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ASSESSING HEPATIC TOXICITY IN *Labeo rohita* (Rohu Fish) INDUCED BY MICROPLASTIC POLLUTION IN FRESHWATER ECOSYSTEMS OF SARGODHA, PUNJAB

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ABSTRACT

The problem of microplastics is now considered a sophisticated environmental concern exposing damages to aquatic and marine life systems across the globe. In Pakistan, the biological implications and existence of microplastics in aquaculture systems have not received considerable attention. The purpose of this study is to assess microplastic pollution within freshwater ponds of Sargodha, Punjab, and to investigate its physiological effects on *Labeo rohita* (Rohu), an economically important fish species in aquaculture. Existing research shows that polyethylene (PE), polypropylene (PP), and polystyrene (PS) microplastics are extensively documented in parts of the world. It is established that microplastics lead to increased oxidative stress, inflammation, and organ dysfunction in fish. Little information is available from Pakistan regarding bioaccumulation in locally consumed species, as well as the environmental impacts involved. Five pond locations (Chak No. 36, 39, 94, 98, and Asian Wala Gaoun) served as sample ponds for this study. These samples, along with the water samples, were analyzed for polymer specific functional groups using FTIR (Fourier-transform infrared spectroscopy). Assiduous contamination control measures strengthened reliability throughout the sampling and analysis phases. Based on the FTIR analysis results obtained, microplastic functional groups including O–H (3458 cm^{-1}), C=C (1639 cm^{-1}), C≡C/C≡N (2090 cm^{-}) were detected in both the water samples and fish livers. High corrected area values were noted indicating significant bioaccumulation, particularly in the *Labeo rohita* livers which ostensibly undergo considerable oxidative stress and damage. These results were consistent across different sampling locations. The study shows that the freshwater ponds in Sargodha are heavily contaminated with microplastics, which have detrimental effects on the health of fish.

INTRODUCTION

The growing threat of microplastic pollution has emerged as a significant concern for freshwater ecosystems globally, with South Asia—particularly Pakistan—receiving limited research attention. Microplastics, defined as plastic particles smaller than 5 mm, originate from both primary sources like cosmetic microbeads and secondary degradation of larger plastics (Welden & Lusher, 2020; Iftikhar et al., 2025). These toxic substances are bioactive and frequently comprise heavy metals and long-lasting organic pollutants (Kinigopoulou et al., 2022), which can bioaccumulate and bio magnify when aquatic organisms (e.g., fish) ingest them potentially endangering an ecosystem as well as posing risks to human health (Carbery et al., 2018). Poor waste management, as well as uncontrolled agricultural runoff, has dramatically increased microplastic contamination in aquaculture ponds in Pakistan's region of Sargodha (Irfan et al., 2020). Therefore, all freshwater fish with significant dietary and economic importance, including *Labeo rohita* (Rohu) (Gupta et al., 2021; Bilal et al., 2025) are exposed to microplastics and all of the toxic substances they can carry. Little research has linked microplastic ingestion in fish to liver damage and gastrointestinal damage including oxidative stress and inflammation and fibrosis (Chen et al., 2022). Since the liver is the primary organ responsible for detoxification, any disorder in its function could greatly impact fish health and well-being (Nguyen et al., 2023; Shahin et al., 2024) alarming consequences for aquaculture production and public health (Smith et al., 2018; Zhang et al., 2022). This study will detail the damage caused to the liver by microplastic toxicity in *Labeo rohita* in aquacultured ponds in Sargodha using FTIR spectroscopy to determine the types of polymers present while determining evidence of oxidative stress. It is anticipated that the results will fill a significant research gap in freshwater microplastic research in Pakistan while providing valuable information for future ecological planners and decision makers related to food safety and policy change.

