

Tissue-Specific Microplastic Burden, Concentration and Size Distribution in *Labeo rohita* from Taunsa Barrage, a Ramsar Wetland in Pakistan

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Abstract

Microplastic burden, mass-normalised concentration and particle size can rank the tissues of a fish in different orders, so reporting only the first of the three gives a partial account of where contamination sits. We examined all three in *Labeo rohita*, a cyprinid of major dietary and commercial importance in South Asia, sampled at Taunsa Barrage, a Ramsar-listed wetland on the Indus system in South Punjab, Pakistan. The gastrointestinal tract, gills and dorsal muscle of thirty individuals were digested in potassium hydroxide, separated in sodium iodide, fractionated between 500 and 50 μm , screened by stereomicroscopy and Nile Red fluorescence, and characterised by Fourier-transform infrared spectroscopy. Absolute burden was highest in the gastrointestinal tract (10.17 ± 4.60 items per fish), intermediate in the gills (4.20 ± 2.30) and lowest in muscle (2.20 ± 0.85). Concentration reversed that order: the gills carried 3.52 ± 1.93 items per gram, six times the gastrointestinal concentration and more than two hundred times that of muscle. Particle size partitioned as sharply as concentration did. Coarse particles were concentrated in the gastrointestinal tract, whereas 49% of the particles recovered from muscle were smaller than 50 μm , against 10% in the gastrointestinal tract. Within individual fish, tissue burdens were not significantly correlated with one another, which is more consistent with separate uptake routes than with sequential movement of the same particles from gut to muscle. Fragments dominated every tissue, and polypropylene and polyethylene were the principal polymers throughout. The same four patterns were reported for the neighbouring Panjnad Barrage by this group, and their recurrence at closely similar magnitudes at a second, independently sampled site is evidence that tissue partitioning of this kind is a regional feature rather than a local artefact. A single-tissue, count-based survey at either site would have missed both where microplastics are most concentrated in this species and which size fraction reaches the tissue people eat.

1 Introduction

Microplastics — plastic particles smaller than 5 mm, arising either as manufactured microparticles or through the progressive breakdown of larger debris (Onaji et al., 2025; Bettencourt et al., 2021) — are now a near-ubiquitous contaminant of aquatic environments. Primary microplastics, such as exfoliating particles in personal-care products and pre-production pellets, are distinguished from secondary microplastics generated by the oxidative and mechanical breakdown of larger items (Hidalgo-Ruz et al., 2012), and rising global plastic production has increased the supply along both pathways (Hasan et al., 2023). The durability and chemical resistance that make plastics useful also allow them to persist in sediments, surface waters and the tissues of aquatic organisms long after the parent material has fragmented (Banaee et al., 2025). Beyond their physical presence, microplastics act as vectors for other contaminants: hydrophobic organic pollutants adsorb readily to the plastic surface (Teuten et al., 2007; Ziccardi et al., 2016), and trace metals associate preferentially with weathered or biofilm-coated particles (Ashton et al., 2010; Turner & Holmes, 2015).

Fish take up microplastics through several overlapping routes — direct ingestion of particles mistaken for prey, incidental intake during feeding or respiration, and transfer from contaminated prey (Banaee et

al., 2025) — and the resulting burden is not distributed evenly across the body. The gastrointestinal tract, as the site of ingestion, typically holds the largest number of particles. The gills filter very large volumes of water across a thin respiratory surface. Muscle, generally the least contaminated compartment, is the one of direct relevance to consumers (Hossain et al., 2024).

Many surveys stop at the particle count. That is a defensible first measure, but it conflates two different quantities. A gastrointestinal tract weighing eighteen grams and a gill arch weighing one gram do not become comparable simply because both have been counted, and a tissue can hold few particles in absolute terms while being the most heavily loaded per unit mass in the animal. Particle size is a second dimension that counts obscure. Size governs whether a particle stays in the gut lumen or crosses an epithelium, so a compartment reached only by the finest fraction has a different exposure history from one dominated by coarse fragments. Reporting burden, concentration and size together separates questions that a count alone runs into one.

