



Microplastics Manifestation and Their Potential Impact on Freshwater Fish Species

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Microplastics: A Global Threat



- **Microplastics, tiny plastic particles less than 5 millimeters in size, have emerged as a significant environmental concern globally.**
- **These ubiquitous pollutants are now found in freshwater ecosystems, posing potential risks to aquatic life, including freshwater fish species.**
- **This presentation delves into the occurrence, pathways, and potential impacts of microplastics on freshwater fish, highlighting the urgency of addressing this growing environmental threat.**

Why Environmental Threat For Aquatic Biota



Up to 10 % of plastic produced/year ends up in the aquatic environment



Converted into smaller plastic by autocatalysis and biodegradation



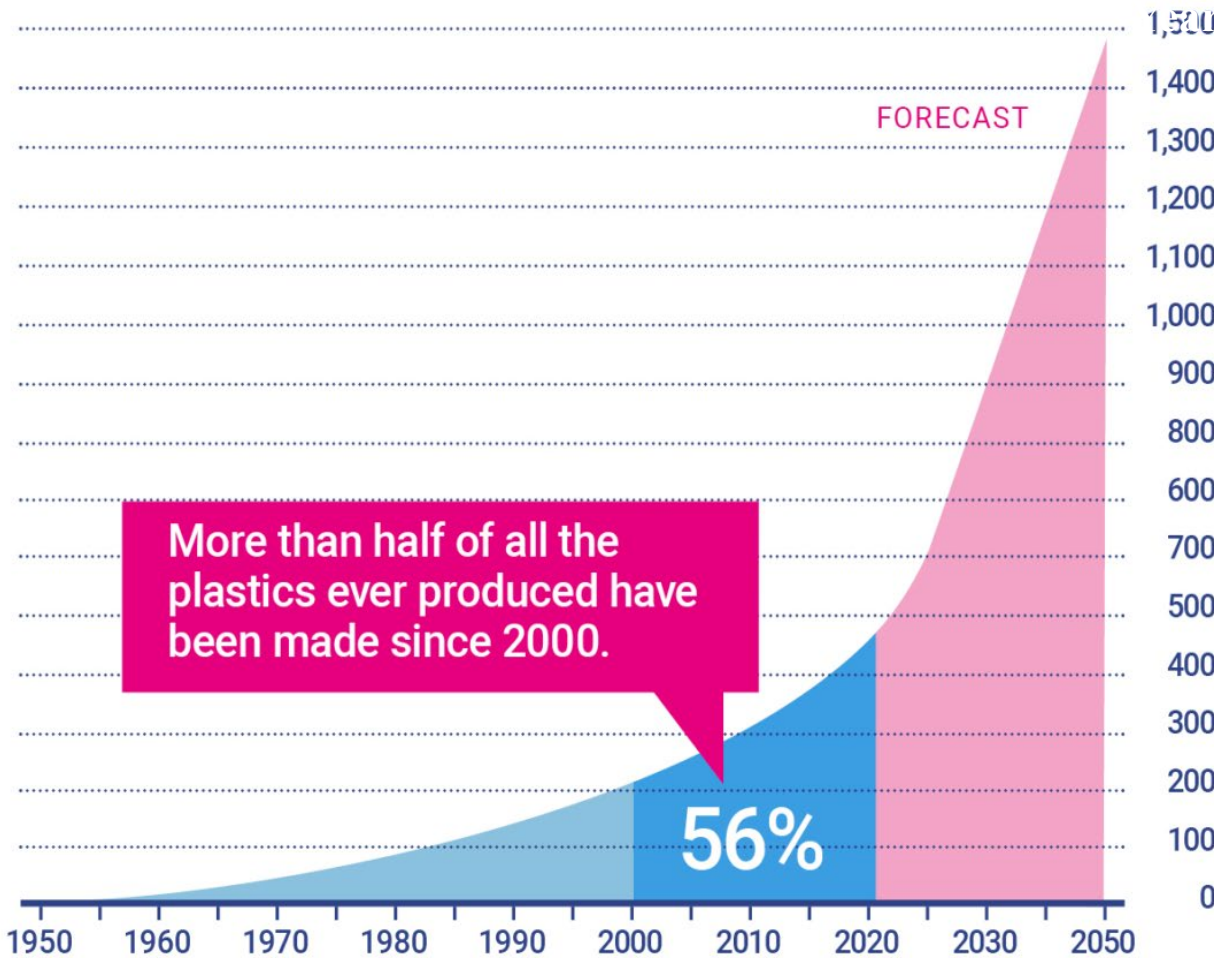
Ingested by aquatic fauna and impose toxic effects on aquatic biota



Global Plastic Production

PRODUCTION OF PLASTIC

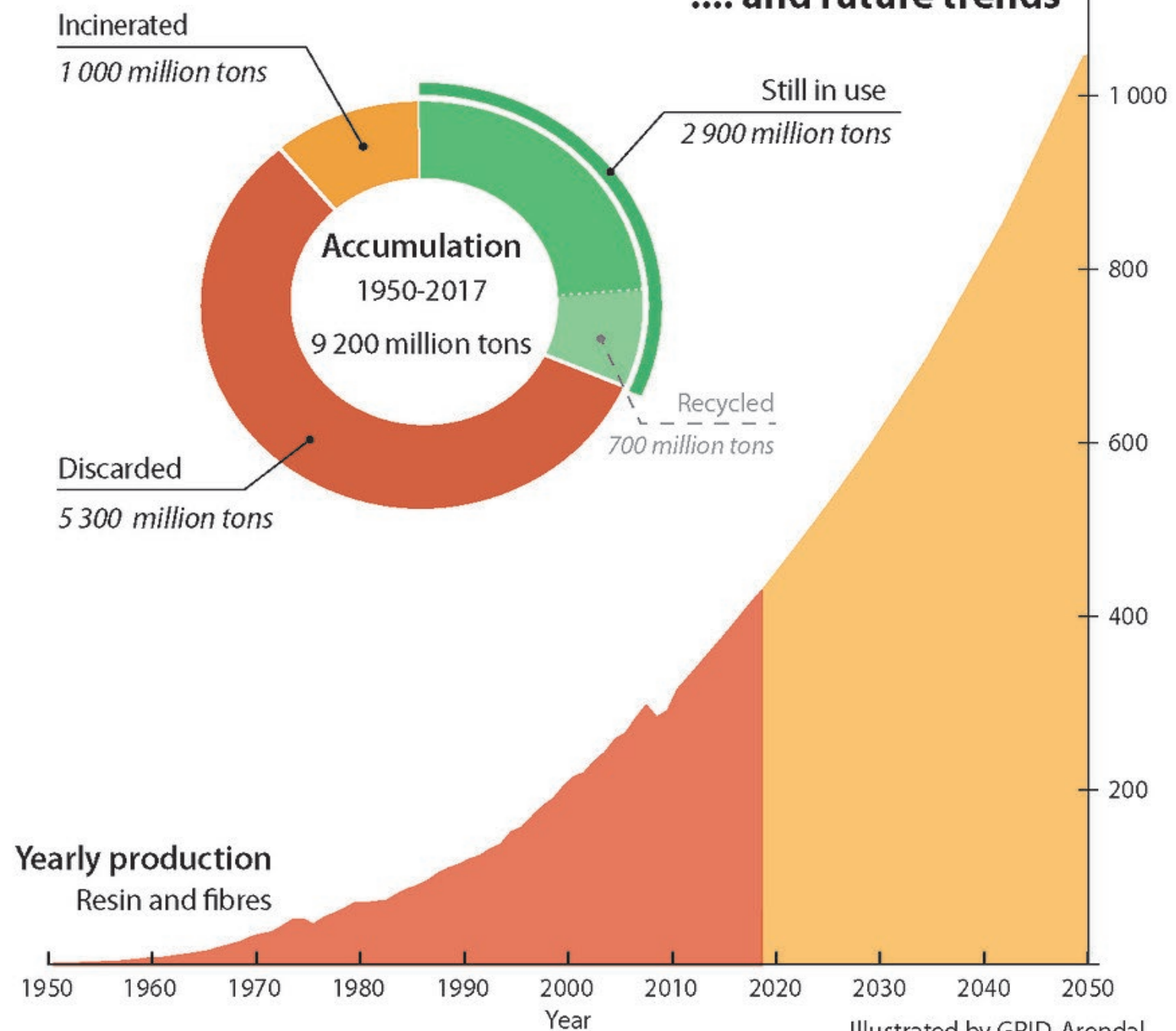
Global annual plastic production in million tonnes.

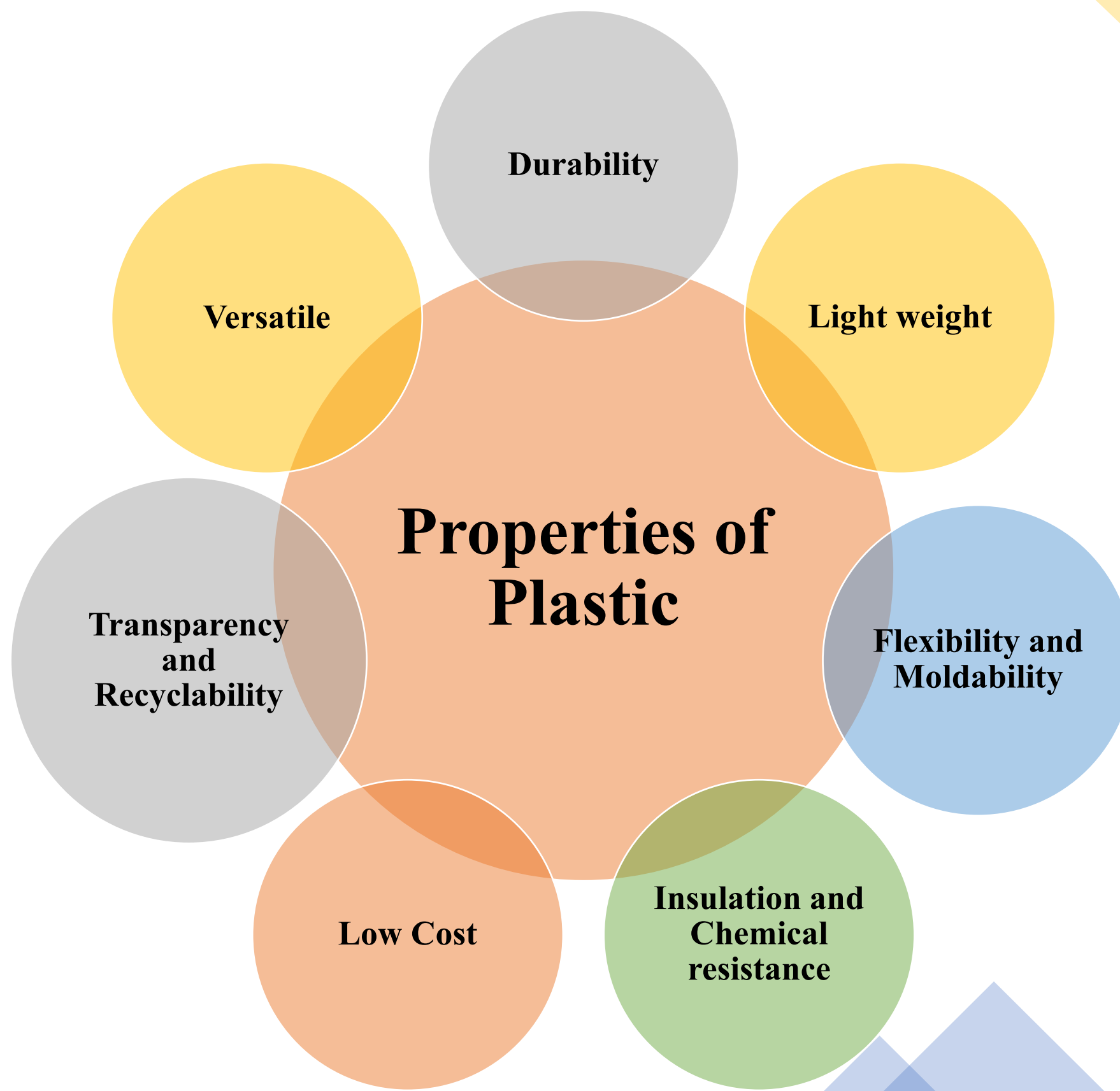


Year	Production
1950	1.5 million tonnes
2015	322 million tonnes
2017	350 million tonnes
2019	374.8 million tonnes
2021	490.7 million tonnes

Global plastic production and accumulation

.... and future trends

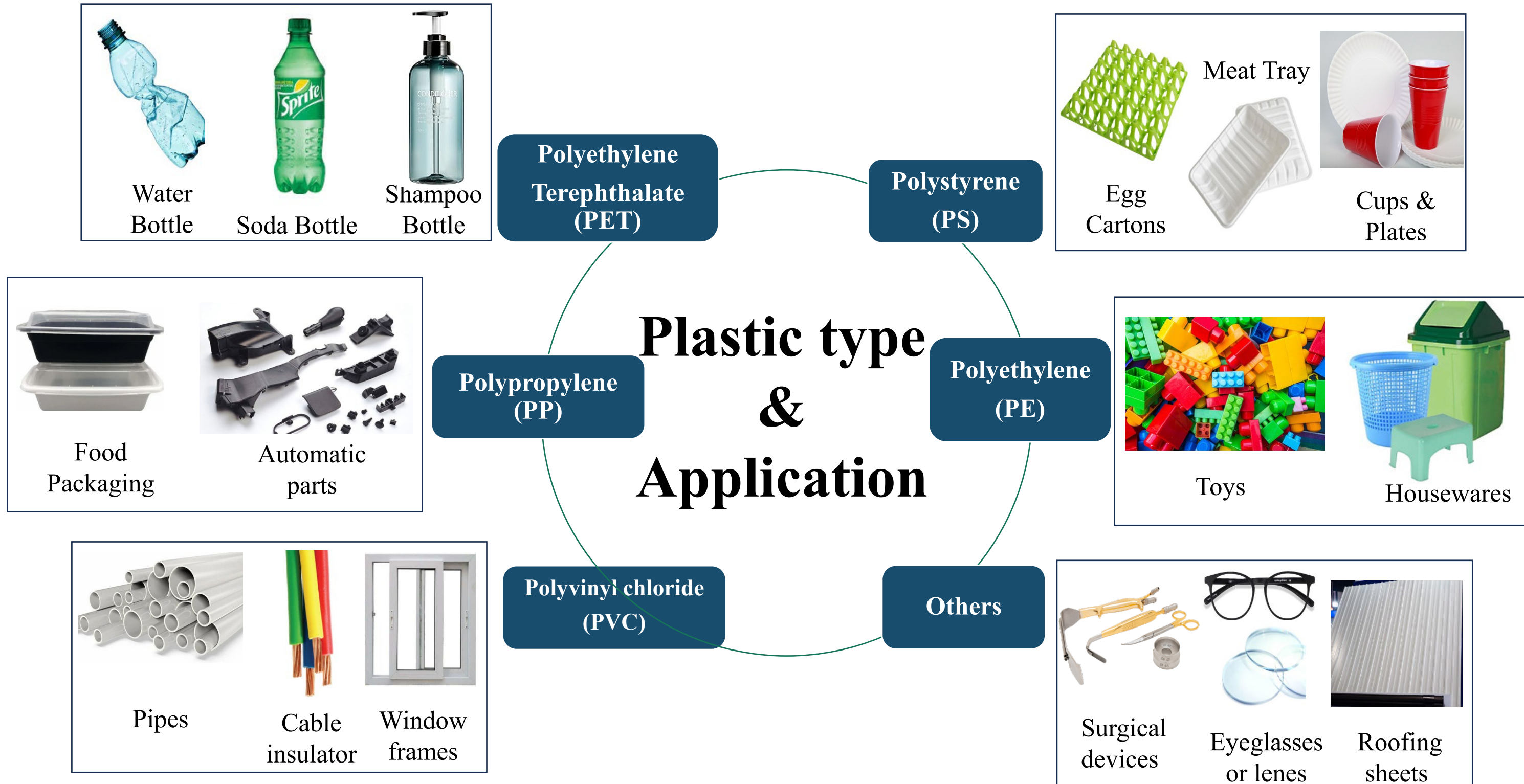


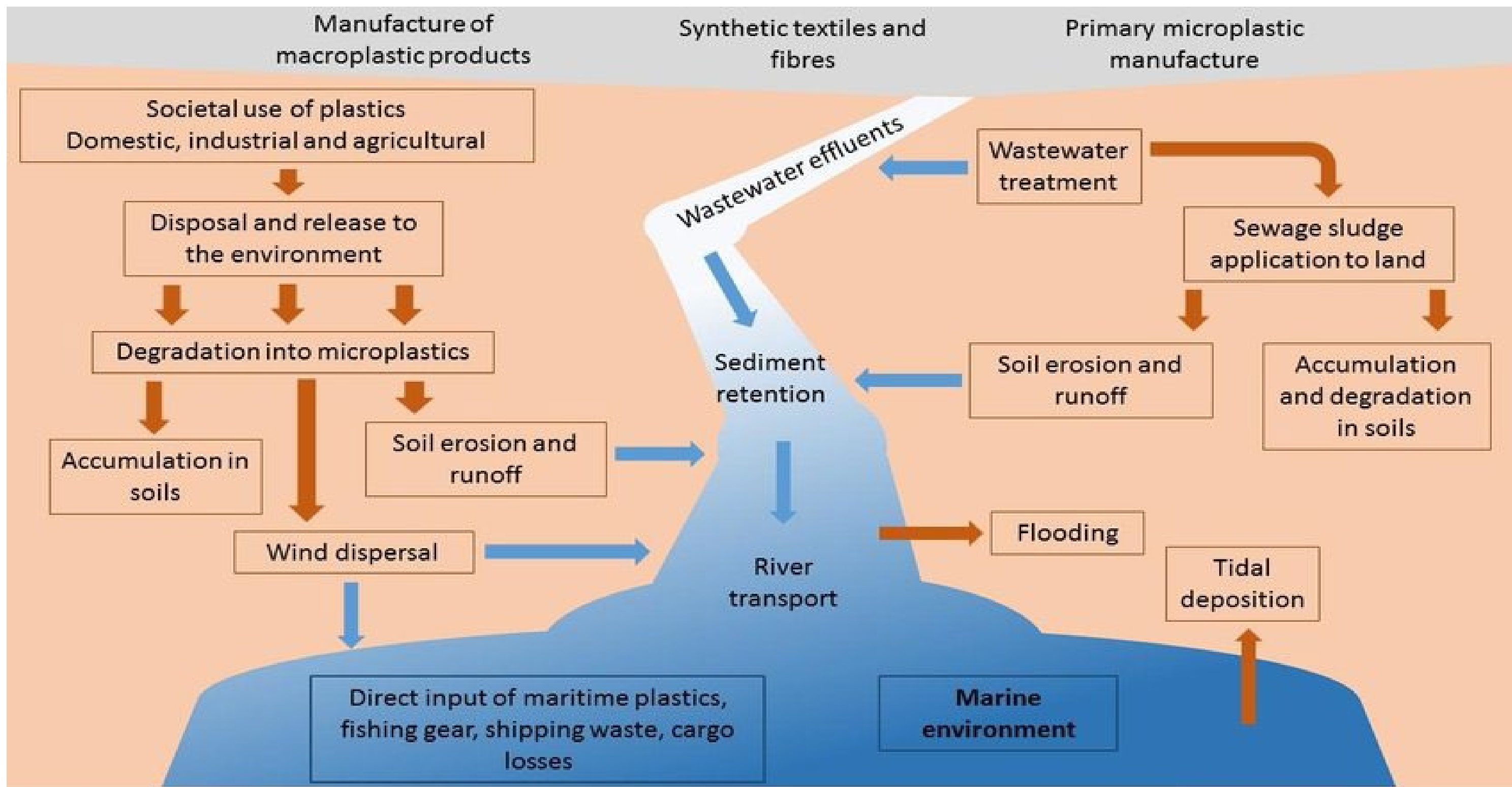


Use of Plastic

- ✓ Health care
- ✓ Medical devices
- ✓ Food packaging
- ✓ Renewable energy
- ✓ Transportation
- ✓ Communication
- ✓ Building and construction







Sources and Pathways of Microplastics in Freshwater Ecosystems

1 Direct Discharge

Sewage treatment plants, industrial effluent, and stormwater runoff contribute significantly to microplastic input into freshwater systems.

2

Fragmentation of Larger Plastics

Larger plastic debris, such as bottles and bags, break down into microplastics due to weathering and UV radiation.

3

Atmospheric Deposition

Microplastics can travel long distances through the air and settle into freshwater ecosystems.

4

Human Activities

Fishing gear, recreational activities, and agricultural practices also contribute to microplastic pollution.



Plastic Waste Categories

Macro



> 25mm

Meso



< 25 - 5mm

Mirco



< 5 – 1mm

Nano

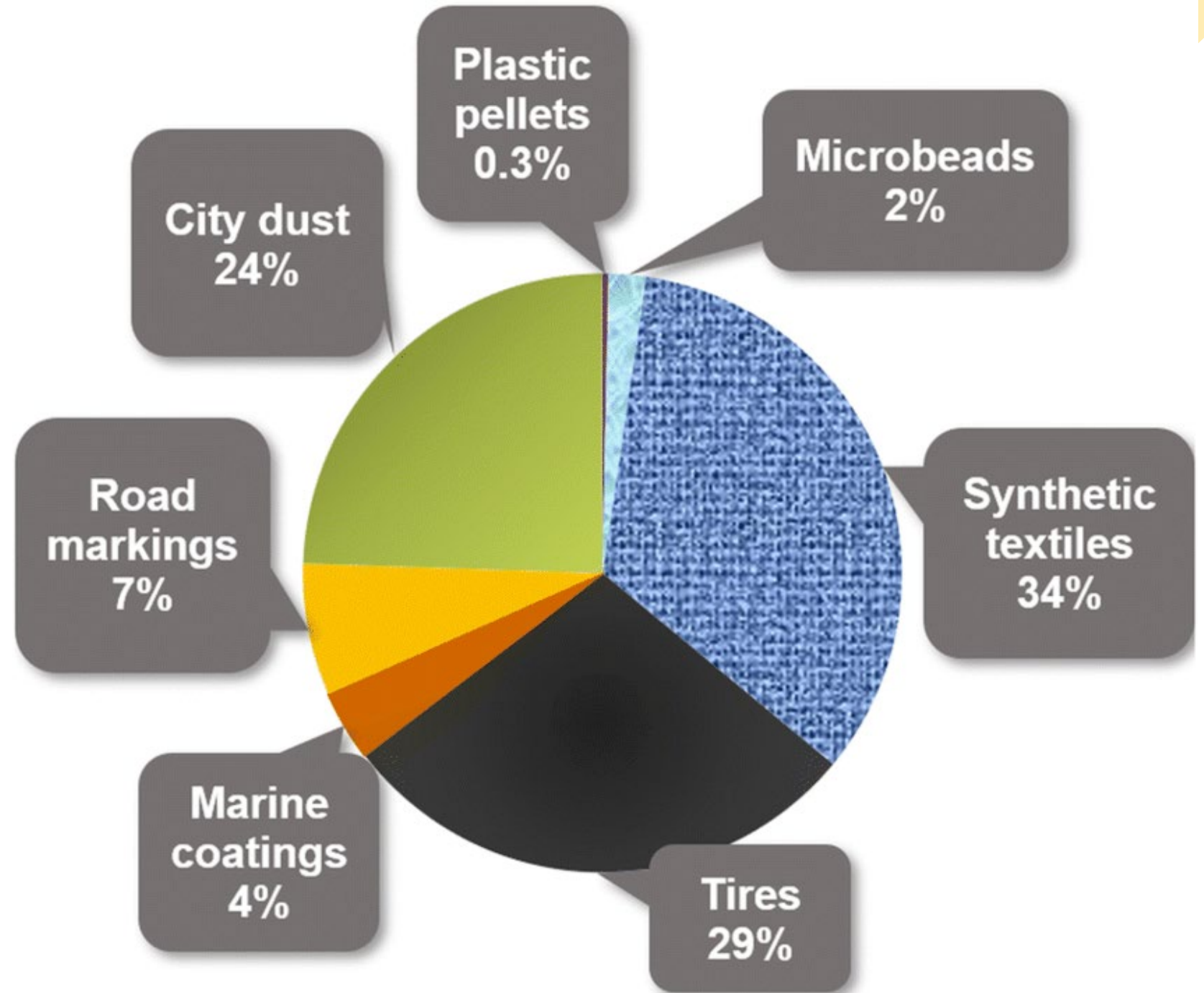


< 100nm

Forms of Microplastic

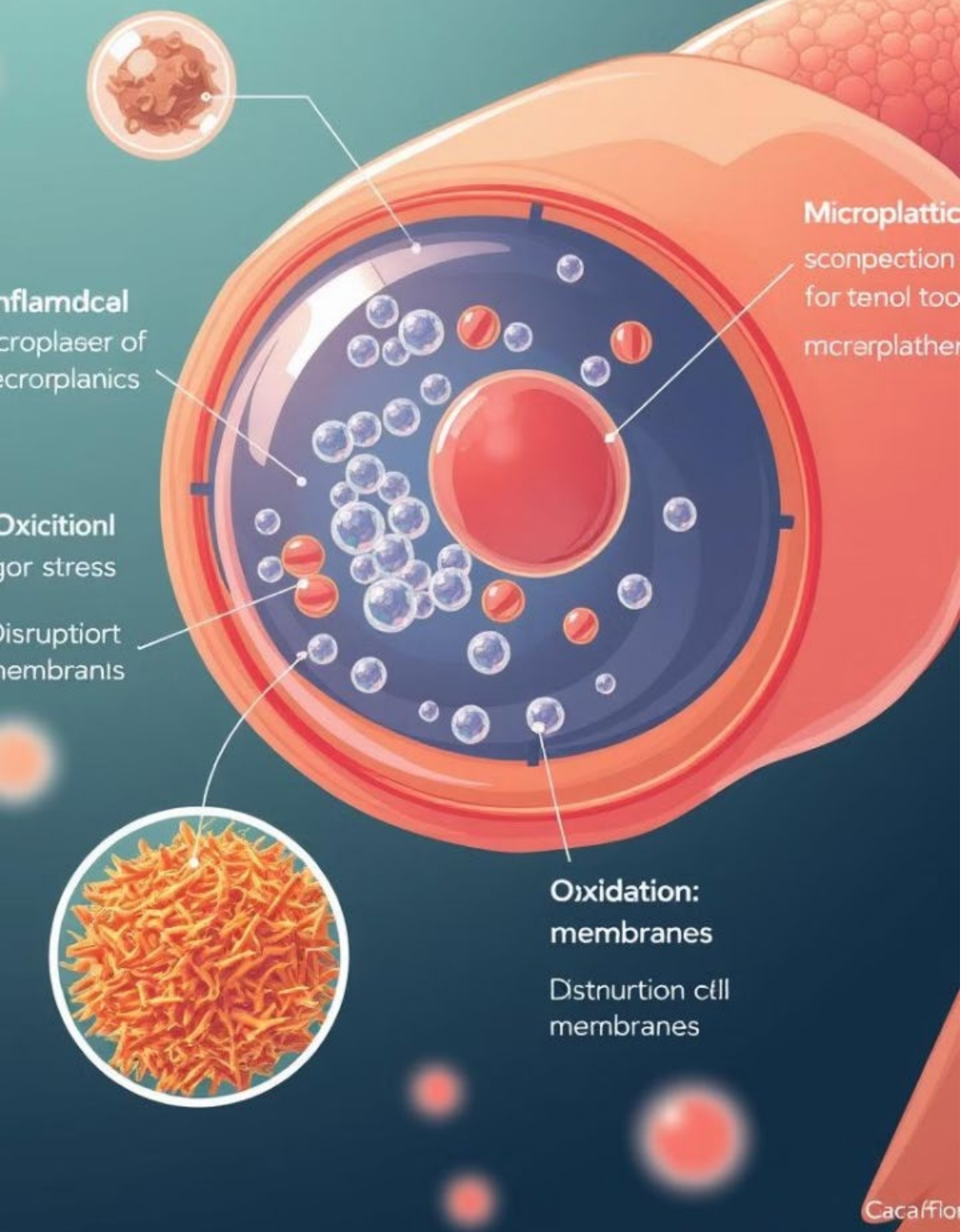


Where do microplastics originate?



Milccoppattecls

our cell : microplastic mechanism of toxicity.



Mechanisms of Microplastic-Induced Toxicity

The mechanisms of microplastic-induced toxicity are complex and involve multiple pathways. Microplastics can interact with cells and tissues in various ways, leading to inflammation, oxidative stress, and disruption of cellular processes.

★ Inflammation

Microplastics can trigger an inflammatory response in fish tissues, leading to tissue damage and impaired function.

✍ Oxidative Stress

Microplastics can generate reactive oxygen species, leading to oxidative stress and damage to cells.

👤 Cell Disruption

Microplastics can disrupt cell membranes, leading to leakage of cellular components and impaired cell function.

Manifestation of Microplastic Contamination in Fish Organs

Microplastic contamination can manifest in various fish organs, including the gills, liver, gut, and muscle tissue. These organs play critical roles in respiration, metabolism, digestion, and movement, making them susceptible to microplastic accumulation and the associated effects.



