure to untreated sewage water may has induced stress in the fingerlings resulting in the suppression of feeding which further leads to increase in the concentration of lipids (glycerol or triglycerides) in the exposed fingerlings. An increase in the concentration of lipids may also cause increase in the concentration of glucose through degradation of lipids into glucose (Azmat and Bibi 2013).

Fishes are the chief source of fatty acids, especially the essential fatty acids such as PUFAs viz. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are not synthesized by the human body itself and humans fulfil the requirement of these essential fatty acids by consuming fish (Tasbozan and Gokce 2017). A large number of biological functions are performed by fatty acids in fish viz. EPA and DHA plays a crucial role in maintaining fish health because these fatty acids are required for regulating development and functioning of neural tissue, endocrine system and immune system; cell formation; ontogenesis; and pigmentation (Tocher 2010; Bou et al. 2017). Fatty acids are also required by the fish to tolerate temperature fluctuations in their surrounding environment and for providing nutrition to the brain of an organism (Giang et al. 2018). The pollutants or toxicants present in the surrounding environment of fish not only pose threat to the health of fish but also deteriorate the quality of meat which may cause health issues in the consumers.

The results of the present study depicted that there was a significant decrease in the percent of polyunsaturated fatty acids (PUFAs) as well as MUFAs (monounsaturated fatty acids); however, significant increase in the percent of SFAs (saturated fatty acids) in the fingerlings exposed to different concentrations of untreated sewage water in comparison to control and treated group. These findings indicate that untreated sewage water may cause devastation of essential fatty acids in fish.

In a study conducted by Sakalli et al. (2018), common carp *Cyprinus carpio* was exposed to pond receiving wastewater from sewage water treatment plant, Vodnany, Czech Republic. Common carp was exposed for the duration of 360 days and after the completion of exposure period, the fish was examined for total lipid content and fatty acid profile of muscles. The total lipid content was significantly higher in muscles of the exposed group in comparison to the control group. In addition, significant alterations were also observed

Table 20 Percent (%) of total saturated fatty acids (SFAs) in *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	$1/10^{th}$ LC_{50} UT	$1/20^{th}$ LC_{50} UT
15 days	$5.45±0.93^{a1}$	$5.37±0.60^{a1}$	$8.90±1.52^{b1}$	$9.93±1.66^{b1}$
30 days	$6.63±2.59^{a1}$	$6.01±2.19^{a1}$	$12.95±4.89^{b2}$	$13.06±4.93^{b2}$
45 days	$6.87±0.41^{a1}$	$6.11±0.40^{a1}$	$13.73±0.12^{b2}$	$14.55±0.90^{b2}$
60 days	$7.22±0.43^{a1}$	$7.30±0.77^{a1}$	$14.49±0.17^{b2}$	$16.48±0.14^{b2}$

Values are Mean±S.E

Values with same numeric superscript (1-2) in columns differ non-significantly ($p>0.05$)

Values with different alphabetic superscript (a-b) in rows differ significantly ($p<0.05$)

Table 21 Percent (%) of palmitoleic acid (C16:1) in *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	$1/10^{th}$ LC_{50} UT	$1/20^{th}$ LC_{50} UT
15 days	0.57 ± 0.01^{a1}	0.58 ± 0.03^{a1}	0.23 ± 0.02^{b1}	0.32 ± 0.01^{c1}
30 days	0.62 ± 0.01^{a1}	0.64 ± 0.02^{a1}	0.17 ± 0.01^{b2}	0.26 ± 0.02^{c2}
45 days	0.65 ± 0.05^{a1}	0.68 ± 0.01^{a1}	0.11 ± 0.01^{b3}	0.19 ± 0.01^{c3}
60 days	0.71 ± 0.02^{a1}	0.70 ± 0.01^{a1}	0.07 ± 0.01^{b4}	0.11 ± 0.02^{c4}

Values are Mean±S.E

Values with different numeric superscript (1-4) in columns differ significantly ($p<0.05$)

Values with different alphabetic superscript (a-c) in rows differ significantly ($p<0.05$)

by them in quantity of fatty acids in exposed group as compared to the values in the control group. They have observed that oleic acid; C18:1 and linoleic acid; C18:2 (n-6) were significantly higher, whereas EPA; C20:5 (n-3), and DHA; C22:6, (n-3) were significantly lower in the exposed group in comparison to the control group. It was also reported that the value of linoleic acid; C18:2 (n-6) was increased after 180 days and then decreased after 360 days of exposure which could be due to reduce synthesis of linoleic acid by muscles under the conditions of stress. The results of the study conducted by Sakalli et al. (2018) also indicated that MUFAs were significantly higher, however, SFAs and PUFAs were significantly lower in the exposed group as compared to the control group. They have also observed that the content of PUFAs (n-3) was significantly lower after 360 days in the exposed group as compared to control group indicating less nutritional value of exposed group muscles for human consumption.