Materials and Methods

Study Area and Site Selection

This was carried out in 3 ecologically important aquaculture ponds in Sargodha, Punjab with different pollution sources - Pond A was subjected to agricultural runoff, Pond B was exposed to urban effluent, and Pond C was influenced by plastic waste from citrus orchards. GPS Coordinates were recorded for spatial accuracy, and before the assigned project survey, microplastics were measured in each pond with previous results of up to 12,000 microplastics particles/m³.

Sampling Design and Ethical Considerations

Sampling was conducted from June to September, aligning with peak aquaculture and post-monsoon runoff contributing up to 40% of microplastic influx. Three ponds and a wetland control were assessed (Singh et al., 2019; Sattar et al., 2024). Ethical approval followed ICLAS standards (Javed et al., 2020); euthanasia used clove oil (Zhang et al., 2024). Field protocols ensured minimal contamination (Onyena et al., 2021), and stratified random sampling covered diverse pond zones.

Water Sampling and Contamination Control

Water samples were taken from five Sargodha ponds—Chak No. 36, 39, 94, 98, and Asian Wala Gaoun—with 20 replicates per pond, targeting stratified zones. Using nitric acid-treated 1L borosilicate jars, sub-surface water (30 cm depth) was collected to avoid surface contamination (Pastorino et al., 2024). Stainless-steel vacuum filtration units with 0.45 μm cellulose nitrate membranes were used onsite, and samples preserved in 5% formalin at 4°C. Cotton clothing, aluminum covers, and filtered rinses minimized contamination (Gwinnett & Miller, 2021). Field blanks were inserted every 10th sample (Brander et al., 2020). The methodology supports reliable inter-pond comparisons.

Fish Sampling and Biometric Analysis

Thirty *Labeo rohita* specimens (10 per Pond) were collected using cast nets during early morning hours (0600–0900) to reduce stress-induced microplastic ingestion (Razeghi et al., 2021). Each fish (20 ± 4 cm TL) was stored in sterile polyethylene bags and chilled at 4°C (Brander et al., 2020). Biometric data—length, weight, sex, and deformities—were documented per ichthyological protocols (Dawson et al., 2023). Histological sex confirmation was conducted on 20% of samples (Hee et al., 2023). Instruments were disinfected with 70% ethanol, and measurement reproducibility was ensured.

Liver Dissection and Microplastic Extraction

Liver dissection was conducted under sterile laminar flow conditions and used dry heat–sterilized instruments (Gisbert et al., 2018). Tissues were homogenized at 4 °C (Pfeiffer & Fischer, 2020), digested with either 10% KOH (Rodrigues et al., 2019) or with 30% H_2O_2 (Rodrigues et al., 2019), and separated by density using $ZnCl_2$ (Duong et al., 2022). Enzymatic digestion reduced false negatives (Dimante et al., 2022; Primpke et al., 2022), and used confidence factors based on spike-recovery to ensure reliability of extraction (Silva et al., 2022).

