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# The occurrence of microplastics in gut contents of endemic barb *Sahyadria chalakkudiensis* (Menon, Rema Devi & Thobias, 1999) inhabiting river systems of Western Ghats, South India

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**Abstract**
*Sahyadria chalakkudiensis,* the endemic barb inhabiting rivers of Western Ghats, S. India has been supporting considerable aquarium trade and is known to dwell in upstream areas of these rivers. A concerted study on its feeding biology from 730 fishes has revealed consistent occurrence of microplastics in their guts, pointing to serious plastic pollution affecting riverine ecosystem. This fish has omnivorous feeding habit as evident from percentage index of relative indices of various prey items as animal matter (62%), filamentous algae (26%), sand particles (4%) and other matter (8%). Among other matter, microplastic fibres were consistently encountered in 86 guts (11.8% of total guts examined) collected in all months except September. The monthly occurrence of guts containing microplastic fibres showed significant correlation to guts containing filamentous algae (r=0.95, $p<0.05$). Further, the mean ± s.d of frequency occurrence of guts with microplastic fibres differed significantly between seasons being lowest in pre-monsoon (3 ± 1guts) and highest in post monsoon (11± 5 guts). However, the occurrence of microplastic fibres was not influenced by feeding intensity which indicated that the fish consumed these microplastic fibres inadvertently. The number of microplastic fibres encountered in each gut varied from 1 to 4, however, guts with one fibre only outnumbered others in all seasons. The present results indicated possibility of micro-litter ingestion during feeding from sediments and from shallow stagnant areas in rivers. The paper discusses challenges of aquatic pollution by plastic litter caused by anthropogenic interventions in protected forest areas and suggests mitigation strategies.

**Keywords:** Micro plastic fibre, river pollution, ornamental fish, habitat management, Western Ghats

## 1. Introduction
One of the most recent anthropogenic impacts adversely affecting environments is plastic litter which has been identified globally as severe threat to different aquatic habitats. The persisting and buoyant nature of discarded and neglected plastic litter accumulated in nature leads to severe environmental hazards that invite increasing research interest in many parts of the world. Nevertheless, plastic production remains increasing to 353million tonnes [1] and plastic litter is more of a concern in terrestrial ecosystems which often gets washed off to rivers, estuaries before ending up in marine habitats [2]. A number of potential hazards of plastic debris in aquatic habitats such as transport of persistent organic pollutants, toxic algae, invasive species etc have been described [3, 4, 5, 6, 7]. This debris undergoes physical, chemical and biological degradation in such environments [8, 9]. Apart from being accumulated as mega and macro litter in water bodies, these plastic debris provide large sources of micro- plastics of less than 5mm size [10].
Anthropogenic sources such as industrial effluents, domestic and urban sewage, consumer products etc., have also been attributed to contain micro plastics which leach out to natural habitats [11, 12, 13]. Occurrence of micro plastics have been reported from a wide array of aquatic environments; from freshwater [14,13] to marine [15], deep sea [16], Antarctic sea [17]. Ingestion of these micro-plastics has been reported in many aquatic biota as zooplankton [18], crustacean [19], fishes [20], sea birds [21], mussel [22] and clams [23]. Ingestion of microplastics also result in health hazards in fishes as evidenced from liver toxicity and pathology through bioaccumulation of chemical pollutants that are adsorbed or associated with plastic degradations [24].

It was recently demonstrated that microplastic ingestion in fishes cause physical abrasions in intestinal wall leading to inflammation duet to leukocyte infiltration, hyperaemia and regressive changes in intestinal tissue [25].

Microplastic ingestion by marine fishes have been reported extensively in contrast to freshwater, in particular, riverine fishes (Table.1).