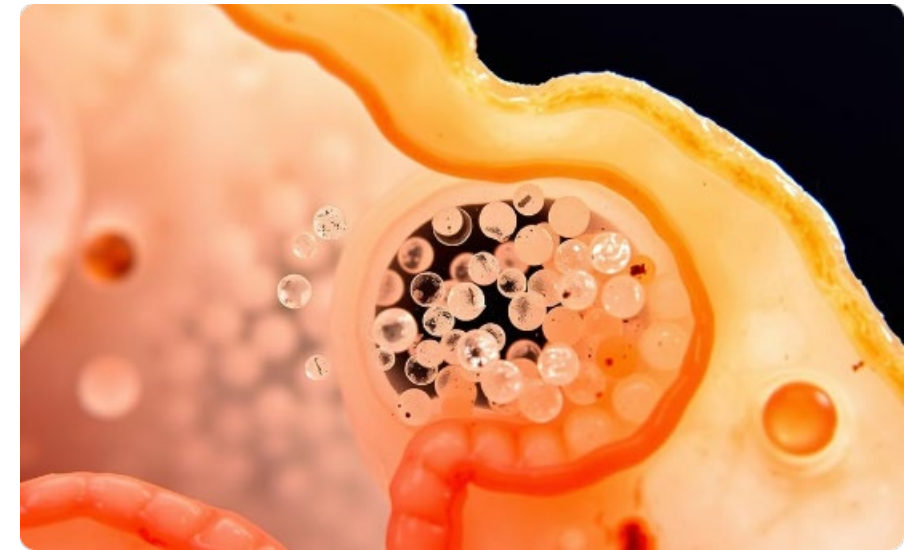
Gills

Microplastics can accumulate in the gill filaments, impairing gas exchange and leading to respiratory distress.



Liver

Microplastics can accumulate in the liver, disrupting detoxification processes and increasing the risk of liver damage.



Gut

Microplastics can accumulate in the gut, interfering with digestion and nutrient absorption, potentially leading to malnutrition.



Harmful Impacts of Microplastics on Fish Health and Survival

Microplastic contamination can have profound and harmful impacts on fish health and survival, affecting growth, reproduction, behavior, and susceptibility to disease. These impacts can disrupt fish populations, potentially altering the structure and function of freshwater ecosystems.

1 **Growth Retardation**

Microplastic exposure can inhibit growth, reducing the size and weight of fish.

3 **Increased Disease Susceptibility**

Microplastics can weaken the immune system, making fish more vulnerable to infections and diseases.

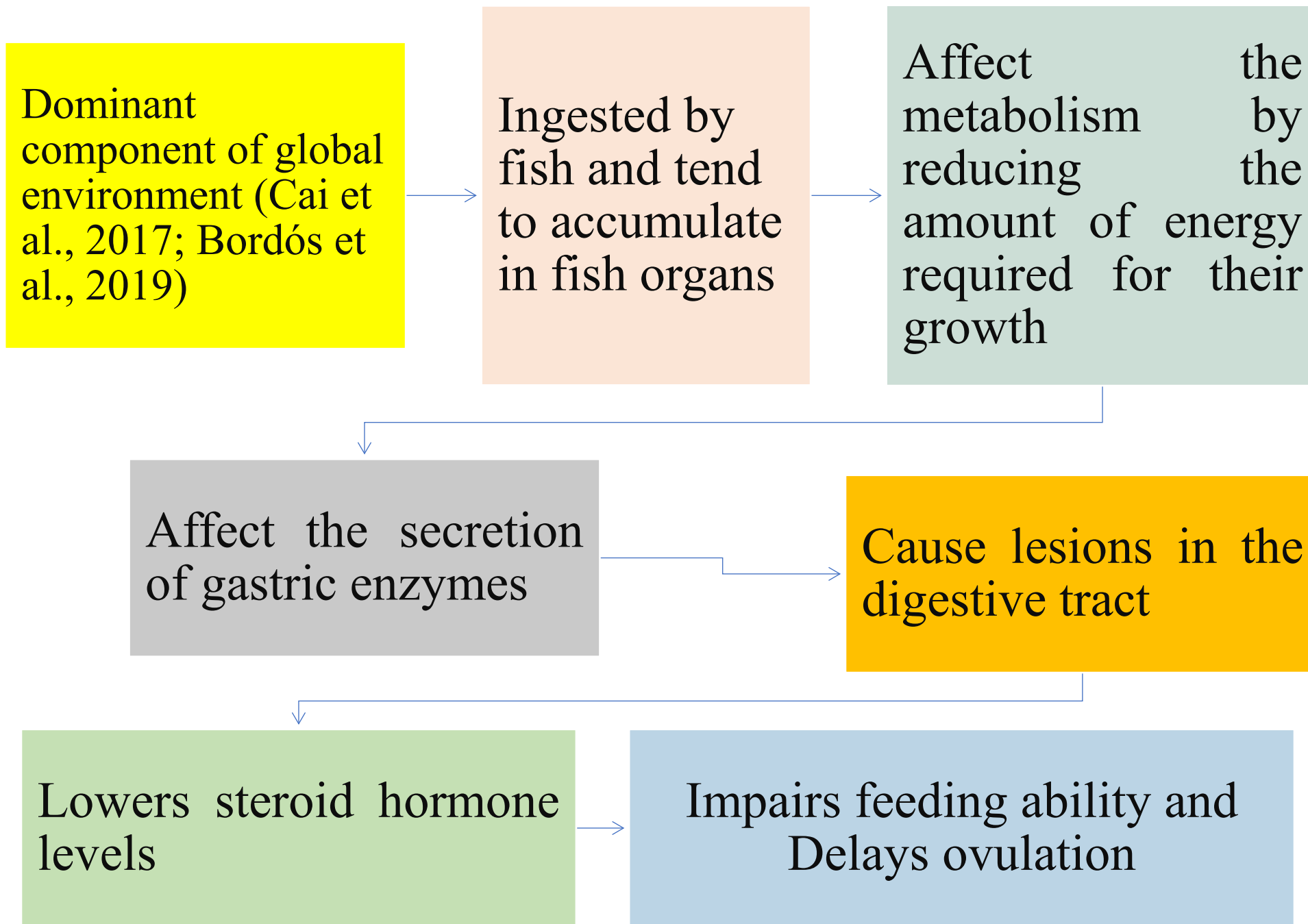
2 **Reproductive Dysfunction**

Microplastics can interfere with reproductive processes, leading to reduced fecundity and impaired offspring development.

4 **Behavioral Alterations**

Microplastic exposure can alter behavior, affecting foraging, swimming, and social interactions.

Microplastic Toxicity





Impacts on Population and Ecosystem

Population Decline

Microplastics can contribute to fish mortality and reduced reproduction, leading to a decline in population size.

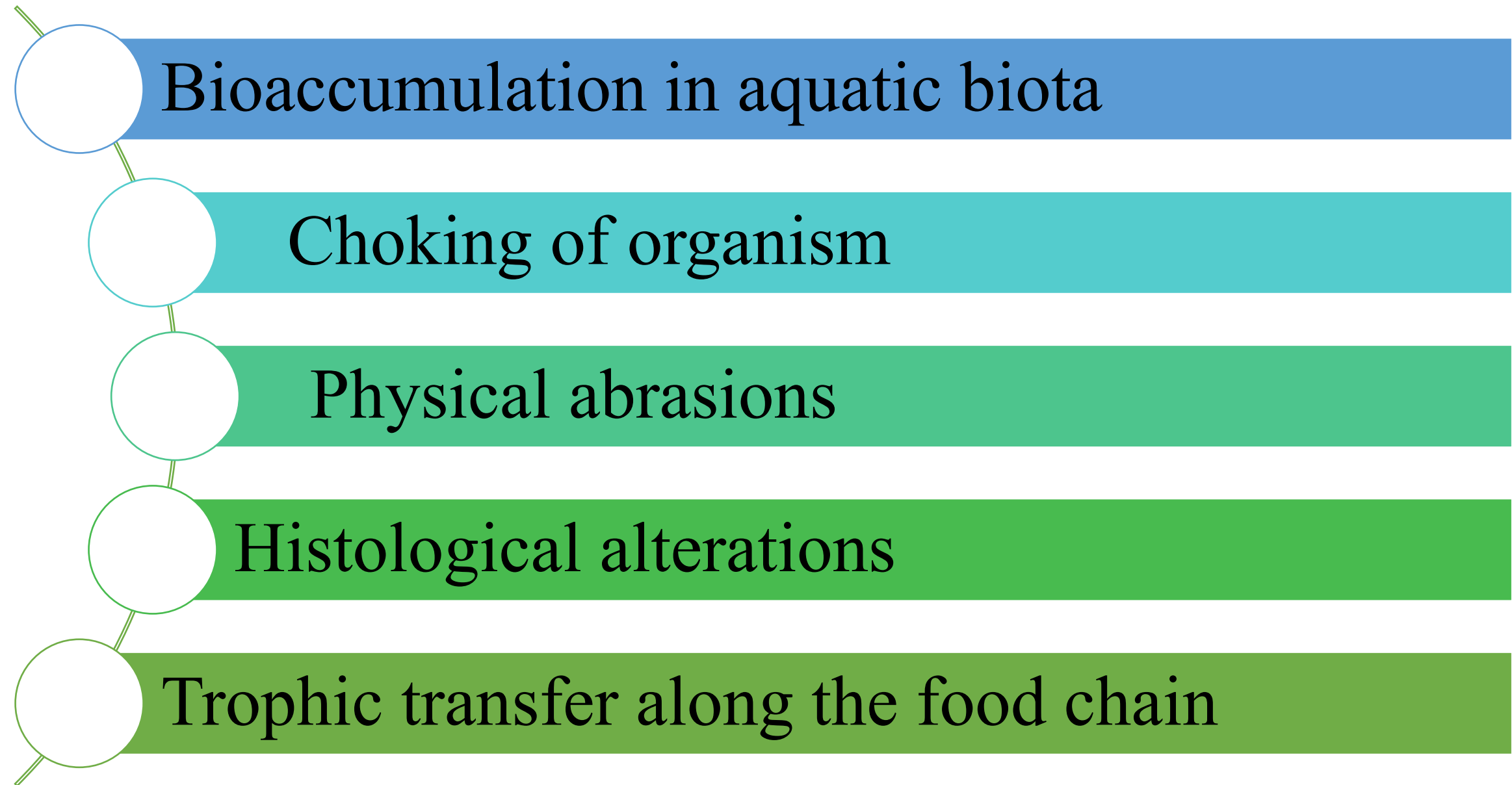
Ecosystem Disruption

Changes in fish populations can have cascading effects on the entire ecosystem, altering food webs and nutrient cycles.

Human Health Implications

The consumption of fish contaminated with microplastics can pose potential risks to human health.

Plastic and Aquatic Environment



Case Studies of Microplastics from Different Rivers of The Punjab Pakistan



MATERIALS AND METHODS

1. Samples Collection

- Water
- Sediments
- Fish

2. samples were collected from three different polluted sites (distance of 100-200 meter) of each River (Ravi, Chenab, Jhelum and Indus)

3. All three samples from each location were analyzed for the detection, identification, and characterization of MPs by using different techniques.



Water Sample Processing



Water sample



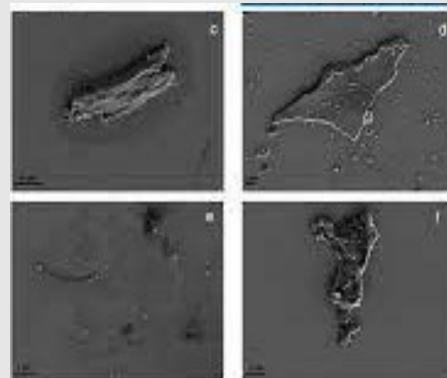
Digestion with 30 % H₂O₂



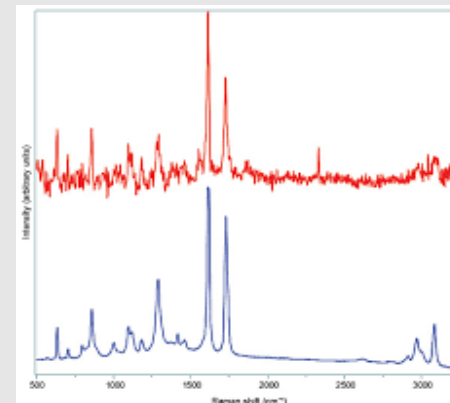
Density separation



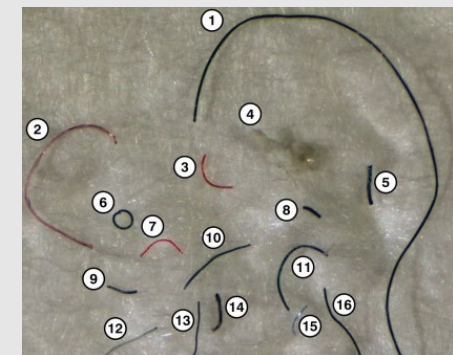
Vacuum filtration



SEM



RAMAN

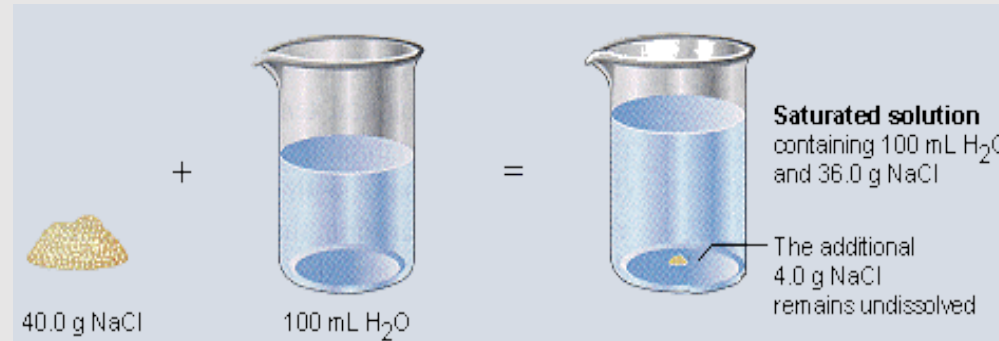


Microscopy

Sediments Processing



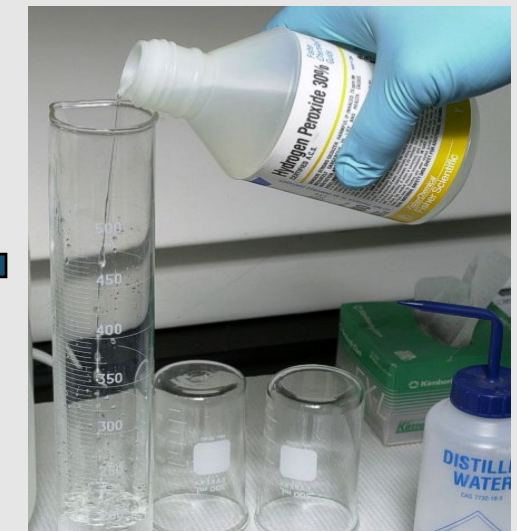
1. Sieving (<5mm sieve)



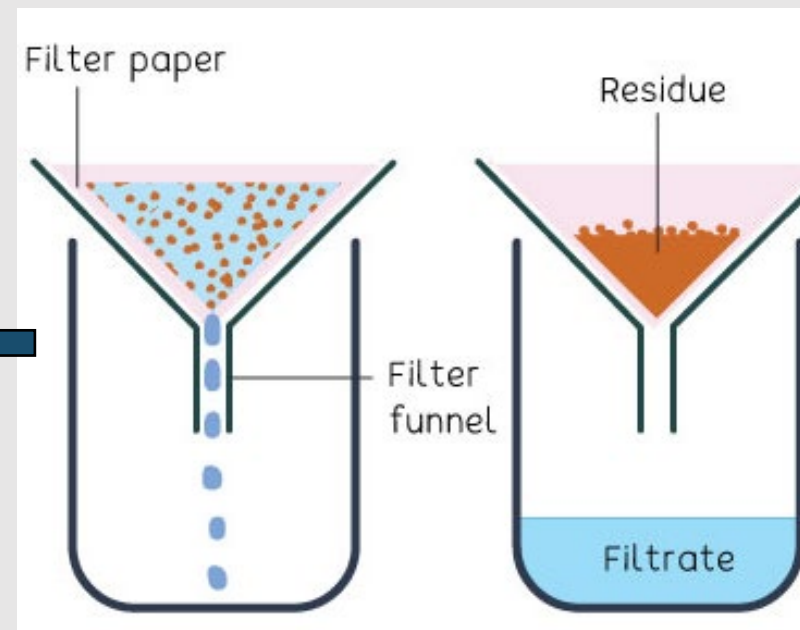
2. Density Separation with saturated NaCl solution



3. Magnetic Stirring



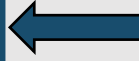
4. Treated with 30% H₂O₂



5. Cellulose Filtration



6. Oven Dry at 50 C



7. Characterization of Microplastics

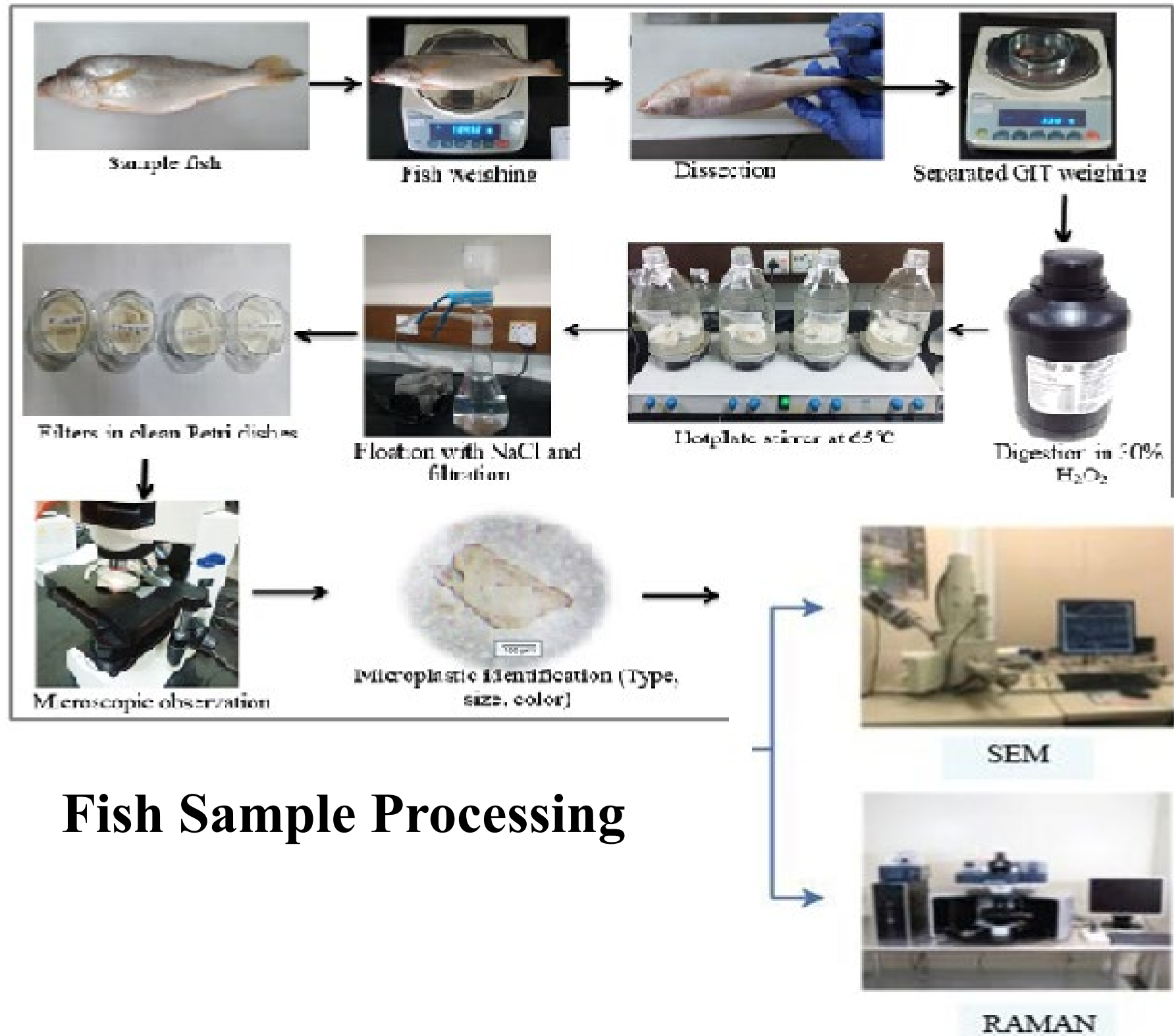
Fish Sample Collection and Processing

Different fish species of different habitats were collected from the polluted sites of each river with the help of local fishermen.

Dissection was performed at the spot after sampling to avoid deterioration of organs.

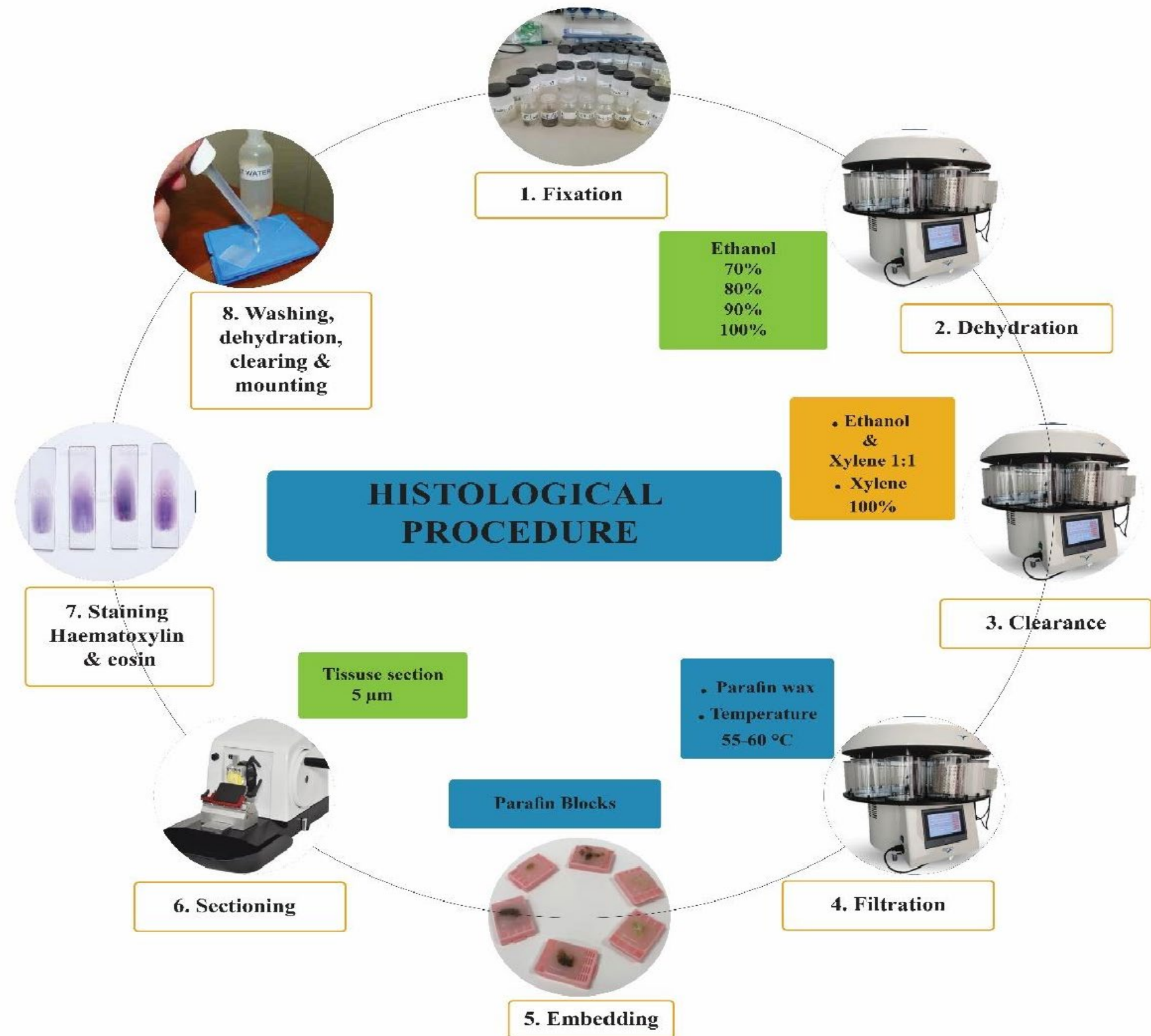
Required organs were collected and stored in 10% formalin solution and were transported for further analysis to the Research Laboratory, Department of Zoology, GC University Faisalabad.





Fish Sample Processing

Histology



Statistical analysis

- **R statistical software** (R Core Team, 2021) through the R integrated development environment in R studio (R Studio Team, 2021).
- **Clustered heat maps** were plotted by customized code (pheatmap) and **correlation matrix** (ggbiplot2) ($p \leq 0.05$) by using R statistical software (R core Team 2019) **Tukey pairwise test.**



Results



The study identified and characterized the microplastics in water, sediments and different tissues of freshwater fish species from the riverine population of the Punjab, Pakistan

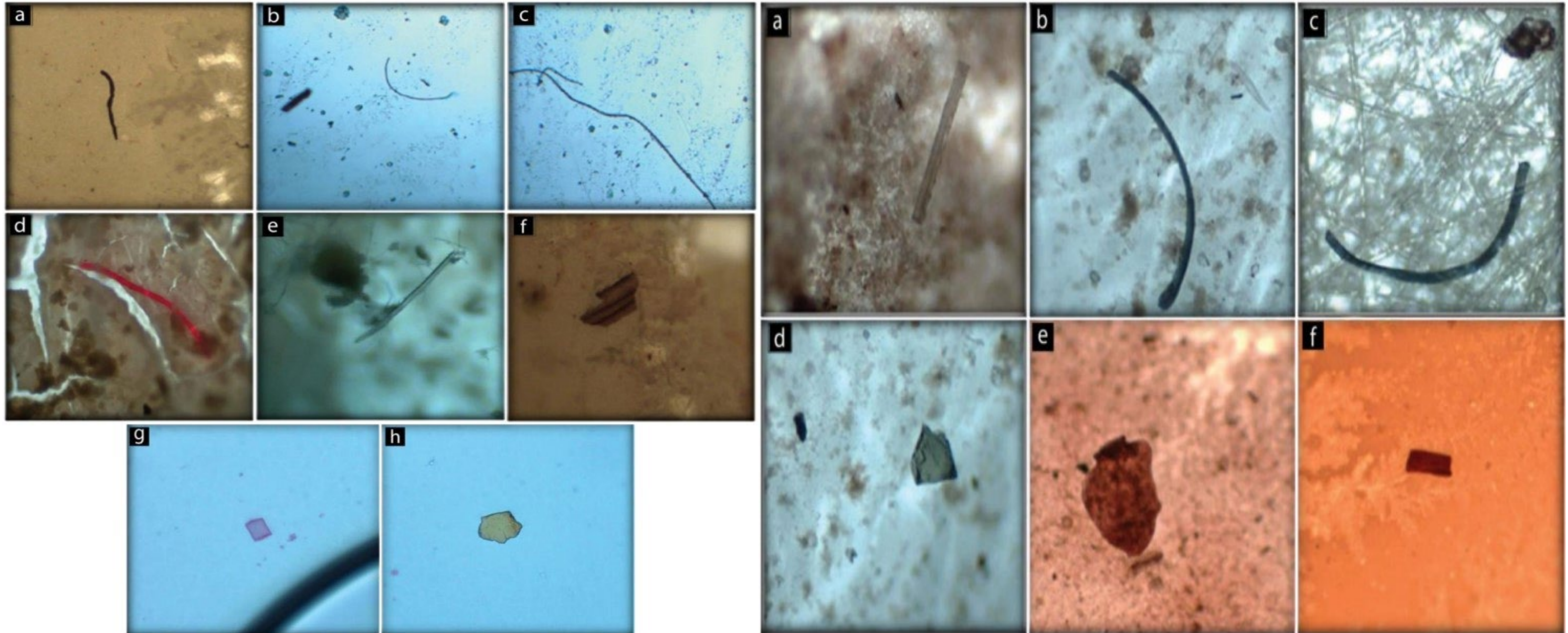


Microplastics were extracted from water, sediments and fish tissues through different analytical techniques and characterized by Raman and Scanning electron microscopy

