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Acute toxic effects of untreated sewage water in *Labeo rohita* (Hamilton 1822)

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Abstract
The present study was designed to evaluate the acute toxic effects of different concentrations of untreated sewage water on survival and growth rate of *Labeo rohita* fingerlings. The fingerlings (7.62±0.25 cm in length, 8.25±0.32 g in weight) were procured from Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) Ludhiana, Punjab, India. Acute toxicity test was conducted for 96 hrs to determine LC_{50} of the untreated sewage water using five concentrations viz. 10%, 25%, 50%, 75% and 100%. Results of the study depict that the fingerlings exposed to 100% concentration of untreated sewage water showed 100% mortality rate in less than 24 hrs. The fingerlings exposed to 10%, 25% and 50% concentration of UT also showed significant decrease ($p<0.05$) in growth rate for the period of 96 hrs. Hence, the study concluded that the untreated sewage water can induce acute toxic effects in *Labeo rohita* which necessitates the treatment of wastewater before its discharge into water bodies.

Keywords: Acute toxicity, mortality, growth rate, *Labeo rohita*, untreated sewage water

Introduction
Anthropogenic activities resulting from modern methods of agriculture, urbanization and industrialization involve the increased release of various chemical pollutants and toxicants, such as industrial effluents, biocides, pesticides and chemicals, which ultimately reach into aquatic environment and become responsible for its degradation [1, 2]. The aquatic ecosystems are threatened due to unregulated discharge of industrial, agricultural and municipal pollutants throughout the world [3]. The impact of chemical compounds depend upon their quantity, toxicological and genotoxic potential and at the same time the characteristics of recipient water bodies (whether flowing or stagnant, sedimentation rate, salinity, temperature etc.). Pollution of water bodies affects the physico-chemical parameters of water bodies, leading to systematic destruction of the community level ecostructure, thus disturbing the delicate food web which in turn is hazardous to human health [4, 5, 6]. The pollution of surface and ground water due to the ever increasing industrial, agricultural and other human activities often induce severe physiological stress in aquatic fauna [4, 7]. Many of these pollutants are non-biodegradable and can accumulate in the aquatic environment and in turn biomagnify in aquatic organisms as well as in the consumers of aquatic products like humans, causing harm to the health of both humans and animals [8, 9]. About 70% of the available water is polluted in India, out of which, 8–16% is polluted by industrial pollution and 84-92% by sewage pollution [10]. Heavy metals, pesticides, pharmaceuticals, cosmeceuticals, nanomaterials and their chemical decomposition derivatives found in sewage wastewater are not well known in most cases. Their unknown toxicity, teratogenicity and carcinogenicity profile associated with lack of monitoring and control measures impose a significant hazard risk on the public health [11].
Biological communities can integrate the effect of changes in chemical, physical and biological factors of environment and hence, are good indicators of ecosystem health [12]. It is not financially or technically feasible to evaluate all the organisms in the entire ecosystem at all times. Fishes are relatively sensitive to changes in their surrounding environment making them potential indicators of the status of a specific aquatic ecosystem. 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 [13]. Fish are on top of the food chain, and through respiration or by ingestion of smaller species, they readily bioaccumulate and biomagnify a variety of contaminants [14, 15]. Since, fish can easily metabolize, concentrate and bioaccumulate water pollutants and hence, it provides an excellent source of material for the study of aquatic toxicity [16].

Furthermore, the aquatic pollutants induce an early response in the fish by causing alterations both at structural and functional levels of different organs including enzymatic and genetic effects, thereby affecting the innate immune system of exposed fish and/or increasing susceptibility to multiple type of diseases. The changes in physical, chemical and biological parameters of water alter the behaviour of fishes besides causing mortality [17].

Acute toxicity tests using fish bioassays give firsthand information about the effect of such discharges on organisms and the ecosystem as a whole. These tests are valuable in creating awareness regarding potential harmful effects of such industrial discharges to the environment [18, 19]. Various studies have been conducted on the river Sutlej as well as its tributary Buddha Nullah, Ludhiana, including assessment of water quality of river Sutlej, Punjab (India) [20], histological changes in gills and liver of fishes in river Sutlej as an effect of Buddha Nullah pollution at Ludhiana [21] and impact of pollutants on water quality of river Sutlej in Nangal area of Punjab, India [22].

Until date, little work has been done regarding the effect of untreated sewage waste water discharged in the water bodies on the health of inhabiting fishes. In view of this, the present study was designed to evaluate the effect of different concentrations of untreated sewage water on survival and growth rate of *Labeo rohita* fingerlings during an acute toxicity test for 96 hrs.