**Table 1:** Previous works carried out by authors on plastic ingestion in fish species

| Authors | Species |
|---|---|
| [26] | *Gadus morhua* |
| [27] | *Cathorops spixii Cathorops agassizii Sciades herzbergii* |
| [28] | *Gerreidae* |
| [29] | *Anguilla anguilla* |
| [30] | *Johnius borneensis* |
| [31] | *Merlangius merlangus Limanda limanda* |
| [32] | *Gobio gobio* |
| [33] | *Thunnus albacares, Lethrinus amboinensis, Katsuwonus pelamis* |
| [34] | *Cyprinella lutrensis Cyprinella venusta Notropis anabilis Notropis volucellus Pymephales vigilax* |
| [35] | *Ammodytes personatus Clupea pallasii* |
| [36] | *Galeus melastomus* |
| [37] | *Rhizoprionodon terraenovae* |
| [38] | *Mullus surmuletus* |
| [39] | *Lates niloticus Oreochromis niloticus* |
| [40] | *Gonostoma denudatum Serrivomer beanie Lampanyctus macdonaldi* |
| [41] | *Lepomis macrochirus Lepomis megalotis* |
| [42] | *Myripristis spp. Siganus spp., Epinephelus merra Cheilopogon simus* |
| [43] | *Thamnaconus septentrionalis Carrassius auratus Cyprinus carpio Hemiculter bleekeri Hypophthalmichthys molitrix Megalobrama amblycephala Harpodon nehereus Pampus cinereus* |
| [44] | *Hoplosternum littorale* |
| [45] | *G. melastomus E. spinax* |
| [46] | *Pagellus erythrinusP. bogaraveo* |

Studies on microplastic pollution and ingestion by fishes in Indian waters are scanty [47,48,49,50]. A concerted study on the feeding biology of an endemic barb *Sahyadria chalakkudiensis* [51] inhabiting rivers originating from the Western Ghats, S.India has revealed consistent ingestion of microplastics by the fish. This paper encompasses extent of microplastic ingestion by endemic fish species inhabiting freshwater rivers of Western Ghats and discusses need for developing habitat management strategies for sustainability and conservation of this species.

## 2. Materials and Methods

### 2.1 Sampling Period and Study area

Two-year sampling was carried out from April, 2015 to March, 2017 in river Pooyamkutty (10°9′39.79″N 76°47′11.94″E) and river Chalakudy (10.2922° N, 76.5149° E) (Fig.1)