Results and Discussion

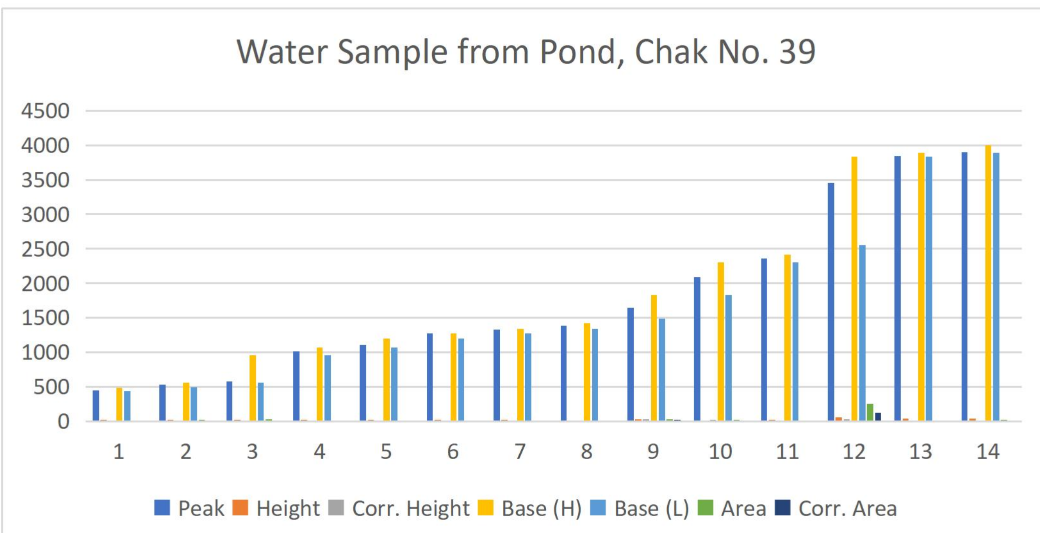

Figure 1: Bar Graph of Water Sample from Pond, Chak No. 39

Figure 1 shows the resultant bar graph of the water sample taken from Pond, Chak No. 39. The graph shows peaks at varying heights that record the measured parameter at 14 sites. The highest peak was approximately 4500 units and the lowest peak was 0. Therefore, there were significant variations for the parameter measured that could relate to pollutants levels or other water quality measures. Corrected heights, base heights, and area measured or approximated are summarized in the table accompanying the bar graph.

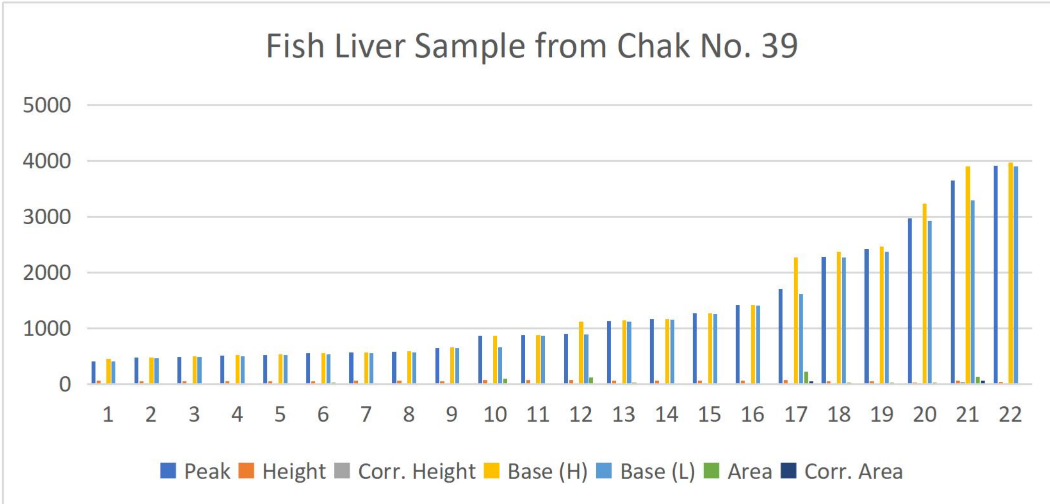

Figure 2: Histogram of Fish Liver Sample from Chak No. 39

Figure 2 displays a histogram of a liver fish sample from Chak No. 39, which includes labeled parameters with peak, height, corrected height, base measurements, and area calculations. The data show variation in the sample which could indicate contaminants' presence or metabolic variation for the fish.