Hussain et al. (2017) conducted a study to examine the fatty acid profile of the freshwater fish *Cirrhinus mrigala* collected from those sites of river Chenab, Pakistan, which were highly polluted by discharge of sewage and industrial waste. The levels of PUFAs and MUFAs were significantly lower in the specimens collected from highly polluted sites, however, the level of SFAs was found to be significantly higher as compared to the specimens collected from less polluted sites. Moreover, certain fatty acids viz. caprylic acid; C8:0, lauric acid; C12:0, palmitoleic acid; C16:1 (n-7), eicosenoic acid; C20:1 (n-9), linoleic acid; C18:2 (n-6) were found to be missing in the specimens collected from highly polluted sites as compared to fish collected from less polluted areas.

Marqueño et al. (2019) represents alterations in the lipid homeostasis of the skeletal muscles using a novel non-invasive method of skeletal muscle lipidomics in the fish (*Barbus meridionalis*, *Squalius laietanus*) collected from Mediterranean River which receives urban and industrial wastewaters. The targeted analysis of skeletal muscles performed in fish collected from polluted sites revealed a decrease in phosphatidylcholines (PCs) -plasmalogens (36:4, 36:6, 38:6) and highly unsaturated PCs (36:5, 36:6, 38:6, 40:6, 40:7) and an increase in lyso-PCs (16:1, 18:1, 22:4), plasmanyl-PCs (36:5, 38:5), and cholesteryl esters (CEs) (16:0, 18:0, 20:4). Alterations in the lipid profiles of fish from polluted sites are indicators of oxidative stress and dysregulation of cholesterol homeostasis (Marqueño et al. 2019). Therefore, the approach of using muscular tissue in the present study for toxicity assessment is relatively novel given that it offers the possibility to collect muscular tissue samples without killing the fish.

Exposure of fish *Oreochromis mossambicus* to hydrogen sulphide (H_2S) a lethal gas which is produced by decomposition of organic effluents from municipal sewage and industries (Sreejai and Jaya 2010) at the level of 4.9 and 6.6 mg/L at the interval of 12, 24, 48, 72, and 96 h was found to induce oxidative stress and lipid peroxidation in fish. The end products of lipid peroxidation such as malondialdehyde (MDA) are formed from the breakdown of polyunsaturated fatty acids

Table 22 Percent (%) of oleic acid (C18:1) in *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	$1/10^{th}$ LC_{50} UT	$1/20^{th}$ LC_{50} UT
15 days	36.98 ± 0.44^{a1}	37.57 ± 0.29^{a1}	24.57 ± 0.19^{b1}	28.72 ± 0.16^{c1}
30 days	39.21 ± 0.65^{a1}	37.77 ± 1.20^{a1}	23.66 ± 1.10^{b1}	28.13 ± 1.10^{c1}
45 days	39.60 ± 0.81^{a1}	39.50 ± 0.71^{a1}	18.16 ± 0.45^{b2}	22.11 ± 1.24^{c2}
60 days	42.24 ± 1.01^{a12}	40.82 ± 0.95^{a12}	16.99 ± 0.67^{b2}	20.67 ± 0.22^{c2}

Values are Mean±S.E

Values with different numeric superscript (1–2) in columns differ non-significantly ($p>0.05$)

Values with different alphabetic superscript (a–c) in rows differ significantly ($p<0.05$)

Table 23 Percent (%) of total monounsaturated fatty acids (MUFAs) in *Labeo rohita* fingerlings after rearing in control, treated, and different concentrations of UT