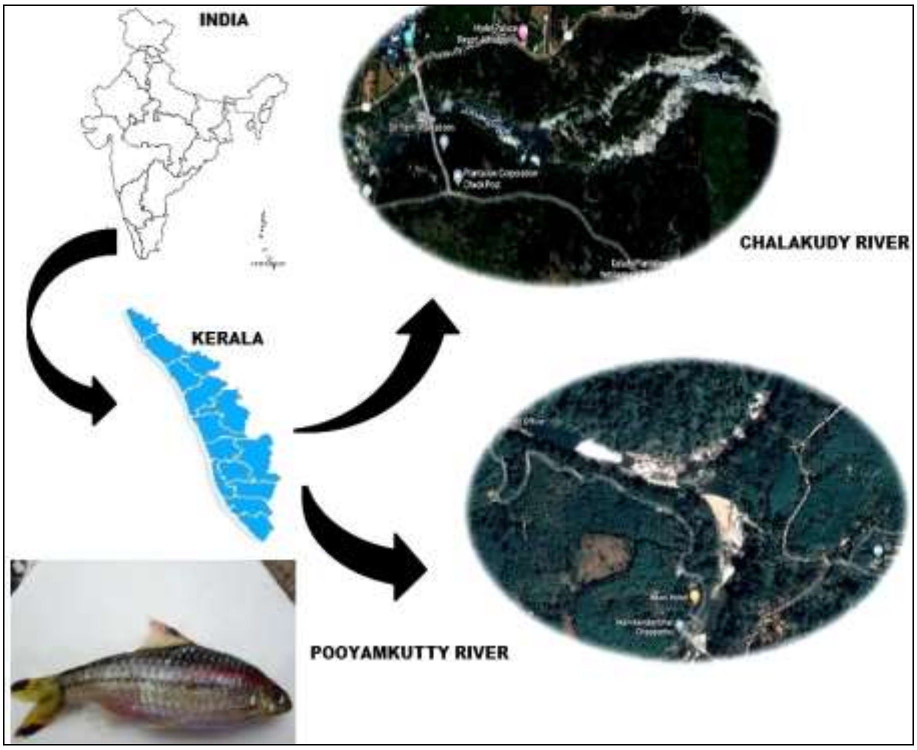


**Fig 1:** Sampling locations-Chalakudy and Pooyamkutty river of Western Ghats, Kerala, India.

### 2.2 Study organism

*Sahyadria chalakkudiensis* is an endemic barb having restricted distribution in Western Ghat river systems. It is a popular food fish among the native communities and is also marketed as a highly valued ornamental fish. *S. chalakkudiensis* was first described by Menon *et al*. (1999) [51] from the upper reaches of river Chalakudy. Apart from Chalakudy river, its presence has also been reported in restricted parts of Achankovil river [52] and Pamba River [53].

### 2.3 Sample collection

478 fishes were collected from native fish vendors in local markets of river Pooyamkutty which included 167 males and 97 females. 252 fishes were collected from Chalakudy river (Male:147and females:105). The total length (TL) was recorded from tip of snout to end of caudal fin to the nearest millimetre. Weight was recorded to the nearest gram on wet weight basis [54]. A small pierce at the end of belly was given and the fish was preserved in 10% formalin for further analysis.

### 2.4 Gut content analysis

The total length (mm) and weight (g) of the fish were determined for each individual. The fish was then dried with a tissue paper, put over a box filled with ice. The belly portion was then cut open and the whole alimentary canal was separated from fish. The length of gut was taken and the stomach portion was separated. Length was taken and the stomach was then separated. The gut contents (stomach and intestine) were examined under a stereoscopic microscope (Lawrence and Mayo make) for the identification of food items and micro plastics. All food items were identified to the lowest possible taxonomic level following the protocol of Hynes [55].

### 2.5 Micro plastic identification

The suspected micro plastic particles were observed to meet all of the following selection criteria, Nor and Obbard (2014) [56]: 1) no visible cellular structures, 2) un segmented nature 3) fibres of consistent width (not tapered) and should have at least two of the additional criteria: 1) brightly coloured coating 2) homogenous texture 3) abnormal shape 4) fibre that remained unbroken if tugged 4) reflective 5) flexible without being brittle.

### 2.6 Sample digestion

The gut contents along with the suspected particles were taken and transferred to a conical flask in which 10% KOH [57] strong oxidising agent was placed and incubated for 5 days so that all the biological compounds disintegrate and the remaining plastic fibres are separated.

### 2.7 Confirmation test

Hot Needle Test was performed for further verification. The fibres were held with a forceps and a hot needle was brought near to the plastic fibre and the plastic fibre started to swirl around and it confirmed the material as plastic [58]. Images and measurements (a micrometre in Motic Plus 2.0) of plastic items recovered were taken with a Motic Image Plus 2.0 (Fig.2).

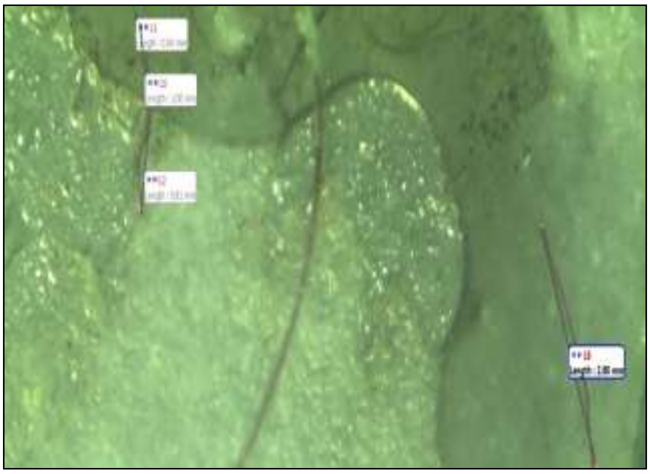

**Fig 2:** Micro plastics obtained from gut and measured using a micrometre in Motic Plus 2.0

### 2.8 Statistical analysis

A descriptive analysis was carried out to describe types of collected micro plastics according to shape categories, size classes and colour opacity. Inferential Analyses were performed and graphical representations were generated with the statistical software SPSS and PAST. Images and measurements of plastic items recovered were taken with a Motic Image Plus 2.0

### 3. Results

A total of 730 guts were dissected for examining the contents. The percentage IRI values worked out in respect of various prey items are depicted in Fig.3.

