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A Study on Toxic Effects of Wastewater from Drain to Freshwater Fish Rohu

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Abstract: This study will explore the acute toxicity of untreated water from Labeo rohita in order to betterunderstand potential health risks. The CA divided the agglomerative hierarchy's four sample seasons intotwo clusters. Based on similarity and distance indices, CA determined that the water quality during therainy season (cluster I) was distinct from that of the other three seasons (cluster II). Three crucialcomponents for temporal water quality assessments were found using the PCAFA. The variables withthe largest factor loadings (>0.90), such as EC, TD, LP, TS, TDS, TSS, N, NH4-N, NO3-N, SO4-N, and Ni,have an impact on PC1, which explains 52.5 of the variation. PC2 accounts for 29.2 of the variationand includes Mg, AK, COD, OG, P, and Cd. According to PC3, pH, TH, and K account for 18.3 of thevariation. Minerals, organic, industrial, agricultural, and environmental contaminants that wereresponsible for the decline in drain water quality were found using PCAFA. On the basis of time andconcentration, scale morphological alterations were seen. Lepidontal breakage and uprooting,destructions at the bases of circuli and radii, complete structural loss in the focal area, neighbouringcirculi and radii, and changes in tubercle structure were also seen at the anterior and posterior regionsof scales.

Keywords: Freshwater, Drain, Toxic Effects, Wastewater, Fish Rohu

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INTRODUCTION

The capacity to respond to both internal and external stimuli is facilitated by an organism's behaviour, making it more equipped to face the challenge of survival in a dynamic environment. Therefore, behaviour is a deliberate reaction that continuously evolves as a consequence of reciprocal influences from the environment's physical, chemical, physiological, and psychological components. Behavioral and morphological changes caused by sublethal toxicity may be hazardous to marine life. Researchers have shown that changes in behaviour and appearance are more reliable indicators of health than mortality. Fish swimming patterns are a highly organised, species-specific response to pollutants that might alter locomotor activity. The effects of aquatic toxins on various fish species are best evaluated by behavioural toxicity experiments, which may be conducted with little disruption to the fish population. [1]

The pollution of the oceans is becoming an increasingly urgent problem around the world. Many aquatic organisms, including fish, have suffered as a result of the increasing quantity and volume of industrial, agricultural, and commercial chemicals released into the aquatic environment. Heavy metal pollution of aquatic systems is a topic of study for scientists in both developed and developing countries. The nutritional and therapeutic benefits of fish have led to a dramatic increase in fish consumption in recent years. Fish accumulate heavy metals through their gills, skin, or diet, and the levels of these toxins in their bodies are a reflection of their past or present exposure. [2]

Surface Water Quality

A decline in water quality and availability, intense flooding, loss of species, and changes in the distribution and structure of the aquatic biota are all consequences of human interference with one of the most influenced ecosystems on Earth: surface water. This makes surface water courses unsustainable in providing goods and services. For instance, the geomorphology and geological formations, physicochemical and microbial quality of the water, hydrological regimes, and the nature of instream and riparian habitats are all factors that affect the health of a river system. Chemical, physical, and biological properties of water are what define its quality and determine its usefulness for different purposes and the maintenance of aquatic ecosystems' health and diversity.[3]

The Environment and Ecology

The term "aquatic ecosystem" refers to the groups of creatures that live in bodies of water and are reliant on one another and their surroundings. Freshwater ecosystems and marine ecosystems are the two basic kinds of aquatic ecosystems. The marine environment is one of the biggest aquatic ecosystems on Earth, and it is characterised by its salinity-rich waters. It consists of coral reefs, sea grass beds, mangrove forests, lagoons, tidal zones, marshes, and estuaries. A subset of the aquatic ecosystems on Earth, including lakes and ponds, rivers, streams, springs, bogs, and wetlands, is the freshwater environment.[4]