Groups/Duration	Control	Treated	1/10th LC_{50} UT	1/20th LC_{50} UT
15 days	18.77 ± 0.18^{a1}	19.07 ± 0.18^{a1}	13.40 ± 0.12^{b1}	15.52 ± 0.14^{c1}
30 days	19.91 ± 0.19^{a1}	19.20 ± 0.18^{a1}	10.91 ± 0.11^{b2}	13.19 ± 0.13^{c2}
45 days	20.12 ± 0.19^{a1}	20.09 ± 0.19^{a1}	8.12 ± 0.10^{b3}	11.60 ± 0.12^{c3}
60 days	21.47 ± 0.20^{a1}	20.76 ± 0.21^{a1}	6.53 ± 0.74^{b4}	9.39 ± 0.10^{c4}

Values are Mean±S.E

Values with different numeric superscript (1-4) in columns differ significantly ($p<0.05$)

Values with different alphabetic superscript (a-c) in rows differ significantly ($p<0.05$)

in fish (Sreejai and Jaya 2010). Likewise the present study, the breakdown and depletion of polyunsaturated fatty acids in fish may disturb cellular integrity and might lead to cell damage/death. Also, unsaturated fatty acids play a crucial role in maintaining physiological homeostasis in fish, for example, arachidonic acid is the precursor of eicosanoids in fish (Bell et al. 1994), promotes fish growth and survival, provides resistance to stress (Koven et al. 2001) and also plays a major role in cellular signal transduction. The eicosanoids derived from the arachidonic acid have negative cardiovascular effects viz. vasoconstrictions and platelet aggregation. Therefore, the present alterations in the concentration of unsaturated and saturated fatty acids in the muscles of fingerlings exposed to untreated sewage water indicate that the untreated sewage water has a potential to disturb physiological equilibrium of an organism.

Monitoring the physiological status of fish by using histopathological examination serves as an early warning signal to detect disease and long-term injury of cell or tissue due to aquatic pollution (Sumi and Chitra 2017). Similar to the findings of the present study, *Clarias batrachus* revealed predominant alterations in muscle tissue viz. broken myofibrils, absence of myoseptum, disorganization of epidermis, disintegration of myotomes and lesions on exposure to 50% and 100% concentration of untreated sago industry effluent (Francis and Nagarajan 2013). Disintegration of myotomes, hyperplasia, edema, and lesions was observed in the fishes exposed to 100% concentration of untreated sago effluent (Francis and Nagarajan 2013). Fish inhabiting polluted water also showed degeneration of musclular bundles and epithelial lesions in muscle tissue which could be because of invasion by microorganisms present in the surrounding environment of the fish (Saad et al. 2012). Sub-lethal exposure to environmental pollutants may result in changes in the histological structure of cells and the occurrence of pathological changes, which can significantly change the function of tissues and organs (Olarinmoye et al. 2009; Poleksic et al. 2010).

In corroboration to the findings of the present study, histopathological alterations viz. edema, necrosis, atrophy, and intramyofibril spaces were examined in the muscles of fish *Ictalurus punctatus* which was collected from a drain which receives untreated sewage and industrial wastes from Faisalabad city, Pakistan (Shahid et al. 2021). The contaminated water from El-Rahawy drain of Egypt was also observed to cause edema, splitting of muscle and hyaline degeneration in the muscular tissue of fish *Clarias gariepinus* (Ibrahim and Ramzy 2013). Various histopathological alterations viz. necrosis, intra- muscular edema, and shortening of muscle bundle were induced in the muscular tissue of the fish *L.rohita* by the industrial pollutants discharged in the river Cauvery (Pakistan) (Dhevakrishnan and Zaman 2012). Heavy metal loaded contaminated water of ponds

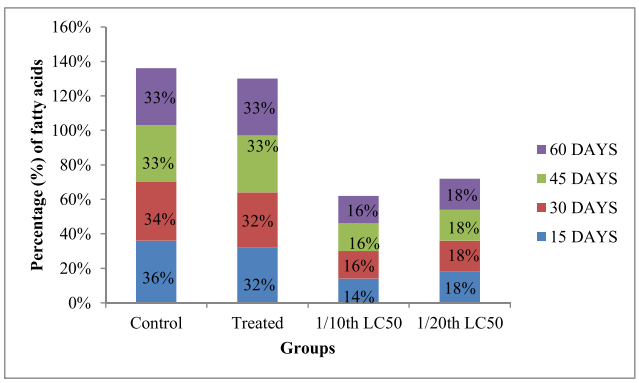

Fig. 1 Relative contribution of PUFAs (n-3) to total (%) of fatty acids in fingerlings of control, treated, 1/10th LC_{50} UT and 1/20th LC_{50} UT groups at 15, 30, 45, and 60 days of exposure. Key to abbreviations: ut: untreated sewage water