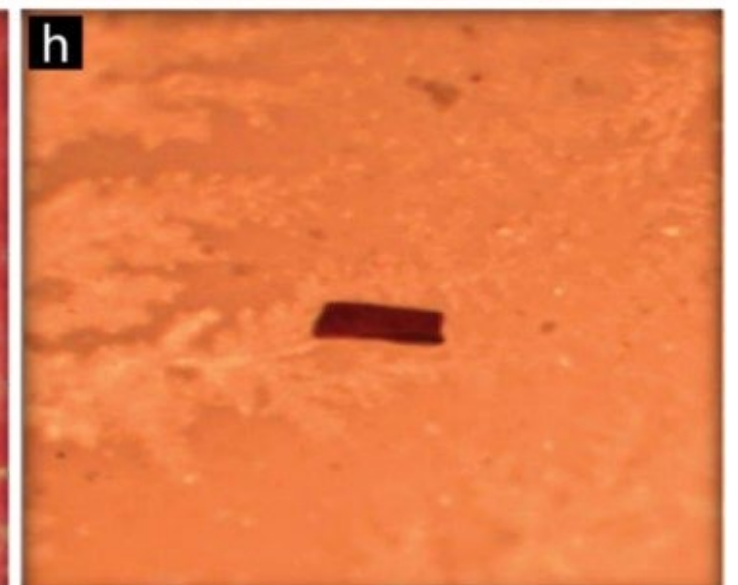
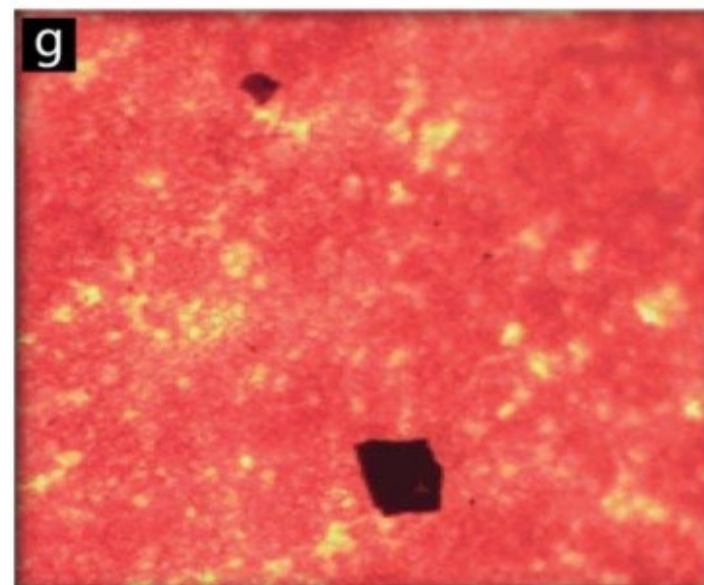
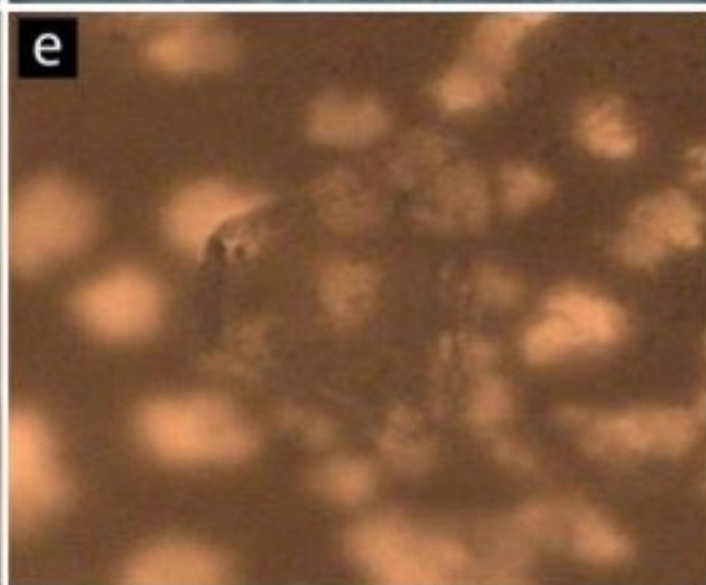
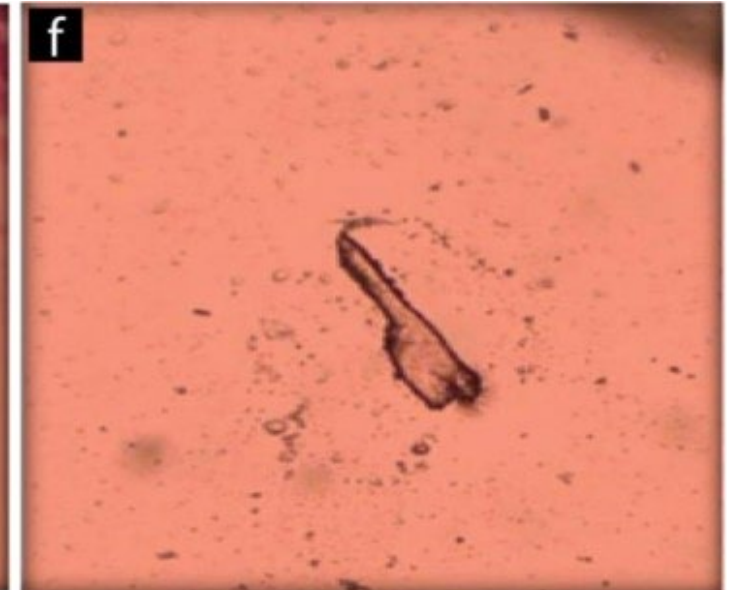
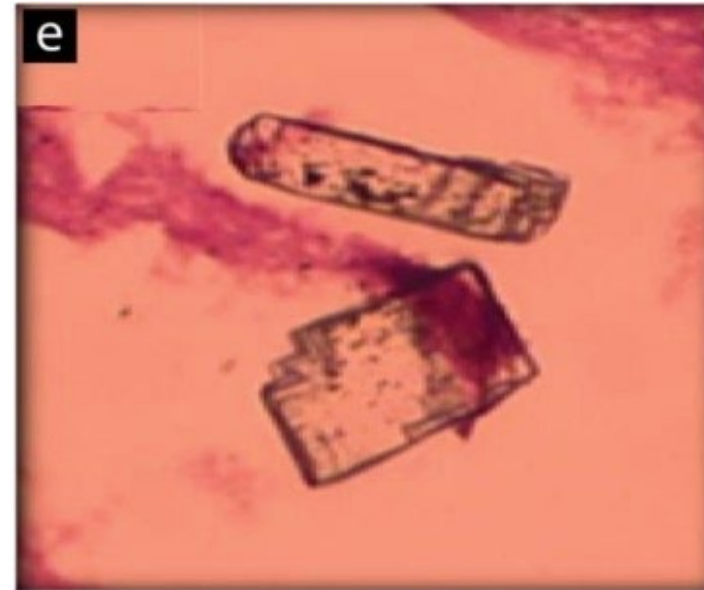
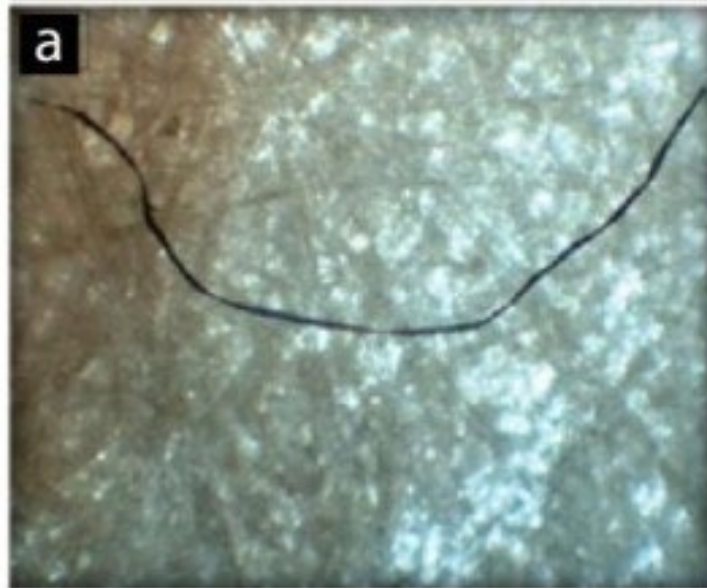


The effect of microplastics on selected organs (liver, gills, gut and muscles) was studied through histological analysis

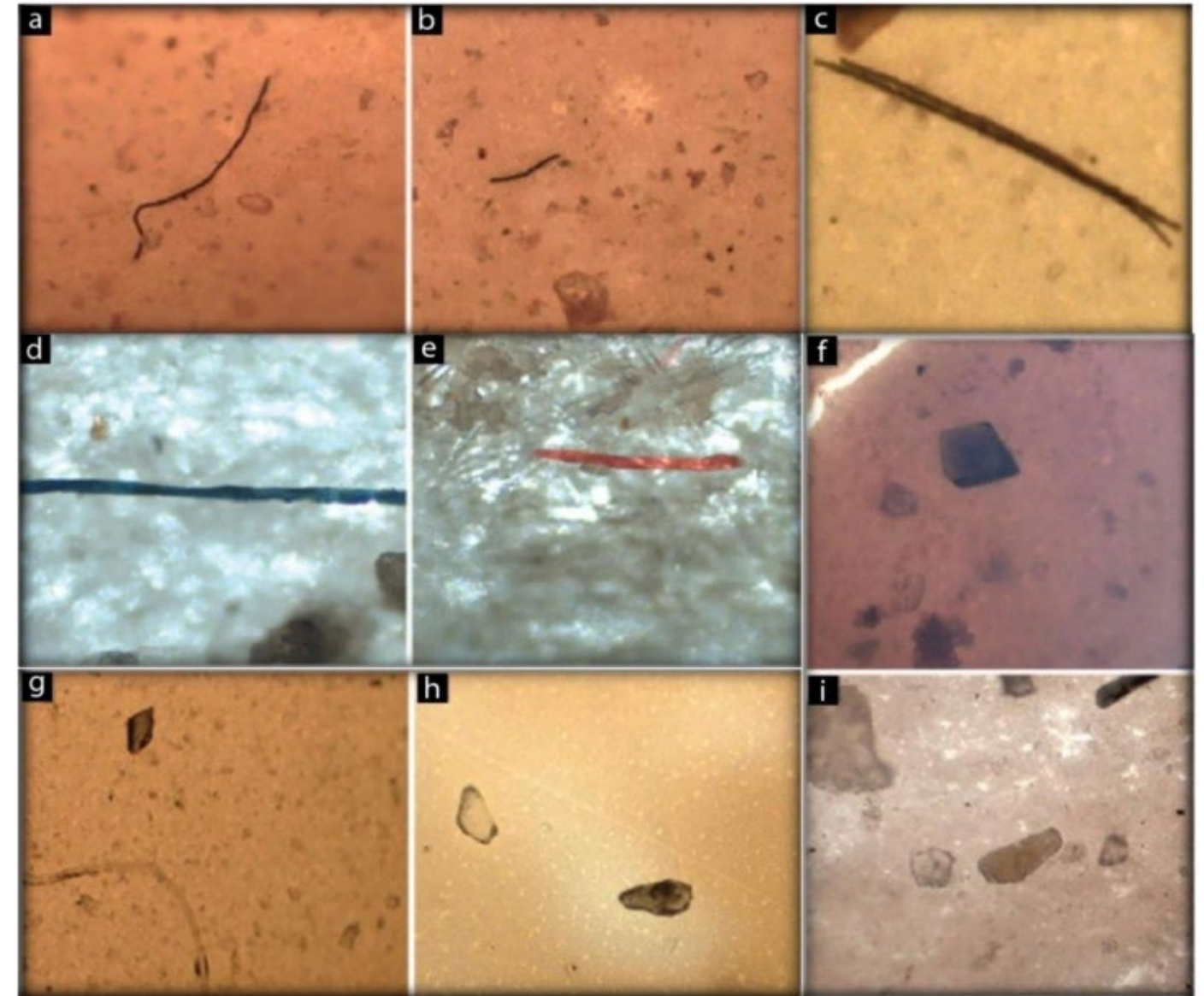
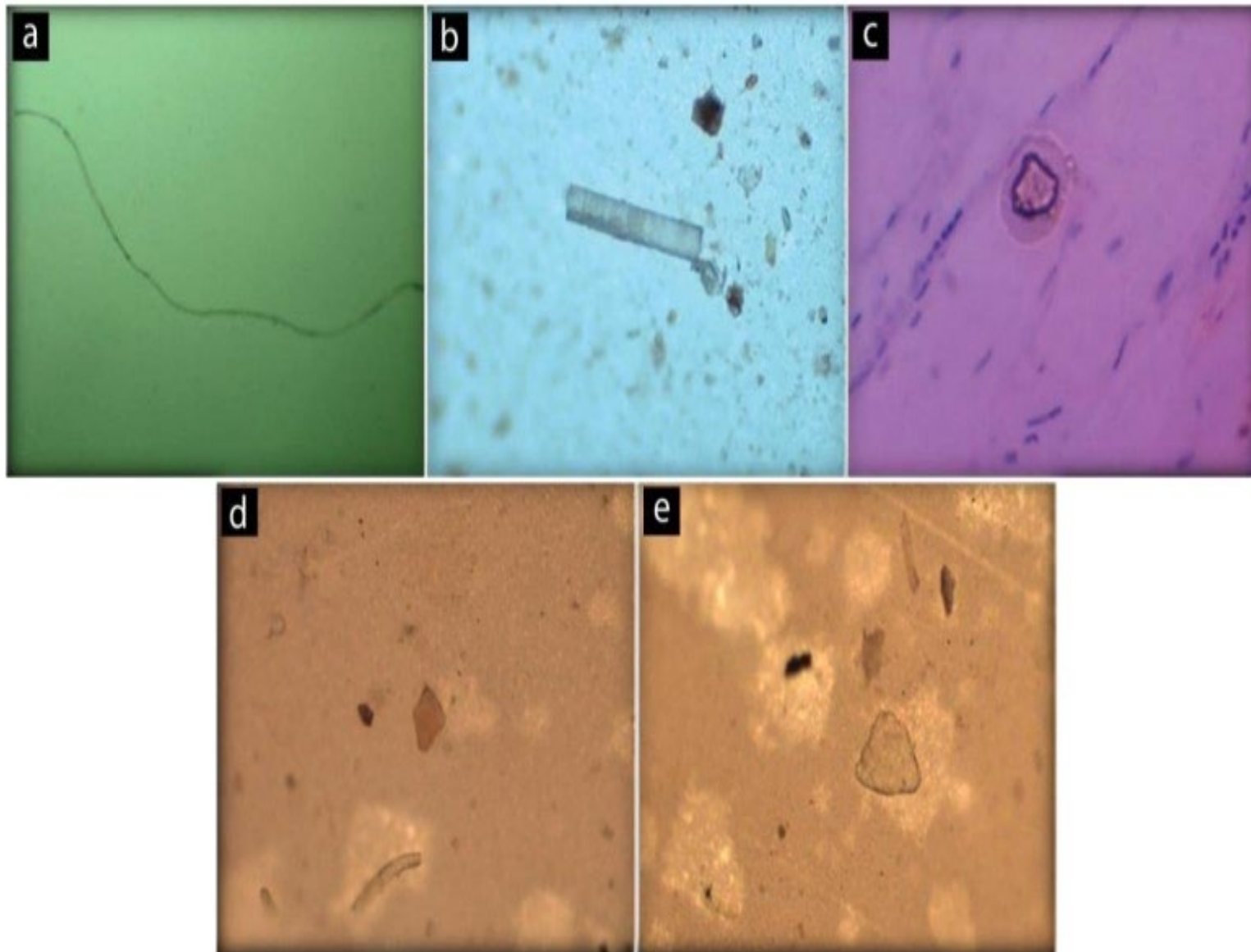
Morphotypes of MPs in water samples from River Ravi & Chenab



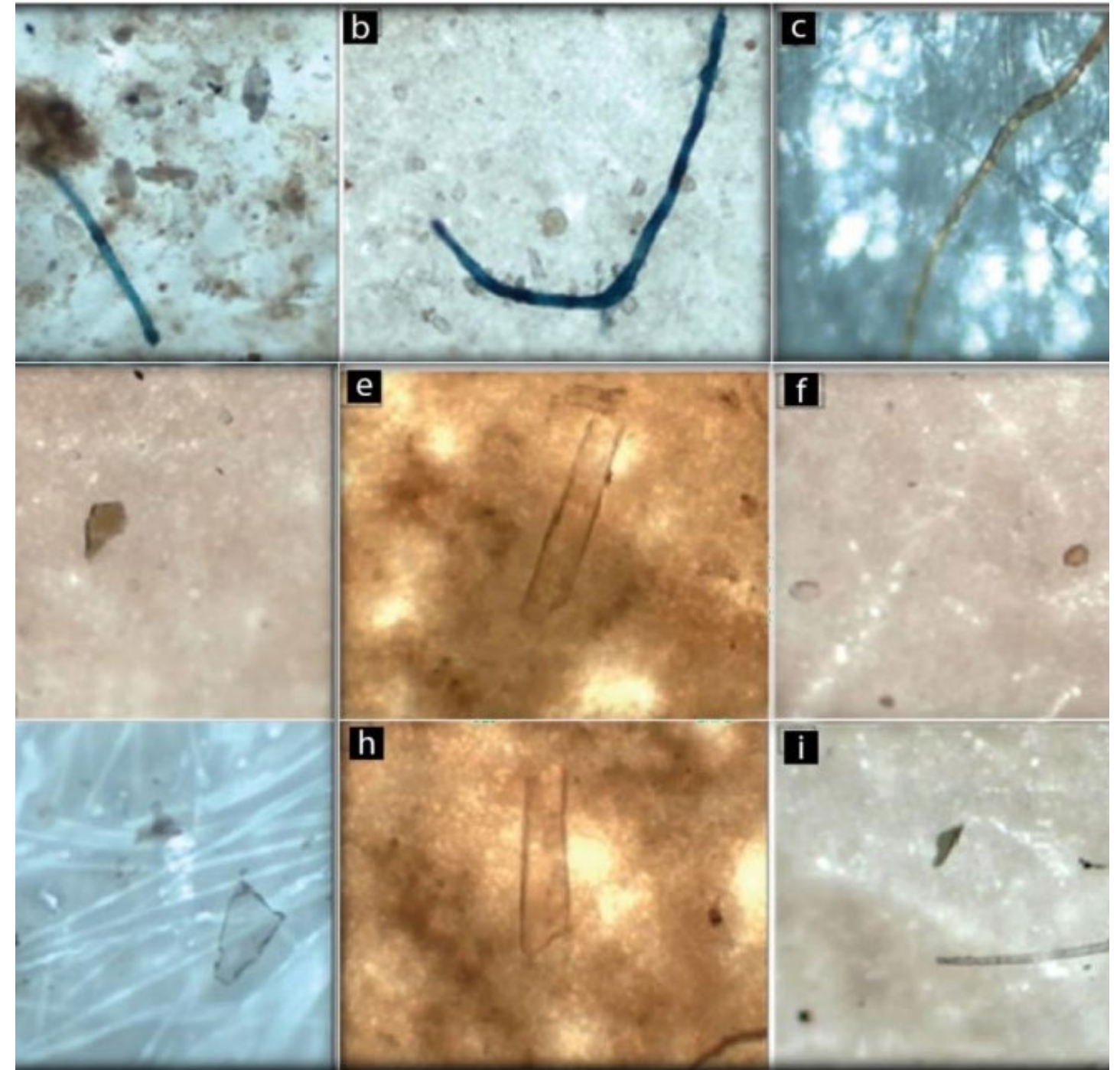
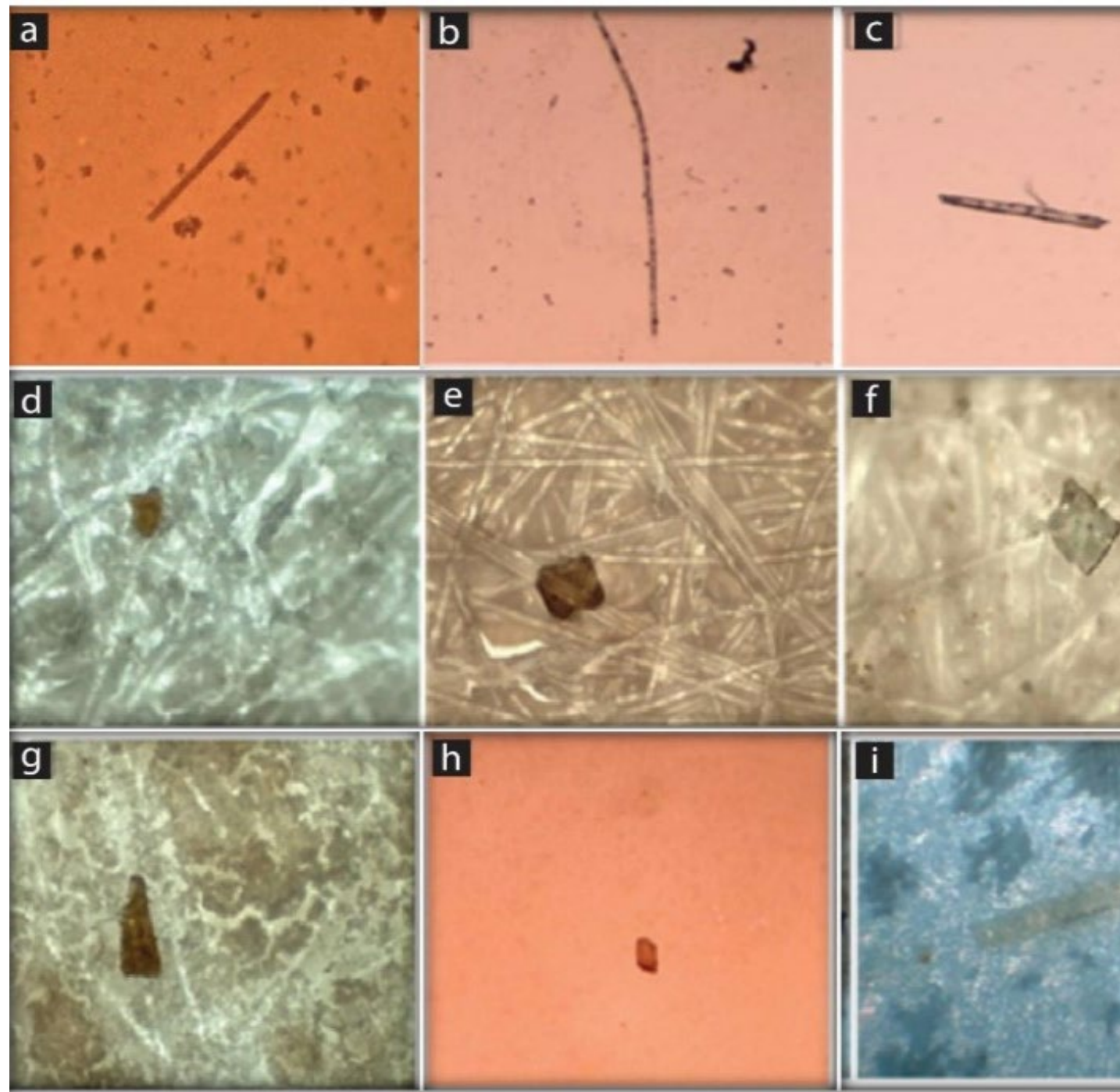
Morphotypes of MPs in Sediment samples from River Ravi & Chenab



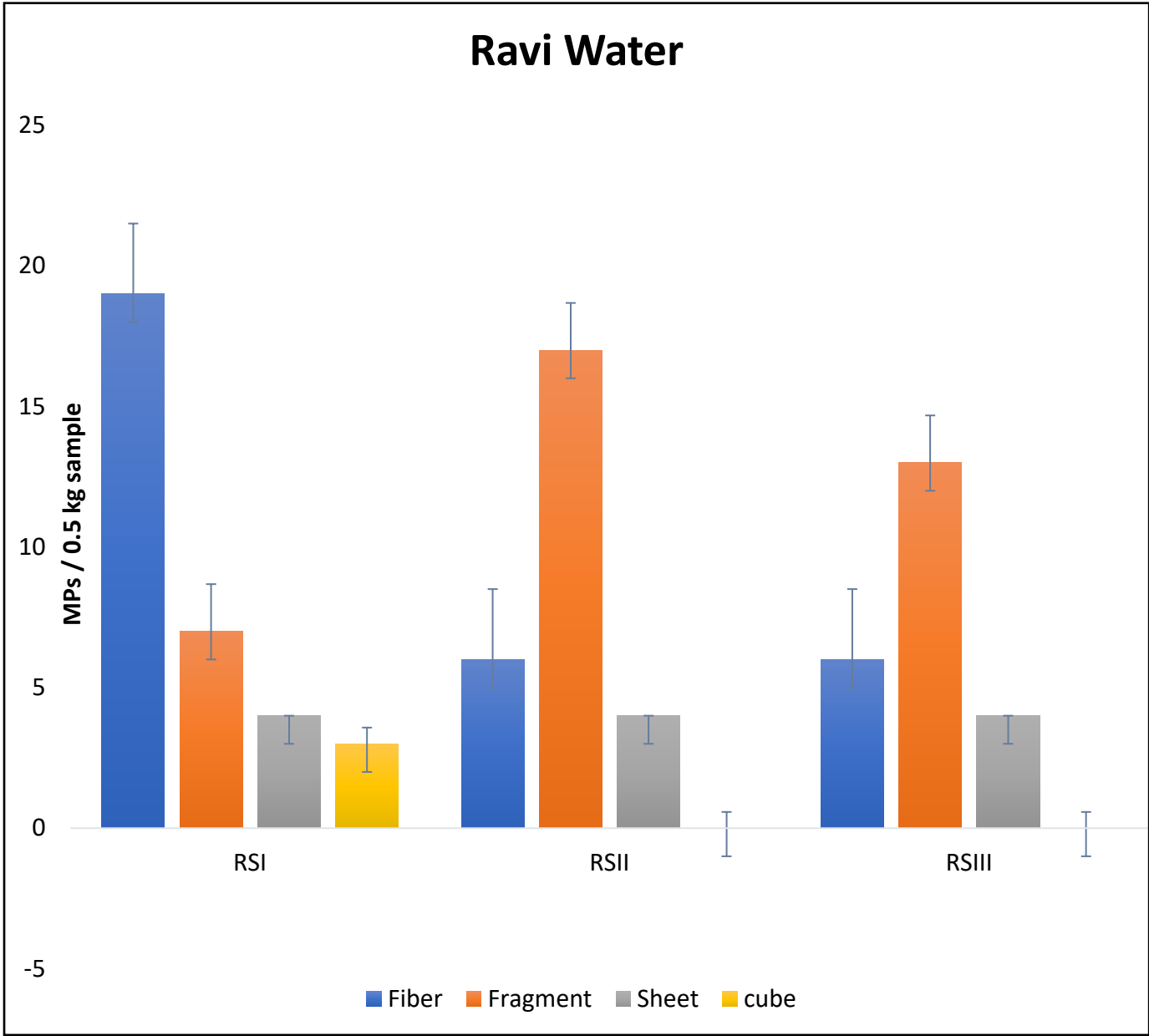
Morphotypes of MPs in Sediment samples from River Jhelum & Indus



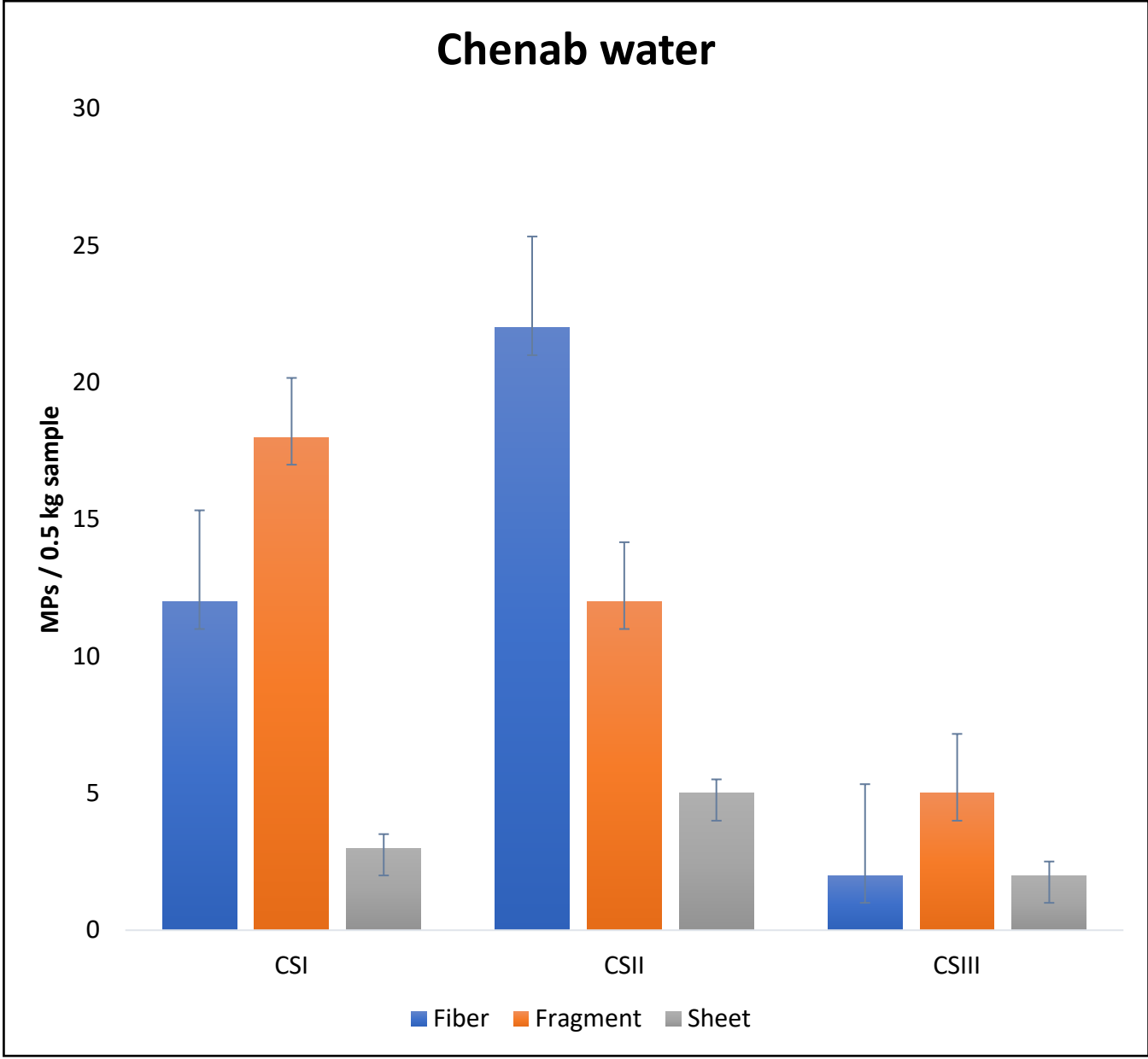
Morphotypes of Microplastics in different tissues of fish species from selected rivers



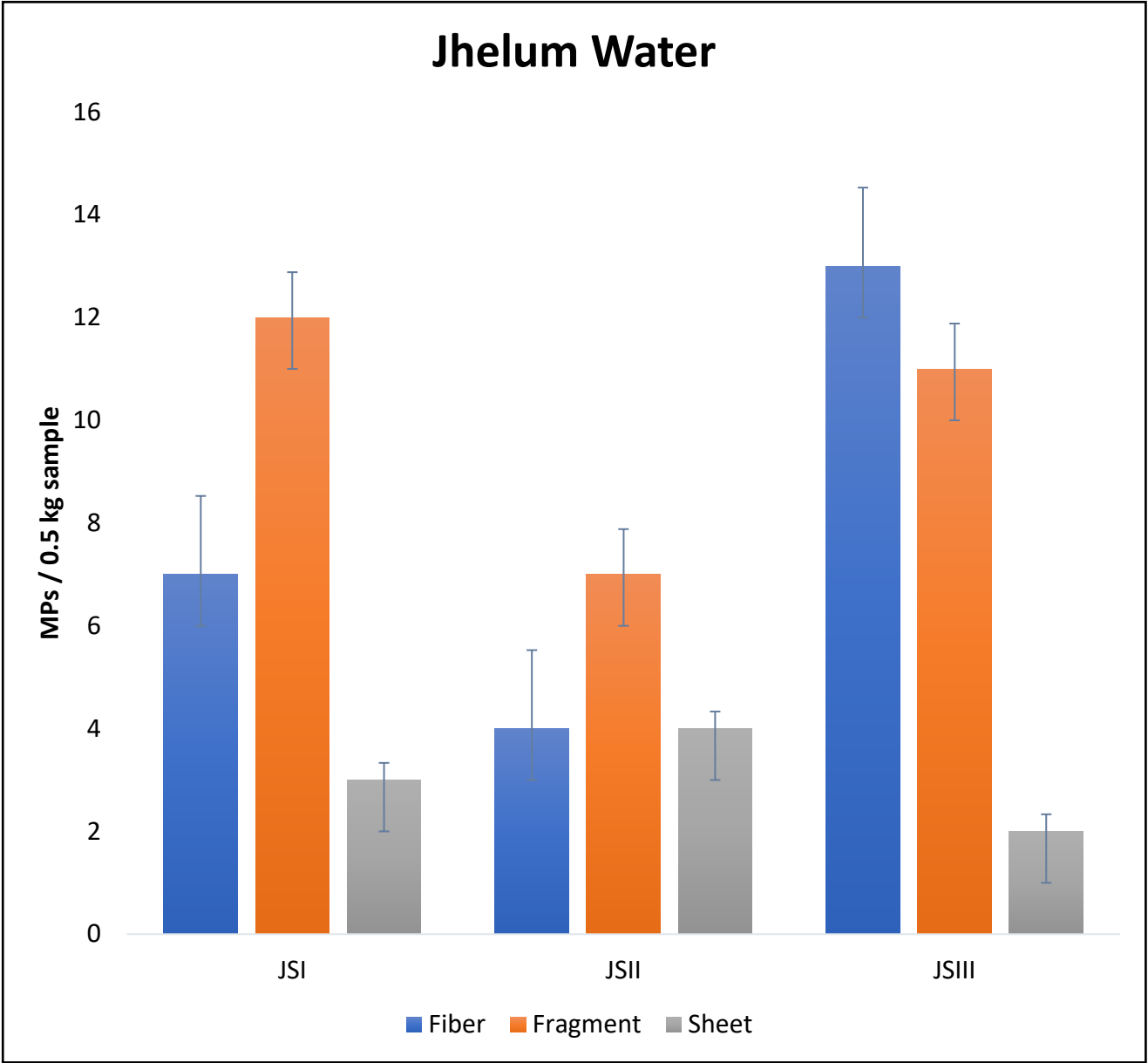
Fiber dominance at RSI (Nano Dogar) and **fragments abundance at RSII** (Jhamra) **and RSIII** (Mari patan), respectively.