While salt-water habitats have significant salinity (around 3.5%), freshwater ecosystems are found in inland waterways with relatively low salt concentrations (0.05%). The most important water sources are rivers and lakes. Over millions of years, a variety of species have developed and adapted to aquatic environments that provide the food, water, shelter, and space necessary for the survival of aquatic animals and plants. Many of these kinds of animals and plants are aquatic; some, like fish, spend their entire lives beneath the water, while others, like toads and frogs, may only utilise surface waterways while reproducing or when they are young. Limnology is the study of watery ecosystems. Evaporation, transpiration, precipitation, and runoff are continual processes that cause water to travel until it reaches the sea.[5]

Water-Waste-Resource Management

Everyone has the right to live in an atmosphere that is free from danger and filth, and as a result, we have an obligation to make a concerted effort to reduce levels of pollution and ensure that this world is preserved for the benefit of subsequent generations. There is still a significant number of commercial enterprises that dispose of their sewage directly into waterways or landfills without first diluting it. However, as a direct result of the Water Act of 1974, every company is currently in the process of constructing its very own in-house treatment facilities, the vast majority of which can be found in the South Gujarat region. The fact that many inorganic and synthetic organic pollutants found in industrial wastewater are resistant to biodegradation presents a challenge for treatment processes.[6]

- **Primary treatments**

Mechanical methods or gravity are examples of primary treatments, both of which are used to remove the waste's floating and suspended particles. In initial treatment operations, fine screens and sedimentation tanks are often used. The raw effluent will be screened manually using a bar screen chamber for bigger particles and leaves. Grease and oil are present in effluent, which stink when released into the water and are challenging for sludge digestion tanks to break down. Skimming tanks are used to remove them. Next, lime will be added to the effluent to reduce its pH in a flocculation tank, where it will be treated.[7]

- **Secondary treatments**

After the first treatment, biological techniques must be utilised to further reduce the suspended and dissolved particles in the liquid effluent. Before entering the exchanger system, effluent water from the primary treatment will be delivered to filter feed pumps, where it will pass through pressure sand filters to remove any suspended particles. By exchanging certain cations and anions for sodium, hydrogen, or other ions in a resinous substance, ion exchange is a technique for treating wastewater.[8]

LITERATURE REVIEW

Elzen, G.W. (2019) Vengaiah lake and Yellamallappa Chetty lake were chosen for the examination of trace metals including arsenic, aluminium, cadmium, lead, mercury, iron, copper, and zinc in water samples near Krishnarajpuram-Hoskote taluk, Bangalore, Karnataka. Labeo rohita, a species of freshwater fish, was bred and grown in these water systems, and its bioaccumulation of trace metals in muscle and gill tissues was studied. Hebbal fish farm was utilised as a standard for water and fish quality testing (control site). Atomic absorption spectroscopy was used to quantify trace metal concentrations in water and fish samples, with the data then being compared to FAO/WHO guidelines. Lake B water samples revealed varying concentrations of trace metals including Al, As, and Hg due to the lake's complex toxicology and the wide range of potential sources. Al, Pb, and Cd contents in Lake B water samples were strongly correlated with concentrations in fish tissues such as muscle and gills. Fish tissues and water samples from all bodies of water had detectable levels of Cu, Zn, and Fe. In Lake B, fish gills had a greater concentration of metals. [9]

Anita Susan, T. (2016) Following the spraying, three days later, rynaxypyr showed the greatest percentage reduction in the population of Helicoverpa armigera, followed by spinosad and indoxacarb. In the population of Helicoverpa armigera, seven days after spraying, rynaxypyr showed the greatest parentage reduction, followed by spinosad and indoxacarb. In addition to this, he discovered that even a minute amount of exposure to spinosad was enough to cause an excitation of the insect nervous system, which resulted in involuntary muscle contractions, prostration with tremor, and ultimately paralysis. It is very effective against pests of the lepidopteran, dipteran, and thysanopteran orders, as well as certain species of coleopteran, homopteran, hymenopteran, and orthopteran pests.[10]