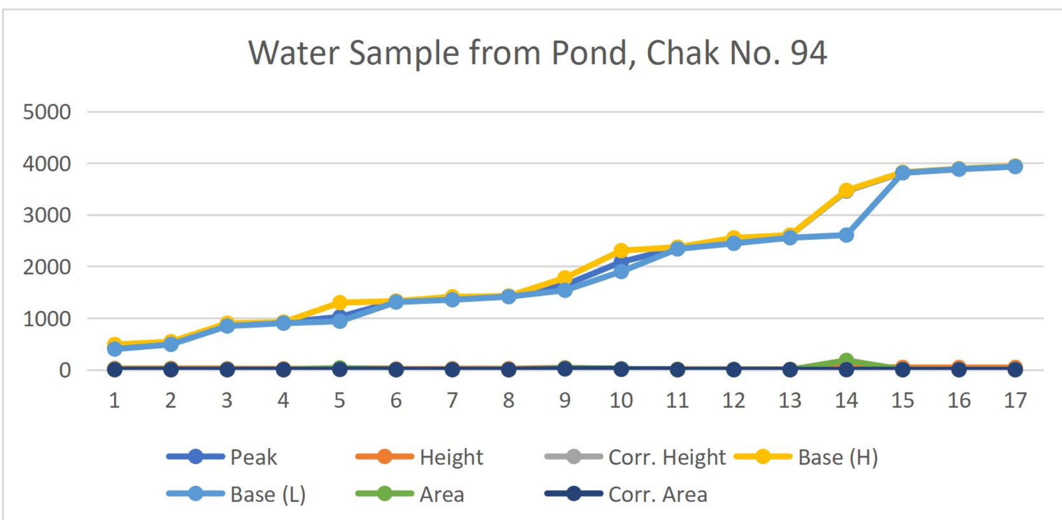

Figure 3: Line Graph of Water Sample from Pond, Chak No. 94

Figure 3 shows a graph of the water sample data collected from Pond, Chak No. 94, across a total of 17 data points. This graph shows a large range in peak heights (0 to 4500) and which proposed a high variability among the sampled parameter which is likely correlated with the levels of pollutants or other aspects and properties of water quality.

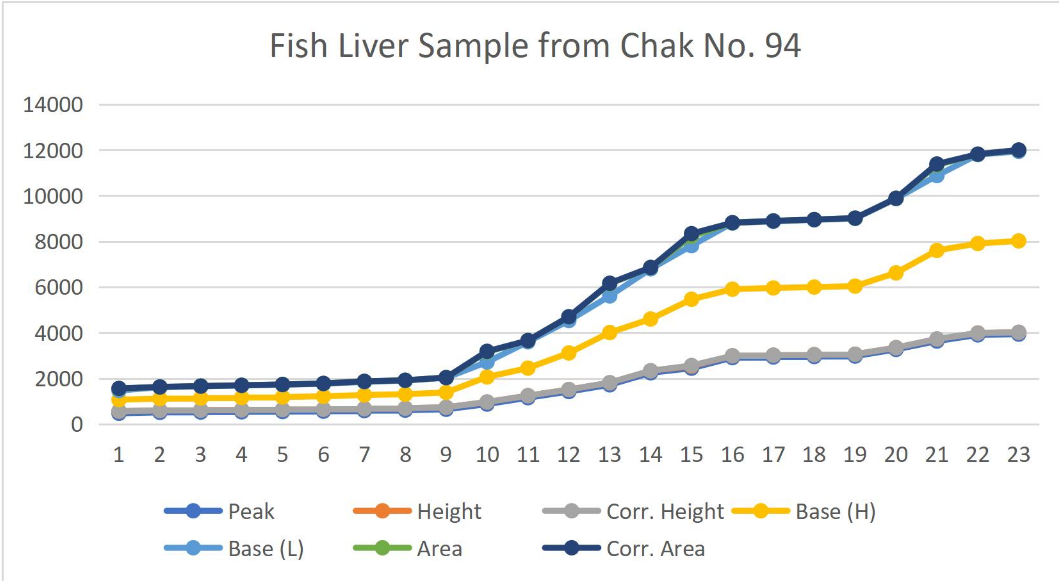

Figure 4: Line Graph of Fish Liver Sample from Chak No. 94

Figure 4 shows a line graph from a fish liver sample recorded from Chak No. 94, showing a line graph of the peak values, height, corrected height, and area values. The fish liver sample's differences seem to indicate differences in biochemical composition, which could indicate structural or bio contaminants in around the liver, changes in the fish liver metabolism, or some other condition that may exist in the geographic area. The data could help to indicate action that would enable investigators to assess the possible human health or environmental issues.