Freshwater systems have received less biomonitoring attention than marine environments, despite supplying a substantial share of global inland fisheries production. Taunsa Barrage, a Ramsar-listed wetland on the Indus system in South Punjab, Pakistan, supports a regionally important fishery that includes *Labeo rohita* (rohu), a major aquaculture and capture-fisheries cyprinid across South Asia (Khan & Arshad, 2014). The surrounding catchment receives agricultural runoff and domestic wastewater, both plausible sources of plastic input. Tissue-resolved microplastic occurrence has been characterised for the nearby Panjnad Barrage using exactly this three-measure approach (Raheel et al., 2025). That study reported the highest particle count in the gastrointestinal tract, the highest concentration per gram in the gills, an enrichment of particles below 50 μm in muscle, and polypropylene and polyethylene as the dominant polymers. No equivalent data have been published for Taunsa Barrage, some 200 km upstream on the same system, and whether the Panjnad pattern holds at a second site has not been tested.

This study applies the same measures to *L. rohita* at Taunsa Barrage: tissue mass, microplastic burden, mass-normalised concentration, particle size distribution, morphology, colour and polymer composition across the gastrointestinal tract, gills and dorsal muscle, together with an examination of whether burdens in the three compartments covary within individual fish. There are two aims. The first is a site-specific baseline for a Ramsar-listed wetland not previously sampled. The second is a test of whether the Panjnad partitioning pattern recurs at an independently sampled site — a question no single-site study, however detailed, can answer on its own.

2 Materials and Methods

2.1 Study site and sample collection

Thirty *Labeo rohita* were obtained from Taunsa Barrage (South Punjab, Pakistan) with the assistance of local anglers, from sites spanning a gradient of agricultural and other anthropogenic activity. Species

identity was confirmed using the field key issued by the Government of Punjab. Fish were anaesthetised with MS-222 (Sandoz) following capture, then placed in ice-packed bags with the gastrointestinal tract and gills intact and transported to the Fisheries Laboratory, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, where they were held frozen until dissection.

2.2 Contamination control and quality assurance

Glassware was washed in detergent, immersed in chromic acid overnight, rinsed with purified water, oven-dried, wrapped in aluminium foil and stored in insulated containers between uses. Glass was used in preference to plastic at every step where a choice existed. Laboratory personnel wore 100% cotton laboratory coats and nitrile gloves, and surfaces and equipment were cleaned with deionised water followed by 70% ethanol before each session. Ten procedural blanks, carried through digestion and density separation without tissue, accompanied every batch of thirty samples to record background contamination. In addition, two cellulose nitrate membrane filters were left exposed in open glass Petri dishes at the processing and observation stations throughout each session to record airborne deposition.

2.3 Dissection and digestion

Fish were thawed, blotted dry with filter paper, and the eviscerated body weighed to the nearest 0.01 g on an electrical balance (Chyo, Japan); total length was measured from the tip of the maxilla to the tip of the caudal fin ray to the nearest 0.01 cm. The entire gastrointestinal tract, the gills and a portion of dorsal muscle were dissected with sterilised instruments, adherent fatty tissue was excised, and each organ was weighed and transferred to a 250 mL glass beaker. Organs were rinsed with distilled water and incised to expose their contents, and particles large enough to be recognised by eye at this stage were isolated and set aside. The remaining tissue was digested in 10% potassium hydroxide (laboratory reagent grade, S.D. Fine Chemicals) at a 1:3 tissue-to-solution ratio and held at 60°C for 24 h, a treatment that removes organic matter, including lipid-rich tissue, while leaving polymer particles intact.