Cavaş, T. and Ergene-Gozukara, S. (2018) Larvae of the fourth instar of the species Spodoptera littoralis (Boisd.) were used in this study to investigate the insecticidal, biological, biochemical, and histological effects of the bioagents spinosad, diple 2x (Bacillus thuringiensis var kurstaki), and a pyrethroid compound respectively. According to the results of the LC50 concentration, cyclomethrin seemed to be the most poisonous of the three to S. littoralis. All of the treatments resulted in a substantial reduction in the longevity, fecundity, and fertility of the females as compared to the controls. It was found that the total protein content, carbohydrate content, phosphatase activity, and carbohydrase levels all underwent shifts that were not only variable but also significant. Malathion, an organophosphorus, was discovered to be the most toxic of the insecticides that were tested against the Chrysoperla carnea that was studied in this article, while spinosad was found to be the least toxic. In addition to this, a number of ovarian histology structures that were aberrant were found.[11]

H.F. Dahi and Y.A. El-Sayed (2018) The histological changes that were examined included epithelial swelling and lifting, leukocyte infiltration, an increase in epithelial cells, and fused and dilated lamellae. Histopathological changes that occurred in fish O. mossambicus after exposure to paper mill effluent included hyperplasia, epithelial lifting, cell swellings, congestions, the bending of secondary lamellae, the formation of edematous spaces that became infiltrated with red blood cells and leukocytes, and finally whole epithelium became degenerated. [12]

Hackenberg, D. (2017) An increased number of epithelial cells, an aneurysm, eroded epithelial cells, fused gill lamellae, and congested blood sinuses were observed in the gills of Mystus cavasius exposed to effluent from an electroplating industrial process. When researchers looked at the gills, they saw these alterations. The epithelium of C. lazera fish exposed to dyestuff and chemical wastewater exhibited hyperplasia, proliferation, lamellar fusion, epithelium lifting, and a change in the surface structure of epithelial pavement cells. The researchers say that the toxicity of the pesticide, the safety measures taken during its application, the dosage, the pesticide's adsorption on soil colloids, the post-treatment environment, and the pesticide's persistence in the atmosphere all play significant roles in determining the final outcome.[13]

METHODOLOGY

Information about test species, acute (LC50, behavioral, and morphological studies), and chronic (histopathology, micronuclei, and scale studies) bioassays and their methodologies are included in the materials and methods. The sampling site location, collection, instrumentation, and wastewater analysis are all thoroughly described.

Study Area

Tung Dhab Drain (31°67′612″ N 74°74′280″ E) is the location of the present investigation's sample site. Tung Dhab drain stretches over 20 kilometres, with a catchment area of 208.83 square kilometres, a capacity of 53 cubic metres per minute, and a bed width of either 13.72 metres (at the outfall) or 1.22 metres (at starting point). The drain is a natural storm water drain, but industries are using it to dump their untreated waste. In addition to collecting effluents from milk factories, iron foundries, and woollen dyeing mills through the Verka and Gumtala drains, the drain also receives sewage water from the city of Amritsar. As there is currently no treatment plant set up along the drain, the level of pollution is quite high. The PWSSB study states that the Amritsar North zone's sewage (91.98 MLD) is currently being disposed of using temporary disposal facilities and is being fed into the Tung Dhab Drain (2006). It travels through Verka, Fatehgarh Shukarchak, Nashera, Othian, Mahal, Gumtala, and Wadala Bhitewadh before joining the Hudiara drain at Khiala Khurd and finally emptying into the River Ravi at the international boundary.

Sampling and Physico-chemical Examination of Wastewater

During the 2011-2012 dry (January, March) and wet (June, August) seasons, monthly samples of drain water were collected (between 0500 and 0700 hours) at the sampling site. Sludge was collected in 120 L polypropylene cans and sent to a facility for both acute and chronic bioassay testing within fifteen minutes of its origin. Physical and chemical testing samples were collected in sterilised, properly labelled plastic and glass bottles, frozen as soon as possible, and analysed according to the APHA/AWWA/WEF methodology

Data Transformation

The variables in this study followed a normal distribution, as determined using Kolmogorove-Smirnov (KeS) statistics. The water quality datasets were standardised using a z-scaling transformation (34 variables), and then analysed using one of three multivariate methods: principal component analysis, cluster analysis (CA), and factor analysis.

