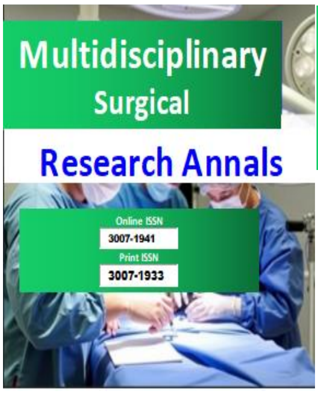

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Effects of Heavy Metals, Water Quality and Temperature Fluctuation on the Growth Parameters of Fish (Labeo rohita) In River Indus

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Abstract

Fish is totally depending on the water. The increasing environmental strain is exacerbated by water pollution and scarcity, with the depletion of natural water sources contributing to this limited availability. Fish

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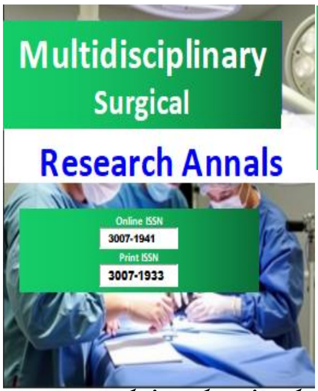
growth effected by environmental pollution. This research will explore technological and scientific ways to diagnose the water quality, heavy metals accumulated in body of fish in order to safe Labeo rohita. Heavy metal contamination (e.g., arsenic, cadmium, lead, etc.) in aquatic environments has a significant impact on fish developmental and physiological processes, such as organ development, breeding and spawning, resulting in a decrease in offspring quantity and quality. Temperatures exceed tolerance limits, fish experience stress that disrupts various physiological, metabolic, and molecular processes, as well as their behavioural responses. For this purpose, the research was conducted on Labeo rohita (Rahu). It was taken as primary tool for this research took place in the premonsoon 2024 and postmonsoon 2023. Heavy metals inter in the fish body through gill and mouth cause bioaccumulation in gills, liver and kidney. Histology of gills clearly indicate necrosis abnormality and the histology of liver clearly indicate completely damage the liver tissue. fish losses its immunity lysozyme level both in mucus and blood serum clearly indicate decrease in this research, it was concluded that the exposure of water quality temperature fluctuation and heavy metals contamination causes the



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histological changes in Labeo rohita. The present research will aid to find new ways to safe rahu from adverse effect of heavy metals and water contamination and open new areas for further research.

Key words: Heavy metals (Cd, Pd, As), temperature, gill, kidney, liver, Labeo rohita, lysozyme, haematology, histology

Introduction

Today, the significance of water for the environment is recognized as an essential requirement globally. Fish is totally depending on the water. Fish growth effected by environmental pollution (Carolin, 2017). Labeo rohita, commonly known as rohu, is a species of freshwater fish with the optimum temperature range of 28 °C to 32 °C (Mridul, 2024) . It is native to the rivers of South Asia, particularly the Indus, Ganges, and Brahmaputra river systems. As an omnivorous species, rohu feeds on algae, zooplankton, and decaying organic matter in its juvenile stage. As it matures, it becomes more herbivorous, consuming mostly phytoplankton and aquatic plants (Asghar, 2023) . Rohu is typically found in freshwater river, lakes and ponds.it thrives in subtropical environment with temperatures ranging from 18oC to 37oC, making it highly suitable for aquaculture in warm regions (Tahir, 2024).

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Pakistan is very rich with natural water resource in the form of Rivers, streams, estuaries, man-made reservoirs, lakes and ponds. the River Indus is one of the longest river systems of the world that flows southward until it drains into the Arabian Sea in Sindh Province and forms the Indus Delta. these regions have number of large basins for growth and nesting of many commercial fishes (Sheikh, 2017). Labeo rohita fish is the most copious freshwater specie in River Indus Pakistan which ranks as the 21st largest river globally in terms of annual flow (Memon, 2010).

Heavy metals are classified in essential and non-essential elements. Metals including copper, cobalt, chromium, manganese, zinc, nickel, iron are essential for living organisms and play vital role in various processes, while others are non-essential for living organisms including arsenic, cadmium, lead, mercury, and have acute to chronic toxic effect (Saad, El-Sikaily, & Kassem, 2014). Some of the pollutants like lead (Pb), arsenic (As), mercury (Hg), chromium (Cr) specially hexavalent chromium, nickel (Ni), barium (Ba), cadmium (Cd), cobalt (Co), selenium (Se), vanadium (V), oils and grease, pesticides, etc. are very harmful, toxic and poisonous even in ppb (parts per billion) range

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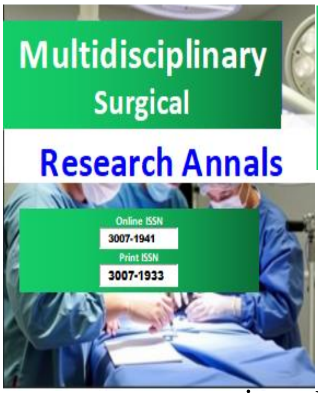
(Verma & Dwivedi, 2013). Heavy metals can be released into various environmental compartments through both natural and human activities (Edelstein & Ben-Hur, 2018).

Heavy metal contamination (e.g., arsenic, cadmium, lead, mercury, etc.) in aquatic environments has a significant impact on fish developmental and physiological processes, such as organ development, breeding and spawning, resulting in a decrease in offspring quantity and quality. The intake of waterborne heavy metals by a fish results in abnormalities and disruptions in the function and structure of many tissues and organs (Rabbane, Kabir, Habibullah-Al-Mamun, & Mustafa, 2022).

Among heavy metals, arsenic, lead, Cadmium of the most prominent toxicants in the aquatic environment, polluting water severely (Kumari et al., 2017). The World Health Organization (WHO) has classified arsenic as one of the most dangerous chemicals to public health. Arsenic levels have been documented up to 800 and 2500 ppm in many countries, including Chile and Bangladesh Previous research has demonstrated the negative effects of arsenic on fish growth, mortality, development, RNA: DNA ratio, histopathology and genetic



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expression. Fish exposed to high concentrations of arsenic experience changes in body physiology, including effects on growth, mortality, ion exchange, immune system, reproduction, enzyme activity, histology and gene regulation (Rabbane et al., 2022).

The release of this hazardous heavy metal into the aquatic ecosystem through trash has resulted in severe anemia and significant changes in haematological parameters in the Labeo rohita (Praveena, Sandeep, Kavitha, & Jayantha Rao, 2013). According to the pollution WHO giving the permissible limit of heavy metals in the water as shown in Table 1 (Singh et al., 2022).

Heavy metals ions	WHO' s permissible limit (mg l_1)
Se	0.02
Hg	0.001
Mn	0.02
Ag	0.1
Cd	0.05
Cr	0.003
Pb	0.01
Zn	3.00

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Fe	0.30
Cu	0.02

Table 1: Possible limit of heavy metals in water

Water is the second most important need for life to exist after air. As a result, water quality has been described extensively in the scientific literature. Water quality is a measure of the condition of water relative to the requirements of one or more biotic species and/or to any human need or purpose" (Omer, 2019).

Study of the physio-chemical parameters of an aquatic ecosystem is basic for understanding its biological productivity (Rajkumar, Ojha, Saini, & Sharma, 2018). In developed and developing countries, the water quality is incredibly uneven, reflecting their social, economic, and physiological factors (Tabrez, Zughaibi, & Javed, 2022). The gill was selected for study because it considered to the organ most exposed to water quality while the liver tissue was selected because it is considered as the storage of heavy metals in fish body and the kidney was selected because it is considered as the filtration process in the fish (Malik, Hussain, & Waqas, 2020).

Lysozyme is an antibiotic peptide and growth enhancer with positive

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bacteriolytic ability that can used in aquaculture and poultry industry (Abdel-Latif et al., 2017; Gong et al., 2017) In this study, dietary lysozyme supplementation generally increased the growth performance parameters and survival rate of fish although no significant different was observed between treatment groups. Lysozyme is a growth promoter. Chen et al., (2013), some factors like stress, season, sex, water temperature and quality, low pH and sedimentation can change lysozyme activities in fish (Shakoori, 2018).

Water temperature is a fundamental parameter that affects the health of aquatic organisms. DO will also fluctuate due to temperature, salinity and pressure changes. DO refers to the level of free, non-compound oxygen present in water or other liquids. An excess of decaying organic matter leads to a shortage of oxygen, which can prove fatal for fish. DO is necessary to many forms of aquatic, fish need DO for respiration. If DO concentrations drop below a certain level, fish mortality rates will rise. Areas with little to no dissolved oxygen are known as dead zones as aquatic organisms cannot survive there. (Ahmad & Kamran, 2020). Fish erythrocytes have the potential cues for any aberrant level and clinical indicators because they include

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haemoglobin, which carries oxygen to all organs. Thus, haemoglobin levels in blood are commonly utilized to evaluate fish anemia and physiological condition (Asghar, 2023).