2.4 Density separation and size fractionation

Digested material was brought to a 5 M sodium iodide solution (approximately 6 g NaI per 20 mL), heated to 75°C until the salt dissolved, then covered with aluminium foil and left overnight for solids to settle. Buoyant material was recovered on a 0.3 mm sieve, with the beaker rinsed repeatedly with distilled water to transfer all solids, and air-dried for 24 h under foil. Supernatants were then passed through a graded series of 500, 300, 150 and 50 µm sieves to separate size fractions, and each fraction was filtered onto filter paper. Filters from the gastrointestinal tract, gills and dorsal muscle were stored separately in sealed glass Petri dishes at room temperature.

2.5 Staining, screening and polymer identification

Nile Red (1 mg mL⁻¹ in chloroform) was applied to each filter, which was then covered and incubated in a fume hood for 24 h before examination under a fluorescence microscope. A positive Nile Red result was treated as grounds for further screening rather than as confirmation of polymer identity, since the stain

also binds some natural and biogenic material. Candidate particles were then subjected to a hot-needle test, in which melting or shrinkage on contact with a heated probe was taken as evidence of a synthetic thermoplastic (De Witte et al., 2014); this separates plastic from non-plastic debris but does not identify polymer type. Shape, colour and size of hot-needle-positive particles were recorded by stereomicroscopy using ImageJ. Polymer identity was determined for a subset of particles by Fourier-transform infrared spectroscopy at the Husein Ebrahim Jamal Research Institute of Chemistry, International Center for Chemical and Biological Sciences, with spectra assigned against polymer reference standards and the identification criteria of Jung et al. (2018). The reference spectra and band assignments established for this analytical chain are those published by Raheel et al. (2025) and were used unchanged here.

2.6 Statistical analysis

Tissue mass, microplastic count and microplastic concentration were compared among the gastrointestinal tract, gills and muscle by one-way analysis of variance with body part as the fixed factor, followed by Tukey's honestly significant difference test for pairwise comparisons, with significance taken at $P \leq 0.05$. Within-fish associations among the three tissue burdens were examined by Pearson correlation across the thirty individuals. Analyses were performed in SPSS v.18.0 (IBM Corp., Armonk, NY, USA); figures were prepared in Microsoft Excel 2016.

One feature of this design should be stated plainly. The three tissues were dissected from the same thirty fish, so the ninety measurements entering each analysis of variance are not fully independent, and the error term does not separate variation among individuals from variation among tissues within an individual. A repeated-measures or mixed-effects model with fish as a random effect would be the stricter treatment, and the Pearson correlations reported in Sect. 3.2 are given partly because they use the paired structure that the analysis of variance sets aside. The differences reported below are large relative to their standard errors, and we do not think the conclusions turn on the choice of error term, but readers should weigh the results with the design in mind.

3 Results

3.1 Tissue mass, microplastic burden and concentration

Microplastics were recovered from all three tissues of every fish examined. The three compartments differed by two orders of magnitude in mass, from 1.19 g of gill tissue to 146.65 g of muscle, and this difference drives much of what follows (Table 1).

Absolute burden was highest in the gastrointestinal tract, intermediate in the gills and lowest in muscle, and all three differed significantly from one another ($F(2,87) = 56.91$, $P < 0.01$; Table 2). Summed across compartments the pooled mean was 16.57 items per fish, or approximately 497 particles across the sample.

Once burden was expressed per gram of tissue the ranking reversed. The gills carried 3.52 ± 1.93 items g^{-1} , six times the concentration in the gastrointestinal tract and roughly two hundred times that in muscle ($F(2,87) = 83.30$, $P < 0.01$). The gastrointestinal tract and muscle did not differ significantly in concentration despite differing by a factor of nearly five in absolute count (Table 2), because the gastrointestinal tract's larger particle load is distributed through a correspondingly larger mass of tissue. The gill is the smallest compartment in the fish and the most heavily loaded per unit mass.