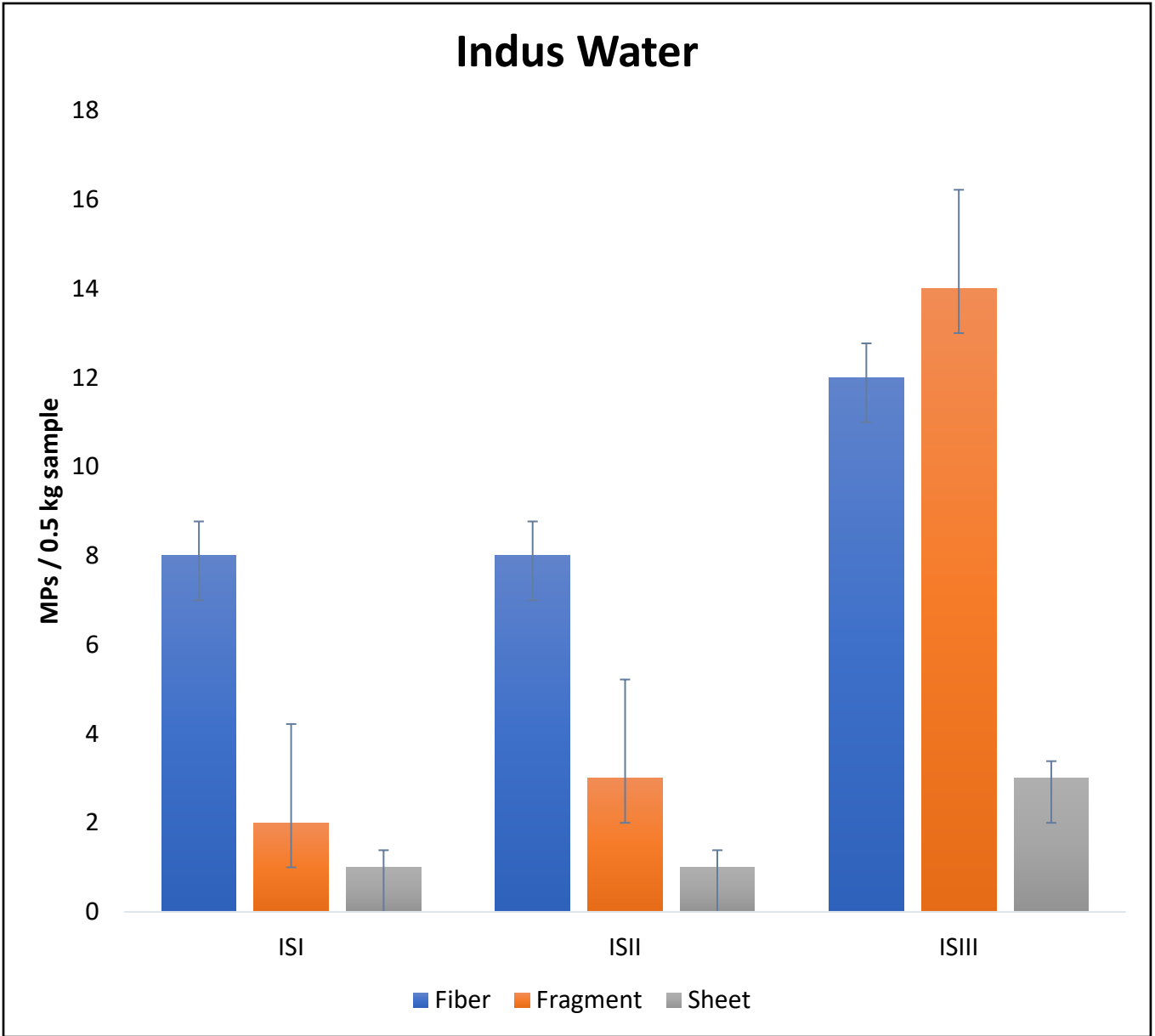
Fiber dominance at CSII (Chiniot) followed by **fragments** and **sheets** while fragments dominance at **RSI** (Chun pul) & **RSII** (walu shah)



MPs distribution in water samples of river Jhelum showing **fragments abundance at JSI (Patan machyana) and JSII (Sakhira)** while **fibers are dominant at JSIII (Sawa Nankana)**

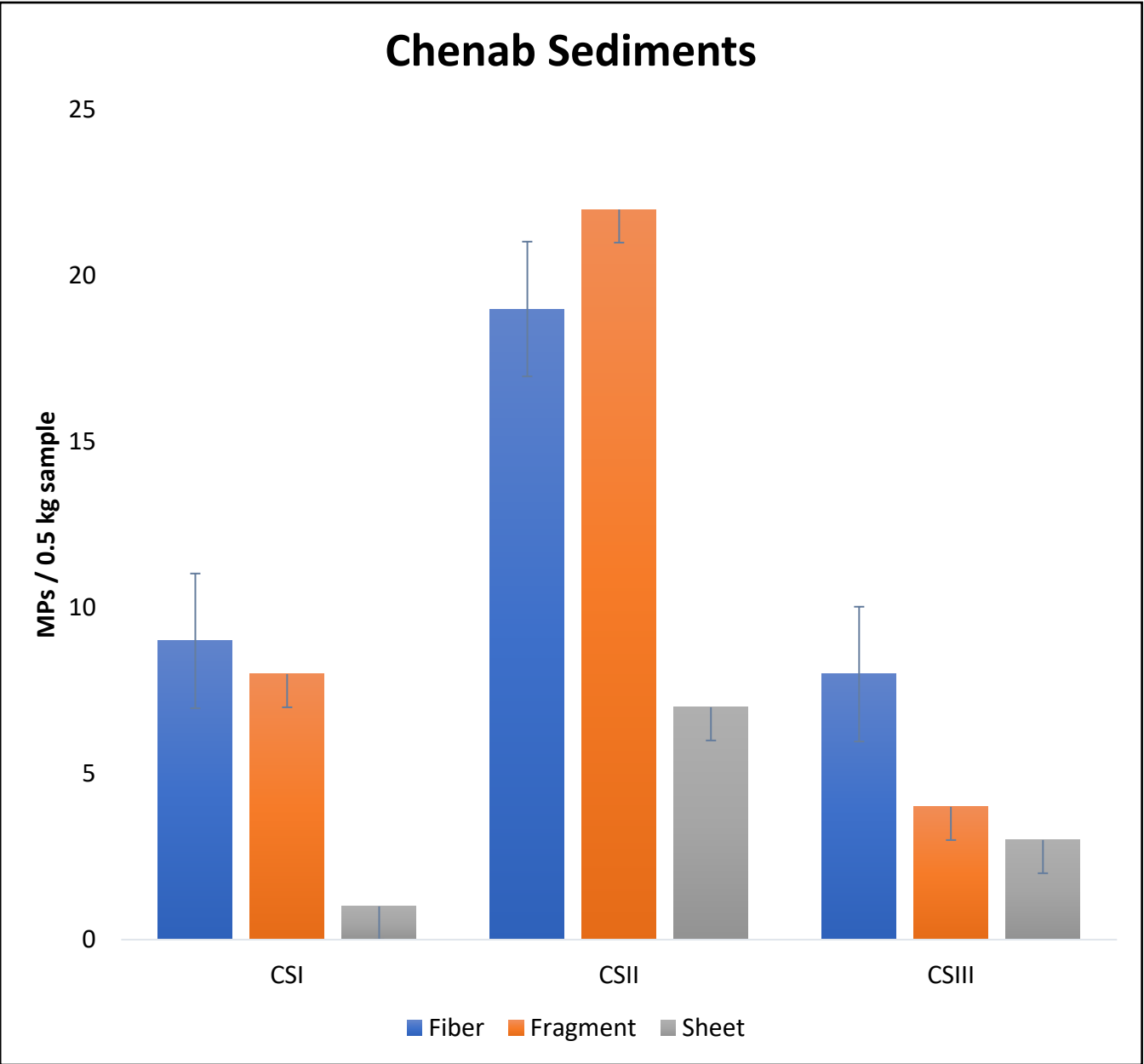
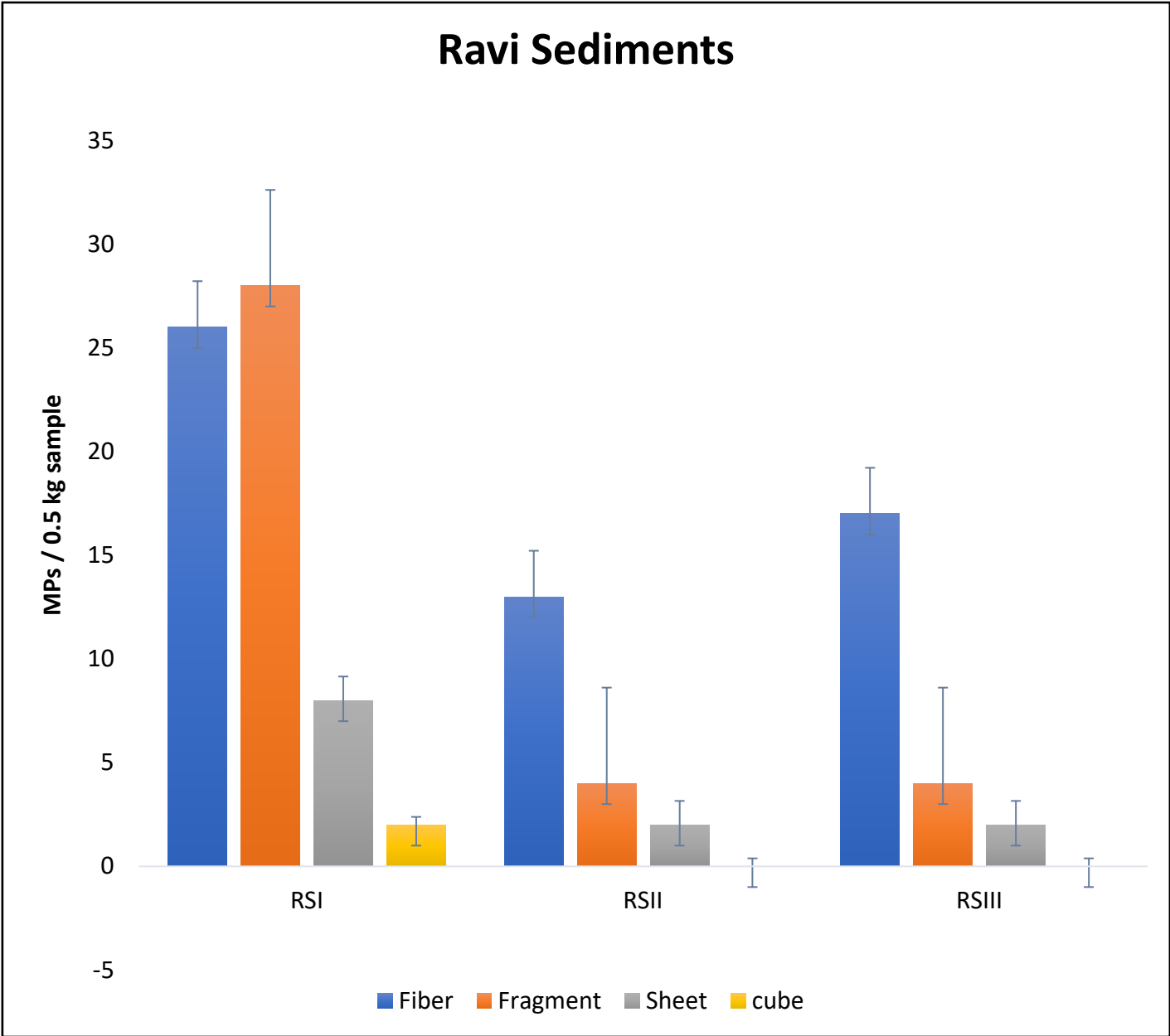


Fiber dominance at ISI (Gazi Ghat) and ISII (Piplan) while **fragments dominance at site 3 ISIII (Alu wali)**

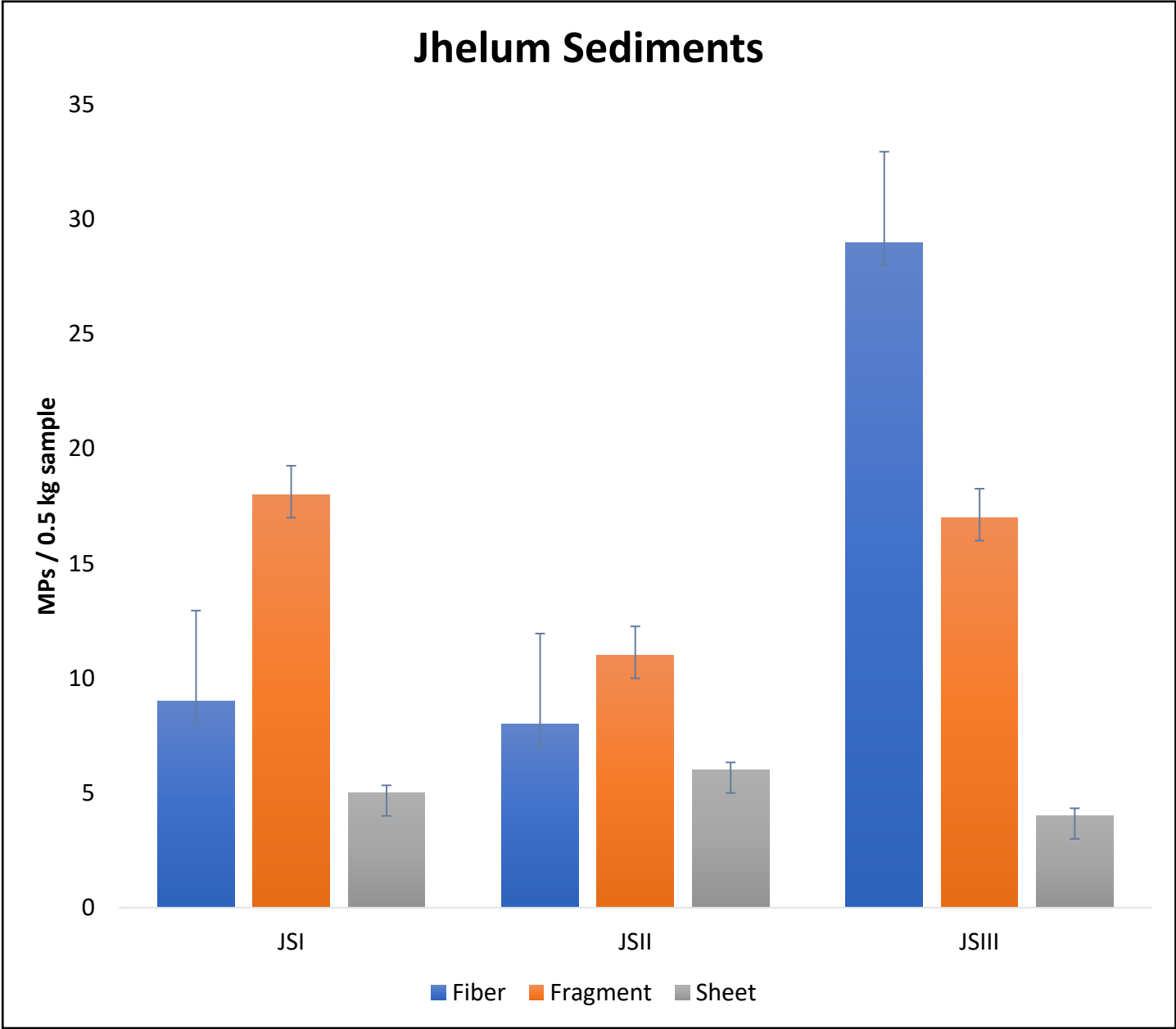


Cube plastic only at RSI as in water samples of RSI. Sheet being least abundant at all selected sites

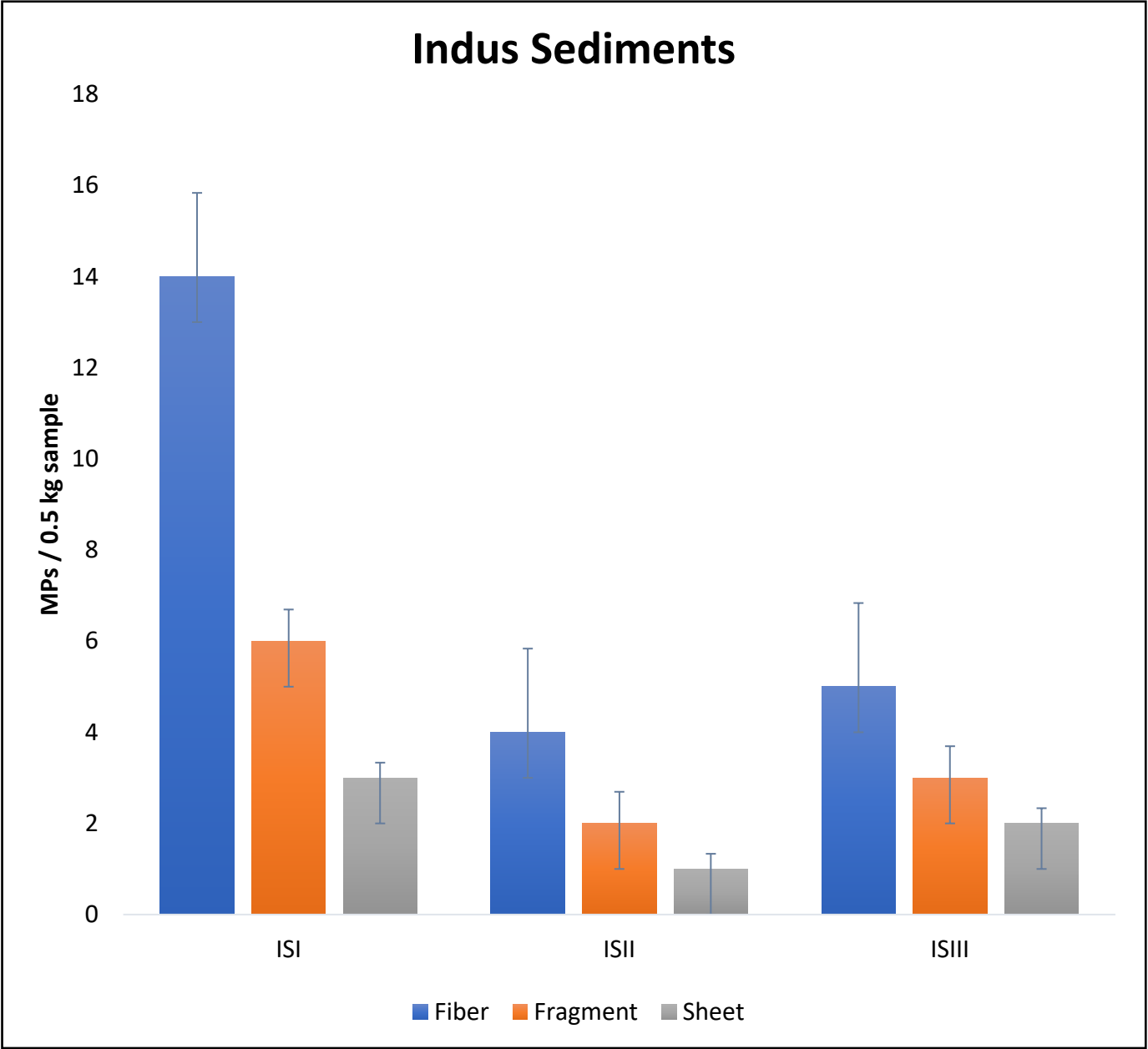
Fragment abundance at RSI (Nano Dogar) at river Ravi and CSII (Chund pul) at river Chenab

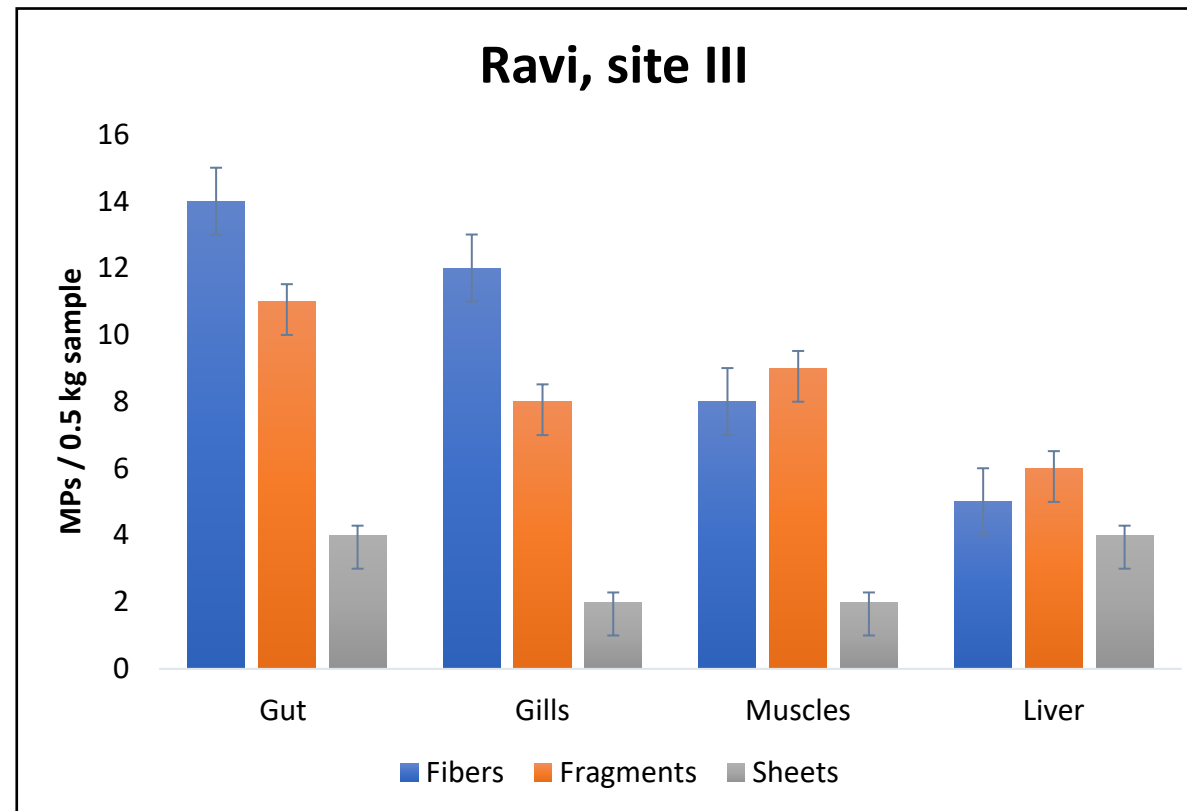
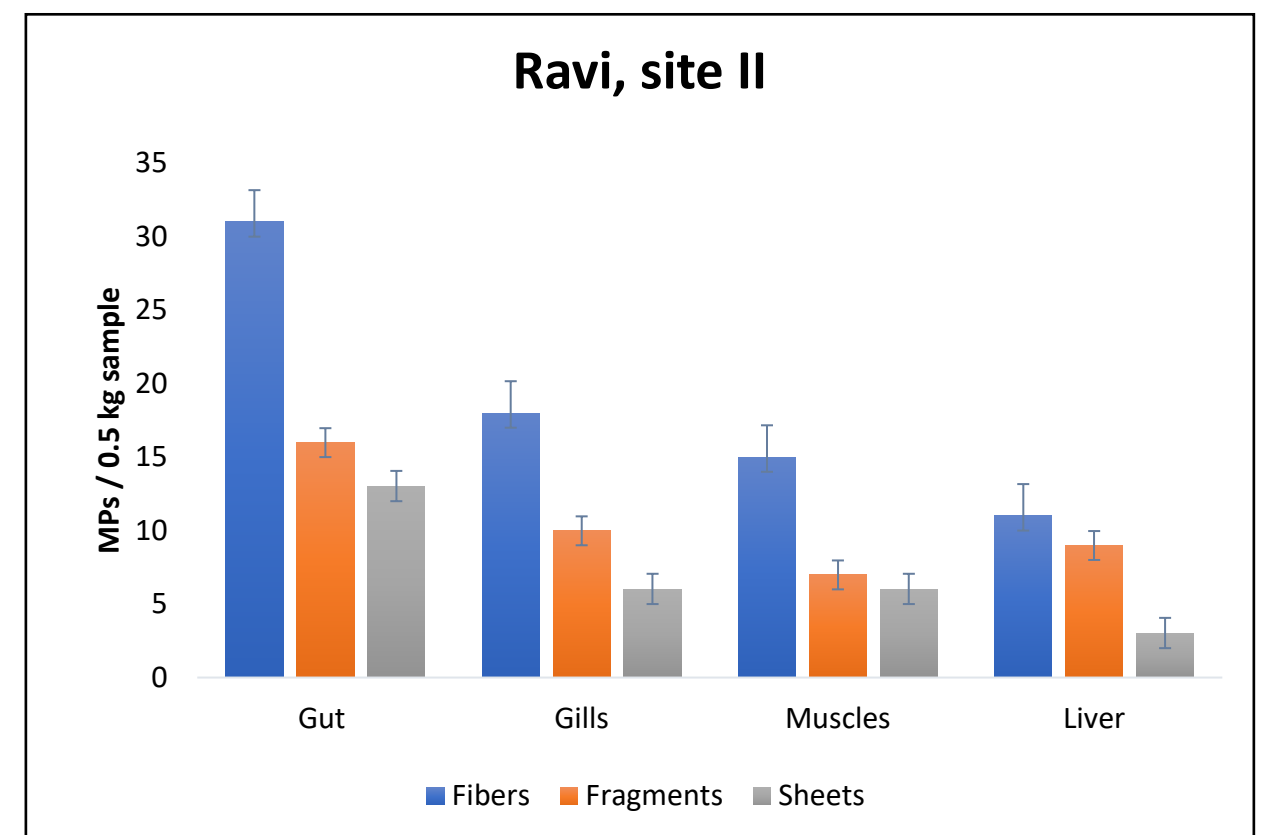
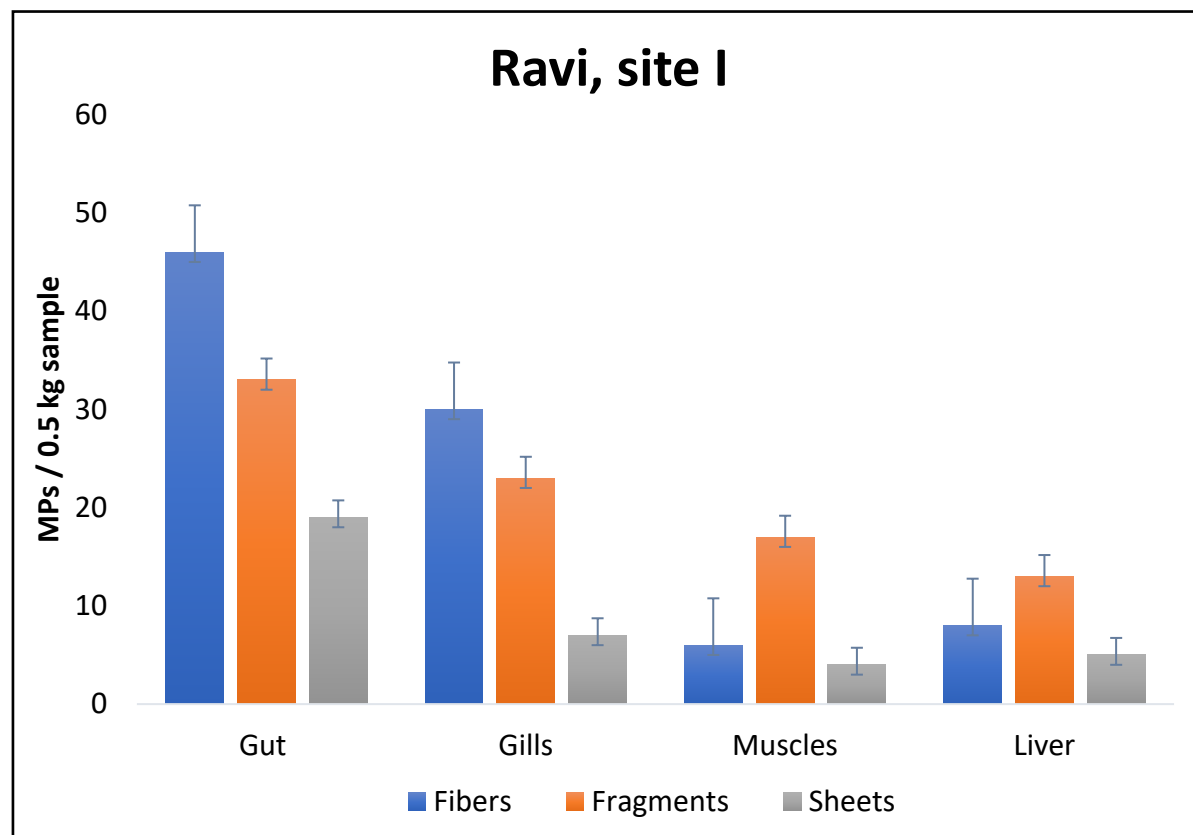