Test Species

Figure1: Test organism: *L. rohita*

Major carps L. rohita, C. catla, and C. mrigala constitute 87% of India's freshwater fish output. Due to its great commercial worth, L. rohita is a highly-priced food fish with strong market demand that is widely cultivated across India. It is indigenous to Bangladesh, India, Myanmar, Nepal, Pakistan, and Sri Lanka and may be found in the majority of local freshwater environments, including rivers, ponds, and reservoirs.

Fish Procurement and Acclimatization

L. In aerated polythene bags, rohita fingerlings weighing 8.4 g and measuring 7.7 cm at length were acquired from the government fish farm in Rajasansi, Amritsar. The fish were securely wrapped to protect them from harm while being transported to the lab. In order to prevent fish deaths brought on by changes in tank temperature, aerated bags were utilised (63x39x63cm). By submerging it in a potassium permanganate solution (2 mg/L) for four hours, the whole fish supply was rendered sterile. Each tank received a total of 1 gramme of fish mixed to 1 litre of water. The necessary amount of dissolved oxygen was maintained throughout the experimentation period with the use of aerators. Fish were given commercial fish food ad libitum for one day before to the experiment, after which they were left without food

RESULT

Wastewater Analysis

The physicochemical characteristics of wastewater from the Tung Dhab drain in Amritsar, India, were analysed using conventional methods (Trivedy and Goel, 1986; APHA/AWWA/WEF, 2005). The mean and standard deviation for 34 physicochemical characteristics of the wastewater from the Tung Dhab drain that were examined over the course of four seasons are shown in Table 4.1. Average concentrations of heavy metals like Cr, Mn, Ni, Pb, Zn, and As, as well as TD, TSS, BOD, COD, OG, NO3-N, and TSS were much higher than those that the Environment Protection Amendment Rules for discharge into inland waterways recommended. All of these show discernible temporal variations over several months. The results showed that the DO values varied from 0.19±0.02 to 0.83±0.02 mgL-1 (Fig. 4.1A); TD values ranged from 126.17±1.59 to 306.22±1.83 mgL-1 (Fig. 4.1B); TSS values varied from 117.23±1.27 to 422.17±0.49 mgL-1 (Fig. 4.1C); NO3-N values ranged from 3.50±0.56 to 15.67±0.46 mgL-1 (Fig. 4.1D); COD values varied from 181.33±1.45 to 283.00±1.53 mgL-1 (Fig. 4.1E); BOD values ranged from 135.0±1.15 to 224±1.15 mgL-1 (Fig. 4.1F); OG values varied from 262.33±1.20 to 343.67±2.73 mgL-1 (Fig. 4.1G). Values for heavies like Cr, Mn, Ni, Pb, Zn, and As were all over the map from 0.42±0.33 to 0.77±0.02 (Fig. 4.1H); 2.08±0.01 to 2.65±0.01 (Fig. 4.1I); 1.67±0.008 to 3.43±0.01 (Fig. 4.1J); 1.66±0.01 to 3.73±0.08; 1.70±0.62 to 9.63±0.60 (Fig. 4.1L) and 0.02±0.005 to 0.20±0.11 mgL-1 respectively.

Table 1: Physicochemical characteristics of water samples

Parameters	Sampling Seasons				EPAR MPL
	Summer	Spring	Rainy	Winter	
	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	
Temp	31.63±0.30	25.36±0.60	28.33±0.29	22.46±0.26	20-35
pH	7.68±0.01	6.92±0.01	7.08±0.005	7.72±0.01	5.5-9
DO	0.19±0.02	0.56±0.01	0.83±0.02	0.31±0.01	-
EC	1038.70±1.20	1065.0±1.15	958.67±1.20	1082.7±1.45	-
TD	172.43±1.07	283.13±1.17	126.17±1.59	**306.22±1.83**	**300**
LP	9.23±0.09	9.38±0.02	10.26±0.01	8.53±0.20	-
AD	108.30±1.33	181.93±0.81	71.00±1.42	145.97±1.49	-
AK	752.77±1.07	664.67±0.78	375.20±1.89	539.20±0.64	-
CO_2	83.56±0.41	92.53±0.59	35.43±0.69	75.58±0.74	-
TH	300.50±1.57	215.17±1.34	241.77±1.07	285.67±0.92	-
Ca	65.64±0.50	77.49±0.79	76.33±0.64	96.25±0.64	100