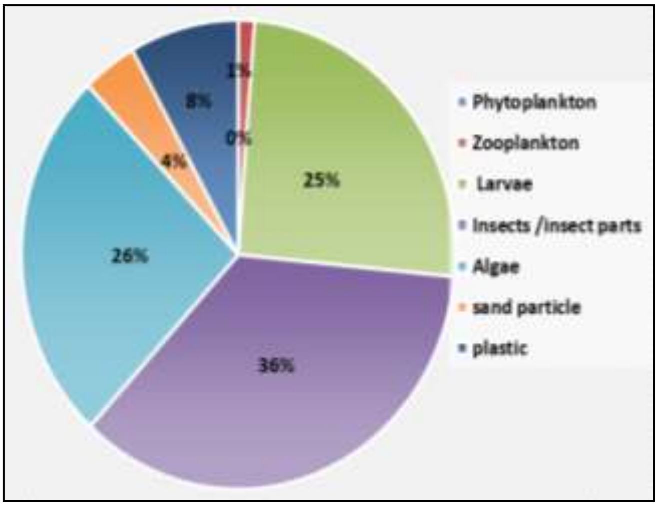


**Fig 3:** Percentage IRI recorded in major food items of *S. chalakkudiensis*

It could be noted that the fish feeds mainly on animal matter comprised mainly of insect parts (36%) and larvae (25%). Higher percentage IRI could also be recorded in filamentous algae (26%) and sand particles (4%). Other minor matter included very small proportions of phytoplankton, zooplankton and undigested matter.

Among undigested matter, a number of fibres like matter could be observed, which on further examination were identified as microplastic fibres. Such fibres were observed in 86 guts which formed 11.8% of total guts examined. Elongated pale red coloured fibres of approximately 2.6mm could be identified in the guts collected in all months except September. The lowest % occurrence of microplastic fibres among gut contents could be noticed in March (7%) while it was highest in October (25%). The mean ± s.d gut frequency having microplastic fibres in pre-monsoon (February – May), monsoon (June to September) and post monsoon (October to January) are depicted in Fig.4.

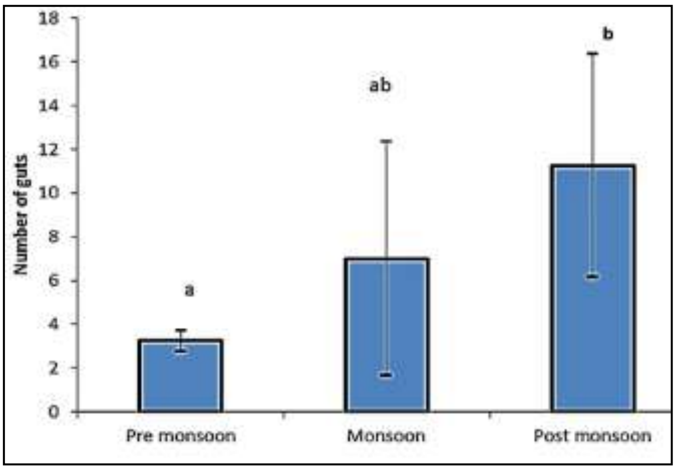


**Fig 4:** Season wise percentage of guts containing micro plastic fibres.

It could be noticed that mean number of guts with occurrence of microplastic fibres was significantly different between seasons (F= 5.49, $p<0.05$) which increased from pre-monsoon (3 ± 1guts) to monsoon (7±5 guts) and post monsoon (11± 5 guts). It could also be noted that the occurrence of microplastic fibres was not influenced by feeding intensity of the fish as these fibres were present in guts with considerable amount of food and guts having traces of food. The proportion of guts with food and plastic and guts with trace of food and plastic were not statistically significant in pre-monsoon ($chi^2$=3.6 $p>0.05$), monsoon ($chi^2$=3.7 $p>0.05$) and in post monsoon ($chi^2$=1.0 $p>0.05$).