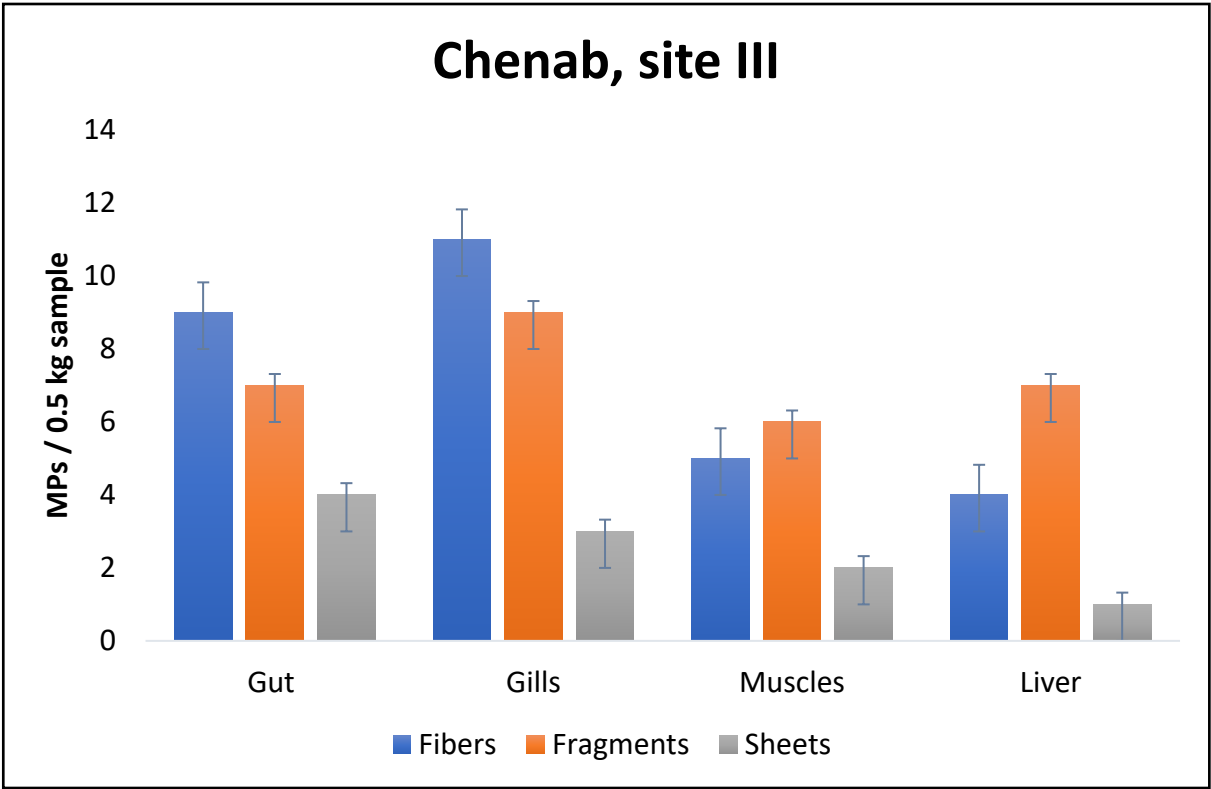
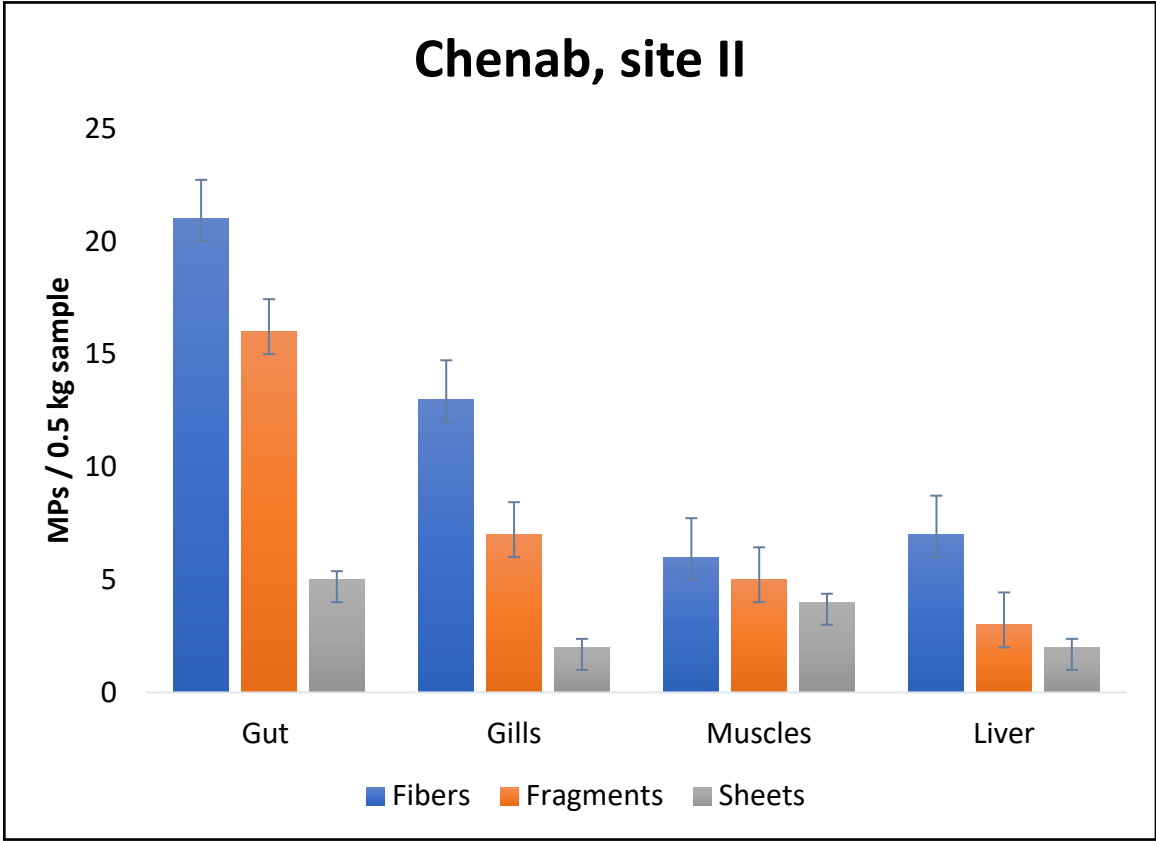
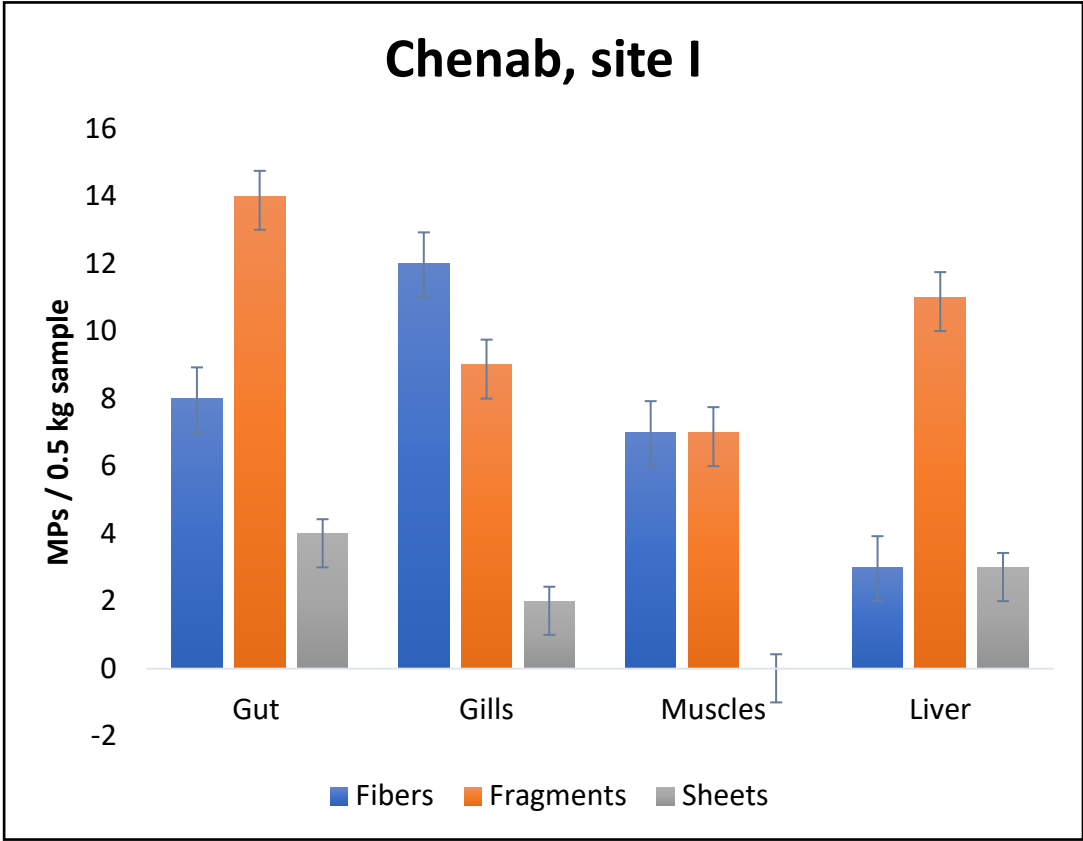
**Fragment abundance at JSI (Patan machyana) and
Fiber at JSIII (Sawa Nankana) at river Jhelum and**

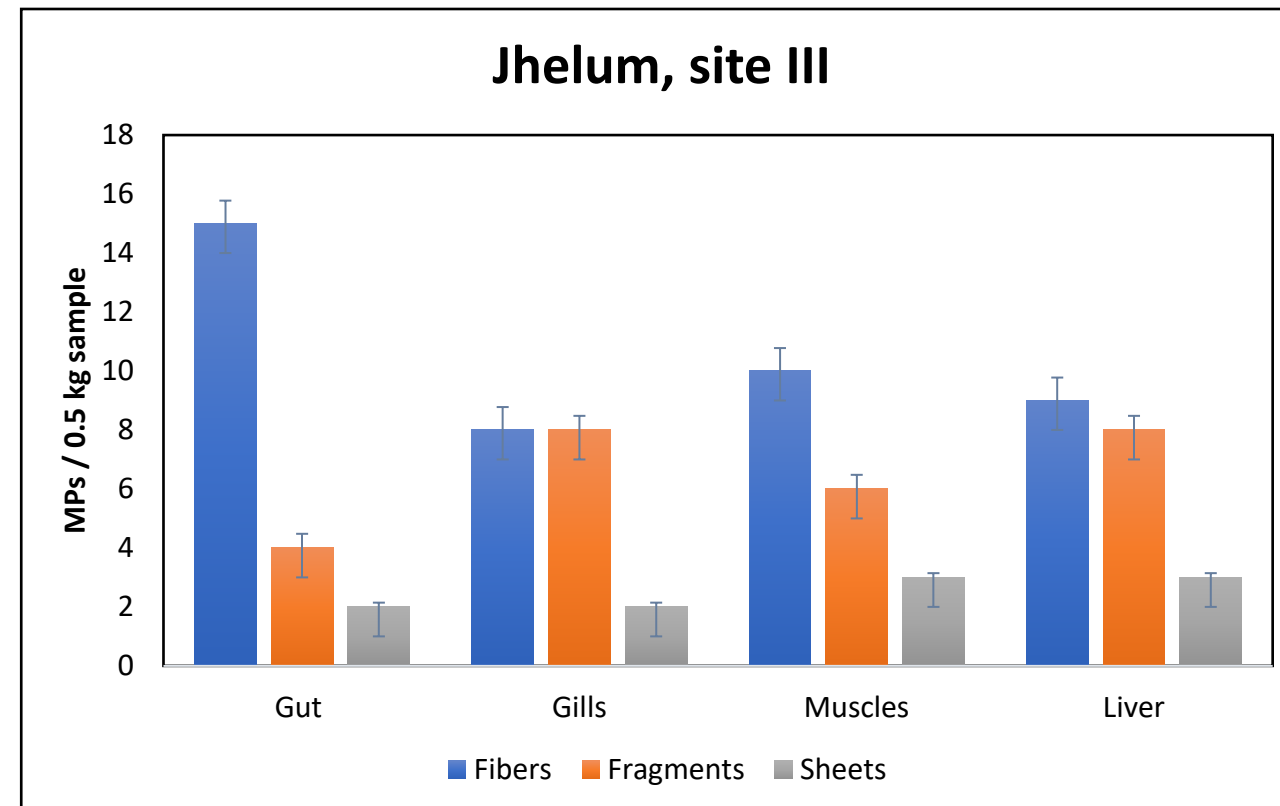
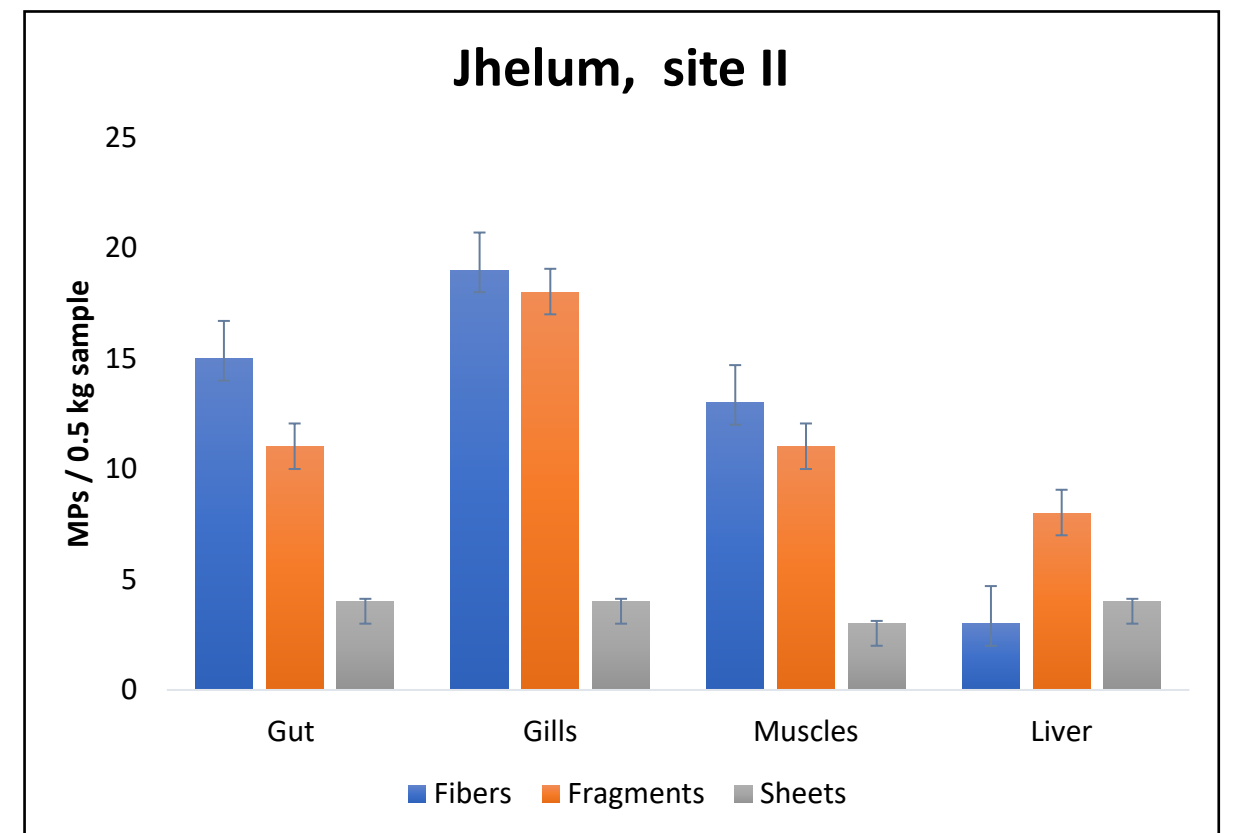
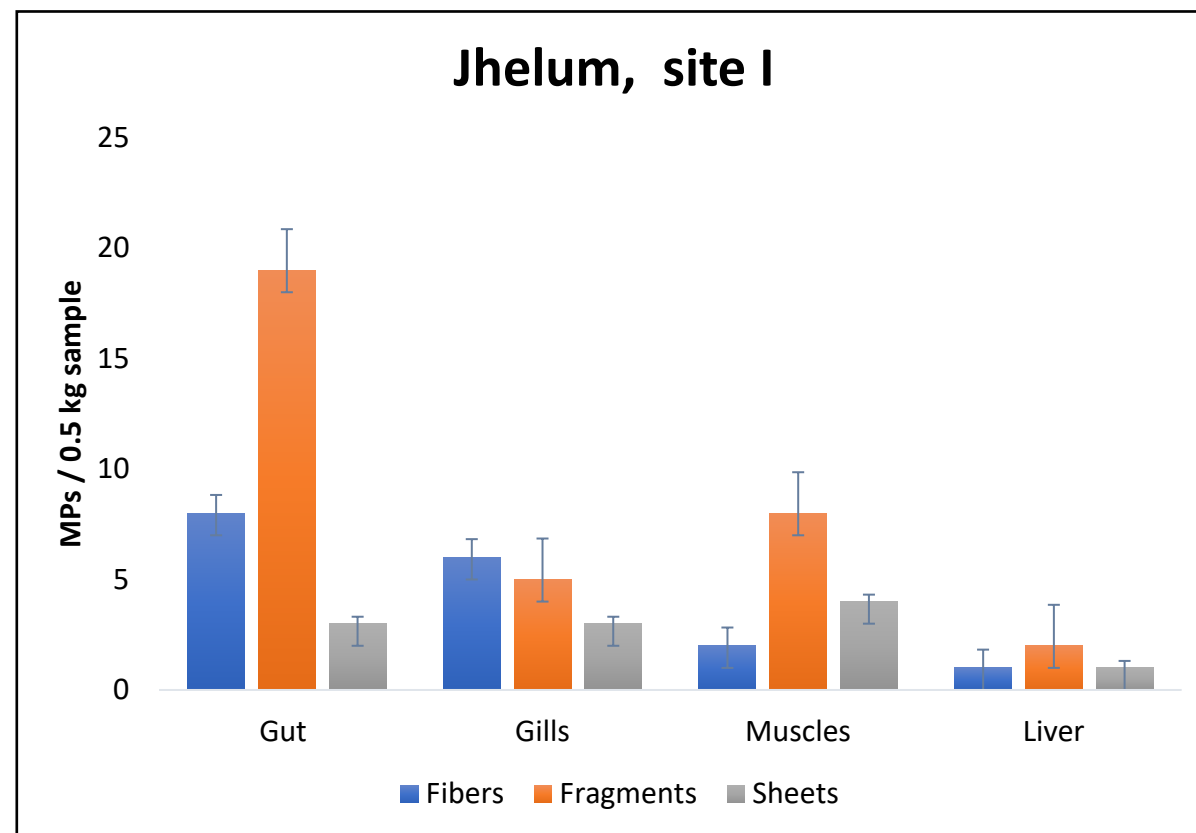


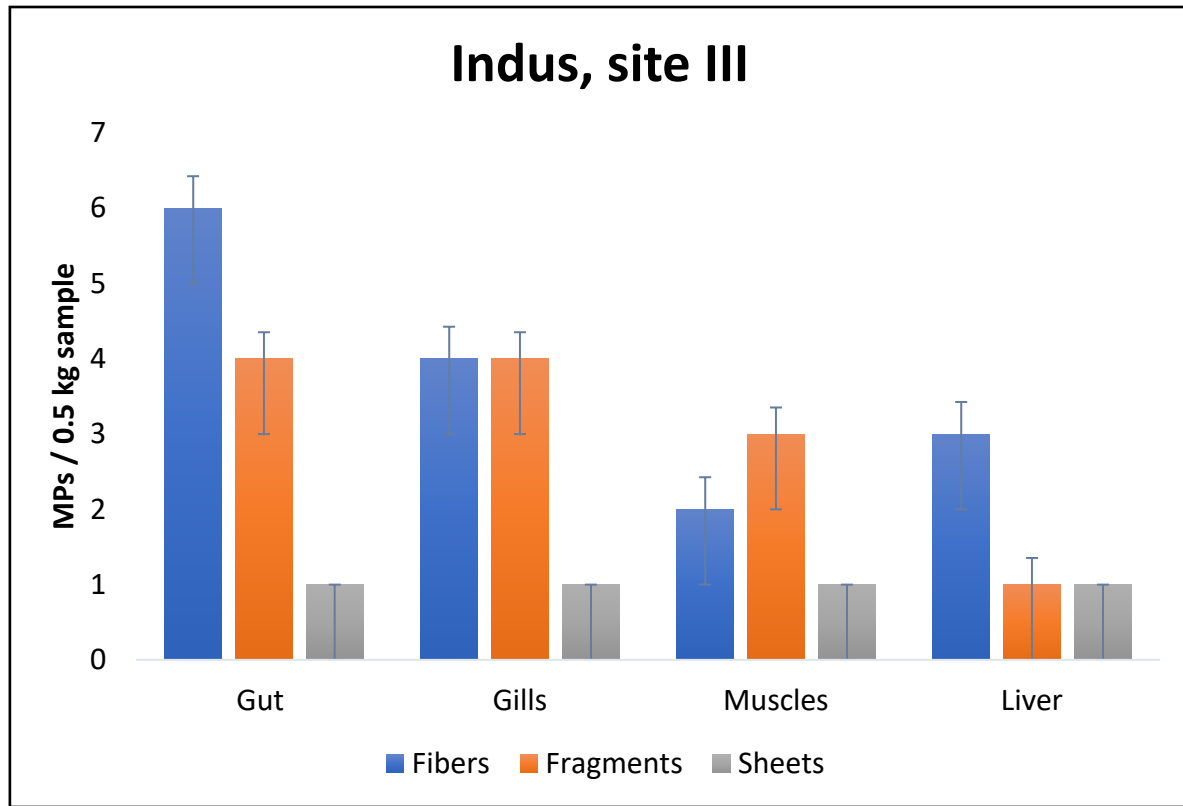
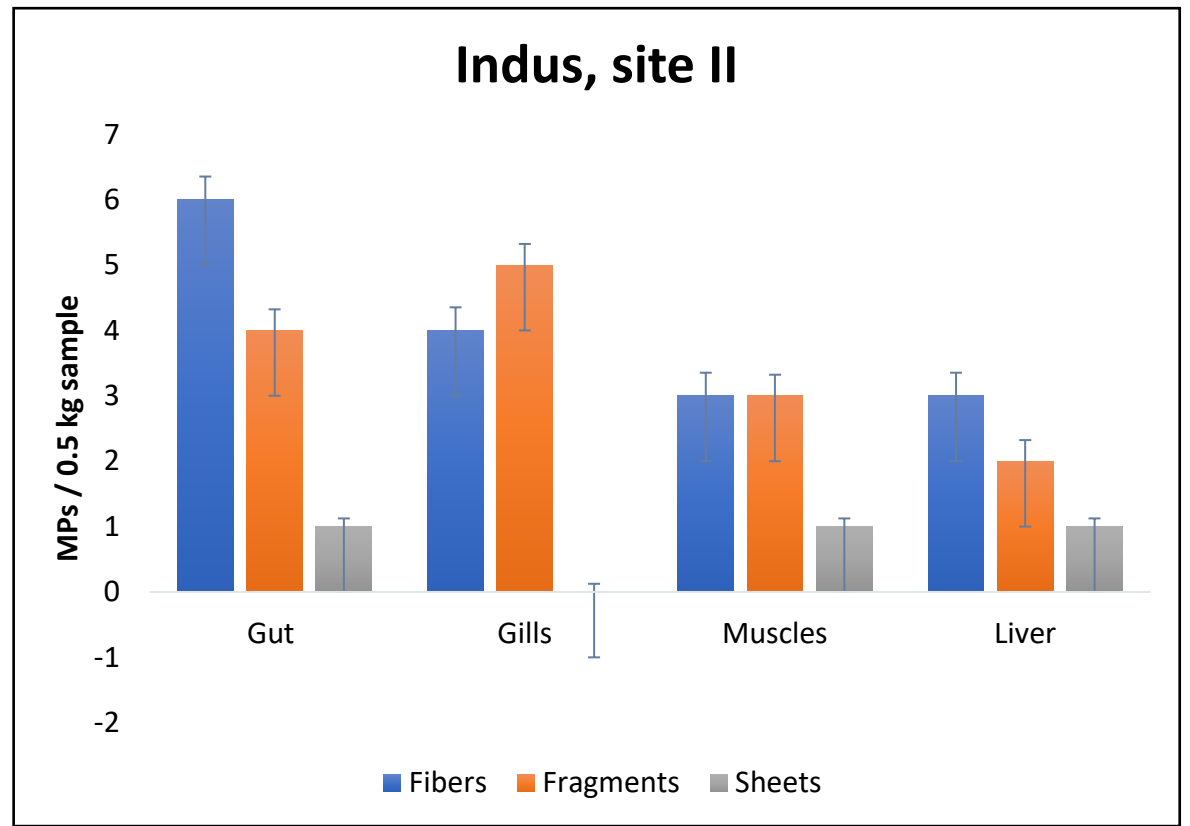
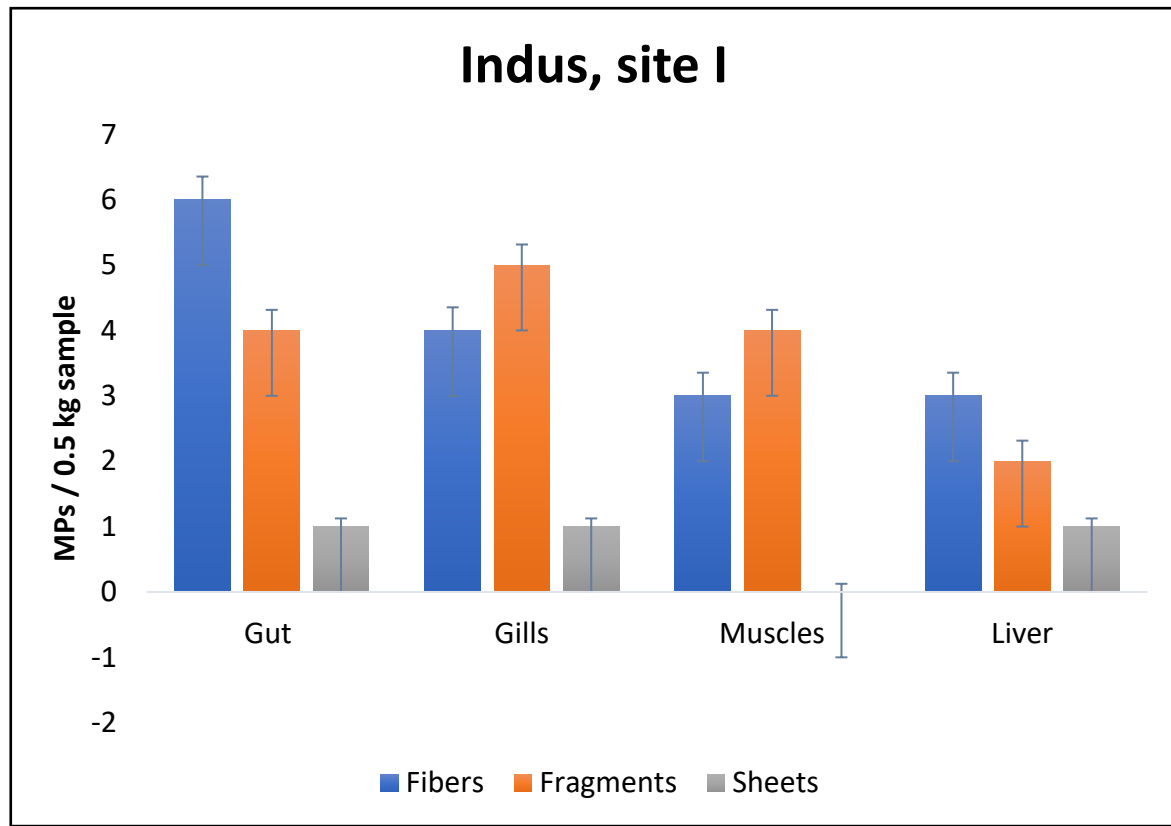
**Fiber abundance at all selected sites of river Indus.
Sheet being least abundant at all selected sites**





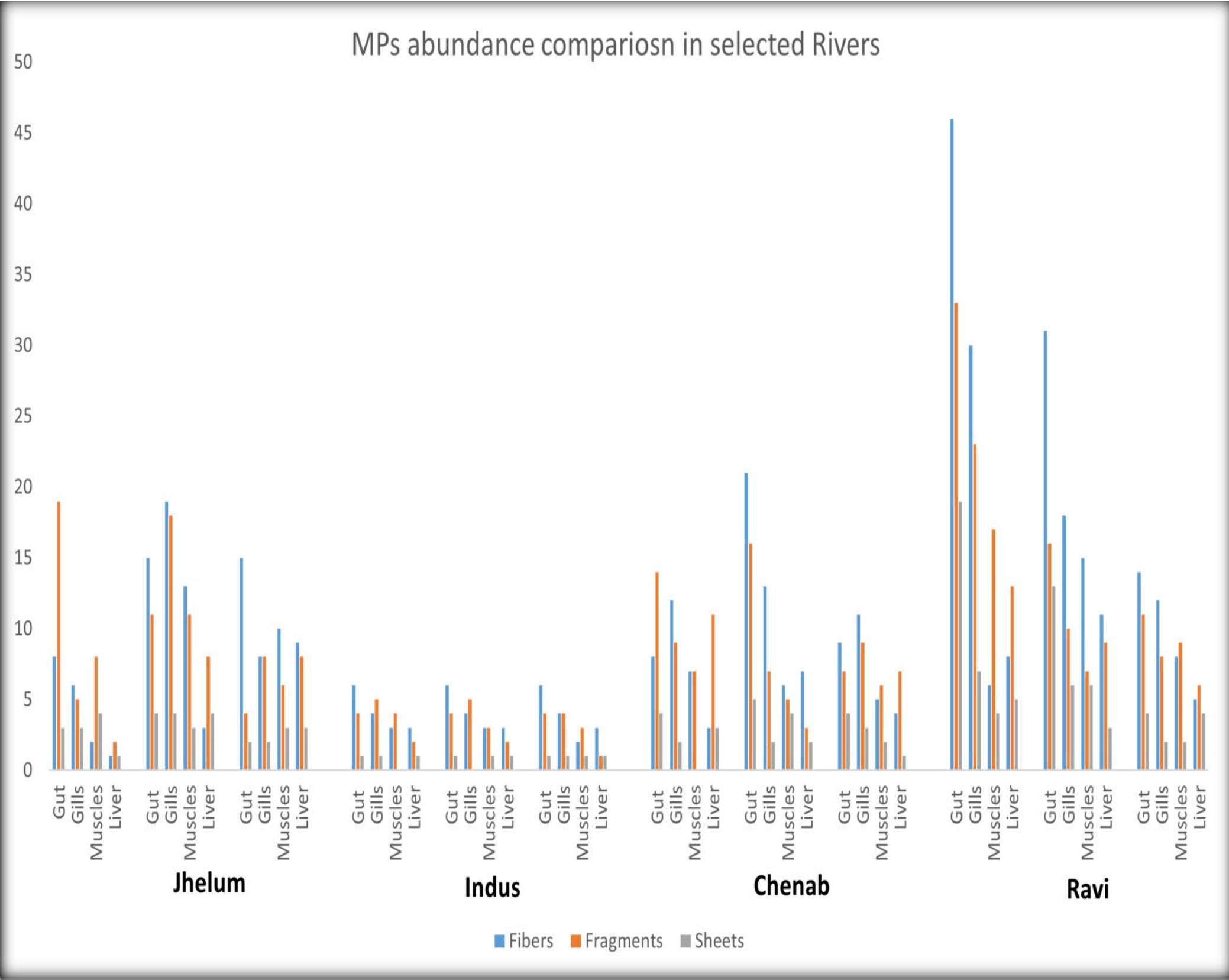






Microplastic abundance comparison in fish tissues from all selected rivers denoted

The highest load was observed **in the gut** tissues and among selected rivers **Ravi** showed more microplastics. In contrast, the **river Indus** showed **the least burden** of microplastics.