Table 1

Tissue mass, microplastic burden and microplastic concentration in *Labeo rohita* from Taunsa Barrage (n = 30 fish)

Tissue	n	Mass (g)	Burden (items fish ⁻¹)	Concentration (items g ⁻¹)
Gastrointestinal tract	30	18.22 ± 5.64 b	10.17 ± 4.60 a	0.59 ± 0.26 b
Gills	30	1.19 ± 0.11 b	4.20 ± 2.30 b	3.52 ± 1.93 a
Muscle	30	146.65 ± 57.51 a	2.20 ± 0.85 c	0.017 ± 0.011 b

Values are mean ± standard deviation. Means within a column sharing a letter do not differ significantly (Tukey's HSD, $P > 0.05$). One-way analysis of variance on body part: mass $F(2,87) = 171.89$; burden $F(2,87) = 56.91$; concentration $F(2,87) = 83.30$; all $P < 0.01$.

Table 2

Tukey pairwise comparisons of microplastic burden and concentration among tissues

Variable	Comparison	Mean difference	95% CI	P
Burden (items fish ⁻¹)	GIT vs. gills	5.97	4.12–7.82	< 0.01
	GIT vs. muscle	7.97	6.12–9.82	< 0.01
	Gills vs. muscle	2.00	0.15–3.85	< 0.05
Concentration (items g ⁻¹)	Gills vs. GIT	2.93	2.24–3.63	< 0.01
	Gills vs. muscle	3.50	2.81–4.20	< 0.01
	GIT vs. muscle	0.57	–0.12–1.26	ns

GIT gastrointestinal tract; ns not significant. Intervals are Tukey 95% simultaneous confidence intervals computed from the analysis-of-variance error mean square (9.055 for burden, 1.270 for concentration) with n = 30 per tissue.

3.2 Within-fish associations among tissue burdens

If the particles found in muscle arrived there by moving out of the gut or across the gills, fish carrying heavier gastrointestinal or gill burdens might be expected to carry heavier muscle burdens as well. That was not the pattern. Across the thirty individuals, none of the three pairwise correlations among tissue burdens reached significance: gastrointestinal tract and gills $r = -0.215$ ($P = 0.253$), gastrointestinal tract

and muscle $r = -0.062$ ($P = 0.745$), gills and muscle $r = 0.192$ ($P = 0.311$). Burden in one compartment therefore carried almost no information about burden in another within the same animal, which is easier to reconcile with three partly independent exposure routes than with a single ingested load redistributing itself through the body.

3.3 Particle size distribution

Size partitioned across tissues as sharply as concentration did (Table 3). Mid-range particles of 150–50 μm were the largest single class in every compartment, but the tails differed markedly. Coarse particles of 500–300 μm made up 16% of the gastrointestinal total and only 4% of the muscle total, while particles below 50 μm accounted for 49% of the muscle total against 10% of the gastrointestinal total. The gills fell between the two in both tails. Put simply, the coarser the particle, the more likely it was to be found in the gut; the finer the particle, the more likely it was to be found in muscle.

Table 3
Size distribution of microplastics recovered from *Labeo rohita* tissues, Taunsa Barrage (percentage of tissue total)

Tissue	500–300 μm	300–150 μm	150–50 μm	< 50 μm
Gastrointestinal tract	16	31	43	10
Gills	11	29	33	27
Muscle	4	12	35	49

Percentages are rounded to the nearest whole per cent. The four classes are exhaustive; the 300–150 μm value is the balance of the remaining three.

3.4 Particle morphology

Fragments were the most common morphology in every tissue (Table 4), consistent with a predominantly secondary origin, whereby most particles derive from the breakdown of larger debris rather than from the direct release of manufactured microplastic. Particle shape alone is an imperfect proxy for origin, so this is best read as a plausible inference rather than a demonstrated source attribution (Agharokh et al., 2022; Ayyamperumal et al., 2022). Pellets and beads formed a notably larger share of the muscle total (27.2%) than of the gastrointestinal (12.5%) or gill (10.7%) totals, which fits the size pattern in Table 3: beads are compact and, at the small end of the range, among the shapes most likely to pass an epithelium.