Materials and Methods

Collection and analysis of municipal sewage water

Municipal sewage waste water was collected from Sewage Water Treatment (STP) plant located at village Bhattian district Ludhiana, Punjab, India. This collection site (30º57ˈ57ˈˈN and 75º49ˈ54ˈˈE) is within the municipal limits of Ludhiana located at the distance of 10.7 Km from Punjab Agricultural University, Ludhiana, Punjab, India. Two STPs are located at Bhattian covering an area of 36.42 hectares with 5.7 Km length, using two different technologies viz. Sequencing Batch Reactor (SBR) and Upflow Anaerobic Sludge Digestor (UASB) for the treatment of untreated sewage wastewater. In the present study, the fingerlings were reared in the water obtained from the plant using SBR technology with capacity of treating 50 million litres per day (MLD) with 90% efficiency of effluent removal. The control, untreated and treated water was analyzed for analysis of physico-chemical parameters viz. pH, temperature, Biochemical oxygen demand (BOD), Dissolved oxygen (DO) and free carbon-dioxide (CO_2), using standardized methods given by American Public Health Association (APHA) [23].

Fingerlings collection and acclimatization

Fingerlings of *Labeo rohita* (Hamilton 1822) (7.62 ±0.25 cm in length, 8.25±0.32 g in weight) were procured from Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) Ludhiana, Punjab, India. The fingerlings were acclimatized to the laboratory conditions for 10 days in tanks (35 ltrs. capacity) containing chlorine-free tap water with adequate values of temperature, conductivity, dissolved oxygen and biochemical oxygen demand. A normal photoperiod (12-h light:dark cycle) was maintained during acclimatization period and during experimentation. Fingerlings were fed *ad libitum* with commercial fish food throughout the acclimatization period. The feeding was suspended 1 day prior to the experiment. The tubs were continuously aerated with electrically operated aerators (2 aerators/35 ltrs. tub) and filters (2 filters/tub).The experimental protocol met the Organisation for Economic Co-operation and Development (OECD) guidelines [24].

Acute toxicity test

Two batches, each of seven healthy fingerlings (n=7), were exposed to dechlorinated tap water (taken as control group), treated sewage water and different concentrations viz. 10%, 25%, 50%, 75% and 100% of untreated sewage water for the period of 24, 48, 72 and 96 hours. Per cent mortality rate of the fingerlings was recorded after 24, 48, 72 and 96 hours of exposure in exposed groups. None of the fingerling was found to dead in control and treated water, however, mortality was observed in the fingerlings exposed to different concentrations of untreated sewage water. The fish was considered dead when it did not respond to the probing with a glass rod. The dead specimens were removed from the tubs at the earliest after being noticed. POLO software given by Robertson *et al.* [25] was used to calculate the lethal concentration (LC_{50}) value of untreated sewage water to *Labeo rohita* for 96 hrs at different concentrations of untreated sewage water.

Statistical analysis

Multifactor analysis of variance (ANOVA) was used to determine significant difference among different groups using CPCS I software. Significant differences at $p<0.05$ were determined using Tukey's test as the post hoc analysis. Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 20.0 in consultation with the Department of Mathematics, Statistics and Physics, Punjab Agricultural University, Ludhiana, Punjab, India.

Results

In the present study, the fingerlings were exposed to control, treated and different concentrations of untreated sewage water (UT) for the period of 96 hrs. The per cent mortality rate and per cent growth rate in fingerlings was observed at the interval of 24, 48, 72 and 96 hrs. It was observed that there was no mortality in the fingerlings exposed to control, treated and 10% concentration of UT for 24, 48, 72 and 96 hrs. However, at 25% concentration of UT, two fingerlings were found dead after 96 hrs of exposure, at 50% concentration of UT, four fingerlings were observed to be dead after 24 hrs of exposure and six fingerlings showed mortality after 48 hrs of exposure, however, after 72 hrs of exposure none of the fingerling was found dead. At 75% concentration of UT, six fingerlings were observed to be dead after 24 hrs and eight fingerlings showed mortality after 48 hrs of exposure. At 100% concentration of UT, all the fingerlings exposed to water became dead in less than 24 hrs. The per cent mortality in case of control, 10%, 25%, 50%, 75% and 100% was observed to be 0, 0, 14.28, 71.42, 100 and 100%, respectively (Table 1). After the analysis of mortality rate in fingerlings, the lethal concentration (LC_{50}) of UT was calculated using POLO software. The value of LC_{50} of UT was calculated as 51.70 mg/ltrs (Table 2).