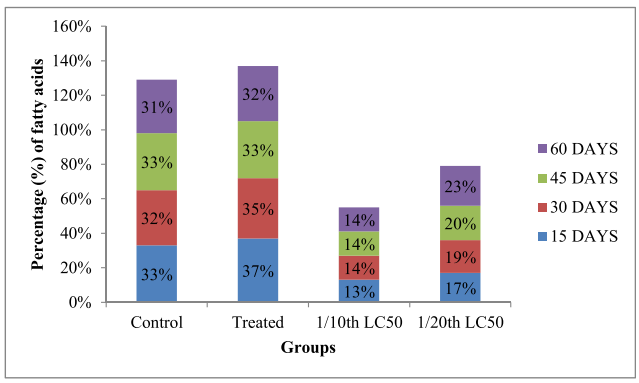

Fig. 2 Relative contribution of PUFAs (n-6) to total (%) of fatty acids in fingerlings of control, treated, 1/10th LC_{50} UT and 1/20th LC_{50} UT groups 15, 30, 45, and 60 days of exposure

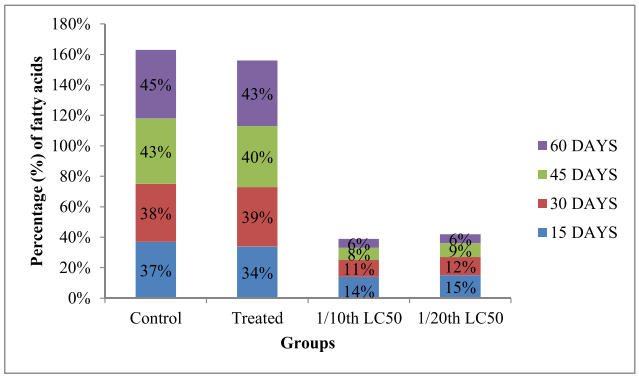

Fig. 3 Relative contribution of PUFAs (n-9), i.e., eicosadienoic acid (C20:2) to total (%) of fatty acids in fingerlings of control, treated, 1/10th LC_{50} UT and 1/20th LC_{50} UT groups at 15, 30, 45, and 60 days of exposure

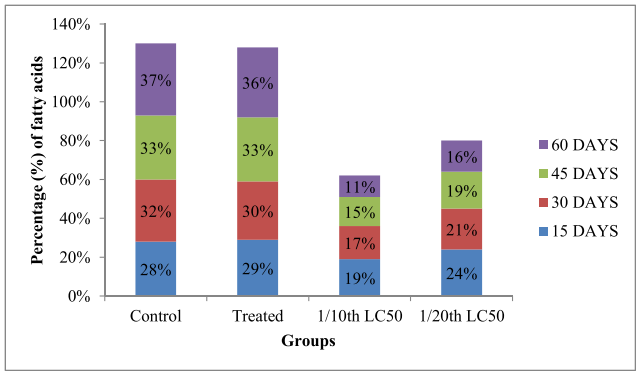

Fig. 5 Relative contribution of MUFAs to total (%) of fatty acids in fingerlings of control, treated, 1/10th LC_{50} UT and 1/20th LC_{50} UT groups at 15, 30, 45, and 60 days of exposure

(Ludhiana, India) was found to induce alterations in the histology of muscular tissue viz. separation and thickening of muscle bundle, internal edema, atrophy, degeneration, vacuolization, and necrosis of fish *Cyprinus carpio* (Ullah et al. 2014).