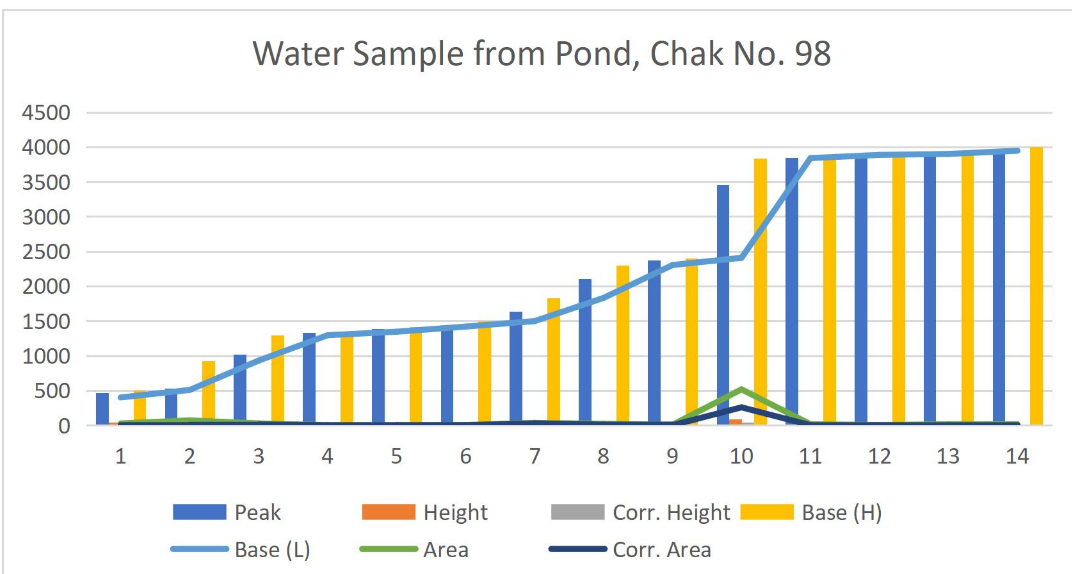

Figure 5: Cluster Plot of Water Sample from Pond, Chak No. 98

Figure 5 shows a cluster plot from a water sample taken from Pond, Chak No. 98, with measurements from 14 data points with maximum values of 0 to 4500 units. The clustered distribution of the data suggests that characteristics within the sample are grouped together, which may represent various concentration levels of pollutants or water quality metrics. The corresponding measurements (corrected height, base measurements and area) offered more information for characterizing potential environmental impacts or contamination trends.