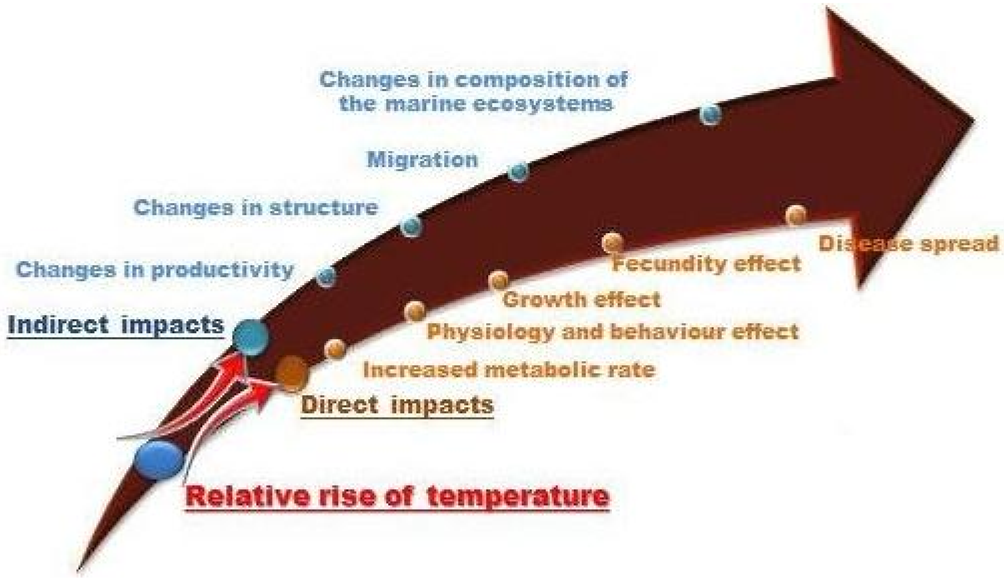
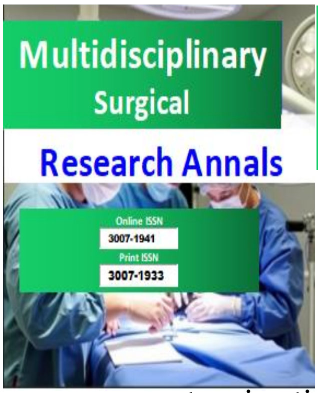


Figure 1: Impacts of Temperatu

Water quality (heavy metals, fish fluctuation) poses a significant environmental threat, and its impact on the aquatic ecosystem, including the contamination of aquatic organisms, is a matter of growing concern.in the case of the Indus, one of the largest rivers in the world, there is a pressing need to investigate the extent of water contamination in fishes inhabiting this ecosystem. This research aims to address the problem by focusing on the identification and quantification of immunohistochemical biomarkers associated with heavy metals



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contamination in River Indus fishes. The overarching question to be explored in this thesis is: To what extent does water quality in the river Indus impact the immunohistochemical biomarkers of fish Labeo rohita, and how can this information contribute to our understanding of the environmental health and conservation of this vital aquatic ecosystem.

The aim of the research is to comprehensively examine how water quality parameters, heavy metal contamination, and temperature fluctuations affect the growth, survival, and overall health of Labeo rohita (Rohu) in the Indus River, Pakistan, with the ultimate goal of understanding the environmental factors that impact fish populations and providing recommendations for sustainable fisheries management.

Research Methodology

Study Area

Water samples collected from three key barrages (1) Chashma Barrage (Punjab), (2) Taunsa Barrage (Punjab), (3) Sukkur Barrage (Sindh) in the Indus River in Pakistan. These locations were selected due to their significance in water regulation and fish activities along the Indus River, as well as their varied ecological condition to study different environmental impacts on the Labeo rohita.

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Study Design

Research Lab: The research work was carried out in Qurtuba University of Science & Information Technology, D.I. Khan.

Sample size: 30 fish of Rahu.

Sample wight:1200 to1300

Time/ Duration: October to November 2023 June to September 2024. (June to September premonsoon, October to November postmonsoon)

Study Animal: Labeo rohita (Rohu)

Water Sampling

Water and sediments samples were collected in the premonsoon and postmonsoon. Plastic bottles with double stoppers having a volume of 500 ml of each and labelled with barrage name were used for collection of water samples. Before sampling, the bottles were cleaned by washing with detergent solution and rinsed with deionized water, and dried. Water samples were collected from each sampling sites(barrage). Samples was transported to the laboratory PHED (Public Health engineering Department) of D I Khan to measure the heavy metals, dissolved oxygen hardness suspended solids. And other water quality parameters measured the pH, temperature during sample collection.

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Fish Sampling

30 number of fishes (Labeo rohita) purchased from the fisherman in the premonsoon and post monsoon from each sampling site. The fish samples were fastly washed with tap water to remove the dirt before preserving them in sterile polythene bags transport them in a cooling box at about -20 oC to the Lab on the same day. Then the samples were dissected fish (Kidney, liver, gill) with the sterile scissors and washed with the distilled water put on the filter paper and kept in the prerinsed plastic bottles, labelled and kept for digestion and further analysis.

Figure 2: Samples of fishes from different barrages.

Samples Collection

Chashma barrage: In this sample collected the water and fish from

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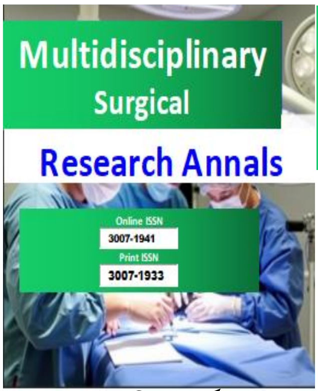
chashma barrage in premonsoon and postmonsoon.

Taunsa barrage: In this sample collected the water and fish from Taunsa barrage in premonsoon and postmonsoon.

Sukkur barrage: In this sample collected the water and fish from Sukkur barrage in premonsoon and postmonsoon.

Figure 3: fishes collect from three sites.

Fish blood collected from the caudal vein by using hypodermic micro syring (Jiangsu Zhiyu Medical Instrumented Co. Ltd, Taixing City, China) with EDTA (Ethylenediaminetetraacetic acid) anticoagulant for measurement of (WBCs), Red Blood Cells (RBCs), (PLT), Mean Cell Volume, Mean Cell Hemoglobin (MCH) and Hemoglobin (Hb) by using Celltac a hematology analyzer (MEK-6500K Nihon Kohden).



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Samples were placed in a digestion apparatus, followed by the addition of 2.5 ml of rigorous sulfuric acid and 4. ml of rigorous nitric acid. This mixture initially reacted vigorously, and the reaction was allowed to subside. The solution was then gently heated on a hot plate, with three to four drops of hydrogen peroxide added. This heating and addition process were repeated several times until the solution became clear. Subsequently, the mixture was heated at 150°C for another 20 minutes and then permissible to cool to 37oc. Once cooled, the solution was filtered into a 50 ml volumetric flask and diluted to the mark using deionized water. Metal concentrations in both water and fish samples were determined using a Perkin Elmer atomic absorption spectrophotometer, model 3100.

Activity of lysozyme was evaluated using a Turbidimetric assay. (Demers and Bayne, 1997).

Procedure

Briefly,50ul solution of the bacterium Micrococcus luteus (Sigma, St Louis, MO) was mixed with a 50ul sample of blood serum (0.3 mg/ml of lyophilized cells was diluted in 40 mM sodium phosphate buffer pH 6.5) the solution was incubated at 30oC for 15 minutes &variation in

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absorbance at 450 nm was monitored after 0 and 15 minutes in microplate reader (BioTeKELx808 instruments, USA).

Histology

After sampling fish was killed humanely and dissected to observe the modification in the tissue of organs i.e. kidney gills and liver. Slides was prepared by following the method explained by Amin et al. (1991).

Slides preparation   Collection of the sample

After fish dissection the dissected organs were taken i.e. kidney liver and gills.

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Figure.4. Dissection of fish

Dehydration of the Sample

After proper fixation, the next crucial step in tissue processing is dehydration, which effectively removes water from the tissue block. This is typically achieved through a series of graded alcohol solution.

First, immerse the tissue in 50% alcohol for one hour. This initial step begins the dehydration process by gradually introducing alcohol to the tissue. Next, transfer the tissue to 70% alcohol for one hour, allowing further removal of water. This step is repeated with another hour in 70% alcohol to ensure adequate dehydration.

Following this, the tissue is moved to 85% alcohol for one hour, further concentrating the alcohol and continuing the dehydration process. Subsequently, the tissue is placed in 95% alcohol for an

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additional hour, enhancing the drying effect. Finally, the tissue is immersed in 100% alcohol for one hour, completing the dehydration process and preparing the tissue for infiltration with embedding mediums. Each step in this method plays a critical role in ensuring the integrity and preservation of the tissue for subsequent analysis.

After dehydration, the tissues are to be cleared with clearing agent to remove alcohol prior to paraffin embedding. The clearing agent should be miscible both in alcohol and paraffin. The commonly used cleaning agents are:

Xylene = (3-4 mm thick tissue can be cleared in 30-60 minutes for two changes each) Chloroform = (Clearing time – Overnight, but less harmful than xylene for the tissue) Cedar wood oil (Histological GR) = (It is good for cleaning of delicate tissue).

The tissue sample specimen is now prepared for cutting into sections for putting on a slide. To reveal the tissue, the block's surface is cleared of wax.

Prior to sectioning, blocks are chilled for ten minutes on an ice tray or refrigerator plate.

Very thin tissue slices are cut off the block in the shape of a ribbon

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using a microtome.

Although the microtome can be programmed to cut at various thicknesses, most tissues are cut at a thickness of about 5 µm. Here, I learn more about slicing tissue sections.

The tissue ribbons are carefully moved to a warm water bath after being cut. Here, they are permitted to float on the water's surface before being lifted into a slide that is submerged. For this procedure, charged glass slides are ideal because they enhance tissue adherence to the glass and lessen the possibility of sections washing off the slide while staining. To gently melt the extra paraffin wax and preserve the tissue section, slides should be properly labelled and then left to dry upright at 37°C for a few hours.

Figure5 a: Tissues of fish

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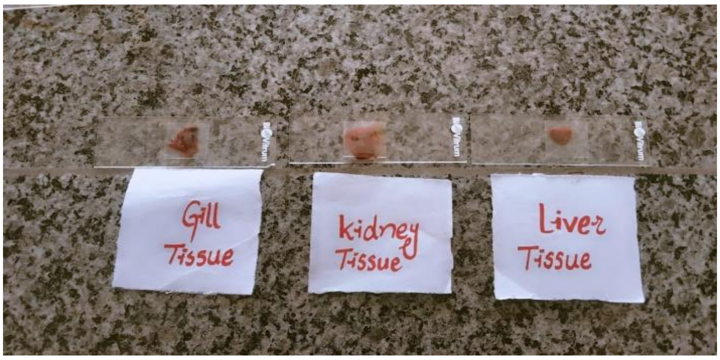


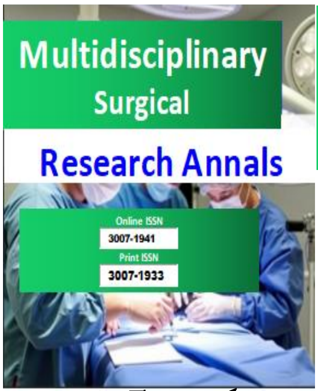
Figure 5 b: Tissues of fish covered Staining

Water Quality Analysis

Water quality parameters, such as pH, dissolved oxygen (DO) Water temperature and dissolved oxygen (DO) were measured by Oxygen-Thermometer apparatus YSI model 58(Yellow Spring Instrument Co., Yellow Springs, Ohio, USA)., electrical conductivity, and turbidity, will be measured using standard analytical instruments (e.g., pH meter, DO meter, conductive meter) following protocols from the APHA 2017 (American Public Health Association).