Mean ($\pm$ SE) values of microplastics (particles/0.5L) among different water samples and river sites

River	Site I	Site II	Site III
Ravi	11.50 $\pm$ 0.68 ^{Aa}	9.00 $\pm$ 0.74 ^{Ba}	7.67 $\pm$ 0.54 ^{Bb}
Chenab	11.00 $\pm$ 0.91 ^{Aa}	13.00 $\pm$ 0.98 ^{Aa}	3.00 $\pm$ 0.04 ^{Cc}
Indus	3.67 $\pm$ 0.08 ^{Cb}	4.00 $\pm$ 0.18 ^{Cb}	9.67 $\pm$ 0.85 ^{Aa}
Jhelum	7.33 $\pm$ 0.40 ^{Bb}	5.00 $\pm$ 0.31 ^{Cb}	8.67 $\pm$ 0.61 ^{Aa}

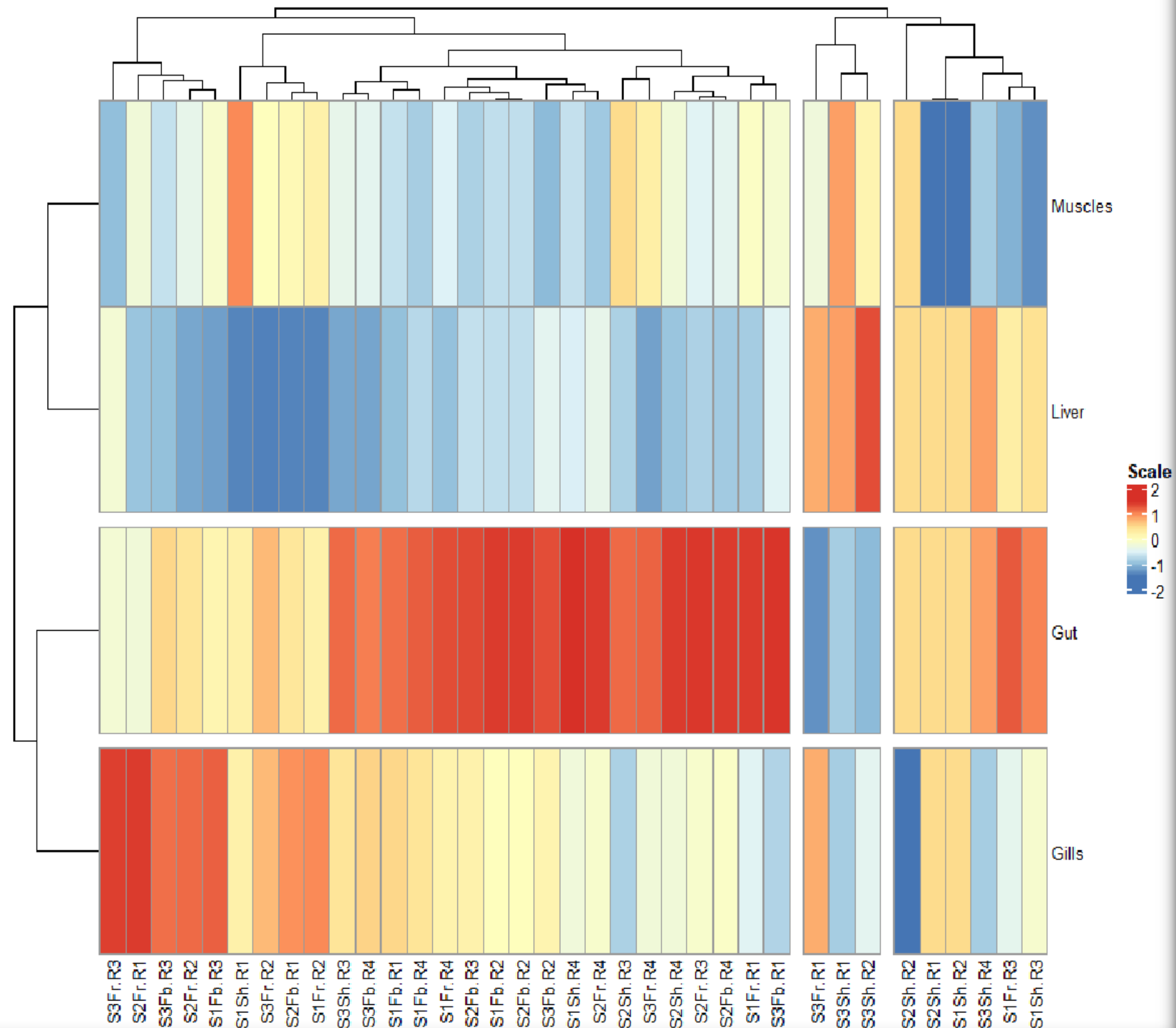
Mean ($\pm$ SE) values of microplastics (particles/0.5kg) among different sediment samples and river sites

River	Site I	Site II	Site III
Ravi	20.67 $\pm$ 1.48 ^{Aa}	6.33 $\pm$ 0.38 ^{Bb}	7.67 $\pm$ 0.30 ^{Bb}
Chenab	6.00 $\pm$ 0.11 ^{Cc}	16.00 $\pm$ 1.48 ^{Aa}	5.00 $\pm$ 0.53 ^{Bb}
Indus	7.67 $\pm$ 0.34 ^{Ca}	2.33 $\pm$ 0.02 ^{Cc}	3.33 $\pm$ 0.04 ^{Cc}
Jhelum	10.68 $\pm$ 0.84 ^{Ba}	8.33 $\pm$ 0.45 ^{Bb}	10.67 $\pm$ 0.97 ^{Aa}

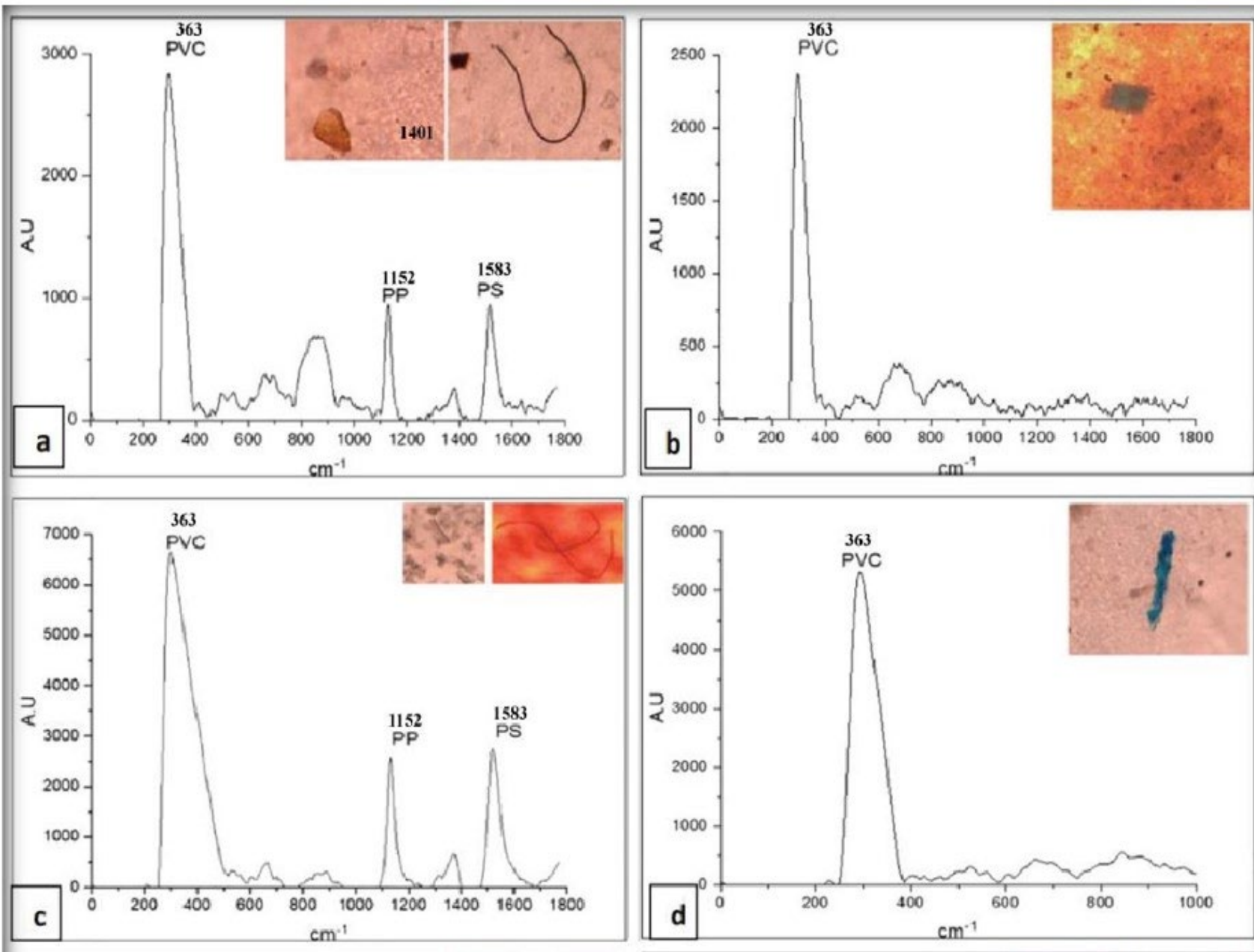
Mean ($\pm$ SE) values of microplastics among different tissues of fish sampled from different sites of selected rivers.

River	Site I	Site II	Site III
Ravi			
Gut	32.67 $\pm$ 2.80 ^{Aa}	20.00 $\pm$ 1.57 ^{Ab}	9.67 $\pm$ 0.96 ^{Ac}
Gills	20.00 $\pm$ 1.57 ^{Ba}	11.33 $\pm$ 0.99 ^{Bb}	7.33 $\pm$ 0.81 ^{Bc}
Muscles	9.00 $\pm$ 0.67 ^{Ca}	9.33 $\pm$ 0.69 ^{Ca}	6.33 $\pm$ 0.59 ^{Bb}
Liver	9.67 $\pm$ 0.73 ^{Ca}	7.67 $\pm$ 0.71 ^{Cb}	5.00 $\pm$ 0.58 ^{Cc}
Chenab			
Gut	8.67 $\pm$ 0.73 ^{Ab}	14.00 $\pm$ 1.01 ^{Aa}	6.67 $\pm$ 0.65 ^{Bc}
Gills	7.67 $\pm$ 0.71 ^{Ba}	7.34 $\pm$ 0.68 ^{Ba}	7.67 $\pm$ 0.71 ^{Aa}
Muscles	4.67 $\pm$ 0.34 ^{Ca}	5.00 $\pm$ 0.58 ^{Ca}	4.34 $\pm$ 0.20 ^{Ca}
Liver	5.67 $\pm$ 0.67 ^{Ba}	4.00 $\pm$ 0.09 ^{Cb}	4.00 $\pm$ 0.09 ^{Cb}
Indus			
Gut	3.67 $\pm$ 0.45 ^{Aa}	3.67 $\pm$ 0.45 ^{Aa}	3.67 $\pm$ 0.23 ^{Aa}
Gills	3.34 $\pm$ 0.20 ^{Aa}	3.00 $\pm$ 0.07 ^{Aa}	3.00 $\pm$ 0.07 ^{Aa}
Muscles	2.34 $\pm$ 0.20 ^{Ba}	2.33 $\pm$ 0.18 ^{Ba}	2.00 $\pm$ 0.11 ^{Ba}
Liver	2.00 $\pm$ 0.58 ^{Ba}	2.00 $\pm$ 0.58 ^{Ba}	1.67 $\pm$ 0.67 ^{Cb}
Jhelum			
Gut	10.00 $\pm$ 0.83 ^{Aa}	10.00 $\pm$ 0.83 ^{Ba}	7.00 $\pm$ 0.54 ^{Ab}
Gills	4.67 $\pm$ 0.89 ^{Bc}	13.67 $\pm$ 1.04 ^{Aa}	6.00 $\pm$ 0.40 ^{Ab}
Muscles	4.67 $\pm$ 0.76 ^{Bc}	9.00 $\pm$ 0.67 ^{Ba}	6.33 $\pm$ 0.59 ^{Ab}
Liver	1.33 $\pm$ 0.33 ^{Cc}	5.00 $\pm$ 0.53 ^{Ca}	6.67 $\pm$ 0.86 ^{Aa}

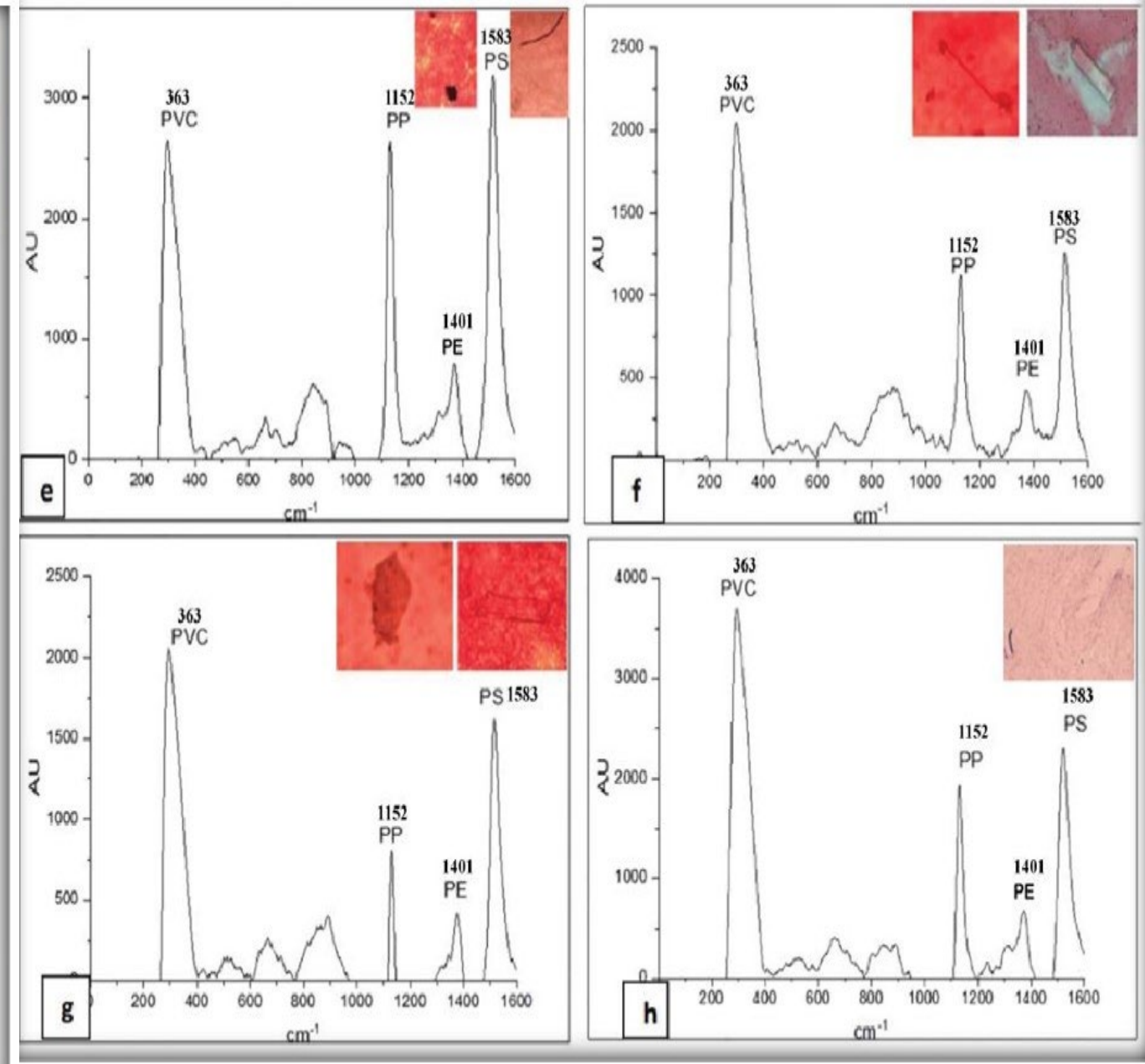
- **Fibers and fragments depicting a strong association with gut and gills.**
- Fb (fiber); Fr (fragment); Sh (sheet) at selected rivers [R1 (Ravi), R2 (Chenab), R3 (Indus), R4 (Jhelum)]; S1, S2, S3 (site I, site II, site III, respectively)

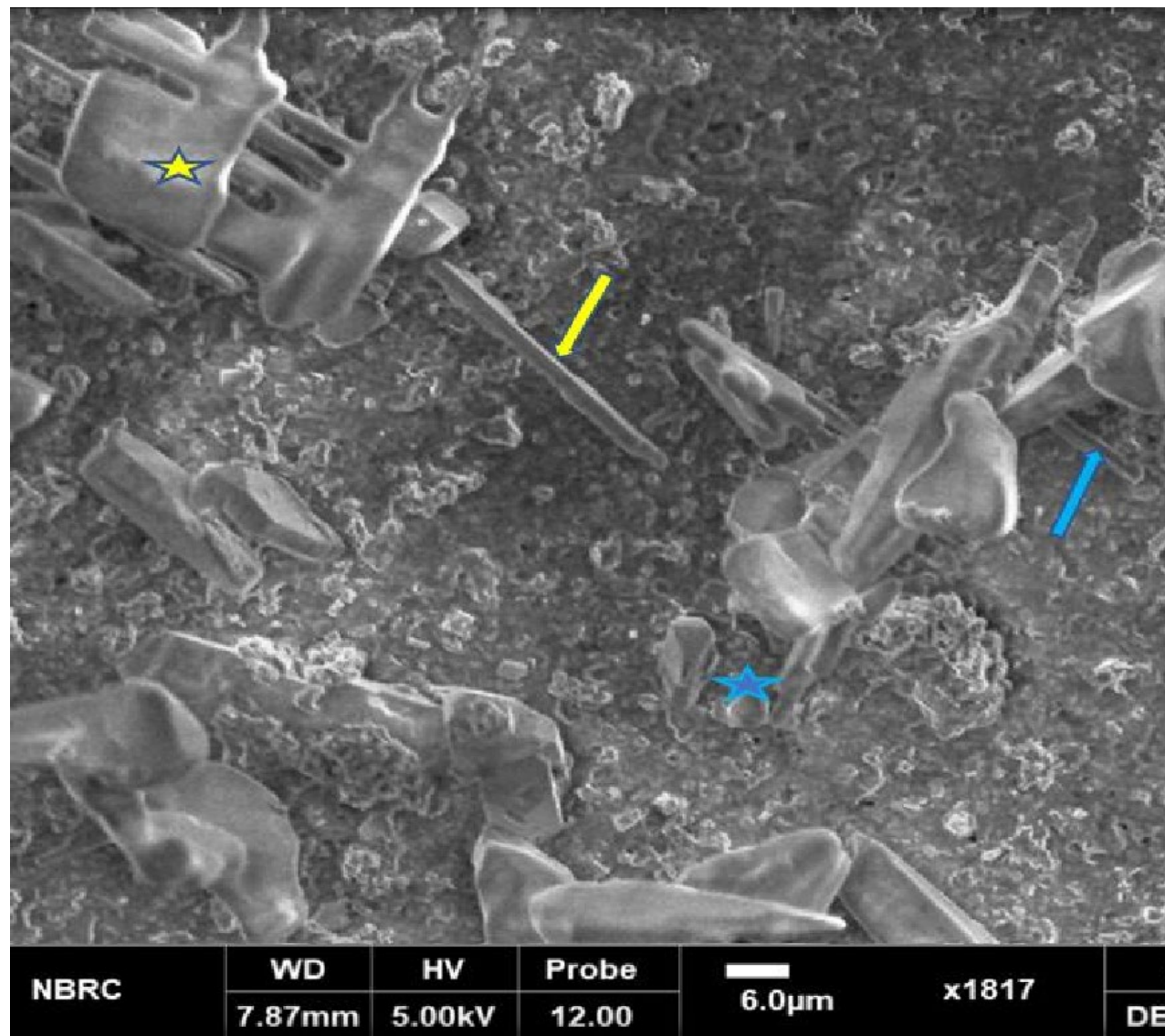


Raman spectra of water (a & b), sediments(c & d)from selected rivers

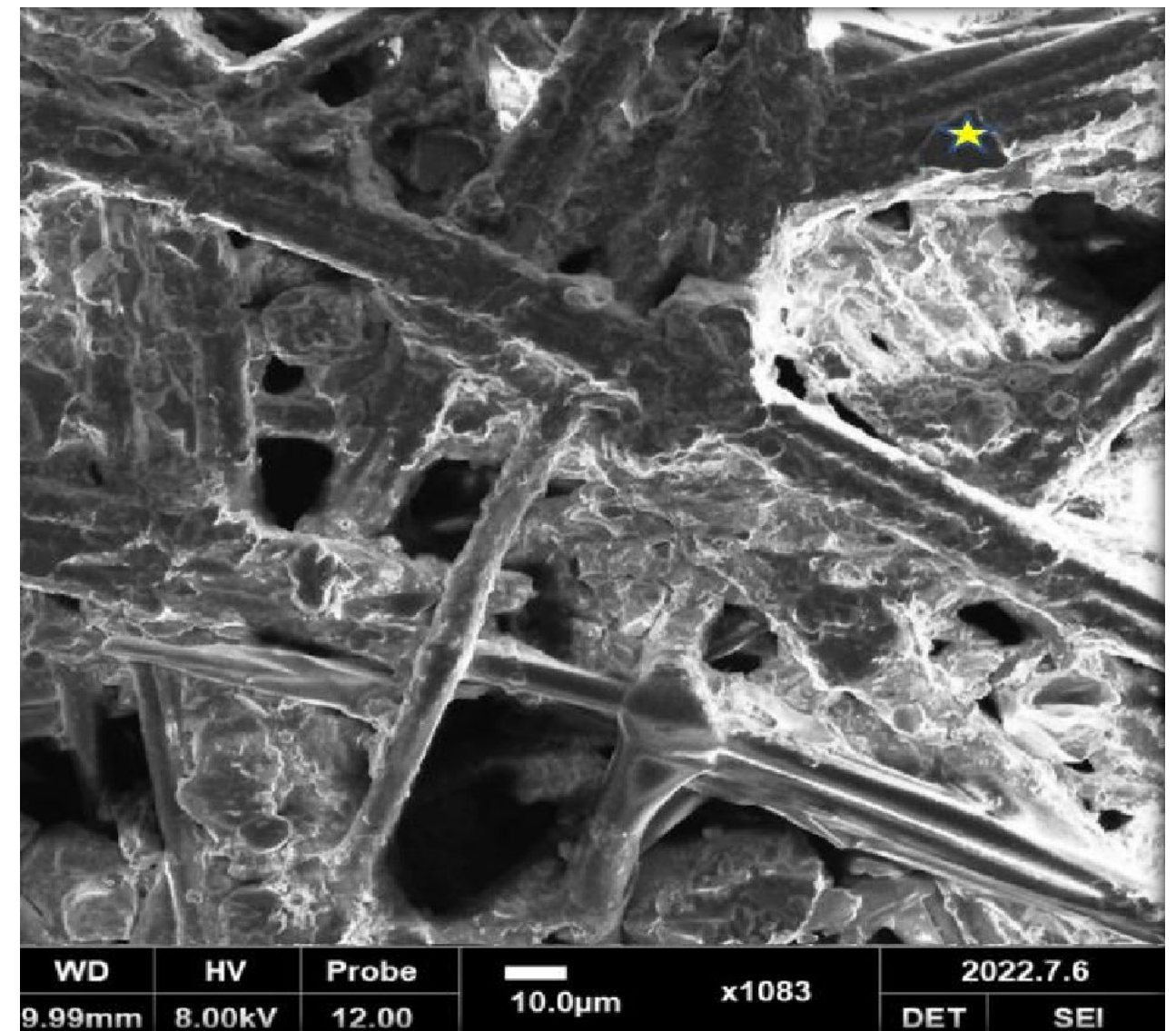


Raman Spectra of fish tissues (e-h) e (gut), f(gills), g (muscle),h(liver) from the selected rivers