Table 4
Morphological composition of microplastics recovered from *Labeo rohita* tissues, Taunsa Barrage (percentage of tissue total)

Tissue	Fibre/line	Fragment	Foam	Film/sheet	Pellet/bead
Gastrointestinal tract	31.2	44.5	7.3	4.5	12.5
Gills	28.2	51.1	6.8	3.2	10.7
Muscle	21.7	37.9	8.8	4.4	27.2

Percentages are rounded to one decimal place; rows sum to 100%, subject to rounding.

3.5 Particle colour

White particles were the most common colour in the gastrointestinal tract and muscle, but the gills departed from that pattern: red particles accounted for 47.3% of the gill total against roughly 20% elsewhere (Table 5). Since the gills are a filtration surface rather than a feeding organ, this is not readily explained as visual selection during feeding, and is better treated as an observation requiring explanation than as one already explained (Lusher et al., 2013; Lim et al., 2022).

Table 5
Colour composition of microplastics recovered from *Labeo rohita* tissues, Taunsa Barrage (percentage of tissue total)

Tissue	Yellow	Red	Green	Orange	White	Black	Transparent
Gastrointestinal tract	11.1	19.9	7.2	5.6	37.2	5.2	13.8
Gills	9.5	47.3	6.1	4.1	18.8	4.2	9.9
Muscle	13.0	20.8	8.2	6.5	36.7	5.1	9.8

Percentages are rounded to one decimal place; rows sum to 100%, subject to rounding.

3.6 Polymer composition

Fourier-transform infrared spectroscopy confirmed polyethylene, polypropylene, polyethylene terephthalate and polyvinyl chloride among the particles analysed, with polypropylene and polyethylene the most frequently confirmed polymers in all three tissues (Table 6). The polymer profile was remarkably stable across compartments — polypropylene between 38.4% and 41.5%, polyethylene between 24.6% and 26.7% — which suggests that the tissues are sampling the same ambient polymer mixture, and that the burden, concentration and size differences reported above reflect how particles are taken up and retained rather than differences in what is available to be taken up. Both polymers are used heavily in regional packaging and single-use items (Seetapan & Prommi, 2023).

Table 6
Polymer composition of microplastics recovered from
Labeo rohita tissues, Taunsa Barrage (percentage of
tissue total)

Tissue	PET	PP	PE	PVC
Gastrointestinal tract	14.6	41.3	24.6	19.5
Gills	17.5	38.4	26.7	17.4
Muscle	16.6	41.5	25.3	16.6

PET, polyethylene terephthalate; PP, polypropylene; PE, polyethylene; PVC, polyvinyl chloride. Percentages are rounded to one decimal place; rows sum to 100%, subject to rounding. Composition reflects the subset of particles subjected to and confirmed by Fourier-transform infrared spectroscopy.

Assignments used the bands established for this analytical chain and reported in full by Raheel et al. (2025): C–H stretching near 2950 and 2850 cm^{-1} for polyethylene; C = O stretching near 1730 cm^{-1} with the terephthalate bands at 1240 and 1124 cm^{-1} for polyethylene terephthalate (Pereira et al., 2017); C–H vibrations between 2850 and 3000 cm^{-1} with methyl bending near 1450 and 1375 cm^{-1} for polypropylene (Fang et al., 2012); and C–H stretching near 3000 cm^{-1} with C–Cl stretching at 844 cm^{-1} for polyvinyl chloride. The reference spectra for this laboratory have already been published and are not reproduced here.