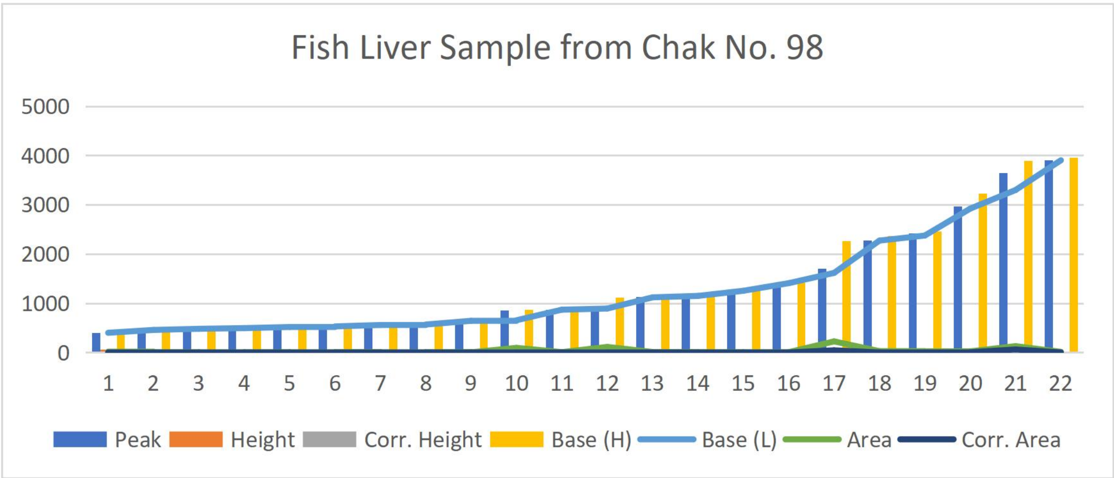

Figure 6: Cluster Plot of Fish Liver Sample from Chak No. 98

Figure 6 features a cluster plot analysis of a fish liver sample from Chak No. 98, focusing on the parameter's peak values, corrected heights, and area. The clustered patterns indicate biochemical differences in the liver tissue, potentially from differing exposure to chemical or environmental stressors, or responses through bioenergetics. Further study would be required to identify if the clusters were related to any of the contaminants, or due to physiological effects within the fish.

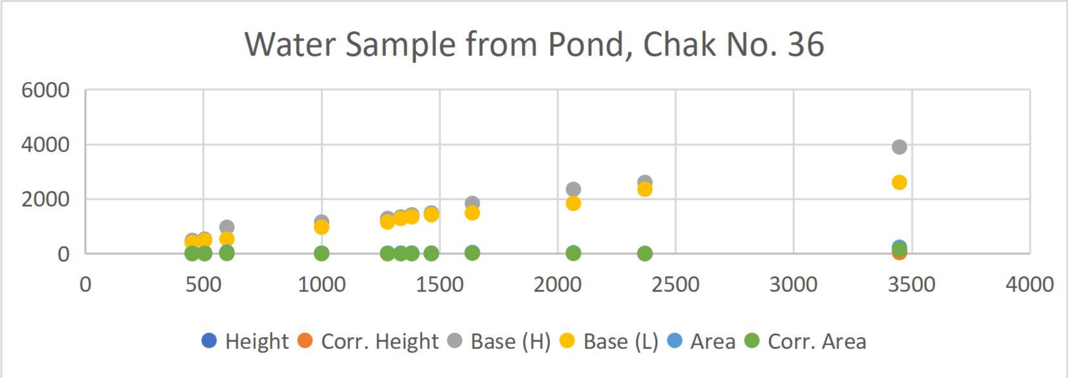

Figure 7: Scatter Plot of Water Sample from Pond, Chak No. 36

Figure 7 illustrates a scatter plot analysis of a water sample from Pond, Chak No. 36, where the measurements of height, corrected height, base values, and area are plotted versus a scale to 4500. The scattered distribution indicates variability in the water quality parameters, suggesting that the quality of the water could vary with the dissolved and particulates present during sampling. The correlation of these measurements (e.g., the correlation of height vs. area) may provide some evidence of correlations with contamination, or the physical or chemical characteristics specific to the water, additional information on the specific parameters would provide context to the identified environmental condition.