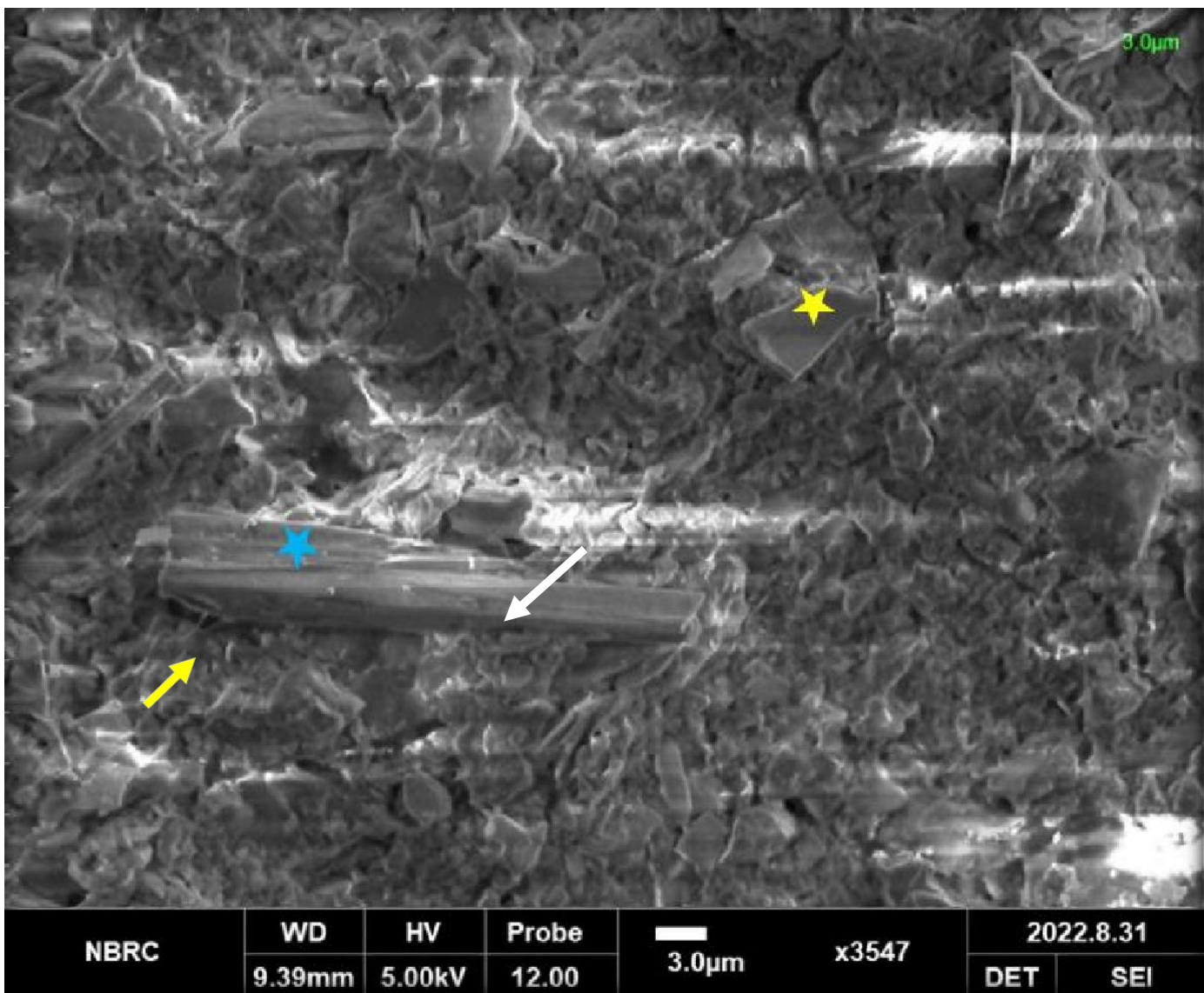




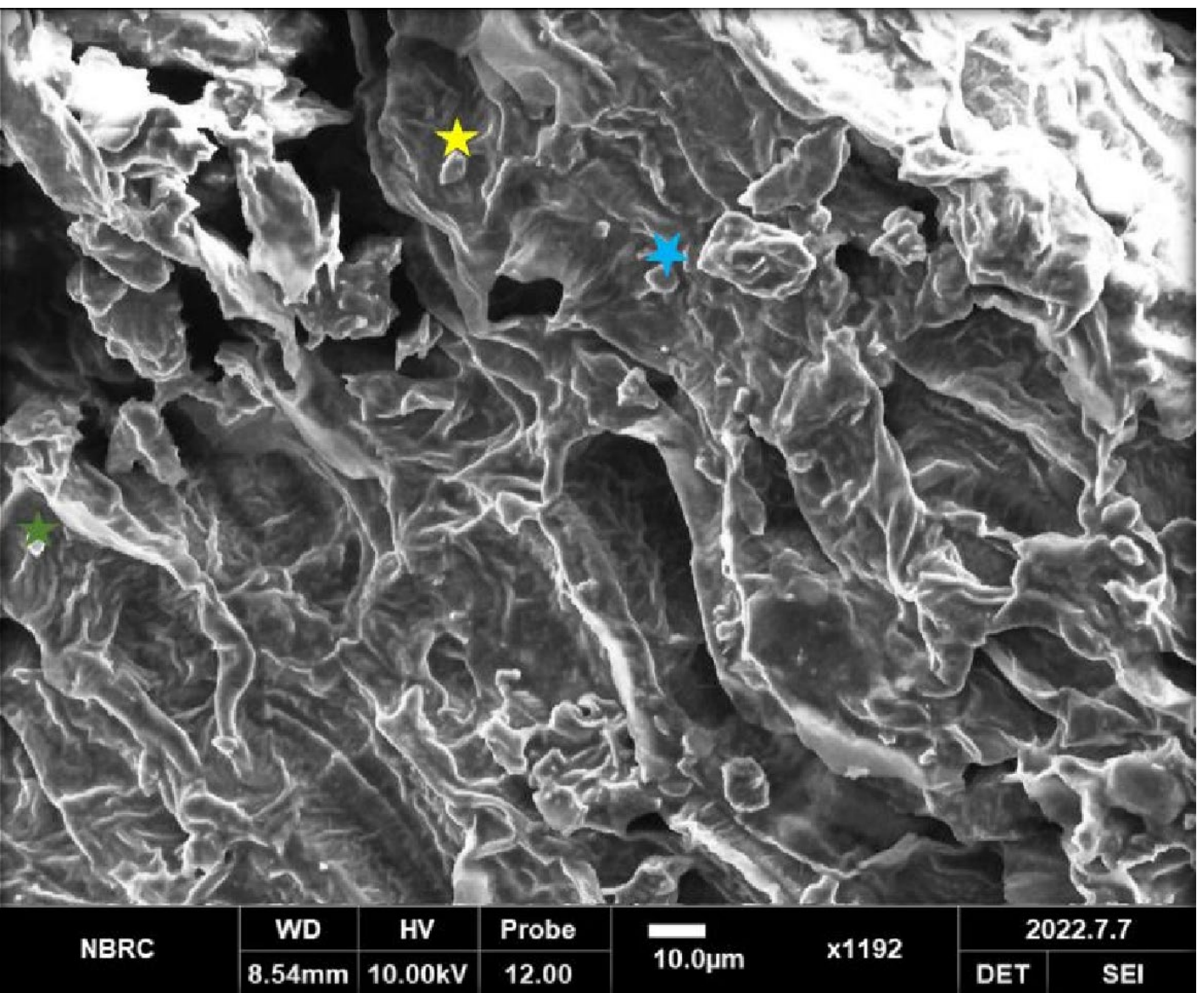
SEM photomicrograph of microplastic in water samples. Fibers (arrow), fragments (asterisk) with surface area and length of $2483 \mu\text{m}^2$ and $218.589 \mu\text{m}^2$ (yellow arrow), $1082 \mu\text{m}^2$ and $72.471 \mu\text{m}^2$ (blue arrow), $567 \mu\text{m}^2$ and $29.530 \mu\text{m}^2$ (blue asterisk), $191 \mu\text{m}^2$ and $190.118 \mu\text{m}^2$ (yellow asterisk)



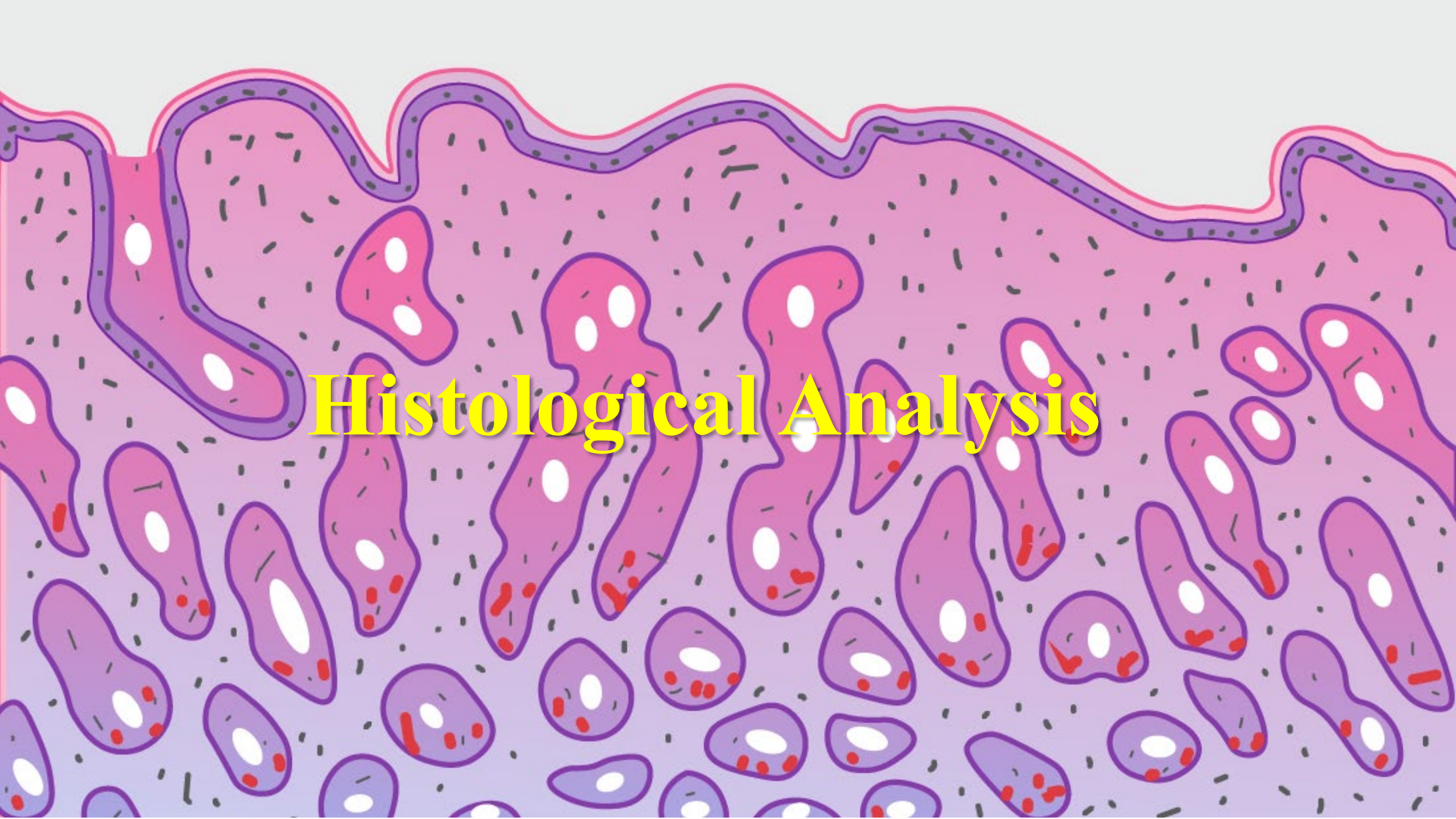
SEM photomicrograph of microplastic in gut tissues (River Jhelum), fragment with surface area and length of $31.364 \mu\text{m}^2$ and $6.367 \mu\text{m}$ respectively (yellow asterisk)



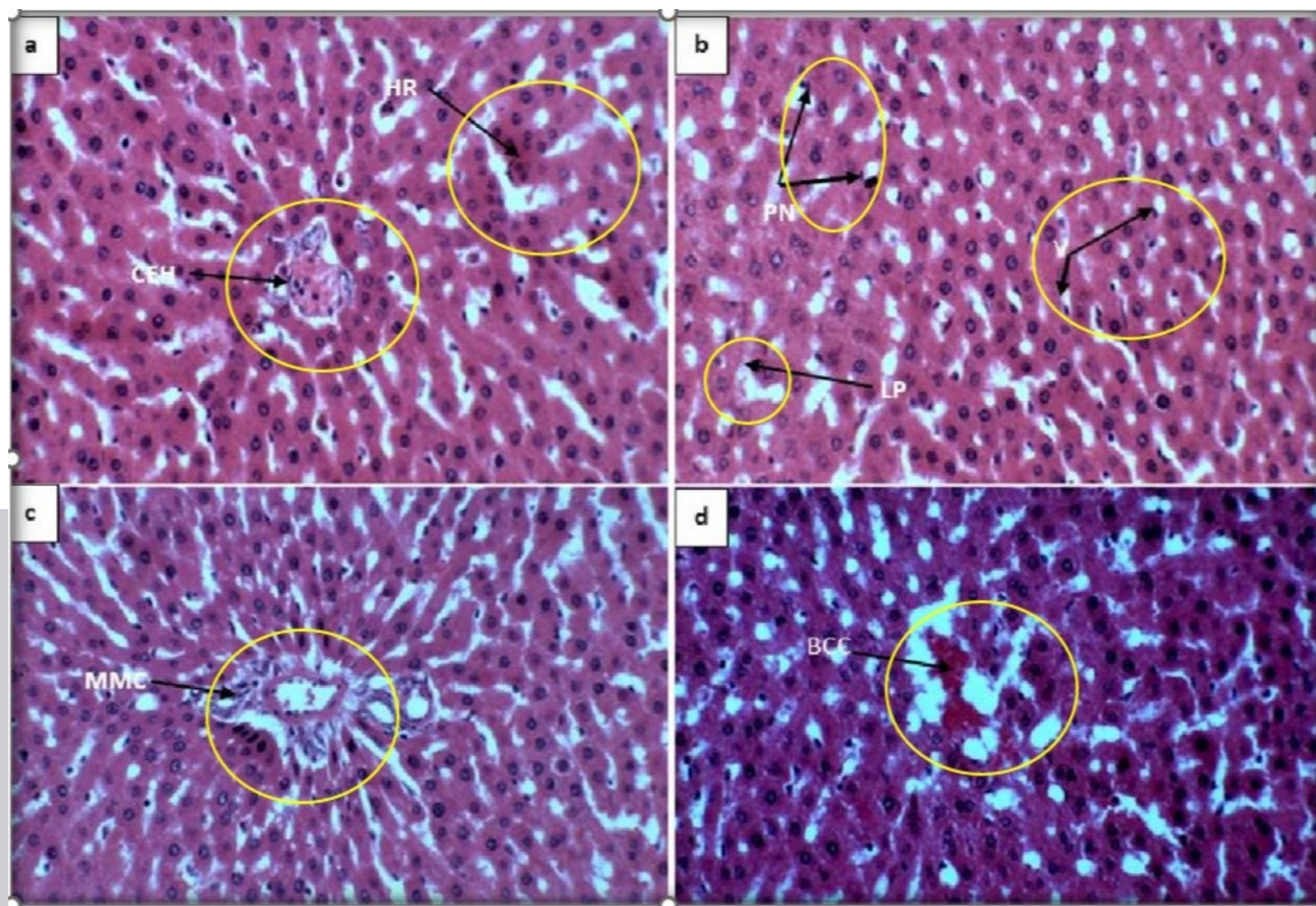
SEM photomicrograph of microplastic in water samples Fibers (arrow), fragments (asterisk) with surface area and length of 2483 μm^2 and 218.589 μm^2 (yellow arrow), 1082 μm^2 and 72.471 μm^2 (blue arrow), 567 μm^2 and 29.530 μm^2 (blue asterisk), 191 μm^2 and 190.118 μm^2 (yellow asterisk)



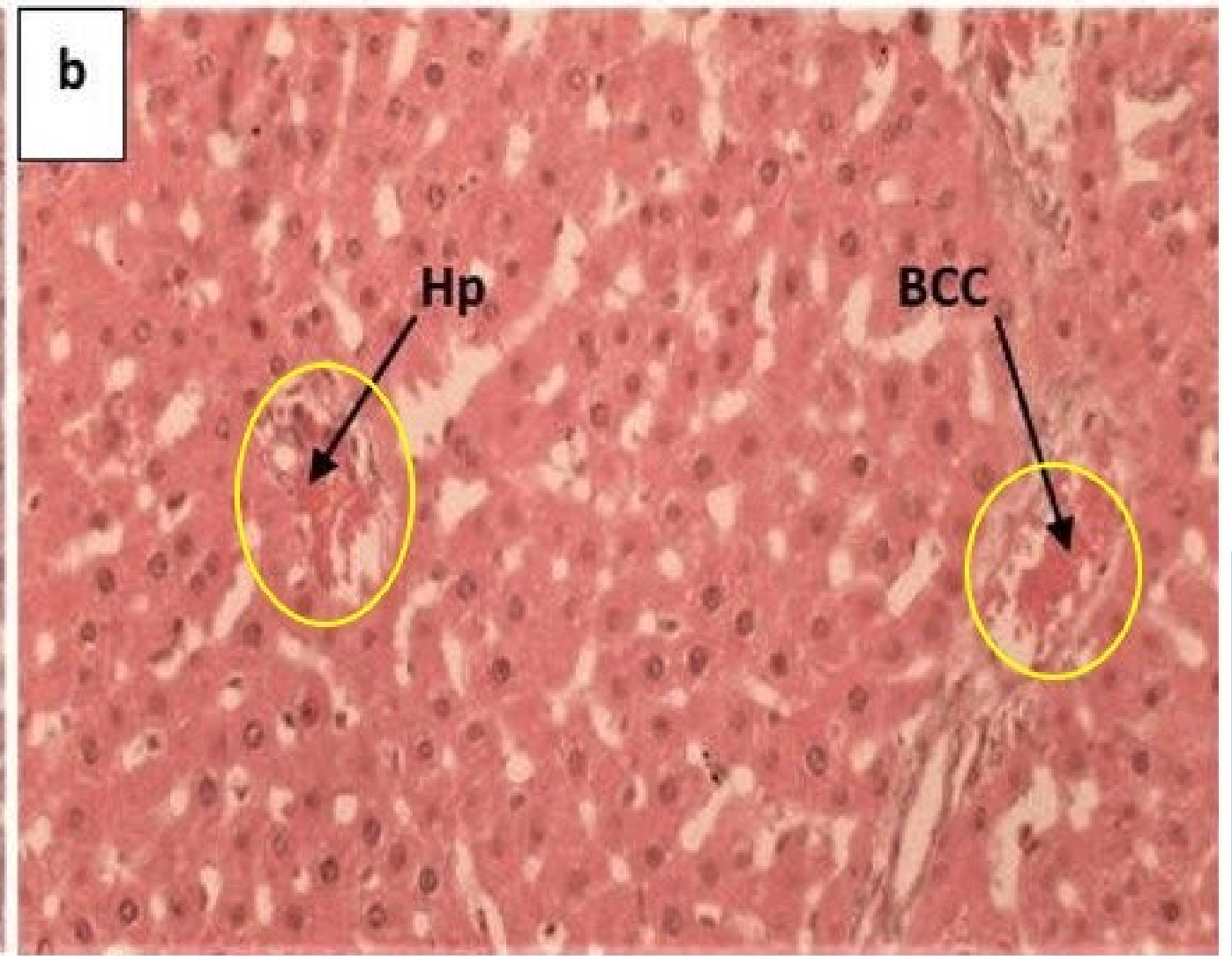
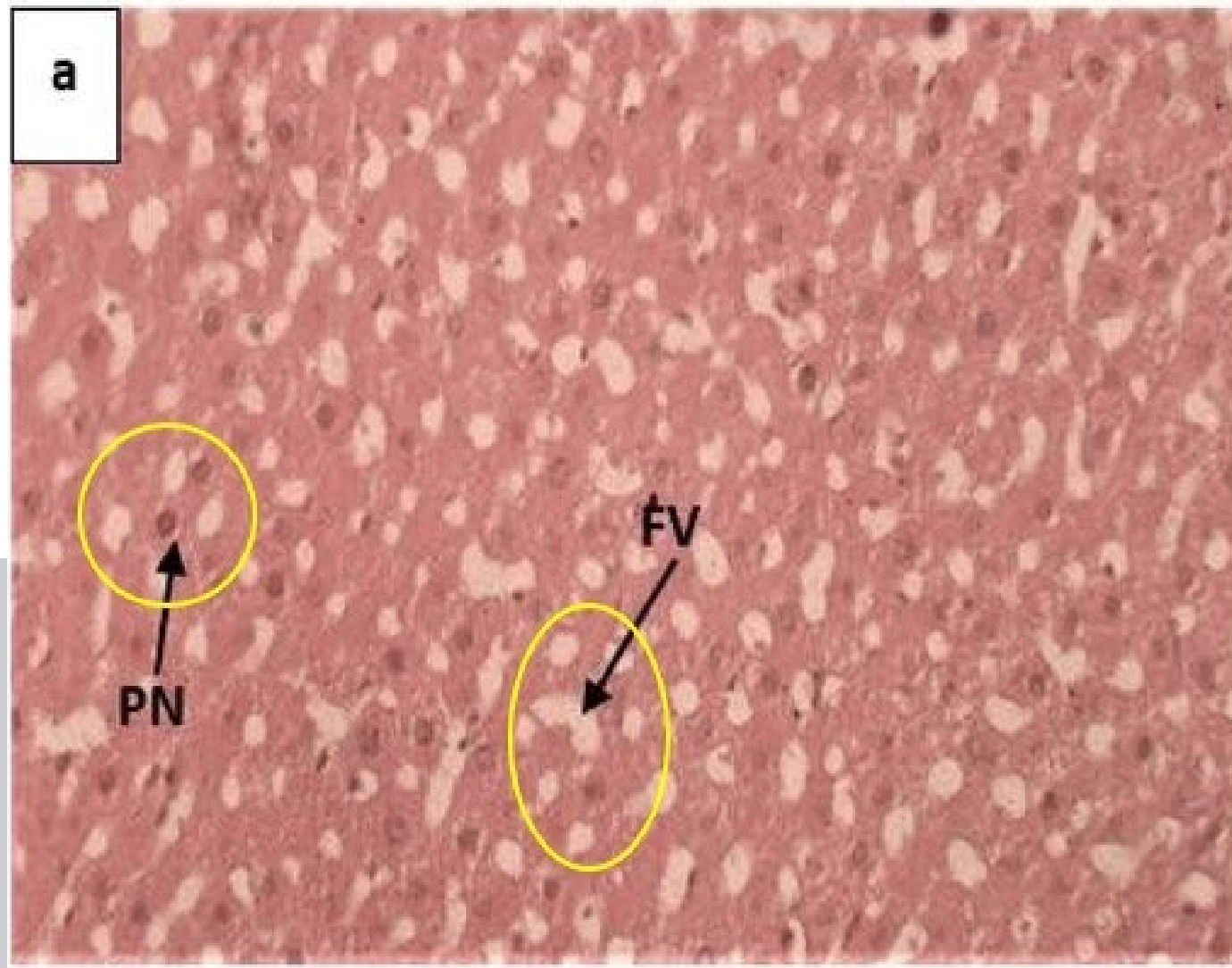
SEM photomicrograph of microplastic fragments in gut tissues with a surface area and length of 22.568 μm^2 and 5.261 μm^2 (yellow asterisk), 17.827 μm^2 and 6.156 μm^2 (blue asterisk) and 11.753 μm^2 and 5.593 μm^2 (green asterisk).



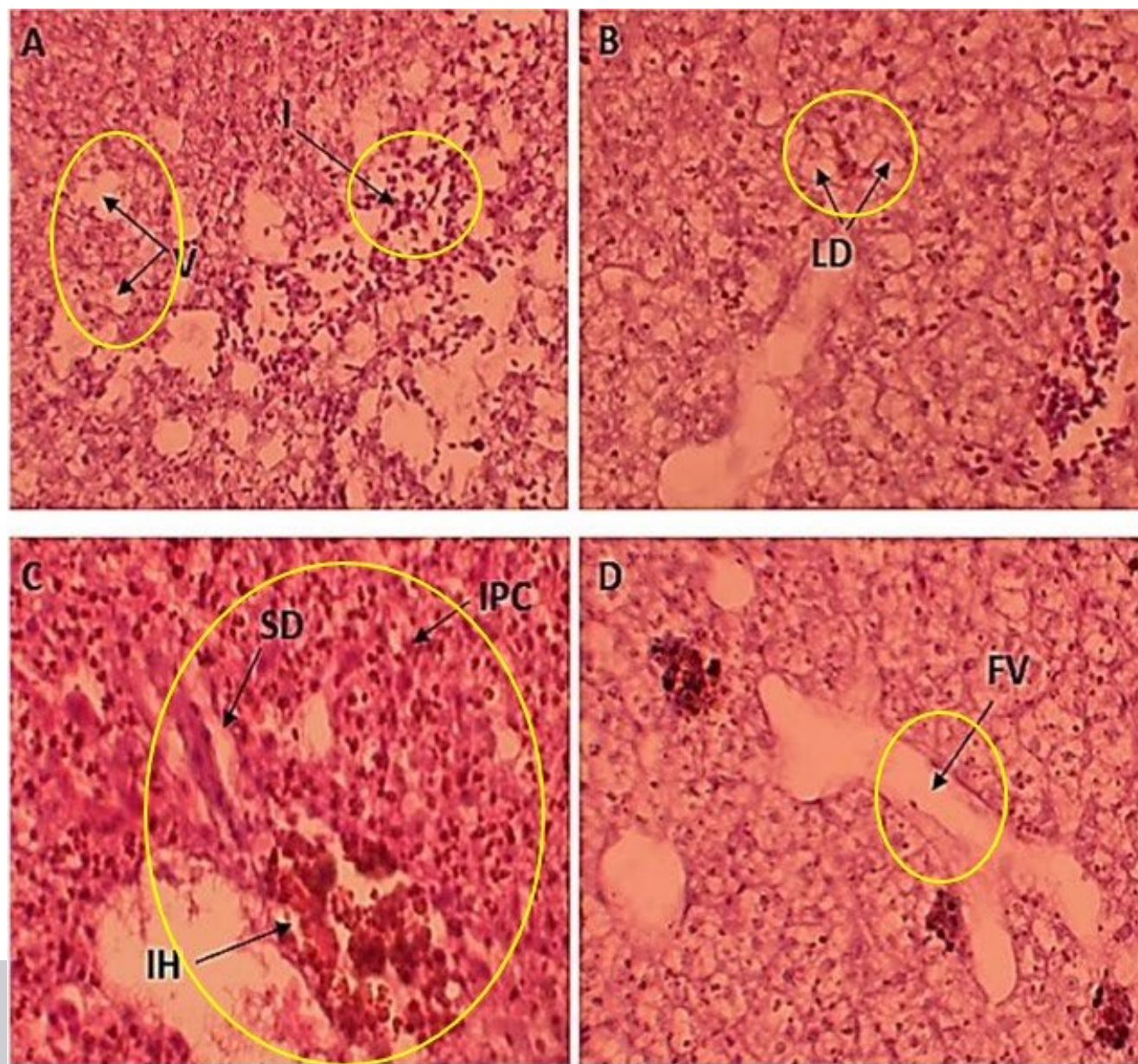
Histological Analysis



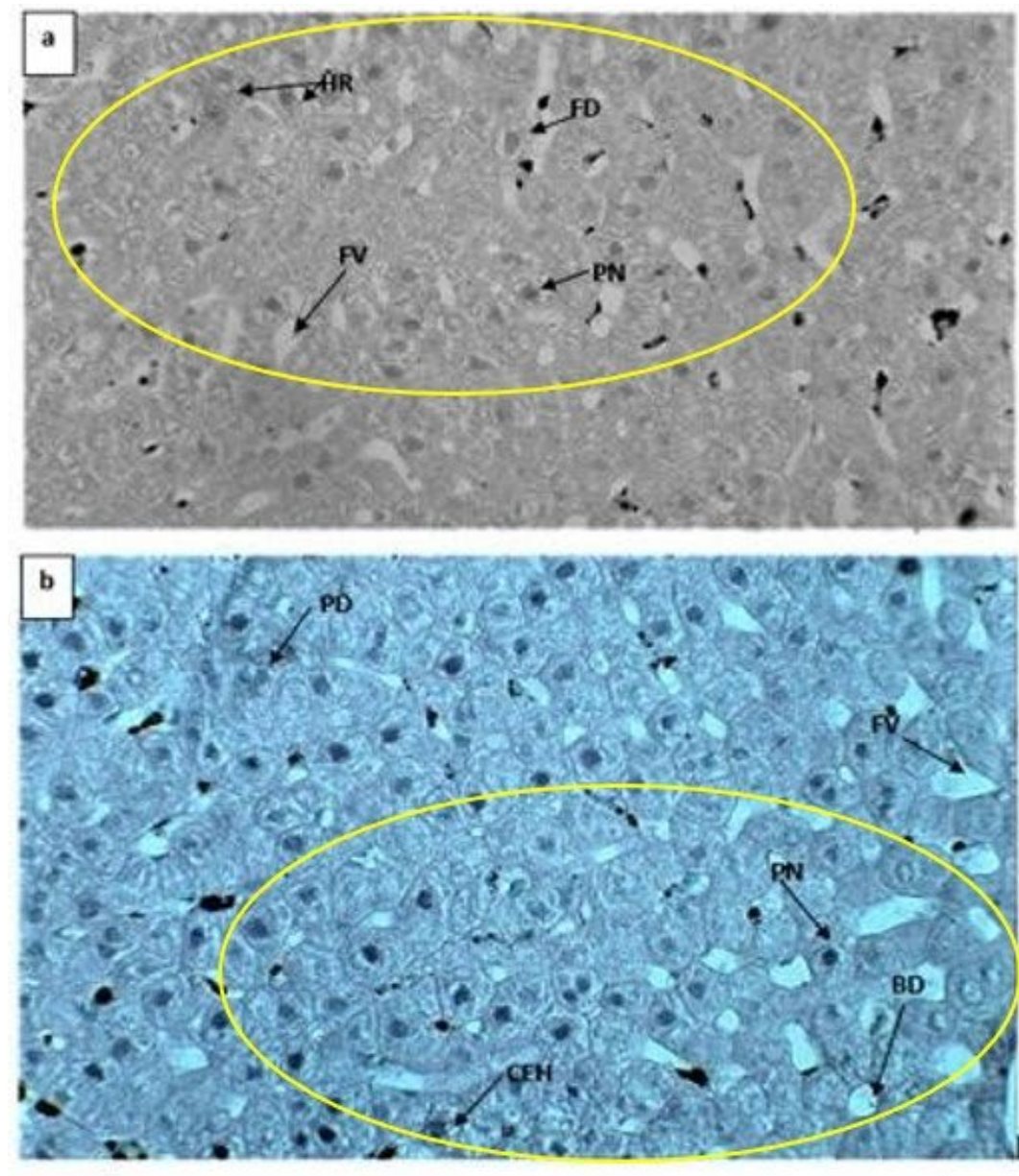
a: CEH: clumps of eosinophilic material in hepatic parenchyma; **HR:** hyperemia; **b: PN:** pyknotic nuclei; **LP:** loose hepatic parenchyma **c: MMC:** melanomacrophage centers **d: BCC:** blood cell congestion



a: **PN:** pyknotic nuclei; **FV:** fatty vacuolation **c:** **Hp:** hyperplasia; **BCC:** blood cell congestion