Growth rate

During pre and post monsoon fish loss the weight due to industrial west, heavy metal and other factor that effect the growth of Labeo Rohita. Effect the growth was estimated according to the following:



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Formula

Growth Rate (GR)

GR = Weight f – Weight i / weight i. x 100

Weight f =final weight

Weight I = initial weight

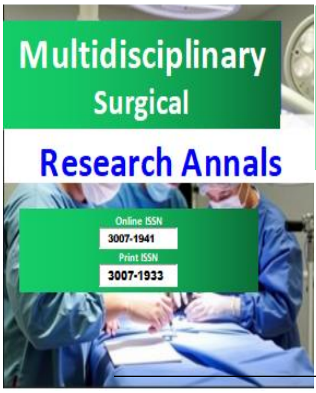
Results

This section categorizes results difference in status of water quality, heavy metals (As, Pb, Cd) and temperature fluctuation that effect growth of Labeo rohita in (chashma, taunsa and sukkur barrages).

Status Of Water Quality Parameters

The results of the analysis of the mean physical- chemical parameter across the three barrages are presented in table. Average temperature in 0C, water pH in ppm, dissolved oxygen in ppm, ammonia ppm, Total dissolved oxygen, Nitrate and nitrite in ppm. Mean of Physico-chemical parameters of 3 barrages. The current study was planned to record water quality in different barrages are listed in table 4a,4b.

Sites	Seasons	Temperature	Ph	DO	Ammonia Ppm
Chashm	Premonsoo	26.5±1.12	7.6±0.1	5.9±0.27	0.01±0.006



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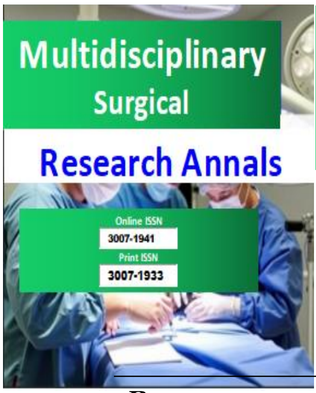
Barrage	Postmonsoon	22.66 ± 7.36	8.09 ± 0.54	7.84 ± 1.62	0.01 ± 0.19
Taunsa Barrage	Premonsoon	37.5 ± 2.5	7.5 ±0.10	4.5±5.5	0.066±0.085
	Postmonsoon	25.58±4.2	7.8±0.18	8.24±1.93	0.24±0.067
Sukkur Barrage	Premonsoon	24.5 ± 7.5	7.2±0.34	9.4 ± 4.2	0.09±0.017
	Postmonsoon	27.6±3.7	7.7 ± 1.2	7.6±2.4	0.079±0.02

Table 2 a: Physico-chemical parameters of water

Sites	Seasons	Total dissolved oxygen	Nitrate ppm	Nitrite ppm
Chashma Barrage	Premonsoon	7.65±7.63	7.14±0.61	0.006±0.003
	Postmonsoon	0.17 ± 0.05	7.29 ± 0.65	0.03 ± 0.05
Taunsa	Premonsoon	11.5±0.76	0.475 ± 0.615	0.475 ± 0.615



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Barrage	Postmonsoon	9.72±0.61	5.9±0.48	0.037 ±0.024
Sukkur	Premonsoon	8.72±0.73	5.55±0.44	0.72±0.32
Barrage	Postmonsoon	0.65±0.02	6.01±0.51	0.64±0.22

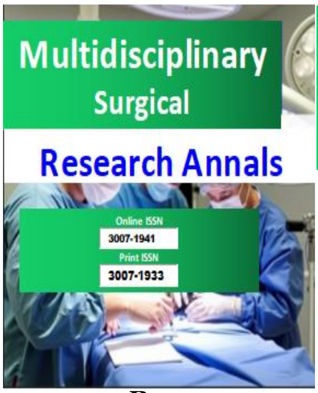
Table 2.b Physico-chemical parameters of water

Effect of the status of water quality parameters on the fish growth

The growth of Labeo rohita (Rohu) is influenced by several water quality parameters including temperature, pH, dissolved oxygen (DO), ammonia, nitrate, and nitrite. The barrage water quality in the table shows differences in these parameters between the premonsoon and postmonsoon seasons, potentially affecting fish growth.

Temperature: Optimal temperature for the growth of Labeo rohita ranges between 25°C and 32°C. In barrages, temperatures range from 22.66°C (postmonsoon at Chashma Barrage) to 27.65°C (postmonsoon at Sukkur Barrage), which are within this optimal range. However, temperatures that are too low or high can slow metabolic processes, affecting growth rates.

The pH Labeo rohita thrives in water with a pH range of 6.5 to 8.5. The barrages have a pH range of 7.8 to 8.2, which is favorable for growth. However, a high pH of 8.24 during postmonsoon at Taunsa



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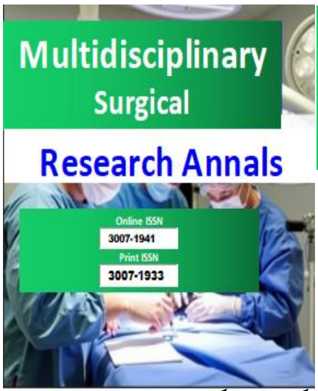
Barrage may begin to stress the fish, affecting feeding and growth.

Dissolved Oxygen (DO): Adequate oxygen levels (above 5 mg/L) are critical for respiration and overall health. DO levels range from 7.84-8.24 mg/L at the barrages, which are sufficient for supporting healthy growth. However, stress may occur if DO levels drop, especially during premonsoon conditions at Taunsa Barrage (4.55 mg/L).

Ammonia: Ammonia levels above 0.02 ppm can become toxic to fish, leading to reduced growth and increased susceptibility to disease. Postmonsoon ammonia levels in Taunsa Barrage (0.24 ppm) are significantly high, which can harm Labeo rohita growth, while other barrages have lower ammonia concentrations.

Nitrate and Nitrite: Elevated nitrate levels above 50 ppm have toxic effects, though the values seen in the table are well below this threshold. However, nitrite, even at low levels, can disrupt oxygen transport in fish blood (brown blood disease). Nitrate and nitrite levels in barrages are mostly safe, but increased levels of nitrite (0.037 ppm at Taunsa Barrage) could cause mild stress, impacting growth.

Barrage waters during postmonsoon seasons, especially Taunsa, show higher ammonia, pH, and nitrite levels, which can negatively affect fish



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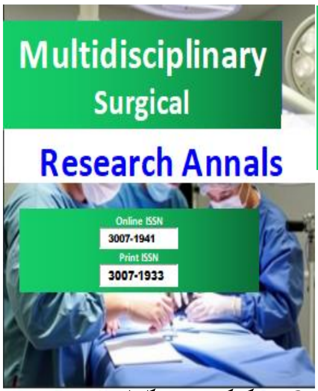
growth and health. Labeo rohita may experience reduced growth rates in these conditions compared to premonsoon waters, which have more moderate values of these parameters. Premonsoon seasons generally offer better conditions for Labeo rohita growth with lower temperatures and ammonia levels, but low dissolved oxygen at Taunsa Barrage (4.55 mg/L) may limit growth.

Postmonsoon water quality in barrages (especially Taunsa) tends to show more adverse conditions for Labeo rohita compared to premonsoon, likely leading to reduced growth rates due to higher ammonia, pH, and nitrite levels.

Heavy metals level in three barrages

Heavy metals	**Chashma barrage Mg/L**	**Taunsa barrage Mg/L**	**Sukkur barrage Mg/L**	**WHO standards for Pakistan (mg/L**
Cd	0.09	0.07	0.006	0.05
Pd	0.35	0.32	0.009	0.06
As	0.34	0.071	0.06	0.05

Table 3: Heavy metals accumulation in water of Indus river



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The table 3 shown the accumulation of heavy metals (Cadmium – Cd, Lead – Pb, and Arsenic – As) in the water of the Indus River across three barrages: Chashma, Taunsa, and Sukkur. The values are compared with the World Health Organization (WHO) standards for safe water quality in Pakistan, which is critical for public health and environmental monitoring.

Cadmium (Cd)

At Chashma Barrage, the concentration is recorded as 0.09 mg/L, Taunsa Barrage shows 0.07 mg/L, and Sukkur Barrage has the lowest level at 0.006 mg/L. However, the WHO safety standard for Cadmium in water is set at 0.005 mg/L. All barrages have levels higher than the recommended limit, with Sukkur being the closest to the permissible level.

Lead (Pb)

The Lead concentration at Chashma is 0.35 mg/L, while Taunsa records 0.32 mg/L, and Sukkur shows a significantly lower concentration of 0.009 mg/L. According to WHO guidelines, the safe limit for Lead is 0.06 mg/L. Both Chashma and Taunsa exceed this limit by a wide margin, while Sukkur's concentration remains well below the

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threshold.

Arsenic (As)

The concentration of Arsenic in Chashma is reported as 0.34 mg/L, Taunsa shows 0.071 mg/L, and Sukkur records 0.06 mg/L. The WHO limit for Arsenic in water is 0.05 mg/L, which is surpassed by all three barrages. Sukkur's concentration is the closest to the WHO standard but still exceeds it. This table serves as a crucial indicator of the heavy metal contamination in the water of the Indus River and highlights the need for intervention to reduce these levels to comply with international safety standards for water quality. The data points to significant environmental concerns, particularly regarding Cadmium and Lead in Chashma and Taunsa, which are alarmingly higher than WHO recommendations.