Table 1: Mortality rate of *Labeo rohita* fingerlings exposed to control, treated and different concentrations of untreated sewage water (UT) at varied time intervals

Groups \ Duration	No. of test fishes	24 hr	48 hr	72 hr	96 hr	Total no. of dead fishes	Per cent mortality
Control	14	0	0	0	0	0	0
Treated	14	0	0	0	0	0	0
10% UT	14	0	0	0	0	0	0
25%UT	14	0	0	0	2	2	14.28
50%UT	14	4	6	0	0	10	71.42
75%UT	14	6	8	-	-	14	100
100%UT	14	14	-	-	-	14	100

Table 2: The value of lethal concentration (LC_{50}) of Untreated sewage water (UT) against *Labeo rohita* for cumulative experimental period of 96 hours

Parameter	Concentration of UT(mg/L)	Slope	Heterogeneity	Degree of freedom
LC_{50}	51.70	51.64±2.45	0.00	3

During the acute toxicity test, the per cent growth rate of the fingerlings was also recorded at an interval of 24 hrs. It was observed that the fingerlings exposed to 10%, 25% and 50% concentration of UT showed significant decrease ($p<0.05$) in per cent growth rate after 24, 48, 72 and 96 hrs of exposure, in comparison to control and treated group. The minimum growth rate was observed in the fingerlings exposed to 50% concentration of UT i.e. 0.55±0.08, 0.60±0.02, 0.71±0.21 and 0.98±0.46 at 24, 48, 72 and 96 hrs of exposure, respectively (Table 3).

Table 3: Per cent growth rate of *Labeo rohita* fingerlings after exposure to control, treated and untreated sewage water (UT) at different time intervals

Duration \ Groups	24 hr	48 hr	72 hr	96 hr
Control	$2.13±0.02^{a1}$	$2.69±0.01^{a1}$	$2.73±0.18^{a1}$	$3.50±0.41^{a1}$
Treated	$2.33±0.12^{a1}$	$2.45±0.09^{a1}$	$2.49±0.16^{a1}$	$3.41±0.09^{a1}$
10% UT	$0.79±0.04^{a2}$	$0.82±0.03^{a2}$	$1.60±0.05^{b2}$	$1.63±0.06^{b2}$
25%UT	$0.75±0.02^{a2}$	$0.80±0.09^{a2}$	$1.24±0.13^{b3}$	$1.98±0.12^{b3}$
50%UT	$0.55±0.08^{a3}$	$0.60±0.02^{b3}$	$0.71±0.21^{c4}$	$0.98±0.46^{d4}$
75%UT	-	-	-	-
100%UT	-	-	-	-

Values are Mean±S.E

CD (5%): A (Groups) = 0.034561, B (Duration) = 0.024561, AB (Interaction) = 0.045361

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

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

Discussion

The results of the present study indicated that the fingerlings showed mortality and decreased growth rate on exposure to different concentrations of UT for 96 hrs. The observed value of LC_{50} of UT depicted that certain toxic impurities/substances were present in untreated sewage water which were responsible for inducing mortality and reduced growth rate in fingerlings. According to a study conducted by Kaur and Dua [26], the value of LC_{50} calculated after exposing *Labeo rohita* fingerlings to the waste water collected from different sites of Tung Dhab Drain, was 72.45% for Tung Dhab drain near a paper mill outlet and 83.20% for another site of Tung Dhab drain near village Mahal. During this acute toxicity test, the mortality rate in *Labeo rohita* fingerlings after 24 hr interval for 96 hr, was found to be increased proportionally with an increase in the concentration of wastewater obtained from Tung Dhab drain, Amritsar. The per cent mortality was recorded as 100% at 100% concentration of wastewater obtained from both the sites. Similarly, an experiment was conducted by Workagegn [27] to evaluate the acute toxicity level of effluents from inlet and outlet of the biological lagoons of Hawassa Textile waste treatment plant using Nile tilapia, *Oreochromis niloticus*, as the test organism. In this study, the data was recorded for 24, 48, 72 and 96 hrs to determine the effects of toxicants of the effluent on behavioural responses and survival rate of *Oreochromis niloticus*. Normal swimming behaviour was observed on the fish stocked at lower effluent concentration, while erratic swimming, gasping and frequent surfacing behavioural responses were observed on the fish stocked at higher effluent concentration. There was no fish mortality in control and 10% (v/v) outlet effluent concentration. The highest percentage mortality was observed at 100% (v/v) inlet effluent concentration followed by 100% (v/v) outlet and 40% (v/v) inlet effluent concentrations. The 96hrs LC_{50} and acute toxicity unit (ATU) values for inlet and outlet wastewater were 30.5% (v/v), 3.279, 71.5% (v/v) and 1.399, respectively. This study revealed that the total efficiency level of the treatment plant to remove toxicants was 57.33% (v/v). However, efficiency of the treatment plant should be improved to use the water for irrigation and other domestic purposes, otherwise, the use of the wastewater at present condition is unsafe.