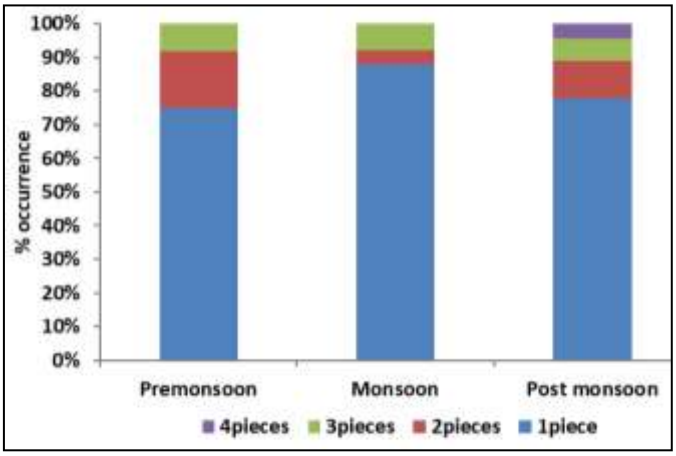


**Fig 5:** Percentage frequency of occurrence of microplastic fibres obtained from guts in different seasons.

The number of micro plastic fibres encountered in each gut varied from 1 to 4 and the frequency of guts with one fibre predominated in all seasons with 75% of guts in pre-monsoon, 88% of guts in monsoon and in 77% of guts in post monsoon (Fig.5). The number of guts with 2 fibres each were high in pre-monsoon (16%) followed by post monsoon (11%) and lowest in monsoon (4%). Number of guts containing 3 micro plastic fibres each did not vary considerably between seasons with 8%, 7% and 8% in pre-monsoon, monsoon and post monsoon seasons. However, guts containing four microplastic fibres were encountered only in post monsoon season.

## 4. Discussion

*S. chalakudiensis* is a fish used for food as well as for aquarium purpose. Other than exploiting live fishes for aquarium purpose, many other important reasons add up to the sustainability issues faced by this fish. The present study has revealed another important issue of environmental pollution, affecting endemic indigenous fishes inhabiting, rivers flowing through protected forest areas after originating in Western Ghats of India. It has been reported that about 70% to 80% of marine litter, most of it plastics, originate from inland sources and are emitted by rivers to the oceans [59] Most of the available information of reports of plastic debris ingestion is for marine species, however studies on freshwater fishes, especially those used as food resource by humans are limited [60].

A primary field report of freshwater fish ingesting microplastics described 12% microplastic in *Gobio gobio* [32, 61]. However, it may be pointed out that such data on micro plastic intake by freshwater fishes is very limited [14]. During the present study, it could be noted that fishes collected from rivers are mostly consumed by natives and tribes and are also sold in nearby markets, which indicated the possibility of microplastic ingested fishes making their way to the next level of consumers. Fishes with microplastics to the tune of 20% have been reported in market-purchased freshwater fish [39]. With each trophic level, bioaccumulation of ingested plastics soon leads to bio magnification, ultimately risking human health [62, 63]. Plastic pollution is carcinogenic to human, it can also cause birth defects, damage immune system, endocrine and reproductive system [64]. Recent studies show how fish health and overall wellbeing of fish are affected by consuming microplastics [65, 66, 67, 68].

If plastic particles become nano-sized, they can cross the blood-brain barrier and can cause brain damage resulting in behavioural changes in organisms [69, 70]. Predators, preferring a greater number of fish with full guts, will generally have higher exposure to microplastics due to simply ingesting more material [71]. A recent study found that microplastic burden varied significantly between species depending up on feeding habits and trophic transfer and top predators contained the highest load of microplastics [72].