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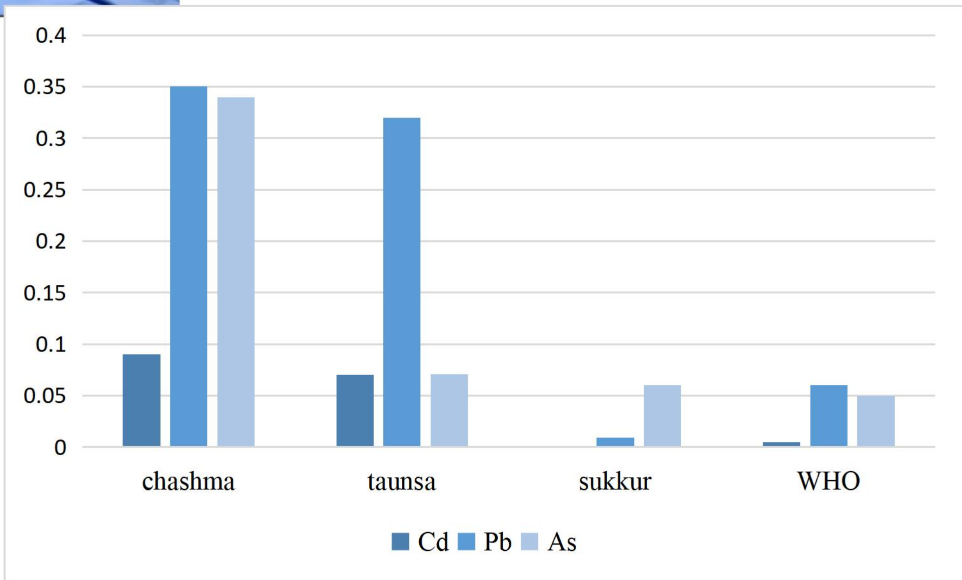

Figure 6 : Heavy metals accumulation in Indus river

Seasonal variations in heavy metals concentration in fish tissue:

Sites	Seasons	Heavy metals	Gill	Liver	Kidney
Chashma		Cd	0.72	1.32	1.05
Barrage	pre	Pb	2.30	3.35	1.13
		As	0.45	0.56	0.35
		Cd	0.94	1.11	1.88
	post	Pb	2.85	3.99	1.76
		As	0.57	0.33	0.79
Taunsa		Cd	4.33	5.2	6.29

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Barrage	pre	Pb	3.46	2.45	1.44
		As	0.54	0.95	0.63
		Cd	5.57	6.5	7.1
	post	Pb	2.99	1.98	1.05
		As	0.82	0.28	1.03
Sukkur		Cd	1.66	2.09	1.43
Barrage	pre	Pb	1.99	2.5	0.87
		As	0.75	0.66	0.79
		Cd	1.29	2.20	1.55
	post	Pb	2.15	2.9	1.77
		As	0.63	0.59	0.43

Table 6 : Heavy metals concentration in fish tissues (pre and post)

From the table 6, the fish in different organs (gill, liver, and kidney) are impacted by varying levels of heavy metals (Cd, Pb, and As).

Chashma Barrage

Gill: The pre-season levels of Cd (0.72 mg/L) and Pb (2.30 mg/L) are high, but post-season, the levels increase to Cd (0.94 mg/L) and Pb (2.85 mg/L), indicating more contamination. As increases from 0.45 mg/L (pre) to 0.57 mg/L (post), which is less drastic.

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Liver: The liver accumulates more Pb (pre: 3.35 mg/L, post: 3.99 mg/L) than other metals. Cd is relatively high at 1.32 mg/L (pre) and 1.11 mg/L (post), with a slight decrease after the season.

Kidney: Post-season, Cd increases substantially from 1.05 - 1.88 mg/L. Pb and As also increase, indicating that the kidney accumulates higher concentrations of heavy metals post-season.

Taunsa Barrage

Gill the Cd levels are extreme in both pre (4.33 mg/L) and post-season (5.57 mg/L). Pb levels decrease post-season from 3.46 - 2.99 mg/L. As remains stable, increasing slightly from 0.82 mg/L (pre) - 0.82 mg/L (post).

Liver: The liver has high Cd levels, increasing from 5.2 mg/L to 6.5 mg/L after the season. Pb levels decrease slightly post-season, but As is consistent, rising marginally from 0.28 mg/L to 0.35 mg/L.

Kidney: Cd levels in the kidney are particularly high, jumping from 6.29 - 7.1 mg/L post-season, the highest in the table. As levels also rise from 1.03 - 1.15 mg/L, indicating more accumulation in the kidney.

Sukkur Barrage

Gill: Cd levels in the gills remain significant, decreasing slightly from

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1.66 mg/L (pre) to 1.29 mg/L (post). Pb levels rise marginally from 1.99 - 2.15 mg/L. As is also present but remains lower than in Taunsa.

Liver: The liver shows a rise in Pb from 2.5 mg/L to 2.9 mg/L post-season, while Cd levels remain almost constant. As levels slightly decrease.

Kidney: Post-season, Cd decreases slightly from 1.43 - 1.55 mg/L, while Pb increases to 1.77 mg/L.

As remains stable, Taunsa Barrage fish are the most affected by heavy metals, especially Cd levels in the gills, liver, and kidney, showing extremely high concentrations, particularly post-season. Chashma Barrage also shows significant contamination, particularly with Pb, especially in the liver post-season. Sukkur Barrage fish are less affected by Cd compared to Taunsa, but Pb and As still remain a concern, especially in the liver and kidneys.

Thus, fish in the Taunsa Barrage are the most affected overall by heavy metal contamination, especially by cadmium, while those in the Chashma Barrage accumulate more lead. The Sukkur Barrage fish show moderate contamination but still pose significant health risks, especially from Pb and As.

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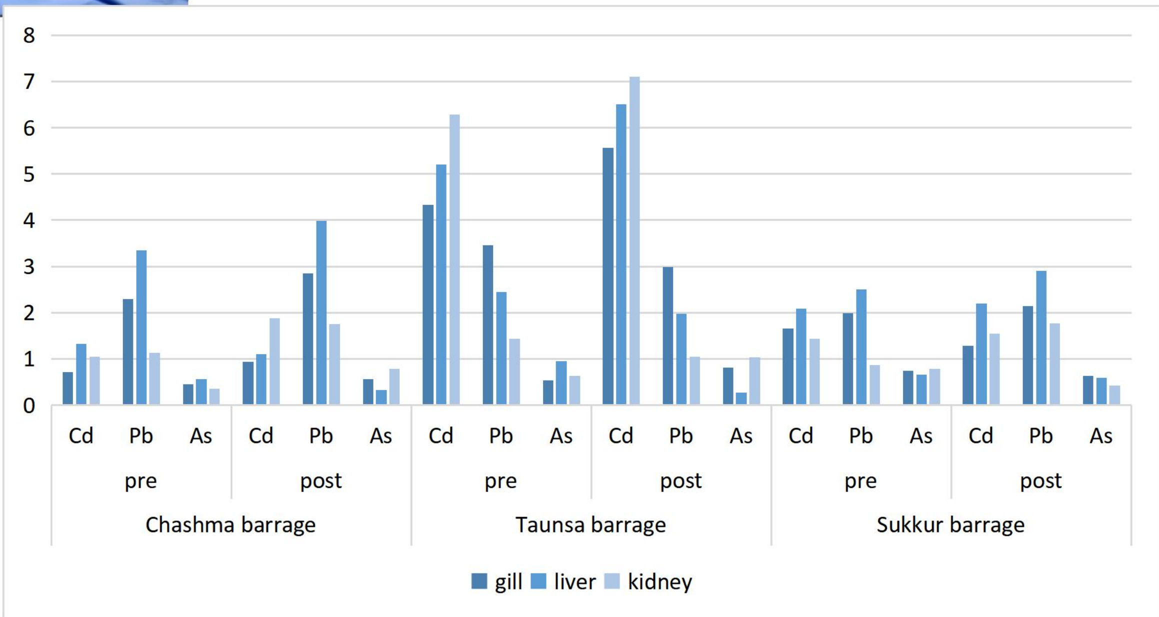

Figure 7: Accumulation of heavy metals in three sites

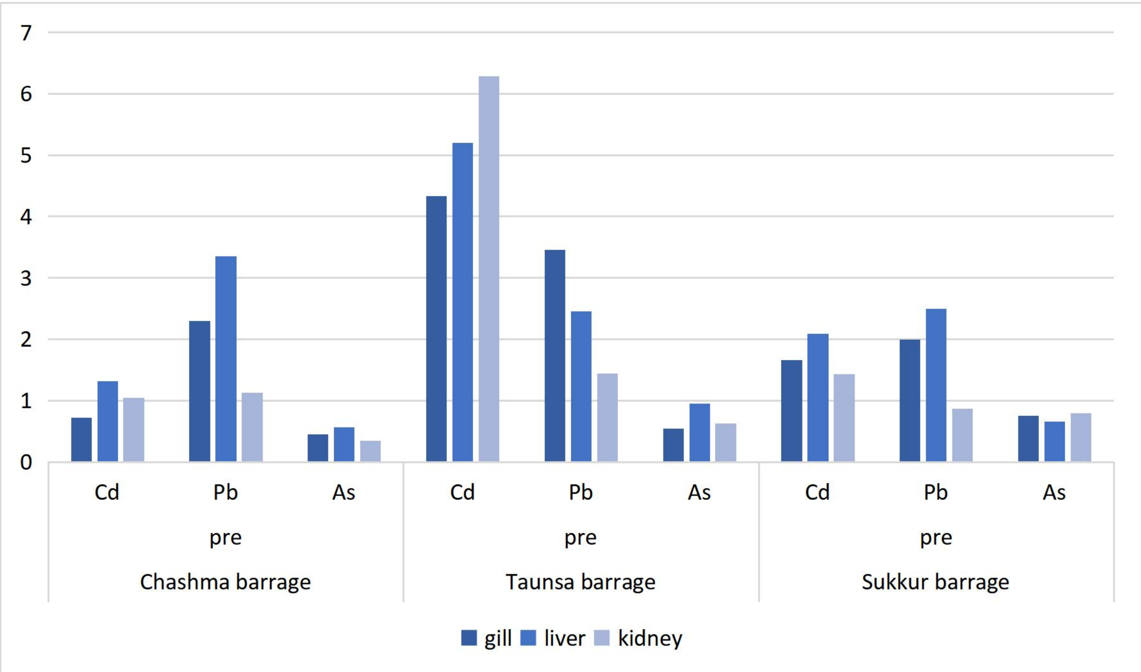

Figure 8 a: Bioaccumulation of 3 heavy metals in fish tissues(pre)