The 96 hrs acute toxicity of the effluent was determined after *C. gariepinus* was exposed to six different concentrations (10-60%) of the effluent collected from pharmaceutical industry [28]. The derived 96 hrs LC_{50} of 17.41% which was 1.89 times more toxic than the 24 h LC_{50} (32.95%) showed that the effluent induced concentration-dependent mortality according to exposure duration. Subsequently, the fish was exposed to sub-lethal concentrations (2.18 - 17.41%) obtained from the 96 hrs LC_{50} for 7 and 14 days and these sub-lethal concentrations were found to cause significant increase in the frequency of micronucleated cells, abnormal nuclear erythrocytes, leucocyte and lymphocyte counts and decrease in total erythrocyte counts, hemoglobin and hematocrit concentrations of treated fish [28].

The LC_{50} values reported in the present study for wastewater are in agreement with the values reported by different workers to *Labeo rohita* when exposed to municipal wastewater of different origins. Yadav *et al.* [17] reported LC_{50} value i.e. 70.0% of industrial wastewater obtained from discharge point of fertilizer industry, Phulphur, Allahabad after exposing *Channa striatus* to this wastewater for 96 hrs. Similarly, LC_{50} - 96 hr test was also carried out by Lopamudra [29], to determine the safe concentration level of sewage water for the survival and growth of *Labeo rohita*. They had collected sewage water from sewage canal situated at metropolitan city of East Kolkata Corporation and to calculate LC_{50} of sewage water, different sub-lethal concentrations viz. 25%, 50%, 75%, 100% were taken. The toxicity of the wastewater was also observed by Chavan *et al.* [30] using fish bioassay on *Lebistus reticulate*. The LC_{50} value of wastewater was observed to be 6% which indicated that the wastewater was toxic in nature. Wastewater exerted more toxicity due to the presence of high organochlorine pesticides (OCs) i.e. 1.719 mg/l, equally sulphide concentration was 17.60 mg/l which is also higher compared to stipulated standard of 2.0 mg/l, which caused odor to the surrounding environment. It also contained oil/grease up to 80 mg/l. Surface layer of floating oil reduced the dissolved oxygen content of the water. All these parameters imparted toxicity to the fish. This study inferred that toxicity evaluation through fish bioassay should be carried out to arrive at a dilution factor for raw wastewater, prior to its discharge into surface water bodies.

With the increase in concentration of sewage water, the growth of *Labeo rohita* increases in the order: 25%<50%<75%<100%, which states that fish growth is dependent on the amount of sewage [31]. However, according to the study conducted by Nwabueze [32], the sequence of growth rate was 100%<75%<50% <25% when fingerlings of *Clarias anguillaris* were exposed to different concentrations of untreated and treated domestic sewage water.

It has been observed by Patro [33] that the growth rate of freshwater fish *Oreochromis mossambicus* decrease significantly after exposure to industrial wastewater effluent for 7, 14, 21 and 28 days and additional 28 days of recovery. The percent decrease in body weight was recorded as 7.2%, 14.96%, 25.06%, 31.89% at 7th, 14th, 21st and 28th of exposure, respectively in comparison to control. No signs of toxicity were observed in the control fish. Recovery was also found to occur non-significantly in the exposed group, instead of any recovery in exposed group, 47.96% decrease was recorded on 28th day of recovery. Reduction in feeding activity, loss of body fluids, loss of ions from body under the conditions of stress, restlessness and agitated movements could cause decrease in body weight and per cent growth rate of fish [34, 35].

Conclusion

The acute toxicity test for 96 hrs was found to induce mortality and to decrease the growth rate of *Labeo rohita* fingerlings on exposure to different concentrations of untreated sewage water. In this study, the observed value of lethal concentration (LC_{50}) of untreated sewage water also reveals the presence of toxic entities in untreated sewage water. Furthermore, the toxic effects in fingerlings were found to increase with the increase in duration of exposure. Therefore, it can be concluded that the untreated sewage has a potential to induce acute toxic effects in *Labeo rohita* fingerlings. This study necessitates the treatment of sewage water before its discharge in the water bodies for improving the health of aquatic fauna or its utilization for agricultural and other activities.

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