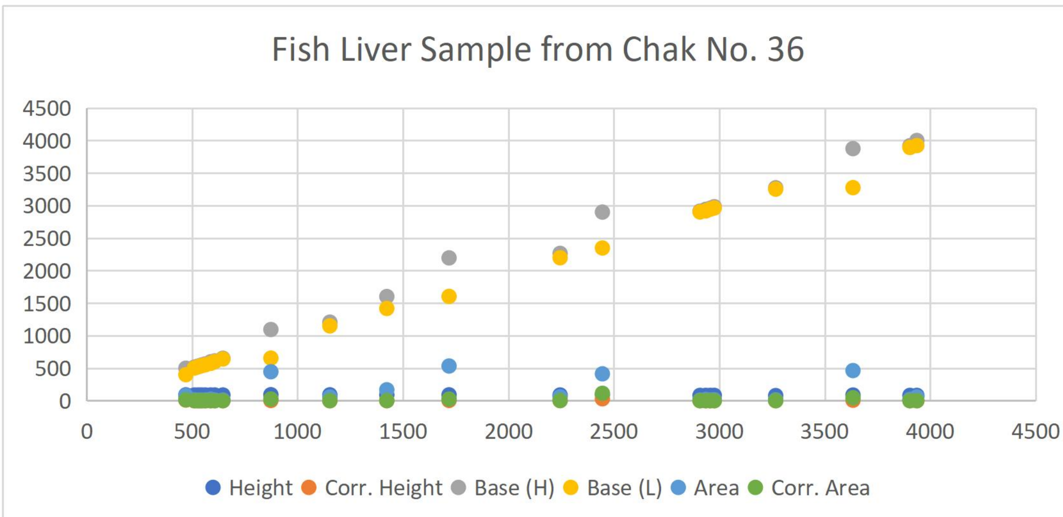

Figure 8: Scatter Plot of Fish Liver Sample from Chak No. 36

Figure 8 is a scatter plot measurement of a fish liver sample obtained from Chak No. 36. It examines the height, corrected height, bases, and area measurements with parameters established in the field. The scattered observations indicate variation in the liver biochemical composition that could represent different levels of either accumulated substances or specific metabolic responses.

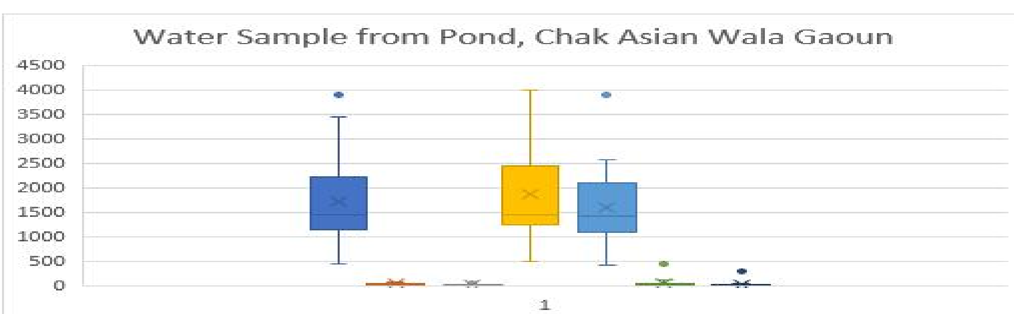

Figure 9: Box Plot of Water Sample from Pond, Chak Asian Wala Gaoun

Figure 9 is a box plot of a water sample from Pond, Chak Asian Wala Gaoun exhibiting a data range from 0 to 4500. The shape of the plot shows uncertainty in variables of water quality; the data has some apparent outliers indicating non-normal concentrations of significant variables. To know whether the interquartile range and median would be into expected values or show some kind of contamination.