The pollutants present in Lake Edku, Egypt, were found to induce significant histopathological alterations viz. vacuolar degeneration, infiltration of macrophages, hyalinized and necrotic muscle fibers, parasitic granuloma, dispersed muscle fibers by edema and connective tissue proliferation between bundles and glycogen granules in the muscular tissue sections of inhabiting fish Nile Tilapia, *Oreochromis niloticus* (Haredi et al. 2020). The muscular tissue sections of the fish *Oreochromis niloticus* and *Lates niloticus* procured from Lake Nasser, Egypt, contaminated with different heavy metals revealed histopathological changes viz. distorted muscular bundles, atrophy, focal area of necrosis and vacuolization in the muscular bundles (Fatma and Mohamed 2008). Vacuolization in the muscular tissue could be a defense mechanism against injury which affecting the health status of fish (Shahid et al. 2021).

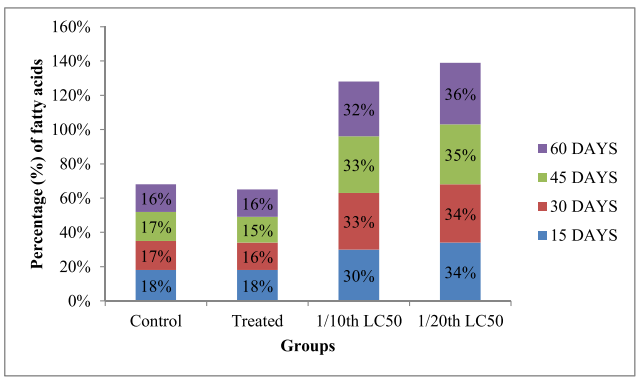

Fig. 4 Relative contribution of SFAs to total (%) of fatty acids in fingerlings of control, treated, 1/10th LC_{50} UT and 1/20th LC_{50} UT groups at 15, 30, 45, and 60 days of exposure

In the present study, the exposure to different concentrations of untreated sewage water depicted progressive damage in the structure of muscle of fingerlings with increasing duration of exposure which could be because of highly stressful conditions in wastewater generated by pollutants or toxicants or this could be a result of defense mechanism adopted by aquatic organisms to protect themselves from the toxicants present in wastewater (Saad et al. 2012). Furthermore, the degradation of fish muscle fibres could be due to the depletion of glycogen synthesis as a result of toxicity induced by pollutants in water (Sumi and Chitra 2017).

Conclusion

The present data indicates significantly decreased muscle-somatic index, decreased level of proteins, increased levels of total lipids, decreased percent of polyunsaturated and monounsaturated fatty acids, and increased percent of saturated fatty acids and histomorphological lesions in the muscular tissue of fingerlings exposed to sub-lethal concentrations of untreated sewage water. These findings revealed the lethal nature of sub-lethal levels of untreated sewage water. The experimental data indicates that no significant alterations were observed in the fingerlings reared in treated sewage water obtained from sewage water treatment plant which emphasizes the utilization of treated sewage water for aquaculture, for irrigation in agricultural fields and other household activities, primarily for the countries in Middle East and Africa which are facing the problem of water scarcity as also in highly populated countries in Asia which could make better utilization of the huge amount of wastewater generated every day. Furthermore, the biochemical and histopathological biomarkers in fish muscles have considerable potential for measuring effect of toxicants present in wastewater on fish health. The study also proposes the utilization of muscular tissue for biomonitoring program in potentially polluted water bodies to assess the health of aquatic ecosystem.

Table 24 Correlation analysis of fatty acids of control group fingerlings

FA	AA	EA	DA	LIA	LA	EDA	LAA	MA	PA	PAA	SA	UA	ADA	POA	OA
AA	1	0.755	0.738	0.878	0.705	0.668	0.758	0.707	0.766	0.774	0.908	0.841	0.816	0.957	0.892
EA		1	0.626	0.697	0.625	0.750	0.631	0.810	0.776	0.621	0.774	0.677	0.814	0.823	0.775
DA			1	0.557	0.619	0.517	0.780	0.617	0.523	0.749	0.867	0.514	0.732	0.833	0.699
LIA				1	0.695	0.805	0.414	0.822	0.708	0.683	0.743	0.770	0.711	0.819	0.603
LA					1	0.818	0.763	0.829	0.875	0.746	0.920	0.933	0.943	0.875	0.855
EDA						1	0.571	0.706	0.830	0.748	0.745	0.671	0.812	0.698	0.632
LAA							1	0.659	0.553	0.742	0.865	0.772	0.880	0.795	0.870
MA								1	0.715	0.579	0.821	0.826	0.846	0.840	0.821
PA									1	0.772	0.665	0.728	0.716	0.693	0.723
PAA										1	0.657	0.819	0.652	0.655	0.690
SA											1	0.831	0.920	0.965	0.826
UA												1	0.712	0.849	0.695
ADA													1	0.864	0.821
POA														1	0.877
OA															1