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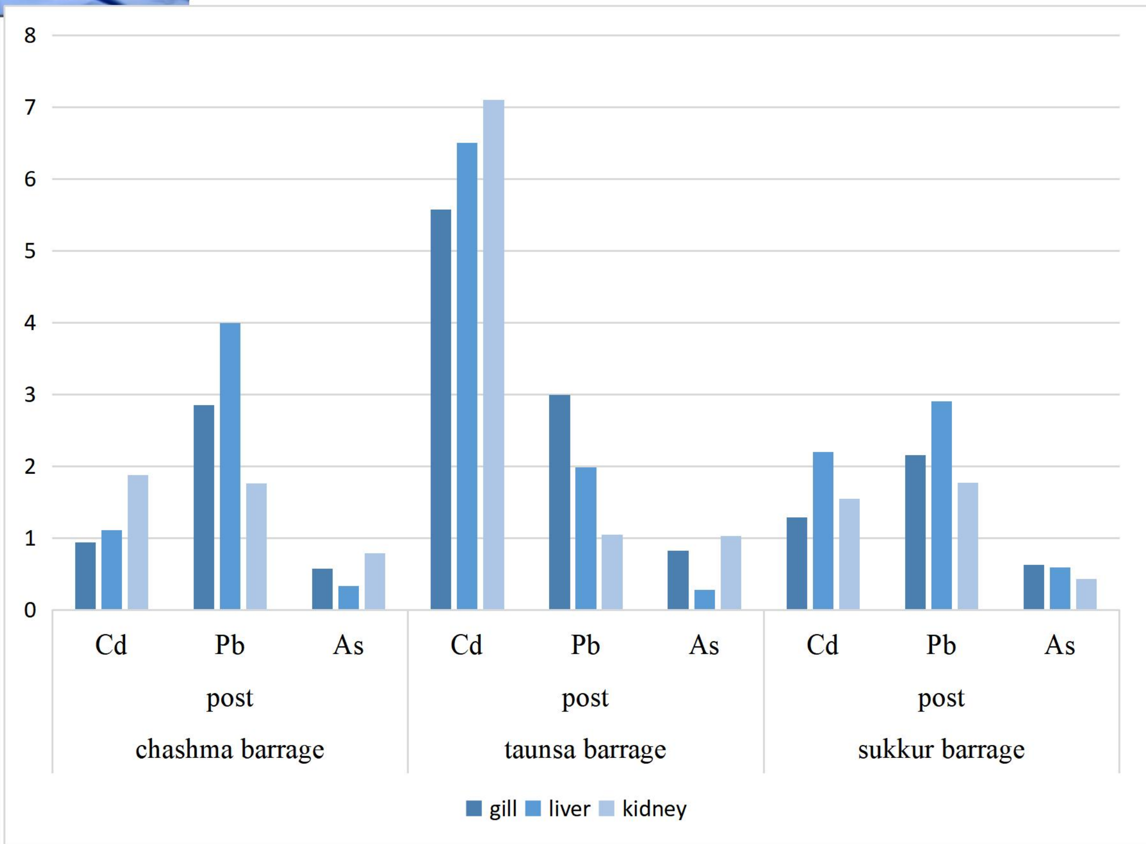

Figure 8 b: Bioaccumulation of 3 heavy metals in fish tissues(post)

Histological Analysis

Histology of Gill

Histopathological changes were observed in the gill of the fish in postmonsoon. The structural details of the gill are show in figure 17 a, b in below. Injuries in gill tissues as reported due heavy metal lead may reduce the oxygen consumption and disruption the osmoregulatory function of fish.

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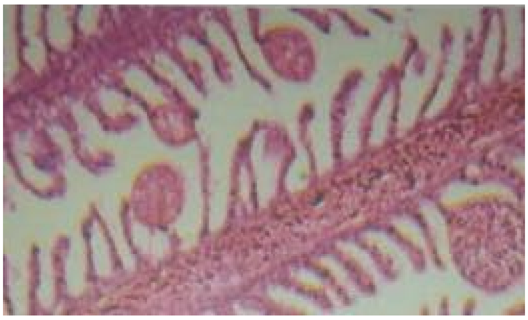

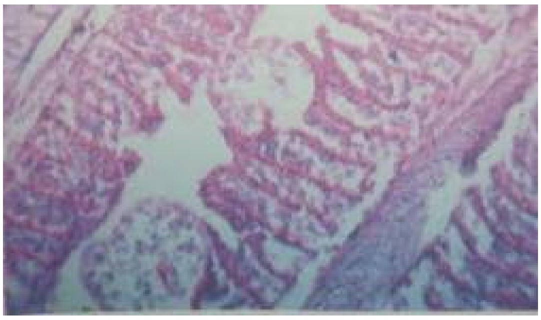

Figure 9 a: Microscopic40x view of gill

Figure 9 b: Microscopic40x view of gill

Histology of kidney

The histopathological changes observed in the kidneys of fish, Labeo rohita (postmonsoon) on being exposed to different concentrations of cadmium, such as degenerated tubular epithelium, shrinkage and degenerating glomerulus indicate kidney failure of the fish.

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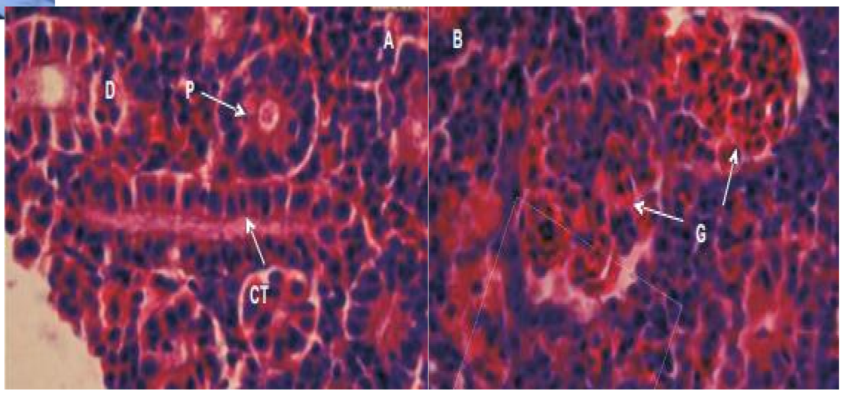

Figure 10 a: Microphotograph of the kidney of Labeo rohita in control. Bar= 20 µm; Abbreviations: P= Proximal tubule, D=Distal tubule, G=Glomerulus, B= Blood vessel; CT= Collecting tubule.

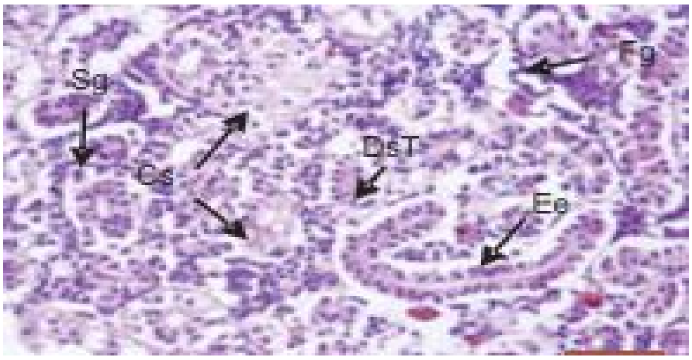

Figure 10 b: Microphotograph of kidney of Labeo rohita -1 on being exposed of Cadmium after 40 days. Bar= 20 µm. Sg = Shrunken Glomerulus, Cs = Cloudy Swelling, Dst = Deshaped Tubules, Ee = Exfoliated Epithelium, Fg = Fragmented Glomerulus.

Histology of Liver

Histopathological changes were observed in the liver of the fish(postmonsoon). Exposed to different concentrations of lead, arsenic

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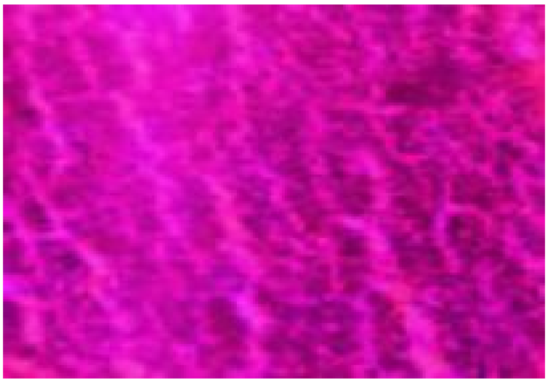
such as degenerated the liver.



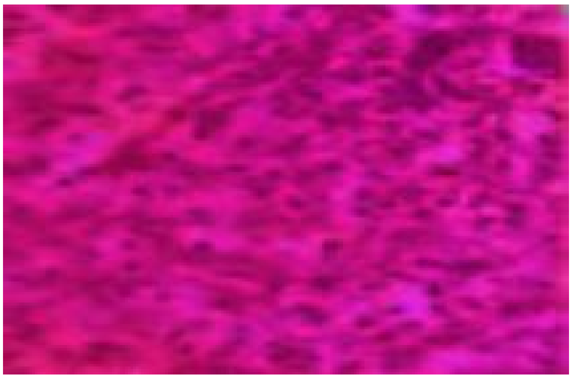


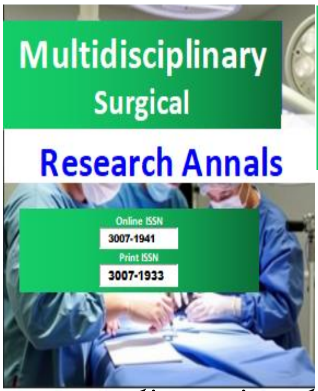
Figure 11 a: Microscopic 10x view of liver Figure 11 b: Microscopic 10x view of liver

Microscopic 10x view of the liver of fish Labeo rohita treated with heavy metals.

Taunsa barrage

Histology of Gill

Microscopic 10x view of gills of fish Labeo rohita in (premonsoon)effected by heavy metals. Heavy metals like cadmium are very high in postmonsoon may reduce the oxygen consumption and



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disruption the osmoregulatory of fish.

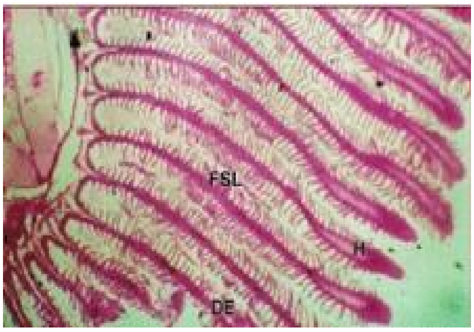

Figure 12 a: Microscopic10x view of gill Figure 12 b: Microscopic10x view of gill

Primary gill lamella Primary gill lamella

Secondary gill lamella Secondary gill lamella

Histology of kidney

The histopathological alterations in kidneys of Labeo rohita in(postmonsoon) exposed to cadmium showed heavy congestion in blood vessels and tubular necrosis of glomerulus.