Mg	32.39±0.64	33.43±0.38	39.63±0.48	45.63±0.64	100
TS	1020.9±1.25	1071.6±1.59	820.93±1.32	1292.3±1.22	-
TDS	750.93±1.85	788.23±0.97	703.70±2.59	870.10±0.72	-
TSS	**270.00±0.63**	**283.70±0.75**	**117.23±1.27**	**422.17±0.49**	**100**
COD	**283.00±1.53**	207.67±0.88	181.33±1.45	213.67±1.20	**250**
BOD	**224.00±1.15**	**138.67±1.20**	**135.00±1.15**	**153.07±1.75**	**30**
OG	**343.67±2.73**	**325.33±0.88**	**289.67±0.88**	**262.33±1.20**	**10**
N	39.67±1.20	49.33±1.45	37.33±0.90	51.67±1.20	-
P	3.09±0.02	3.49±0.03	3.84±0.01	3.92±0.02	10
K	17.63±0.55	21.7±0.69	18.80±0.57	19.40±0.40	-
NH_4-N	6.70±0.30	14.56±0.70	4.93±0.44	19.78±1.01	50
NO_3-N	4.50±0.64	**11.50±0.64**	3.50±0.56	**15.67±0.46**	**10**
Cl^-	68.51±0.66	91.21±0.90	48.98±0.73	83.78±0.83	1000
2- SO_4	62.66±0.88	68.66±0.88	55.00±1.73	81.00±1.15	1000
Cr	**0.42±0.03**	**0.74±0.01**	**0.46±0.02**	**0.77±0.02**	**0.1**
Cd	0.336±0.001	0.236±0.001	0.106±0.01	0.197±0.001	2.0
Cu	1.11±0.02	1.28±0.01	0.14±0.01	1.086±0.01	3.0
Fe	0.59±0.01	0.85±0.02	0.19±0.02	0.260±0.02	3.0
Mn	**2.37±0.01**	**2.17±0.01**	**2.08±0.01**	**2.65±0.01**	**2.0**
Ni	1.83±0.01	2.34±0.005	1.67±0.008	**3.43±0.01**	**3.0**
Pb	**2.28±0.008**	**2.18±0.01**	**1.66±0.01**	**3.73±0.08**	**0.1**
Zn	**5.63±0.13**	**9.63±0.60**	1.70±0.62	3.30±0.49	**5.0**
As	**0.20±0.11**	0.18±0.08	0.020±0.005	0.14±0.04	**0.2**

- **Karls Pearson.s correlation coefficients**

The calculated t values and the very significant correlations (0.90-1.0) between the investigated physicochemical parameters are shown in Table 4.2. TDS and SO42- were found to have the strongest positive correlation (r =1.0, t=23.97), followed by NH4-N and NO3-N (r =0.999, t=4.69), TD and N (r =0.996, t=4.46), TS and TSS (r =0.996, t=22.13), CO2 and Cu (r =0.990, t=5.74), and TS and SO42- (r =0.9). The negative connection between LP and TSS and TS is very significant (r=-0.991, t=4.21; r=-0.976, t=10.73).

- **Cluster analysis**

Cluster analysis was used to generate a dendrogram depicting the relationships between the different seasons in the dataset in terms of their temporal proximity (Bray Curtis method) and separation (Ward's method). After conducting a cluster analysis, the four distinct seasons was separated into two groups: group I included the dry season, while group II included the other three: spring, summer, and winter. The highest resemblance between the spring and summer seasons was 93.3%. Summer and winter were most comparable, with a score of 88.6%, followed by spring with a value of 92.4%. (Fig. 4.2A). The two clusters' reported dissimilarity was 86.4%. The Bray Curtis method's cophenetic correlation was 0.902. The Bray Curtis technique of clustering provided further support for the conclusions from Ward's approach.