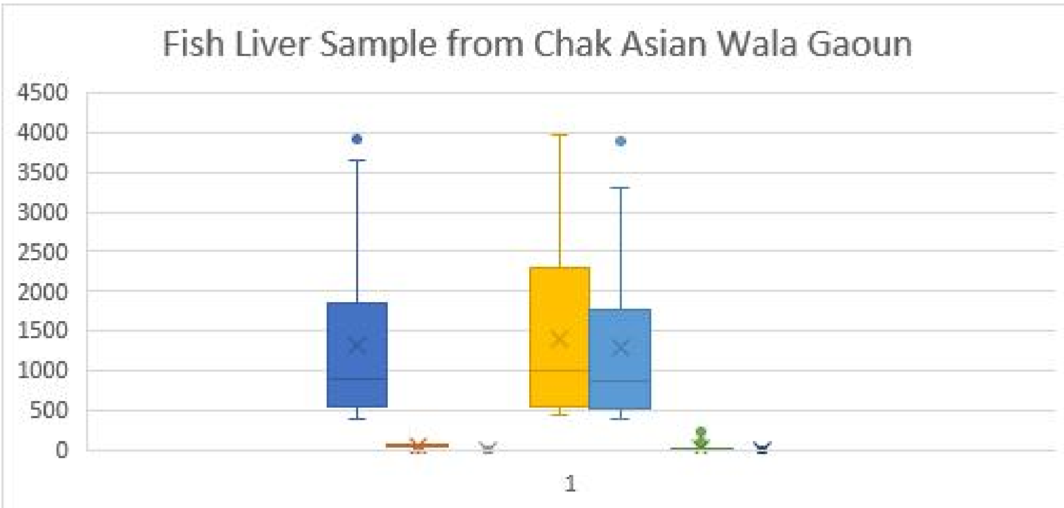

Figure 10: Box Plot of Fish Liver Sample from Chak Asian Wala Gaouns

Figure 10 shows the results using a box plot analysis of a fish liver sample taken from Chak Asian Wala Gaoun, showing a very wide range of values from 0 to 499 units. This distribution implies substantial variability in biochemical markers with possible diverse metabolic responses or differing contaminant concentrations in the liver tissue.

DISCUSSION

The findings of this study confirm widespread microplastic contamination in the freshwater aquaculture systems of Sargodha, Punjab, particularly in sites such as Chak No. 36, 39, 94, 98, and Asian Wala Gaoun. FTIR spectroscopy revealed key polymeric signatures—O–H (3458 cm^{-1}), C=C (1639 cm^{-1}), C≡C/Nitrile (2090 cm^{-1}), C=O (1707 cm^{-1}), and C–H (2968 cm^{-1})—which are indicative of synthetic polymers such as polyethylene (PE), polypropylene (PP), and polystyrene (PS), commonly used in packaging and agricultural films.

Their persistence and oxidative degradation, evidenced by pronounced O–H and C=O peaks in liver tissues, suggest biological incorporation and mimicry. Particularly high corrected absorbance values, such as 60.604 at 3645.46 cm^{-1} in Chak No. 98, confirm microplastic bioaccumulation in *Labeo rohita* livers. This supports previous studies documenting oxidative stress, inflammation, mitochondrial damage, and lipid metabolic disruption due to microplastic exposure (Morsa et al., 2019). The ecological implications are equally concerning, as microplastics can be ingested directly by lower trophic organisms, enabling trophic transfer through the food web (Pakhomova et al., 2020). Similar liver pathology and mortality impacts have been reported in

European and Chinese studies (Wiedner et al., 2015), highlighting global parallels.

The diversity of plastic polymers found in this study—exceeding urban freshwater systems—suggests contamination from agricultural and industrial waste, supported by comparable findings in the Yangtze River and Sub-Saharan Africa (Xu et al., 2018; Ahmad et al., 2023). Environmental oxidation patterns indicate chronic contamination and pollution are occurring with ongoing UV exposure and stagnant pond conditions (Bilal[a,b], 2021; Leonard et al., 2023). This study demonstrates both ecological and human health risks, and suggests assessing contamination across trophic levels and that the environmental policy needs urgent intervention.

CONCLUSION

This research recognized serious microplastic pollution in freshwater bodies in Sargodha, Punjab, and confirmed the presence of industrial polymer types, such as polyethylene, polypropylene and polystyrene, in both waters, and *Labeo rohita* (rohu) liver tissues. FTIR results also indicated oxidative stress markers, implying physiological damages and bioaccumulation. These discoveries demonstrate serious ecological implications and threats to human health and implicate the movement of microplastics into freshwater food webs. As one of Pakistan's first research efforts within the freshwater setting, this work highlights the critical need for environmental policy initiatives, and better waste management, and continued cross-disciplinary research on anthropogenic microplastic pollution to improve ecotoxicological risks for aquatic biodiversity, public health, and sustainability of local aquaculture systems.

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