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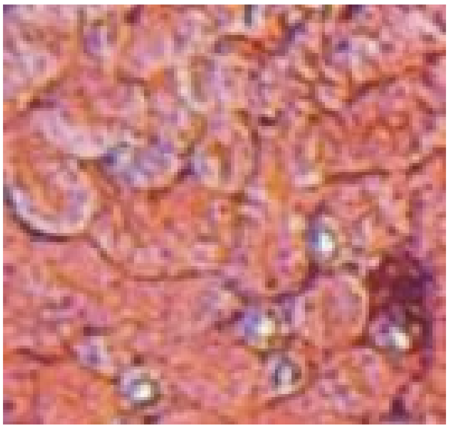


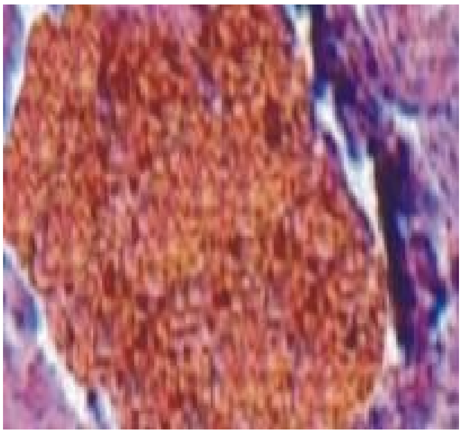
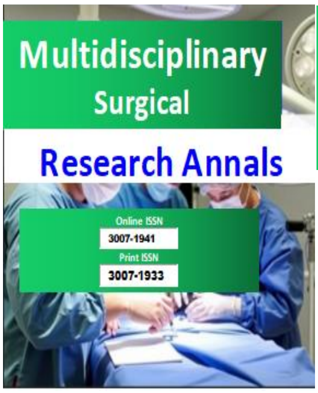


Figure 13 a: Microscopic 10x view of kidney Figure 13 b: Microscopic 10x view of kidney

Histology of Liver

Injuries showed almost normal pattern in liver cells with very slight degenerative changes in cell arrangements premonsoon. Due to heavy metals in postmonsoon showed complete degeneration of liver tissues. Microscopic 10x view of liver of fish Labeo rohita effected by heavy metals.



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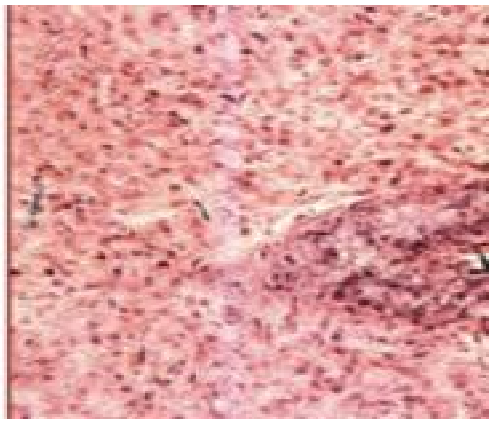

Figure 14 a: Microscopic 10x view of liver

Figure 14 b: Microscopic 10x view of liver

Sukkur Barrage

Histology Of Gill

Microscopic 10x view of gills of fish Labeo rohita treated with accumulation of heavy metals arsenic in postmonsoon and water quality.

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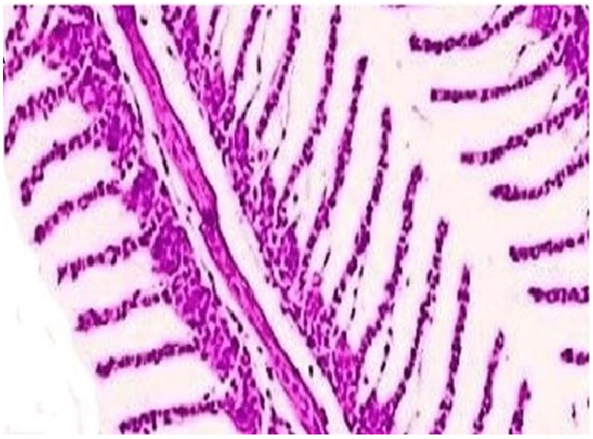

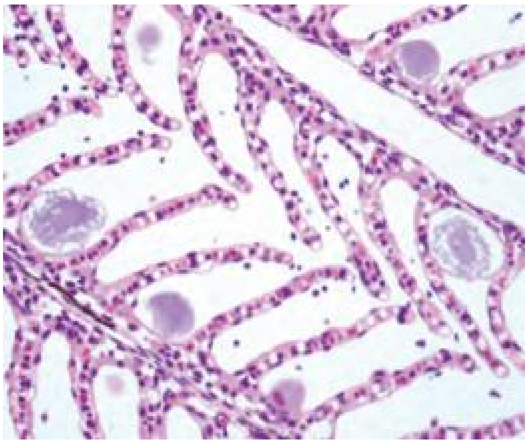

Figure 15 a: Microscopic 40x view of gills

Figure 15 b: Microscopic 40x view of gills

Histology Of Kidney

The histopathological alterations in kidneys of Labeo rohita in postmonsoon exposed by lead, cadmium damage kidney of fish leading to impaired filtration, osmoregulation, waste excretion. Microscopic 40x view of kidney of fish Labeo rohita treated with accumulation of heavy metals and water quality.

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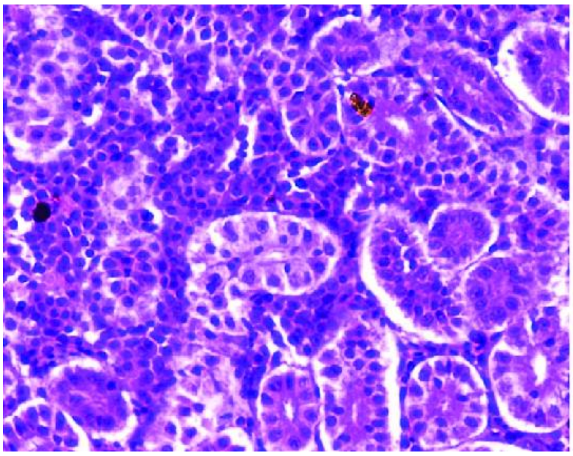

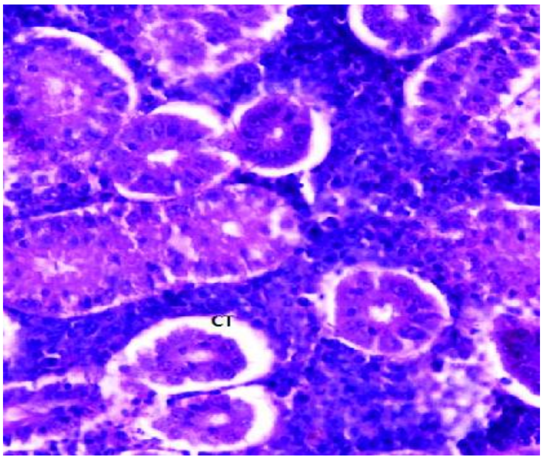

Figure 16 a: Microscopic 40x view of kidney Figure 16 b: Microscopic 40x view of kidney

Histology of Liver

Microscopic 10x view of liver of Labeo rohita in postmonsoon are affected by heavy metals arsenic cadmium lead. Due to these heavy metals are completely damage the liver tissue.

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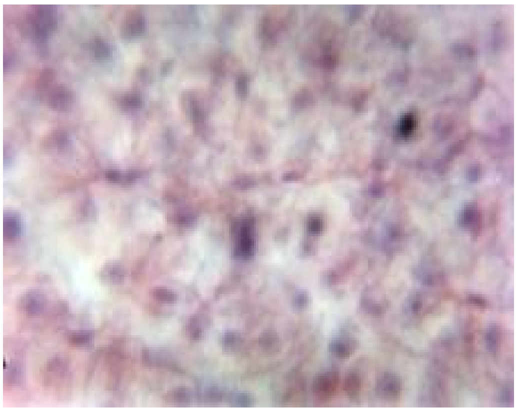

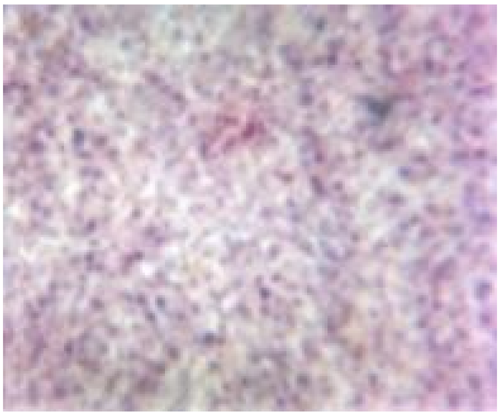

Figure 17 a: Microscopic 10x view of liver

Figure 17 b: Microscopic 10x view of liver

Lysozyme Activity

The verify the presence of immunity of fish lysozyme activity was analyzed in Hematology of Labeo rohita that exposed to heavy metals (cadmium, lead, Arsenic). The was a significantly high the concentration of heavy metals decline the lysozyme activity in blood of fish.

Sites	season	Lysozyme activity
Chashma barrage	premonsoon	31.9

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	postmonsoon	29.2
Taunsa barrage	premonsoon	24.5
	postmonsoon	20.7
Sukkur barrage	premonsoon	34.6
	postmonsoon	32.3

Table 7: Lysozyme activity of fish sample

Lysozyme activity in premonsoon and postmonsoon

The table shows the lysozyme activity (an enzyme important for immune responses in fish) measured in fish samples during the premonsoon and postmonsoon seasons at three different barrages (Chashma, Taunsa, and Sukkur). Premonsoon Season: At Chashma barrage, the lysozyme activity is 31.9. At Taunsa barrage, it is 24.5. At Sukkur barrage, it is 34.6.