Table 4.2 displays the estimated t values and the very significant correlations (0.90-1.0) between the examined physicochemical parameters. The highest positive connection was reported between TDS and SO42- (r =1.0, t=23.97), with subsequent correlations between NH4-N and NO3-N (r =0.999, t=4.69), TD and N (r =0.996, t=4.46), TS and TSS (r =0.996, t=22.13), CO2 and Cu (r =0.990, t=5.74), and TS and SO42- (r =0.9 There is a strong negative correlation between LP, TSS, and TS (r=-0.991, t=4.21; r=-0.976, t=10.73).

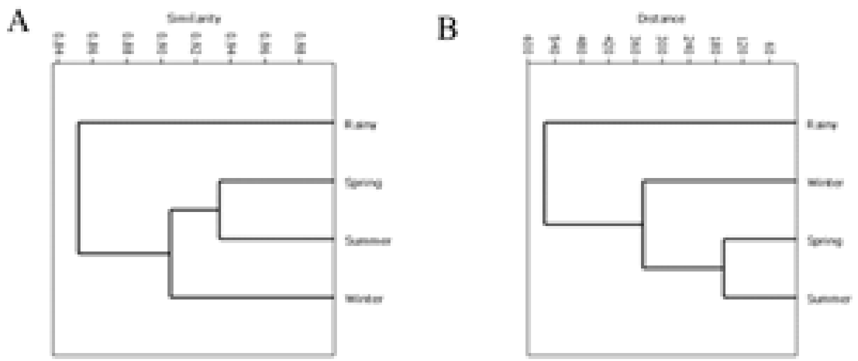

Figure 4.1: Dendrogram (A) similarity and (B) distance clusters

Acute Toxicity Bioassays

To calculate LC50 values and investigate behavioural and morphological changes, acute toxicity experiments were carried out. Prior to the start of the research, samples of the laboratory tap water and wastewater were both evaluated.

- **Analytical results**

Maximum, average, and mean S.E. in Table 4.4 contains the obtained values for the physicochemical properties of diluent water and wastewater. The published findings for the experiment's effluent and dilution water were entirely different. When compared to the standards for effluent discharge into inland surface rivers, wastewater samples did not meet them, even if the findings for dilution water were within permitted levels. The sample water had a pH that was somewhat alkaline, a dark grey colour, and a strong scent. The reported values for a variety of variables, including as TSS, BOD, COD, OG, and heavy metals like Cr (VI), Mn, and Pb, were much greater than the advised discharge limits specified in the Environment Protection Amendment Rules (EPAR, 2012).