Correlation values depict non-significant difference at 0.01 and 0.05 (2-tailed)

FA fatty acids, *AA* arachidonic acid, *EA* eicosapentanoic acid, *DA* docosahexanoic acid, *LIA* linoleic acid, *LA* linolenic acid, *EDA* eicosadienoic acid, *LAA* lauric acid, *MA* myristic acid, *PA* pentadecanoic acid, *PAA* palmitic acid, *SA* steric acid, *UA* undecyclic acid, *ADA* arachidic acid, *POA* palmitoleic acid, *OA* oleic acid

In addition, human beings can directly or indirectly become exposed to genotoxic and cytotoxic agents present in wastewater by consuming fish collected from contaminated water bodies or by purchasing fish from fish vendors who procure fish cultured in farms using unspecified water source. Therefore, the discharge of untreated sewage effluents into water bodies and rearing of fish in untreated wastewater should be strictly prohibited around the world. Future studies are recommended to assess germ cells and germline changes in fish following exposure to sub-lethal concentrations of wastewater.

Table 25 Correlation analysis of fatty acids of fingerlings exposed to treated sewage water

FA	AA	EA	DA	LIA	LA	EDA	LAA	MA	PA	PAA	SA	UA	ADA	POA	OA
AA	1	0.629	0.595	0.659	0.595	0.638	0.719	0.619	0.706	0.582	0.566	0.744	0.613	0.586	0.727
EA		1	0.869	0.965	0.907	0.772	0.759	0.800	0.643	0.862	0.884	0.941	0.929	0.817	0.733
DA			1	0.858	0.753	0.613	0.615	0.638	0.680	0.621	0.661	0.788	0.721	0.675	0.735
LIA				1	0.897	0.895	0.782	0.793	0.758	0.793	0.911	0.884	0.875	0.785	0.738
LA					1	0.730	0.872	0.918	0.707	0.778	0.914	0.954	0.796	0.774	0.709
EDA						1	0.686	0.698	0.664	0.647	0.825	0.683	0.758	0.682	0.669
LAA							1	0.734	0.681	0.708	0.886	0.732	0.616	0.770	0.612
MA								1	0.679	0.719	0.888	0.825	0.740	0.867	0.677
PA									1	0.663	0.541	0.423	0.572	0.650	0.651
PAA										1	0.872	0.815	0.809	0.817	0.715
SA											1	0.831	0.754	0.892	0.629
UA												1	0.899	0.728	0.789
ADA													1	0.769	0712
POA														1	0.736
OA															1

Correlation values depict non-significant difference at 0.01 and 0.05 level (2-tailed)

FA fatty acids, *AA* arachidonic acid, *EA* eicosapentanoic acid, *DA* docosahexanoic acid, *LIA* linoleic acid, *LA* linolenic acid, *EDA* eicosadienoic acid, *LAA* lauric acid, *MA* myristic acid, *PA* pentadecanoic acid, *PAA* palmitic acid, *SA* steric acid, *UA* undecyclic acid, *ADA* arachidic acid, *POA* palmitoleic acid, *OA* oleic acid

Table 26 Correlation analysis of fatty acids of fingerlings exposed to 1/10th LC_{50} of untreated sewage water