In general, higher lysozyme activity during the premonsoon season, like at Sukkur and Chashma, indicates stronger immune defense in fish, making them less susceptible to infections and environmental stressors during this period. The Taunsa barrage shows lower activity (24.5), suggesting that fish in this region might have a weaker immune response before the monsoon, possibly due to factors like water quality,

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pollution, or habitat stress. Postmonsoon Season: At Chashma barrage, the lysozyme activity is 29.2. At Taunsa barrage, it drops to 20.7. At Sukkur barrage, it decreases to 32.3. After the monsoon, there's a slight decrease in lysozyme activity at all sites. This could imply that postmonsoon conditions (like changes in water quality, food availability, or increased pathogen load) impact fish immunity. The Taunsa barrage again shows the lowest activity (20.7), making the fish in that region potentially more vulnerable to diseases in the postmonsoon period. Fish with low lysozyme activity, like those in Taunsa, especially in the postmonsoon period, might experience higher susceptibility to infections and environmental stress. This could be due to pollution, habitat degradation, or other stress factors in the water bodies near the barrage.

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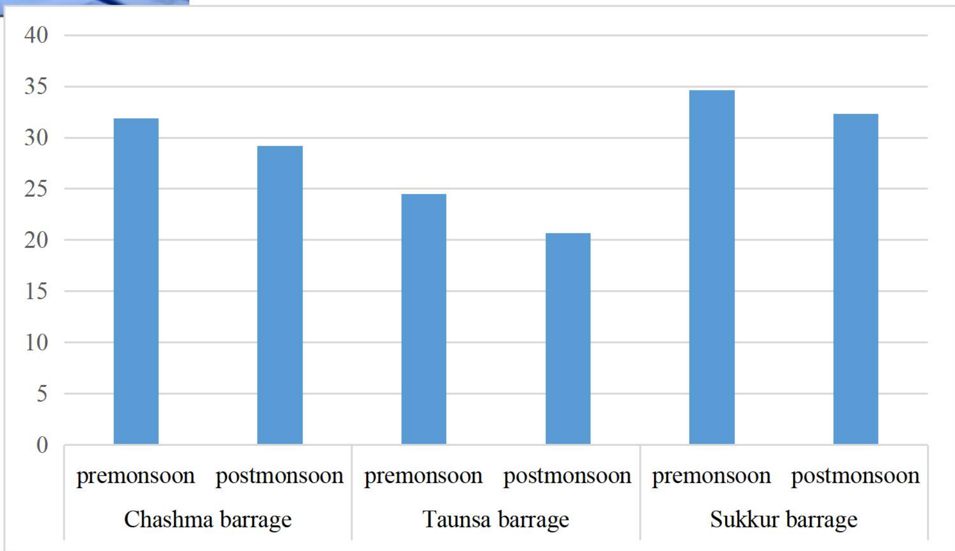

Figure 18. Lysozyme activity of fish sample

Status of Temperature Fluctuation

Temperature	Chashma barrage	Taunsa barrage	Sukkur barrage
Premonsoon	26.7	37.5	24.3
Postmonsoon	23.9	25.6	27.2

Table 8: Effect of Temperature Fluctuation in three sites:

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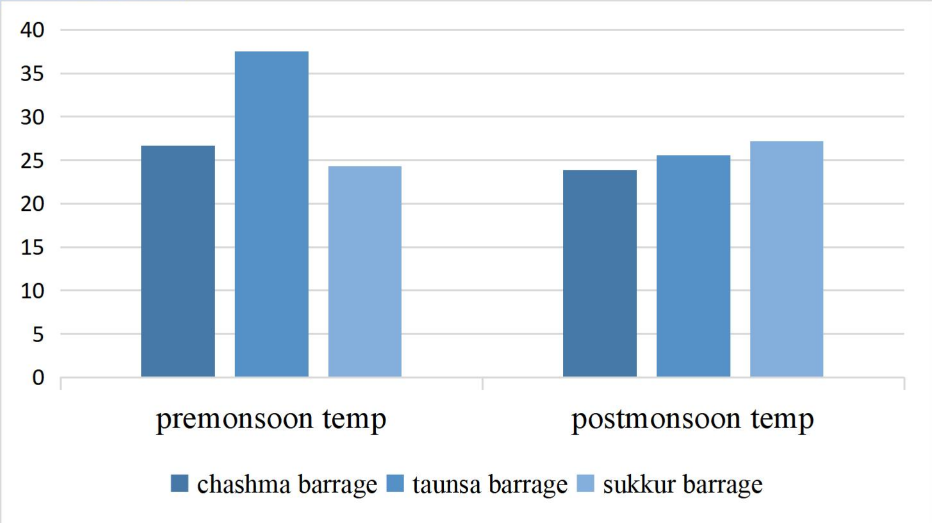
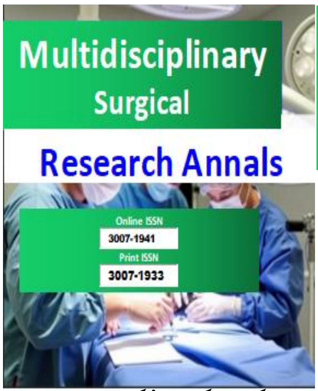


Figure 19: Temperature fluctuation in pre and post in three sites

Fish growth in barrages is highly dependent on maintaining an optimal and stable water temperature. Large temperature fluctuations, like those observed in Taunsa Barrage (12°C difference between pre- and post-monsoon), can stress fish populations, affect their feeding and metabolic rates, reduce oxygen levels, and make them more vulnerable to disease. In contrast, barrages with moderate temperature changes, such as Chashma and Sukkur, may see less drastic impacts on fish growth but still require careful monitoring to ensure favorable conditions for fish populations.

Fish Growth Fluctuation In Response To Environmental Change

Water quality plays a crucial role in fish growth, as it directly impacts their health, metabolism, and ability to thrive. Factors such as pH levels,



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dissolved oxygen, temperature, and the presence of pollutants or toxins like ammonia and nitrates influence fish development.

Heavy metals in water, such as lead, arsenic and cadmium, are high in the postmonsoon in taunsa barrage can accumulate in fish tissues, leading to toxicity that affects their growth, reproduction, and survival. These metals disrupt metabolic processes, impair organ function, and weaken immune systems.

Temperature fluctuations further in taunsa barrage was high stress fish by altering their metabolic rates and oxygen availability. Together, heavy metals and temperature instability create an environment where fish growth is stunted, and their overall health is compromised.

sites	**Chashma barrage**		**Taunsa barrage**		**Sukkur barrage**	
seasons	**Pre**	**Post**	**Pre**	**Post**	**pre**	**Post**
Initial Wight	1245gram	1265gram	1254gram	1287gram	1214gram	1209gram
Final Wight	769gram	808gram	695gram	640gram	837gram	817gram

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Time	30 days	30 days	30 days	30 days	30 days	30 days

Table 9: Effect Growth In Three Sites

The Taunsa Barrage fish loss weight 44% during pre-monsoon, 50% during post-monsoon. The Taunsa Barrage exhibited the highest weight loss followed by Chashma Barrage fish loss weight 38% during premonsoon, 36% during postmonsoon and Sukkur Barrage fish had least weight loss 31% during premonsoon, 32% during postmonsoon. This indicate that fish in Taunsa Barrage experienced the most significant growth challenges, likely due to environmental stressors such as heavy metals, water quality issues and temperature fluctuation

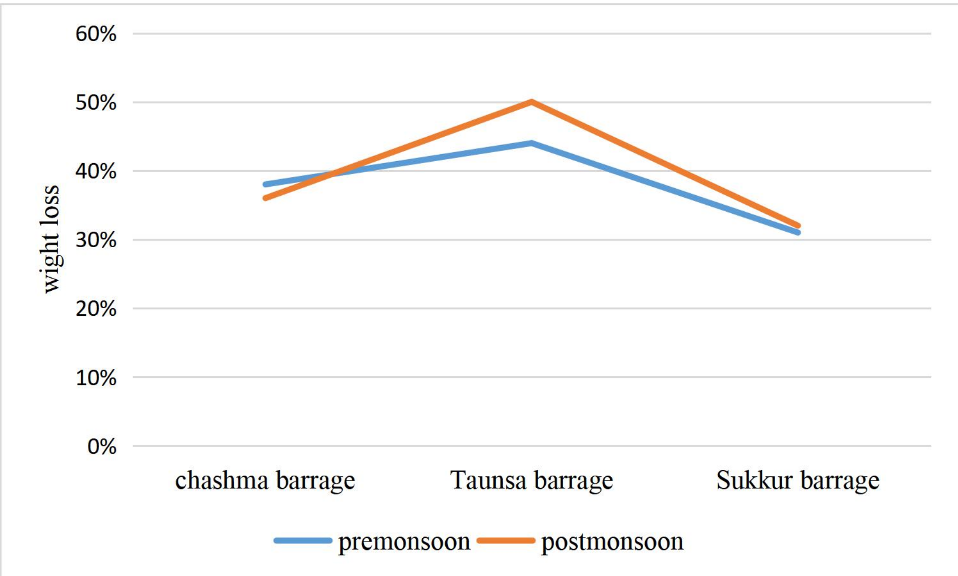

Figure 20: Wight loss of fish in three sites

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Discussion

The quality of the Barrages water in terms of physicochemical parameters in shown in table 4 a, b. The primary importance was given to the temperature. Temperature variation can regulate various metabolic activities of fishes and thus, it has vital importance to study fish diversity. The measured mean values of temperature were within the optimum range in Chashma Barrage (26.5±1.12 oC) in premonsoon (22.66 ± 7.36) in postmonsoon (which supports maximum fish growth rate, resistance to certain diseases, and tolerance under unfavourable conditions). It has also been reported previously that Chashma Barrage has characteristic annual temperature, with increased degrees in premonsoon and decreased degrees in postmonsoon. Increase in temperature in premonsoon has been observed to be correlated with increased parasite infestation (Kalsoom, 2021).