Table 2: Physicochemical parameters of wastewater

Para-meters	Tap water (used for dilutions and running control)			Wastewater			MPL EPAR (2012)
	Limit	Range	Mean ± S.E.	Limit	Range	Mean ±S.E.	
Temp	22.4-25.5	3.1	23.7±0.93	29.0-30.2	1.2	29.7±0.38	20-35
pH	7.00-7.20	0.20	7.13±0.06	7.02-7.08	0.06	7.04±0.02	5.5-9
DO	6.4-7.6	1.2	6.9±0.35	0.32-0.46	0.14	0.39±0.04	-
EC	469-498	29.0	483.3±8.37	958-961	3.0	959.3±0.88	-
TD	0.22-0.30	0.08	0.27±0.02	152.0-159.6	7.60	155.8±2.19	300
AD	29.2-34.2	5.07	31.9±1.49	80-84.37	4.37	82.3±1.26	-
AK	170-189	19	179.7±5.5	537-553	16.0	545.3±4.63	-
TH	128-139	11.0	133±3.21	265.8-283.6	17.8	276.2±5.39	-
Ca	43-52.1	9.10	47.7±2.63	62.8-72.5	4.30	70.53±1.25	100
Mg	24-30.3	5.91	27.6±1.74	34.5-37.9	3.40	36.27±0.98	100
TS	420-450	30.0	436.7±8.82	940.9-957.5	16.6	950.4±4.93	-
TDS	290-310	20.0	300±5.77	698.2-709.3	11.1	703.63±3.21	-
TSS	73-87	14.0	79.7±4.06	242.7-249.3	6.6	**246.7±2.04**	**100**
Cl^-	17-17.9	0.85	17.3±0.28	64.9-69.5	4.6	67.4±1.34	1000
SO_4^{2-}	2.87-3.24	0.37	3.04±0.11	55-67	12.0	60.3±3.53	1000
N	3.8-6.2	2.4	4.8±0.72	32-48	16.0	42.3±5.17	-
K	4.5-5.9	1.4	5.0±0.45	17.7-21.5	3.80	19.4±1.11	-
∑P	0.03-0.06	0.03	0.05±0.008	3.09-3.95	0.86	3.5±0.25	10
NH_4-N	2.2-2.8	0.6	2.5±0.18	5.2-5.9	0.7	5.5±0.2	50
NO_3-N	1.4-1.9	0.5	1.6±0.15	3.8-4.2	0.4	3.97±0.12	10
BOD	8-12	4.0	10±1.15	222.5-237.5	15.0	**230.9±4.43**	**30**
COD	45-48	3.0	46.6±0.88	276.5-288.7	12.2	**282.4±3.53**	**250**
OG	4.3-5.2	0.9	4.8±0.27	248.8-267.0	18.2	**256.4±5.45**	**10**
Cd	0.003-0.007	0.004	0.05±0.001	0.195-0.33	0.14	0.26±0.04	2.0
Cr (VI)	0.05-0.08	0.03	0.07±0.008	0.37-0.41	0.04	**0.39±0.01**	**0.1**
Mn	0.1-0.5	0.4	0.3±0.12	2.07-2.15	0.08	**2.1±0.02**	**2.0**
Ni	0.05-0.08	0.03	0.06±0.009	1.79-1.84	0.05	1.82±0.01	3.0
Pb	0.18-0.23	0.05	0.21±0.01	2.09-2.14	0.05	**2.1±0.01**	**0.1**
Cu	0.05-0.09	0.04	0.07±0.01	1.07-1.13	0.06	1.09±0.02	3.0
Fe	0.017-0.02	0.003	0.02±0.0008	0.35-0.43	0.08	0.39±0.02	3.0

- **Behavioural studies**

The control fish was alert to any slight disturbance near the tank and displayed active feeding, typical schooling behavior, well-synchronized body movements, and normal feeding habits. Because the behaviour of the control groups in the current investigation did not significantly change, it was used as the benchmark for the whole trial. In Table 4.6, experimental fish L. rohita's behavioural responses to exposure to various wastewater concentrations over the course of 1, 6, 24, 48, 72, and 96 hours are compared. When exposed to various wastewater concentrations and exposure times, treated fish showed a variety of changed behavioural responses. The intensity of the increased air gulping and surfacing was not as high as it had been at the beginning of the trial, but it persisted. Fish H. fish L. fossilis exposed to cadmium (Vutukuru, 2005). Rohita was exposed to various quantities of chromium, lead nitrate, tannery effluent, and fish H. The aqueous extract of Buchanania lanzan bark and fossilis following treatment with textile effluent

(Chaudhary et al., 2001) both display this sort of.

Table 3: Comparative and cumulative behavioural responses

Behavioural Response	Control	6.25%	12.5%	25%	50%	100%
Jumping	-	-	+	+	+++	++++
Loss of balance in the column of tank	-	-	-	++	++++	++++
Restlessness	-	-	-	+	+++	++++
Schooling	-	-	-	++	++++	++++
Startle response	-	-	+	++	++++	++++
Un-coordinate Swimming	-	-	-	++	++++	++++

Epithelial cell surface changes in ultrastructure

All three of the treated groups showed differences in the surface ultrastructure of epithelial cells from the control group as a result of wastewater exposure. Three distinct cell types were seen in control fish: pavement cells (PVC), chloride cells (CC), and mucous cells (MC). Both the filaments and the lamellae of PVC contained many microridges and well defined cell boundaries, especially on the main lamellar surfaces. Chloride cells were present in both the filament and lamellar epithelium, however they were primarily confined to the interlamellar gaps. Mucous cells at different embryonic stages were also seen on filament (Fig. 4.11a). The height and density of the microridges considerably reduced after 15 days of exposure to the wastewater. Epithelial cell hypertrophy and hyperplasia, which produced folded and uneven surfaces, were also seen at all three doses. Mucus layers on the filaments' surface showed an increase in the number of MC, whereas the number of CC seemed higher than in the control. The pavement epithelium developed wrinkles after being exposed to the toxicant for 30 days as a result of epithelial cell hyperplasia and hypertrophy, a mild loss of microridges, an abundance of MC releasing their granules, and an abundance of CC that occupied the entire interlamellar space with larger apical surfaces and microvilli. The primary lamellar epithelium of fish exposed for 60 days showed inconsistent thickening, sloughing off, and complete loss of microridges throughout considerable portions.