4 Discussion

4.1 Burden and concentration answer different questions

The two measures reported in Table 1 point in opposite directions, and the disagreement is informative rather than awkward. Counting particles per organ identifies where the largest number of particles sits, and the answer — the gastrointestinal tract — follows directly from ingestion being the principal route of entry. Expressing the same counts per gram of tissue identifies where the tissue is most heavily loaded, and the answer changes to the gills, by a factor of six over the gut and two hundred over muscle. Neither figure is more correct than the other. They describe different things, and a survey that reports only the first will conclude that the gut is the compartment of interest and stop there.

The physiology makes the reversal unsurprising once it is stated. The gill is a large surface folded into a very small mass, and its entire function is to pass a high volume of water across that surface. Particles entrained in ventilated water encounter the lamellae and the mucus layer, and a small quantity of retained material translates into a high concentration simply because there is so little tissue to distribute it through. The gastrointestinal tract holds four times the gill's absolute burden but weighs fifteen times as much. This matters for biomonitoring design: if the object is to detect a change in ambient exposure, the gill offers the strongest signal per gram of tissue processed, whereas the gut offers the largest absolute count and therefore the more stable count statistics.

4.2 Size partitioning and the muscle compartment

The size distribution in Table 3 is the clearest mechanistic result in the dataset. Particles below 50 μm made up half the muscle total and a tenth of the gastrointestinal total, while the coarse fraction did the reverse. Muscle has no external interface, so whatever reaches it must have crossed an epithelium somewhere, and crossing is strongly size-dependent. A compartment reachable only by the finest particles should therefore be enriched in them relative to the compartments where particles first arrive, which is exactly what was observed. The gills, which are both an entry surface and a barrier, sat between the two.

The correlation results in Sect. 3.2 qualify this. If muscle particles were simply the small tail of a load that had passed through the gut of that same fish, individuals with heavy gut burdens should tend to have heavy muscle burdens, and they did not. The absence of any significant within-fish association is more consistent with uptake proceeding along partly independent routes, each with its own retention behaviour, than with a single ingested load redistributing internally. This should be read cautiously: with thirty individuals and a narrow range of muscle counts, the study has limited power to detect a modest correlation, and a null result is weaker evidence than a positive one. It is nonetheless the kind of test that count-only, tissue-averaged surveys cannot perform at all.

4.3 Polymer profile and environmental relevance

Polypropylene and polyethylene dominated every compartment in closely similar proportions, pointing towards packaging film, bags and comparable single-use items as plausible contributors to the local burden, consistent with the production volumes and regional use of both polymers (Seetapan & Prommi, 2023). This is an inference from the polymer profile rather than a source attribution established by the present analysis. Ambient water and sediment were sampled in the wider programme from which these fish were drawn, but those data are reported separately, and pairing tissue monitoring with contemporaneous water and sediment characterisation at the same sites remains the way to test source inferences directly.

4.4 Comparison with other freshwater studies

Reported burdens in freshwater and estuarine fish vary considerably by species, tissue and locality, and the counts recorded here sit above much of the published range (Hossain et al., 2024; Seetapan & Prommi, 2023). Digestion protocol, recovery cut-off and whether fish were wild-caught or market-sourced all affect comparability, and the present data cannot fully separate genuine differences in local plastic loading from methodological differences of this kind. One point is worth making explicitly: because the graded sieve series used here extends to 50 μm , this study recovers a fine fraction that surveys stopping at 300 μm discard, and that fraction is where half the muscle burden sits. Part of the apparent excess relative to coarser-resolution studies is therefore a difference in what was looked for.

The closest comparison is the survey of Panjnad Barrage, some 200 km downstream, which applied the same three-tissue design and the same three measures to *L. rohita* and two other species (Raheel et al.,

2025). The extent of the agreement should be stated plainly rather than left for a reader to discover: that study reported the same burden ranking, the same reversal of the ranking once burden was expressed per gram, the same enrichment of sub-50 μm particles in muscle, and the same dominance of polypropylene and polyethylene. The present study did not discover those patterns. What it establishes is that they recur, at closely similar magnitudes, at a second site sampled independently — 10.17 against 7.80 items in the gastrointestinal tract, 3.52 against 3.17 items per gram in the gills, 49% against 47% of muscle particles below 50 μm . Replication is the contribution. A pattern observed once at one barrage is a local finding; the same pattern at two independently sampled barrages, in the same species, is evidence about how microplastics partition in this fish across the system.