In the present study, it could be noted that this fish feeds mainly on filamentous algae and larva and has preference to slender elongated objects. It may also be inferred that the fish seeks food in shallow stagnant pools and river banks where filamentous algae and insect larvae can be available in plenty. As these areas are shallow and without considerable water flow, permit sediments to accumulate, increasing settlement of microplastic fibres. It is reasonable to infer from present results that *S. chalakkudiensis* adopts bottom feeding as indicated by presence of sand and higher proportion of insect larvae, algae and occurrence of microplastic among gut contents could be indicative of extent of plastic pollution in the rivers. Plastic debris would have mixed with sediment and when fish forage on the bottom, the microplastic fibres could have been unintentionally consumed [73]. Wang et al. [74] found that most microplastic obtained from fish species were coloured and fibrous in nature [75, 76]. More than 80% of microplastics had a size of 2mm. In the present study also, all microplastic encountered were fibrous in nature and were on average 2.6mm in size. Generally, 1- 2 ingested microplastic pieces were encountered in fish guts [77]. In the present study, up to 4 pieces of microplastic fibres could be collected from individual guts. Predators preferably ingest microplastics with colours resembling their prey [78, 79, 80]. The microplastic fibres obtained in present study were red in colour. Coloured plastics have been detected from organisms as well as from habitat [81, 82].

### 4.1 Potential pathways of microplastics in tropical river system

The microplastic pollution in the two rivers of Western Ghat were from untreated sewage, fishing, tourism and industrial waste (Fig.6).

It may be pointed out that urbanisation and population growth are the major reasons for microplastic pollution [83, 84, 85]. House hold waste is a potential source of microplastic fibres as untreated house hold waste consisted of partially digested bags, paints, withered plastic utensils, cosmetics, cloths etc. Untreated sewage may also be an important carrier that conveys fibres to the aquatic system via effluent discharge or surface runoff [86]. Clothing and packaging from surrounding residential areas might be potential sources for these coloured items in the studied areas as reported by Wang et al [74]. In both rivers of present study, natives wash their cloths in river on a regular basis. Washing and dumping of garments discharge microplastic fibres [87, 86, 88]. Lack of proper waste management plan for discharged sewage water from nearby houses contribute to the existing problem of fabric dumping [89].

The tribes and natives inhabiting river banks depend largely on fishing as livelihood means and do supply fish to local markets. Use of modern fishing netting materials is common among them which they frequently replace owing to tearing loss from using in fast flowing waters in rocky areas. Such damaged netting materials are abandoned in rivers which succumb to weathering and biodegradation. Smaller fibres from withered fishing nets are likely to get ingested by fishes. The improper waste management in plantations (rubber, cocoa, plantain and pineapple) result in run off of microplastics during monsoon, result in accumulation of fibres in river waters.