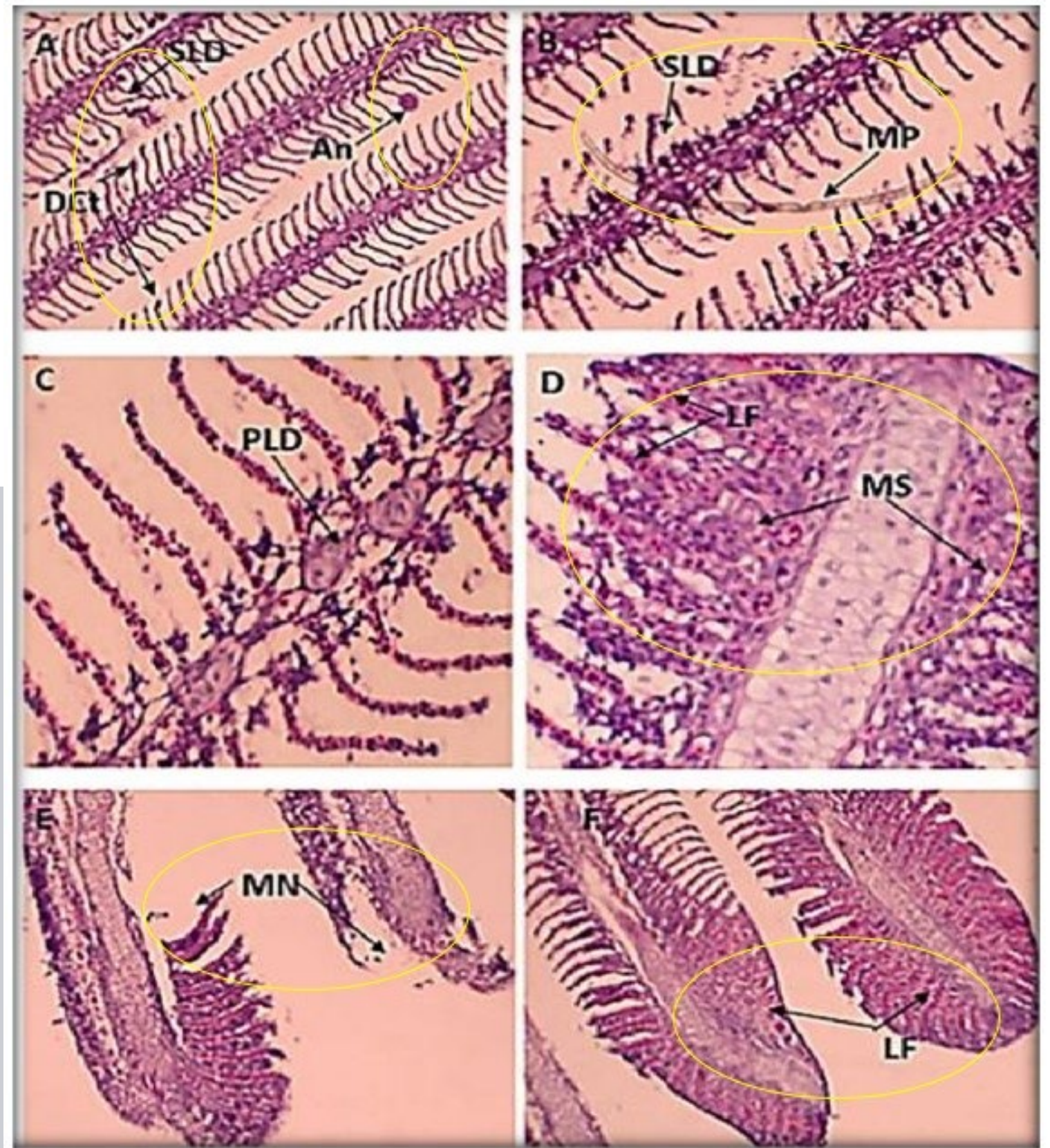


A: **V** (vacuolation) **I** (infiltration) **b** **LD** (lipids droplets) **B:** **SD** (sinusoid dilation) **IPC** (increased pyknotic nuclei) **IH** (Infiltration of hepatocytes) **c:** **LD** (lipid droplets accumulation) **d:** **FV** (fatty vacuolation)

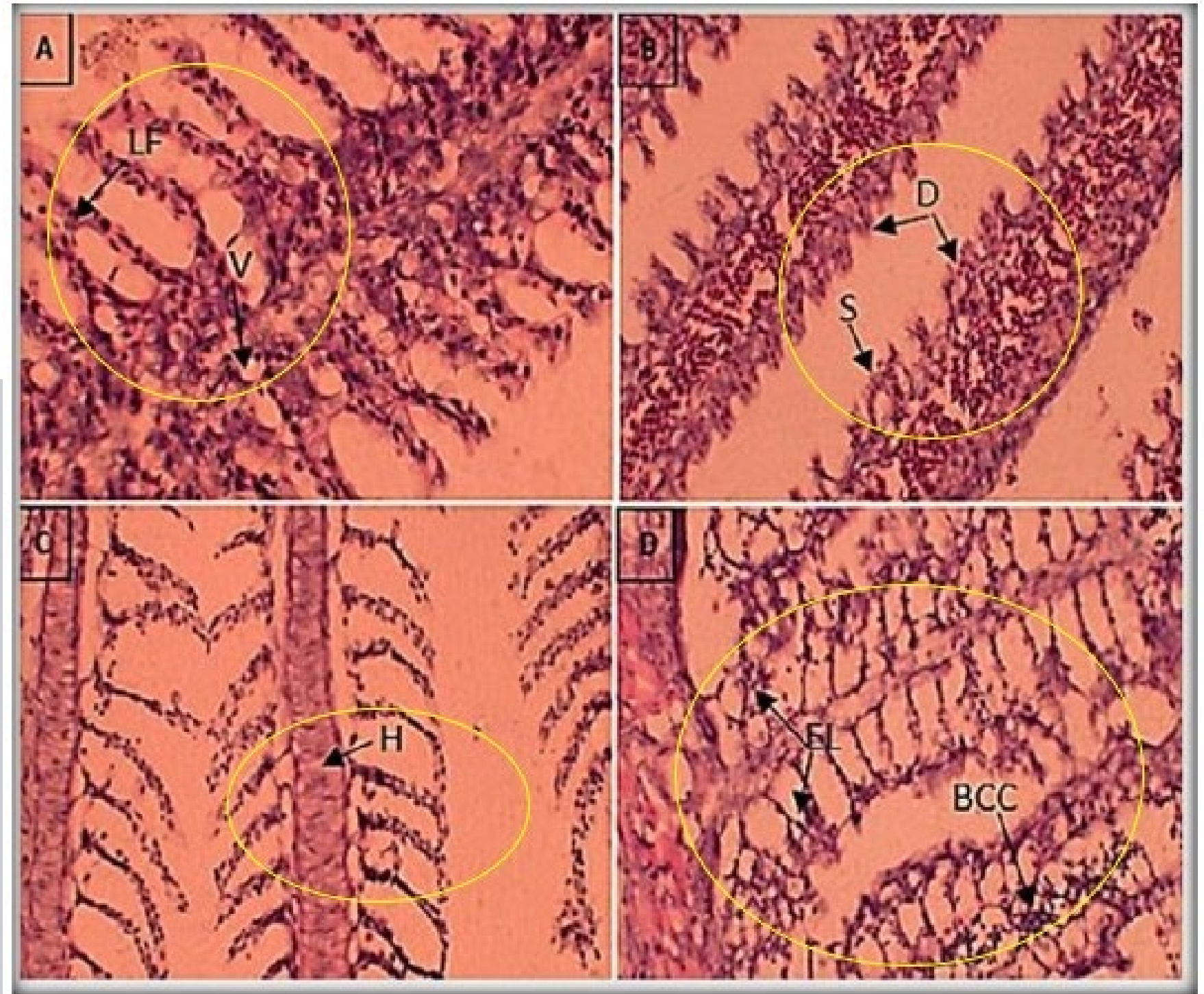


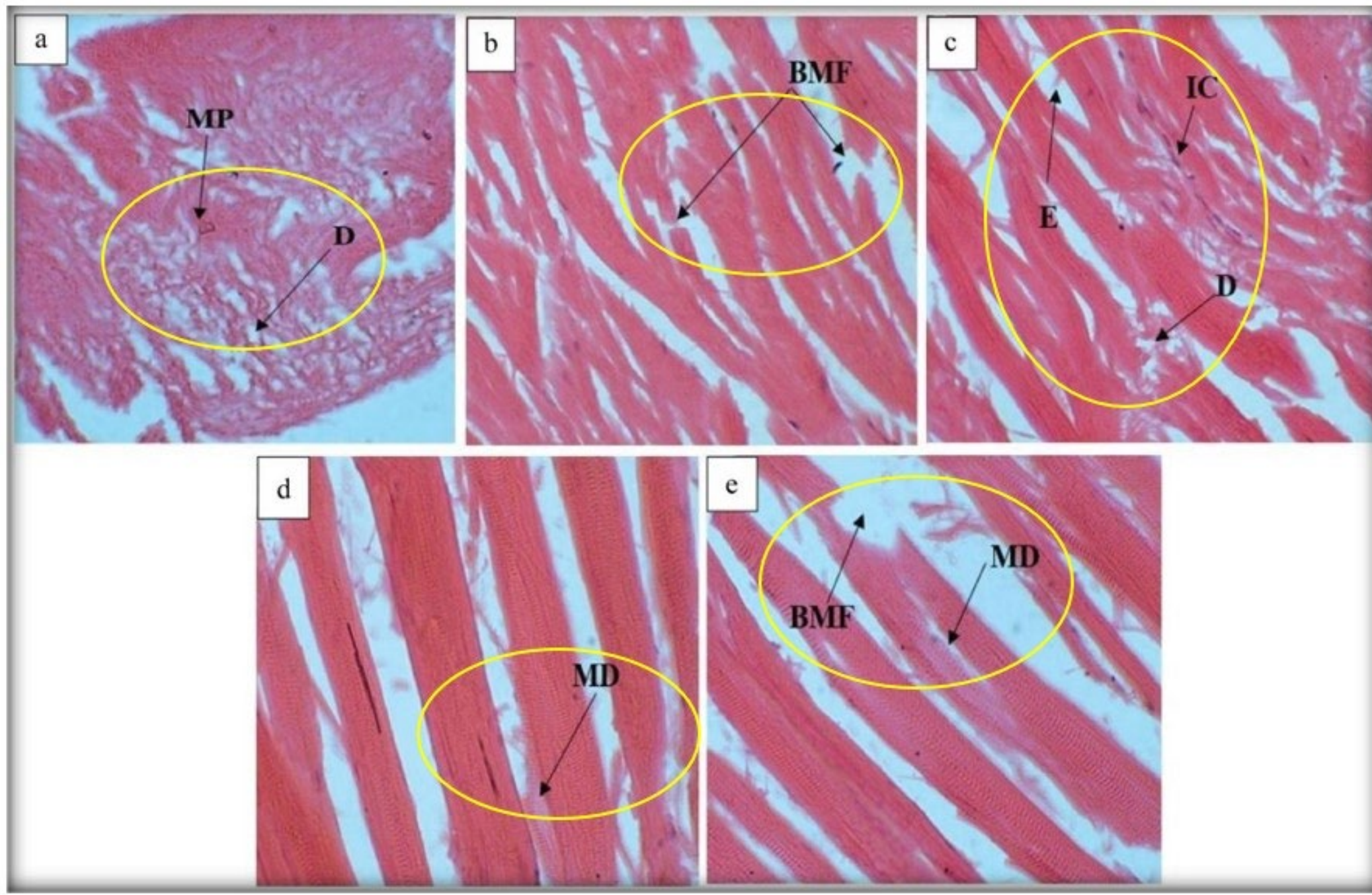
a: **HR** (hyperemia) **FD** (fat deposition) (vacuolation) **FV** (fatty vacuolation) **b:** **PN** (pyknotic nuclei) **BD** (ballooning degeneration) **PD** (hepatic parenchymal degeneration) **CEH** (clumps of eosinophilic material)

A: An (lamellar anuserym)
SLD (degeneration of second order lamellae) **DCt** (dilated and clubbed tip endings) **B: MP** (microplastic fiber deposited) **C: PLD** (disruption of primary lamellae) **D: MS** (excessive mucous secretion) **E: MN** (massive necrotic areas) **F: LF** (lamellar fusion)

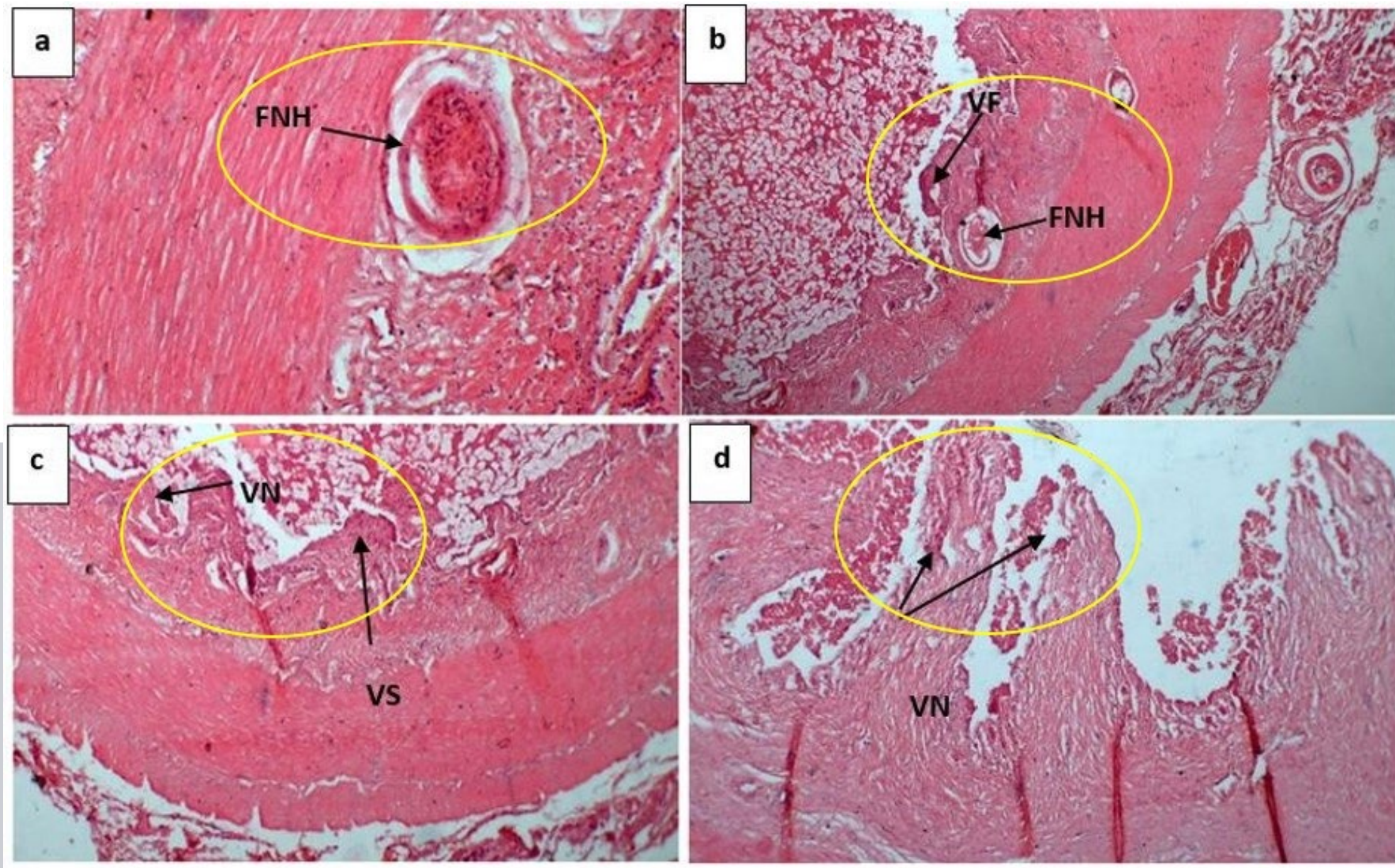


A: **V** (lamellar vacuolation) **LF** (fusion of primary and secondary lamellae) **B:** **D** (lamellar destruction) **S** (lamellar shortening) **C:** **H** (gill core hyperplasia) **D:** **EL** (epithelial lifting) **BCC** (blood cell congestion)



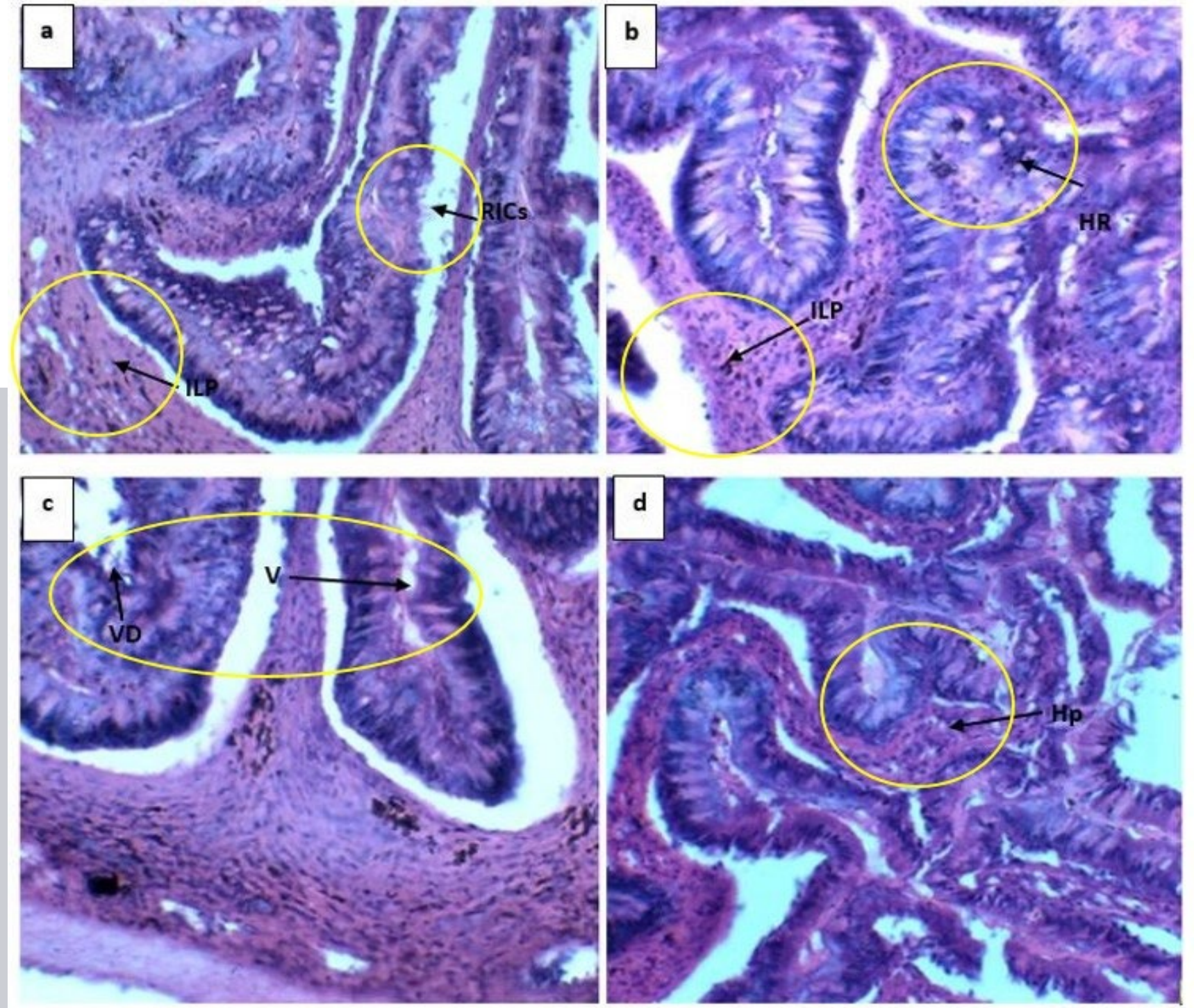


a: **D** (dissociated muscle fiber) **MP** (microplastic fragment) **b:** **BMF** (broken muscle fiber) **c:** **E** (edema) **ICs** (inflammatory cells) **d & e:** **MD** (muscle fiber degeneration)



a & b: **FNH** (focal nodular hemorrhage) **VF** (villus folding) c & d: **VN** (necrosis of villi) **VS** (villus shortening)

a & b: **ILP** (Inflammation of lamina propria) **RICs** (ruptured intestinal cells)
HR (hyperemia) **c: VD** (villus destruction) **V** (vacuolation)
d: Hp (intestinal hyperplasia)



CONCLUSIONS

- The study was a preliminary approach for the identification and characterization of microplastics in the freshwater aquatic ecosystem of the Punjab, Pakistan.
- The study provided the evidence of microplastics contamination and their ingestion by fish species from the surrounding water and sediments.
- Among the morphotypes of microplastics, overall, four different forms of microplastics were found (Fiber, Fragments, sheets and Cubes).
- Among tissues, gut tissues were found to be house of more microplastics due to their direct ingestion and mistakenly as food particles. Overall occurrence of microplastics in tissues was in the following order: gut > gills > muscles > liver indicating the transport of microplastics withing the body of organism.

Conclusion

- Histological analysis of riverine fish tissues (gut, gills, muscles and liver) revealed the toxicological implications of microplastics on the fish population inhabiting freshwater habitat.
- Raman spectrophotometry and Scanning electron microscopy (SEM) provided efficient results about the chemical characterization in the form of vibrational humps or peaks and morphological analysis of microplastics, respectively.
- **Polyvinyl chloride (PVC), Polypropylene (PP), and Polystyrene (PS) in water and sediments samples.**
- **Four different plastic polymers were confirmed in the fish tissues (PVC, PP , PE and PS) in water and sediments samples**
- This study could be beneficial for the assessment of microplastics and their possible toxicity in the freshwater ecosystem.



Characterization and implication of microplastics on riverine population of the River Ravi, Lahore, Pakistan

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Abstract

Microplastic (MP) pollution in the aquatic environment is an emerging subject worldwide. So far, very few investigations have been reported on the riverine fish population. This study investigated the implications of microplastics for three freshwater fish species (*Labeo rohita*, *Cirrihinus mrigala*, and *Sperata seenghala*) as bioindicators of this pollution. Raman spectroscopy was used to confirm MP polymer type and their distribution in water, sediments, and in different organs (gut, gills, liver, and muscles) of *Labeo rohita*, *Cirrihinus mrigala*, and *Sperata seenghala* collected from River Ravi at two sites (site I, Dhand Nano Dogar and site II, Jhamra). These selected sites were situated predominantly near agricultural lands and received polluted water from nearby sewerage and industries that represented potential sources of microplastic pollution. Histological analysis was combined with Raman spectroscopy to assess the effects of MPs on fish organs. MPs were identified in water and sediment samples with an average load (per 0.5 L or per 0.5 kg) of 33 items and 64 items for water and sediments at site I and 27 items and 19 items at site II, respectively. Of total MPs identified, 56.9% were found in bottom feeder *C. mrigala*, 37.91% in column feeder *L. rohita*, and 5.21% in *S. seenghala* at site I while at site II 60% were found in *C. mrigala*, 29% in *L. rohita* and 10.34% in *S. seenghala*. This was linked with more plastic accumulation in sediments from the nearby residential sewerage and industrial effluent flow. In this study, the identified MPs polymers were in the order of polyvinyl chloride (PVC) > polystyrene (PS) > propylene (PP) > polyethylene (PE). Among plastic shapes, fiber (58%) was the dominant plastic in water followed by fragment (21%), sheet (12%), and cube (9%). In sediment, the fragment was the common plastic shape with 51% followed by fiber (28%), sheet (19%), and cube (2%). Fragments (62.9%) in water and fibers (68.4%) in sediments were abundant at site 2. Microplastic mean occurrence in organs was in the order of gut > gills > muscles > liver at both sites. Significant histological alterations were observed in all three species including intestinal edema, hyperplasia, hepatocyte infiltration, accumulation of lipid droplets in the liver, lamellar fusion and breakage in gills, and muscle fiber necrosis. This study showed MP occurrence in the selected freshwater fishes, so further research is needed to assess plastic pollution in the riverine fish population of Pakistan. This study appeared to be the first in the selected area, as no significant information regarding plastic pollution in that riverine system was found when this study was conducted.

Keywords Microplastic pollution · Surface water · Sediments · Raman spectroscopy

Introduction

At present, microplastic (MP) contamination and toxication are believed as one of the major concerns worldwide in the aquatic ecosystem. It is seen to have adverse consequences for the environment and its organisms including muddle, ingestion, and potential deadliness (Eriksen et al. 2014; UNEP 2016). Microplastics in the range of 1 to 5 mm in size

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Mitigation Strategies



Reduce Plastic Consumption

Minimize single-use plastics and opt for reusable alternatives.



Improve Waste Management

Implement proper waste collection and recycling systems to prevent plastic waste from entering waterways.



Develop Filtration Technologies

Invest in technologies to remove microplastics from wastewater and water sources.

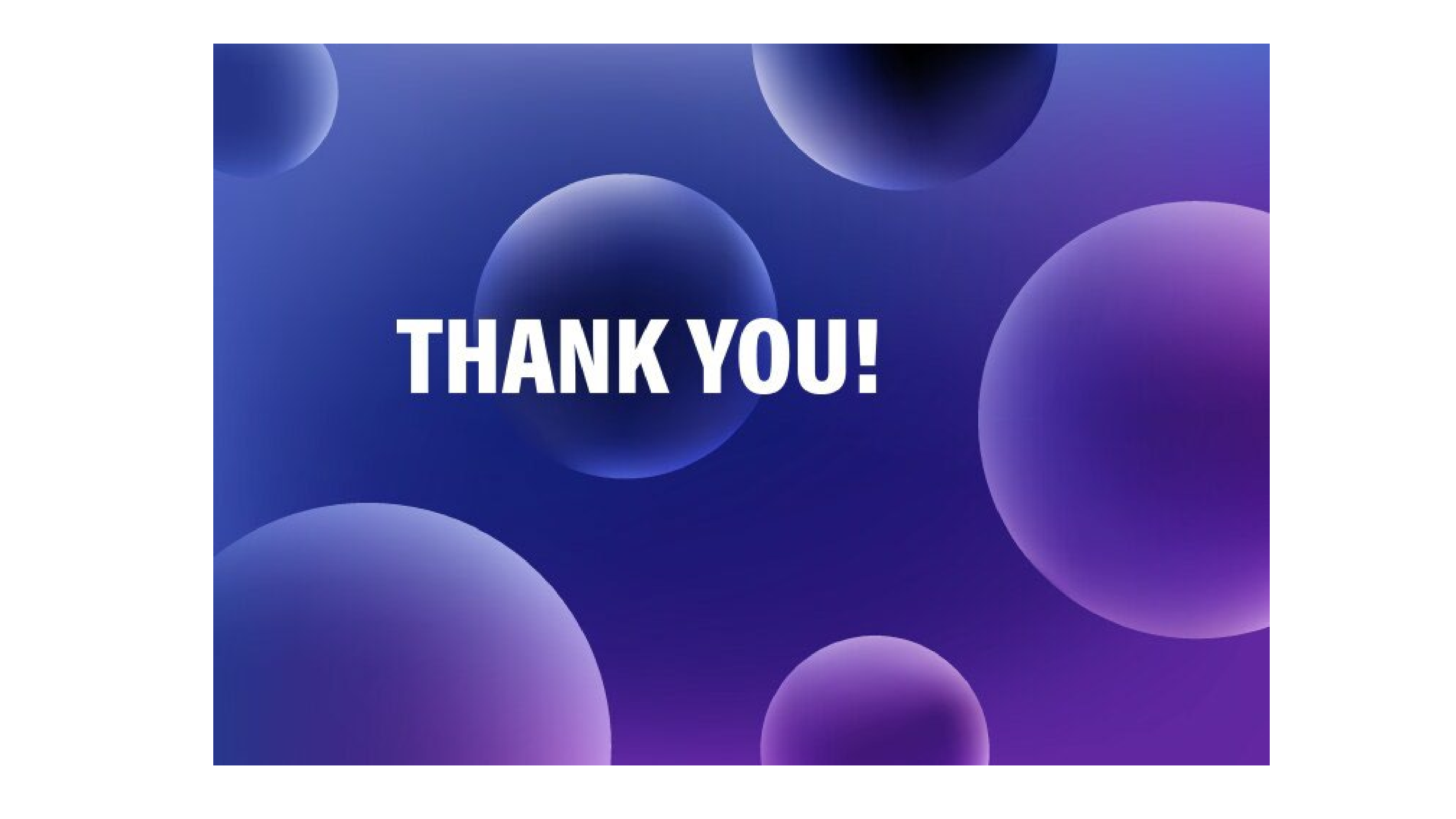


Promote Education and Awareness

Educate the public about the impacts of microplastics and encourage responsible plastic use.

Microplastics pose a significant threat to the environment and Freshwater Fishes.

The future of our planet depends on our collective efforts to protect it from the pervasive presence of microplastics.

The background is a gradient of dark blue and purple, featuring several large, semi-transparent spheres of varying sizes and colors (blue, purple, and magenta) scattered across the frame. The text "THANK YOU!" is centered in a bold, white, sans-serif font.

THANK YOU!