4.5 Implications for edible tissue and monitoring

Muscle is the tissue people eat, and at 0.017 items g^{-1} a 200 g portion of *L. rohita* from this site would carry on the order of three particles. The absolute figure is small. What the size data add is that those particles are not a scaled-down version of what sits in the gut: roughly half of them are below 50 μm , which is the fraction most plausibly able to cross biological barriers and the fraction that coarser protocols would not have recorded at all. A survey reporting only counts, and only above 300 μm , would understate both the number and the relevance of what reaches edible tissue.

This study characterises occurrence. It does not measure particle release under simulated digestion, bioavailability, or any toxicological endpoint, and the results should be read as an environmental-monitoring baseline rather than a risk assessment. Nor are the muscle figures the most robust in the dataset: they rest on small absolute counts and are the most sensitive of the three tissues to procedural contamination.

4.6 Limitations

Several constraints bound what these data support. The sample comprises thirty fish from one wetland and a local gradient of nearby sites; it does not extend to other reaches of the Indus system, and the findings should not be generalised beyond Taunsa Barrage. The number and timing of sampling occasions are not recorded in the source material, so seasonal variation in exposure cannot be evaluated, and a single campaign cannot distinguish a persistent signal from a transient one.

Two analytical limitations bear directly on the numbers. Procedural blanks and airborne-deposition filters were run alongside every batch, but their particle counts were not retained quantitatively, so the values in Table 1 have not been blank-corrected and no spike-recovery test of extraction efficiency is available; the muscle figures, being smallest, are the most exposed to this. And the analyses of variance treat ninety measurements from thirty fish as independent, as set out in Sect. 2.6. Re-analysing the specimen-level data under a mixed-effects model with fish as a random effect, alongside blank-corrected counts from a campaign spanning several sampling occasions, is the obvious next step, and would allow the partitioning pattern reported here — and its agreement with the neighbouring Panjnad dataset — to be tested with the rigour the pattern appears to warrant.

5 Conclusion

Microplastics were present in the gastrointestinal tract, gills and muscle of every *Labeo rohita* examined at Taunsa Barrage, a Ramsar-listed wetland not previously sampled for microplastics. Which tissue appears most affected depends on how the question is asked. By absolute count the gastrointestinal tract leads; per gram of tissue the gills lead by a wide margin, and the gastrointestinal tract and muscle become statistically indistinguishable. Particle size partitions in the opposite direction to burden, with coarse particles concentrated in the gut and half the muscle burden falling below 50 μm , while burdens in different tissues of the same fish were uncorrelated – a combination more consistent with partly independent uptake routes than with internal redistribution of a single ingested load. Polymer composition, by contrast, was almost identical across compartments, indicating that the tissues sample a common ambient mixture and differ in what they retain rather than in what they encounter. Each of these patterns had been reported once before, at Panjnad Barrage on the same river system; what this study adds is that all four recur, at closely similar magnitudes, at a second site sampled independently. The practical implication follows from the replication rather than from either site alone: burden, concentration and size should be reported together, because any one of them by itself gives a partial and, in both these datasets, a misleading account of where microplastics sit in a commercially important freshwater fish.

Declarations

Competing interests

The authors declare that they have no competing interests. A.A.Q. is a co-author of Raheel et al. (2025), the Panjnad Barrage study discussed in Sect. 4.4.

Ethics approval

All applicable national and institutional guidelines for the care and use of animals were followed. The study protocol was approved by the university ethical committee (Cholistan University of Veterinary and Animal Sciences, Bahawalpur during the PhD research duration). Fish were anaesthetised with MS-222 following capture, as described in Sect. 2.1.