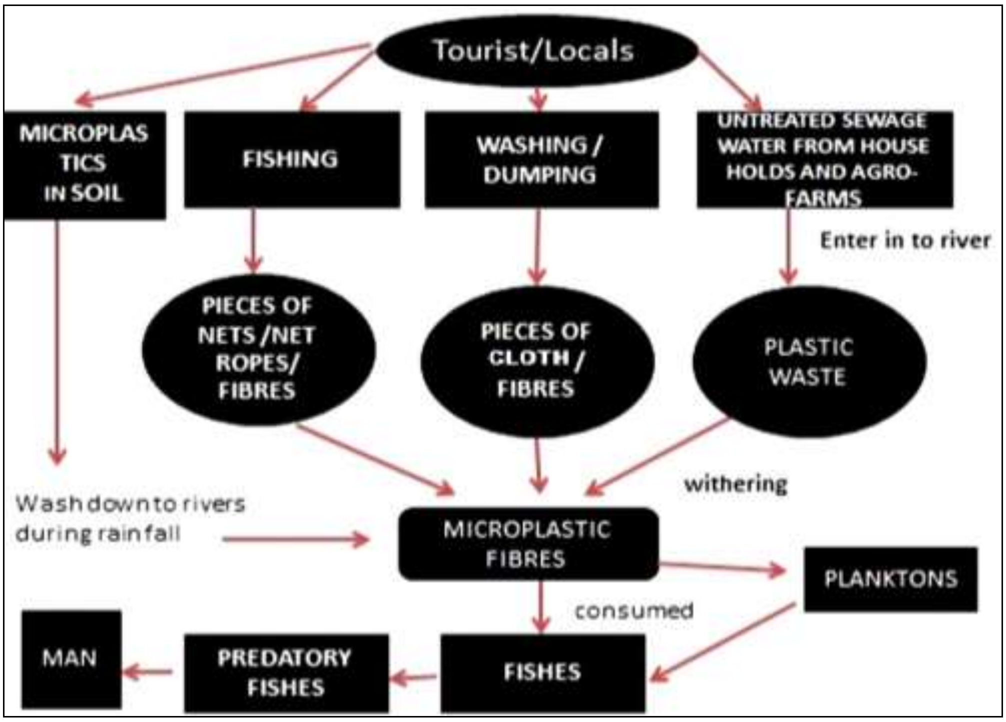


**Fig 6:** Potential pathways of microplastic debris in freshwater fishes of tropical river system

There are some important tourist spots in the rivers Chalakudy and Pooyamkutty. These areas are known for large quantity of plastic debris which due to human negligence gets dumped into these rivers (ie. Cloths, bags, bottles). Need for sustainable eco-tourism plans by giving more thrust on habitat management for healthy sustaining of life have well been recognized by authorities. Reduce, reuse, and recycle strategy might help to resolve the microplastics issue [48].

### 4.2 Strategies to reduce microplastic pollution

The study provides evidence to the fact that even when rivers flow through protected forest they face serious challenge from plastic pollution. Hence, it is important to propose waste management strategies for reducing microplastic pollution. Based on each of the channels of microplastic transmission, habitat management strategies for sustainable maintenance of healthy ecosystem were formulated (Table 2).

**Table 2:** Strategies to reduce microplastic pollution in Western Ghat rivers

| Waste management strategies for reducing plastic fibre discard from household & tourism | Strategies for reducing micro plastic fibre load during fishing | Strategies for reducing micro plastic fibre load originating from textiles |
|---|---|---|
| 1. Educate the communities and official in charge of the area through local government bodies & NGO about the sources of microplastics, its hazards and better management practices during visit and fishing.<br>2. Support plastic litter free zones in upper streams of river and participate in regular river clean-up. Reduce the use of single-use plastics and avoid using cosmetics containing microbeads and paints (road, wall paints) containing microfibers. Encourage the use of natural paints.<br>3. Government must provide effective wastewater treatment facilities in each locality for industrial/household sewage treatment. Advanced biofilter and reverse osmosis filters can be used in public sewage treatment plant.<br>4. Guppy friend washing bags should be introduced to people and its effectiveness and acceptance in rural areas of Kerala need to be subjected to further studies. | 1. Use bio degradable netting/line materials, in fishing gears for fishing.<br>2. Make the stakeholders (native men, fish collectors, tribe's, forest officials) aware about the micro plastics, its potential pathways, probable hazards and good management practices.<br>3. During the months when the water is less the exposed river bottoms must survey and plastic materials should be cleared<br>4. A fishery manager should be entrusted to look after the duties of habitat management in each river. | 1. Encourage the use of natural fibres like silk, cotton, jute and hemp.<br>2. Encourage the installation of ultra-filters in washing machines.<br>3. Create awareness among the people about hazards of washing /dumping cloths in open river |

## 5. Conclusion
The results of present study reveal that even rivers flowing through protected forest are prone to serious challenges from plastic pollution. This paper throws light on the microplastic pollution in Western Ghat river system and consequent challenges to its biota, which necessitate their conservation through implementing better management practices in fish collection and sustainable eco-tourism plans, by giving more thrust on habitat management. Maintaining health of ecosystems is the best way to sustain healthy fish stock in river ecosystems.

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