The temperature was measured in Taunsa Barrage (37.5 ± 2.5) in pre monsoon and (25.58±4.2) in post monsoon showed good agreement with the previous study (ul Arifeen, 2022).The measured mean values of temperature were (24.5 ± 7.5) during premonsoon (27.6±3.7) during postmonsoon of Sukkur Barrage is similar to reported (ul Arifeen,

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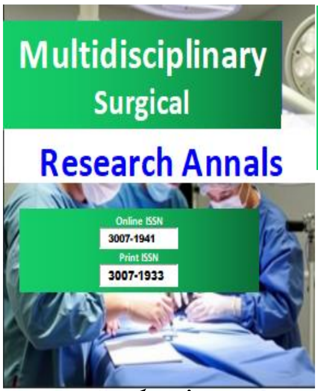
2022).An average pH value of the Chashma Barrage water was observed as (7.6±0.12)during pre-monsoon and (8.09 ± 0.54) during post-monsoon. The pH value indicates slightly alkaline water quality. The observed pH of water during pre and post monsoon in Chashma Barrage showed a very close similarity with other reported studies. (Kalsoom, 2021) .Average pH value of the Taunsa Barrage water was observed as (7.5 ±0.10) during pre-monsoon and (7.8±0.18) during post-monsoon.

An average pH value) of the Sukkur Barrage water was observed (as7.2±0.34) during pre-monsoon and (7.7 ± 1.2) during post-monsoon. The observed pH of water of Taunsa and Sukkur Barrages during pre and post monsoon is showed similarity with the reported water quality assessment of Indus river (Sabir, 2017). The pH values indicated slightly acidic to alkaline water quality. A similar study done at the Chashma Barrage showed consistency with the present study. The river continuously receives the effluent discharge from domestic sewage and nearby industries which ultimately deteriorate the water quality. The dissolved oxygen was recorded as (5.9±0.27) in Chashma (4.5±5.5) in Taunsa (9.4 ± 4.2) in Sukkur Barrage during pre-monsoon and (7.84 ± 1.62) in Chashma (8.24±1.93) in Taunsa (7.6±2.4) in Sukkur Barrage



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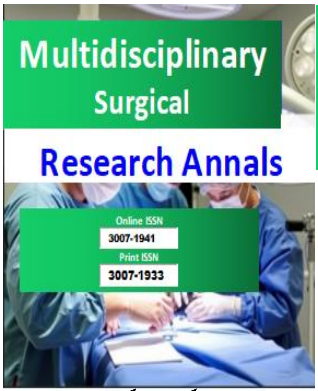
during post monsoon. Similarly, DO found to be the reported water quality assessment of Indus river (Sabir, 2017) .Decreased DO value during pre-monsoon period can be attributed to the rise in temperature level. The observed ammonia level in the (0.01±0.006)Chashma, (0.066±0.085) Taunsa, (0.09±0.017) Sukkur Barrage during premonsoon and(0.01 ± 0.19) Chashma, (0.24±0.067) Taunsa,(0.079±0.02) Sukkur barrage during postmonsoon are very close similar to resent research (Rind, Heavy metal concentrations in water, sediment, and fish species in Chashma Barrage, Indus River: a comprehensive health risk assessment., 2024) .total dissolved oxygen was found to be (7.65±7.63)in Chashma, (11.5±0.76) In Taunsa, (8.72±0.73) In Sukkur during pre-monsoon,(0.17 ± 0.05)Chashma , (9.72±0.61) Taunsa, (0.65±0.02) Sukkur during post monsoon. During both season which was much lower than the reported (Khan, 2014).

The accumulation of heavy metals in fish is influenced by many factors including season, feeding habits, water temperature, pH, salinity, metal interactions, sediment composition, as well as the fish sex and age (MF, 2021) . The concentrations of heavy metals absorbed in various tissues of Labeo rohita in Chashma Barrage are given in (table 6). Cd



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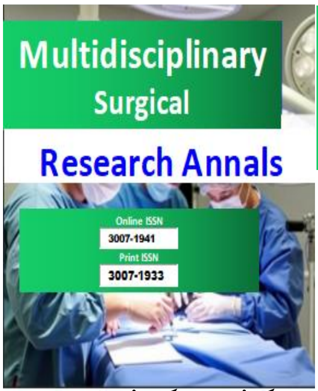
level as 0.72(gills),1.32(liver),1.05(kidney) during premonsoon, 0.94(gills),1.11(liver),1.88(kidney) during postmonsoon, Cd in (pre) liver>kidney>gill (post) kidney>liver>gills. Although absoverd in Chashma Barrage the level of Cd in the tissues of Labeo rohita showed a close similarity with other reported. Lead is cindered as a toxicant element and consumption of Pb contaminated food is known to cause neurotoxicity, nephrotoxicity and other health issues. The concentration of lead in the tissues of Labeo rohita in Chashma Barrage as 2.30(gill), 3.35(liver),0.35(kidney) during premonsoon, 2.85(gill),3.99(liver),1.76(kidney) during post monsoon. Lead in (pre) liver>gill>kidney, (post) liver>gill>kidney.

Although absoverd in Chashma Barrage the level of Pd in the tissues of Labeo rohita showed similarity with that reported in a heavy metal in fish species of Chashma Barrage (Rind, Heavy metal concentrations in water, sediment, and fish species in Chashma Barrage, Indus River: a comprehensive health risk assessment, 2024).Arsenic is a toxic element that is abundant in the aquatic environments such as river and lakes. Heavy metals bioaccumulation and transformation in fish liver, gill, and kidney has emerged from the continuous input of



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industrial wests, pesticides, pharmaceutical compounds and other contaminates into aquatic habitat. As concentration was found as 0.45 (gill), 0.56 (liver), 0.35 (kidney) during premonsoon and 0.57 (gill), 0.33 (liver),0.79 (kidney) during post monsoon.in present study, the observed. As concentration in fish tissues was found several times lower than the reported As concentration at Indus river (Charan, 2023).

Absorbed the cadmium level in the fish Cd 4.33(gills),5.2(liver),6.29(kidney) during premonsoon, 5.57(gills),6.5(liver),7.1(kidney) during postmonsoon of Taunsa Barrage. The amount of Pb was higher in tissues of Labeo rohita in Taunsa Barrage are higher than other Barrages. The concentration of Pd in 3.46(gill,)2.45(liver),1.44(kidney) during premonsoon and 2.99(gill),1.98(liver),1.05(kidney) during post monsoon. The observed Cd and Pb level are higher than recent study (Shaffique, 2024).Although detected As level in fish tissues of Taunsa Barrage are 0.54 (gill), 0.94 (Liver), 0.63 (kidney) during premonsoon 0.82(gill),0.28(liver),1.03(kidney) in this research are several time higher than the reported of (Aamir, 2020). The concentrations of Cd, Pb, and As were recorded low in the Sukkur Barrage than the other

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Barrages. Show the concentration of heavy metals in fish collected from Sukkur Barrage in Table 6.The concentration of Cd 1.66(gill),2.09(liver),1.43(kidney) during premonsoon, 1.29(gill) 2.20(liver)1.55(kidney) during postmonsoon showed higher than the finding (Afzaal, 2022) .accumulation of Pb in Sukkur Barrage showed good agreement with the other reported (Afzaal, 2022).The concentration of As in the fish tissues in Sukkur As 0.75(gill),0.66(liver),0.79(kidney) during premonsoon 0.63(gill),0.59(live)0.43(kidney) during postmonsoon show similar to the finding (Mahessar, 2021).

The overall observed results in the present investigation indicates that marked histopathological changes have been found in the gill, kidney, liver of fish L. rohita. Fusion and shortening lamellae, hypertrophy, degeneration of epithelium and necrosis were found in the gill, kidney, liver of River Indus having high heavy metals contamination. Hemalatha and Banerjee (1977) and Gupta and Kumar (2006) noted similar types of gill lesions in Cd, Pd, As treated Heteropneustes fossils and As treated Cirrhinus mrigala respectively. Kaoud and El-Dahshan (2010) observed severe hyperplasia in

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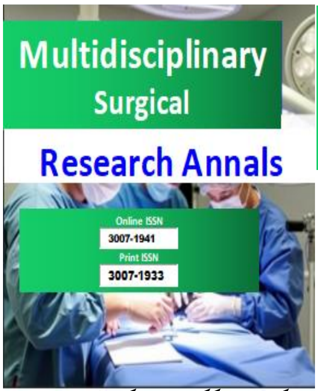
secondary gill lamellae which lead to complete embedding in adjacent lamellae in copper, cadmium, lead and mercury treated Oreochromis niloticus. In the present study, hypertrophy and degeneration of secondary lamellae were observed in L. rohita River indus.

Conclusion

The water quality of the Barrages Temperature changes has an impact on fish metabolism. and growth. The Chashma Barrage is characterized by an annual temperature rise before the monsoon that points to a parasite infection and a decrease in the same during the postmonsoon. In the pre- and post-monsoon, the temperature of the Sukkur Barrage varies between 24.5 and 27.6 C0, while the Taunsa Barrage temperature varies between 37.5-25.5 C0. The quantity of HM absorbed in various L. rohita organs in the Chashma Barrage shows that levels of Cd, Pb, and As during the pre- and post-monsoon followed the liver>kidney>gill trend, which in turn controls fish HM build-up. Significant amounts of Cd were found in the heavy metal concentrations in the tissues of L. rohita in the Taunsa barrage. Conc. of high level Pb & Cd. The present investigation indicates that marked histopathological changes have been found in the gill, kidney, liver of fish L. rohita. Fusion and shortening



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lamellae, hypertrophy, degeneration of epithelium and necrosis were found in the gill, kidney, liver of River Indus having high heavy metals contamination. The present results demonstrate that the lysozyme activity is decreased in fish present in heavy metals in the water. Duration of exposure and concentration of accumulation in water effect enzyme activity by declining fish health.

Recommendation

The acquired information was aided in the development of method for treating heavy metals, water quality and making aquatic environment safe for aquatic life. Monitoring programs to assess environmental health in contaminated area.

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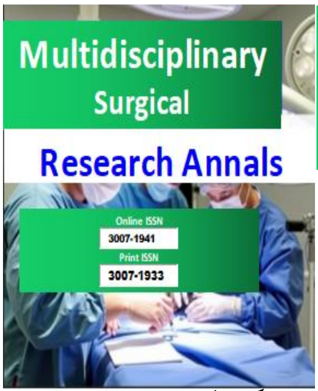

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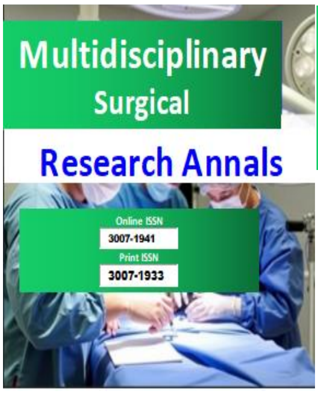

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