Consent to participate

Not applicable. This study did not involve human participants.

Consent for publication

Not applicable. This study did not involve identifiable human data or images.

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Author Contribution

S.M.M.U.R & H.N: conceptualisation, investigation, formal analysis, writing – original draft. A.A.Q.: conceptualisation, methodology, supervision, resources, writing – review and editing. All authors read and approved the final manuscript.

Data Availability

All data reported in this article are provided in the accompanying Online Resource 1.

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Figures

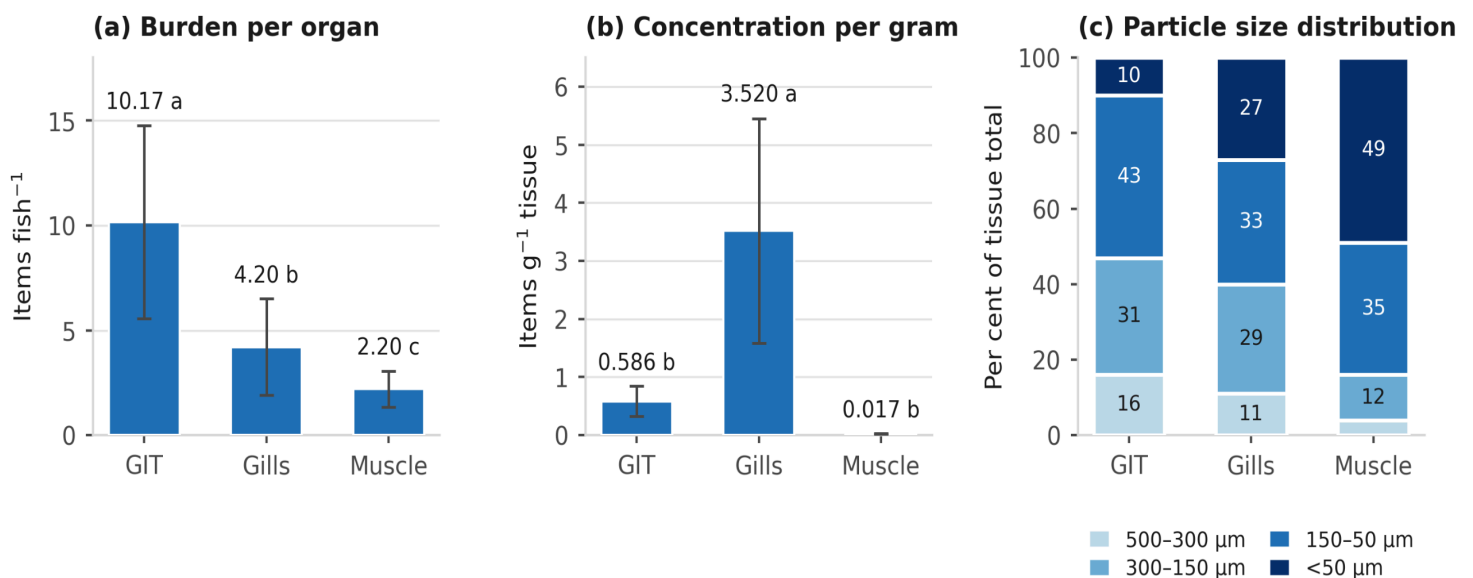


Figure 1

Microplastic partitioning across tissues of *Labeo rohita* at Taunsa Barrage (n = 30). (a) Burden per organ, in items per fish. (b) Concentration per gram of tissue. (c) Particle size distribution as a percentage of the tissue total. Bars in (a) and (b) are mean $\pm$ standard deviation; letters denote Tukey groupings, and bars sharing a letter do not differ significantly ($P > 0.05$). The ranking in (a) reverses in (b), and the size distribution in (c) shifts from coarse in the gastrointestinal tract to fine in muscle

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