FA	AA	EA	DA	LIA	LA	EDA	LAA	MA	PA	PAA	SA	UA	ADA	POA	OA
AA	1	0.651	0.678	0.830*	0.707*	0.656	0.759*	0.878*	0.761*	0.820*	0.839**	0.736*	0.812*	0.798*	0.689
EA		1	0.552	0.732*	0.900**	0.819*	0.779*	0.881**	0.775*	0.872**	0.817*	0.831*	0.857**	0.837**	0.622
DA			1	0.704	0.811*	0.802*	0.884**	0.821*	0.826*	0.738*	0.872**	0.784*	0.778*	0.728*	0.812*
LIA				1	0.764*	0.809*	0.791*	0.851**	0.879**	0.881**	0.906**	0.914**	0.792*	0.759*	0.850**
LA					1	0.960**	0.922**	0.942**	0.901**	0.920**	0.935**	0.910**	0.922**	0.920**	0.823*
EDA						1	0.931**	0.890**	0.951**	0.916**	0.917**	0.949**	0.840**	0.912**	0.920**
LAA							1	0.947**	0.963**	0.916**	0.906**	0.919**	0.822*	0.939**	0.889**
MA								1	0.924**	0.958**	0.949**	0.916**	0.919**	0.938**	0.820*
PA									1	0.962**	0.907**	0.982**	0.779*	0.902**	0.965**
PAA										1	0.907**	0.972**	0.827*	0.904**	0.895**
SA											1	0.918**	0.955**	0.880**	0.856**
UA												1	0.800*	0.861**	0.944**
ADA													1	0.863**	0.684
POA														1	0.804*
OA															1

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

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

FA fatty acids, *AA* arachidonic acid, *EA* eicosapentanoic acid, *DA* docosahexanoic acid, *LIA* linoleic acid, *LA* linolenic acid, *EDA* eicosadienoic acid, *LAA* lauric acid, *MA* myristic acid, *PA* pentadecanoic acid, *PAA* palmitic acid, *SA* steric acid, *UA* undecyclic acid, *ADA* arachidic acid, *POA* palmitoleic acid, *OA* oleic acid

Table 27 Correlation analysis of fatty acids of fingerlings exposed to 1/20th LC_{50} of untreated sewage water

FA	AA	EA	DA	LIA	LA	EDA	LAA	MA	PA	PAA	SA	UA	ADA	POA	OA
AA	1	0.691	0.774*	0.812*	0.733*	0.901**	0.880**	0.890**	0.827*	0.884**	0.822*	0.867**	0.902**	0.767*	0.683
EA		1	0.799*	0.532	0.859**	0.845**	0.847**	0.892**	0.850*	0.805*	0.819*	0.789*	0.761*	0.940**	0.733*
DA			1	0.557	0.804*	0.886**	0.962**	0.884**	0.962**	0.901**	0.974**	0.817*	0.883**	0.942**	0.945**
LIA				1	0.669	0.762*	0.724*	0.759*	0.631	0.831*	0.645	0.873**	0.703	0.527	0.571
LA					1	0.908**	0.884**	0.904**	0.846**	0.907**	0.904**	0.852**	0.827*	0.887**	0.866**
EDA						1	0.942**	0.988**	0.891**	0.953**	0.933**	0.894**	0.978**	0.910**	0.830*
LAA							1	0.942**	0.973**	0.976**	0.985**	0.931**	0.906**	0.941**	0.933**
MA								1	0.917**	0.939**	0.919**	0.885**	0.959**	0.933**	0.814*
PA									1	0.912**	0.960**	0.844**	0.869**	0.957**	0.919**
PAA										1	0.957**	0.974**	0.901**	0.876**	0.950**
SA											1	0.883**	0.906**	0.944**	0.968**
UA												1	0.812*	0.803*	0.827*
ADA													1	0.875**	0.790*
POA														1	0.880**
OA															1

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

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

FA fatty acids, *AA* arachidonic acid, *EA* eicosapentanoic acid, *DA* docosahexanoic acid, *LIA* linoleic acid, *LA* linolenic acid, *EDA* eicosadienoic acid, *LAA* lauric acid, *MA* myristic acid, *PA* pentadecanoic acid, *PAA* palmitic acid, *SA* steric acid, *UA* undecyclic acid, *ADA* arachidic acid, *POA* palmitoleic acid, *OA* oleic acid

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Author contribution Swarndeep Singh Hundal designed this study. Reetu Bhanot conducted experiments and performed all the laboratory work. Reetu Bhanot and Swarndeep Singh Hundal analyzed results. Reetu Bhanot wrote manuscript under the supervision of Swarndeep Singh Hundal.

Data availability Not applicable

Declarations

Ethics approval The experimental protocol met the Organisation for Economic Co-operation and Development (OECD) guidelines (1992). The methods and procedures which were used for handling the animals during the research work were in accordance with the guidelines of Control and Supervision of Experiments on Animals (CPCSEA), India.

Consent to participate Not applicable

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

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