Table 4: Comparison of gill mean DTC values in treated and recovered fish L. rohita

Concentrations (in %)	DTC (Degree of Tissue Change)		
	60 days (Treatment)	60 days (Recovery)	F value
	Mean±S.E	Mean±S.E	
17.7%	77.80±21.40	43.70±14.20	1.75^{NS}
26.6%	93.30±20.40	56.80±19.70	
35.4%	116.20±16.70	72.20±23.50	

Fish testing Fish L's altered gill tissue demonstrates the drain's poor ecosystem. rohita, such as high DTC readings and gill lesions. The detected gill lesions increased between 12.67 (17.7%, 15 days) and 58.1 (35.4%, 60 days) in contrast to the control, with both concentration and time being important factors. The findings of Alazemi et al. (1996), who found that fish gills are among the best markers of water pollution because gill impairment brought on by toxins may have a significant negative impact on fish health, were in agreement with this outcome. Epithelial lifting, hypertrophy, and hyperplasia of epithelial cells caused the pathological lesions seen in treated fish, such as lamellar fusion, which seals the water gaps between the secondary lamellae.

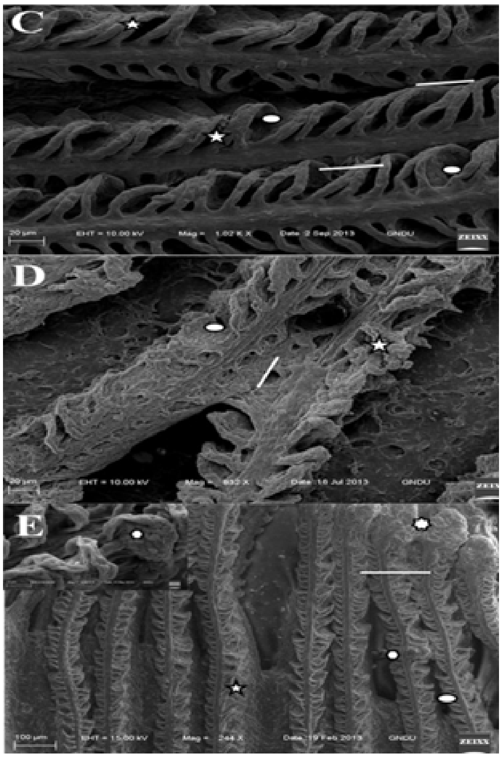

Figure 2: SEM of L. rohita of gill filaments exposed to wastewater concentrations of (C) 17.7%, (D) 26.6% and (E) 35.4% for 15 days.

CONCLUSION

The SEM examination revealed changes in the ultrastructure of the gill surface that were time- and dose-dependent. Aside from minor changes like the fusion of the main and secondary lamellae and normal mean values for the degree of tissue change (DTC), the gills of control fish were functionally normal. The 15 distinct kinds of lesions found included nine, five, and one stage I, stage II, and stage III lesions, respectively. The mean DTC values in the treatment groups were 14.830.48 (17.7%, 15 days) to 116.2016.70 (35.4%, 60 days), and they showed gill damages ranging from mild (reparable) lesions to persistent lesions (no recovery). Lamellar fusion, lamellar telangiectasia, swelling and filament fusion, sloughing of the lamellar epithelium, mucous and chloride cell hypertrophy and hyperplasia, and necrotic lamellae were among the alterations. Although the extent of the damage was less than what was seen after 60 days of therapy, all three forms of tissue damage were still present, which was hopeful in the recovery tests.

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