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MICROPLASTIC CONTAMINATION IS NOW PREVALENT IN EDIBLE FRESHWATER FISHES

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ABSTRACT: Human civilization is moving towards its ends with the ever increasing global warming and pollution. We are witnessing new health hazards every coming day. One of the most concerned issues in present times is the plastic waste. Plastic has reached to the most remote locations in the world and traces of plastic particles are practically everywhere. Large plastic waste is easy to collect and manage but smaller fragments of plastics are emerging as more complicated issue because they largely get unnoticed. Various studies are going on throughout the globe to extract microplastics from soil, aquatic organisms, animals etc. We assessed the presence of microplastic particles in the fishes sold for human consumption in parts of Jaipur, Rajasthan. Fishes with a sample size of n=30 were purchased from the local fish market of Jaipur. All the fishes belonged to same species, Rohu which is one of the most famous and favorite fishes among the non vegetarian population of the area. Fishes were dissected and processed using Alkali-digestion method to digest the organic matter and extract out the microplastics. Following digestion and filtration, microplastics were visually identified and further analyzed using FTIR and SEM techniques. FTIR data showed various peaks indicating the presence of polymers identical to plastics and along with that polysiloxanes were also found in the samples.

Key words: - Microplastics, polysiloxanes, plastic microbeads, microscopy, spectroscopy, fish intake

INTRODUCTION :

Microplastics are the tiny plastic granules of <5mm in diameter but larger than 1μm (Barnes et al., 2009, Hartmann et al., 2019). These are used in cosmetic industries as scrubbers in bulk amount (Kazour et al., 2019). A wide range of daily use items like face wash, toothpaste, face scrubs, soaps, anti-ageing skin creams etc contains micro beads made up of microplastics (Hartmann et al., 2019). Apart from these, microplastics are also formed from breakdown of large plastic items like kitchen utensils, tyres, fishing equipments, paints etc (Wolff et al.,2019). Because of lack of vigilance and unmanaged waste disposal, these waste materials are dumped in open water. These tiny plastic particles get accumulated in the oceans, seas, rivers, lakes and other water bodies (Briassoulis et al., 2019). Studies suggest that while on one hand large plastic debris cause injury to marine flora (Barnes et al., 2009) on the other hand tiny plastic beads are consumed along with food by the fishes and planktons (Moore, 2008). These microplastics get entry in the food chain and ultimately find their way to the human body.

Once a fish ingests the microplastic particles, a) it can cause blockage of gastrointestinal tract leading to feeling of false satiation or b) the plasticizers and additives from the microplastic particles can leach out inside the fish body or c) the third probability is desorption of pollutants which are found bounded with MPs (Strungaru et al., 2019) Other effects which have been reported are changes in feeding rates, gene expression, development, physiology , movement, survival potential etc. (Jovanović, 2017; Wang, X. et al., 2019).

Although researchers throughout the globe are working on this topic but still there is a lack of standard methodology of extracting the microplastics and analysis of the microplastics. Till now no full proof extraction procedure is available through which actual amount of microplastics can be retrieved and analyzing techniques are also ambiguous. Because of lack of universal standard risk assessment becomes more difficult (Toussaint et al., 2019). In short, it can be stated that we are taking baby steps in this direction and this field requires in-depth and continuous studies in future.

In South India, various projects are going on for extraction and evaluation of microplastics in the fishes and other seafood, soil and water also. But no significant work has been done in the Northern and Western states and our study is one of the pioneer studies in this area. Rajasthan is a Western state in India. Most of the part of this state is covered by desert but the Jaipur belt is one of the most habited areas and a significant population of the area is non-vegetarian. People consume fishes and Rohu is one of the most sold out fish in this area. So we choose this species as our research subject. Our study focused on the local market fishes in Jaipur. We collected the sample from local vendor Mashallah Meat Corner, Pratap Nagar, Jaipur. Source of those fishes was Bisalpur dam which is a nearby fish production unit and water quality in that dam is considered to be good and sustainable for aquaculture. We took fishes from there to check the ground reality. Major focus of this study was to show the presence of microplastics in the fish tissues so that further studies can be done on a broader level.

Materials and Methods:

Collection of sample

A total of 30 fishes were purchased from the local fish market of Jaipur, Rajasthan from February to April (a set of 10 fishes at a time). We selected Rohu fish for our study because it is one of the most favored sea foods among the locals due to its reasonable rates and desirable taste. The fishes were wrapped in aluminum foils and put in ice box while being transported to the university lab. After that they were kept at -20°C until further processing.

Contamination check

All the necessary precautions were taken to minimize the contamination of the sample during processing. Working station was cleaned with alcohol before dissection and all other processing. Medical grade nitrile gloves were used throughout. Glassware was thoroughly washed each time before use. Along with this, a quality control blank was also put to check the contamination through air. Not any visible microplastic compound was found over the blank filter paper.

Dissection process and preparation of sample

All the fishes ranged from 30 cm to 38 cm in body length and 260 g to 380 g in body weight. Fishes were washed properly before dissection to remove any kind of external plastic or any other toxic particle from the bodies of fishes. Dissection was done after filleting, that is, removing the scales with the help of sharp razor. Inedible tissues (Gastrointestinal tract and gills) and edible tissues (muscles and skin) were kept in different beakers and covered properly before further processing. Both the tissues from each fish were weighed and transferred to separate conical flask of 250ml each. After that 10% KOH was added to each flask (volume of KOH into each flask was three times more the volume of tissues). For making 10% KOH, firstly 10g KOH pellets were added to 50 ml distilled water and then the volume was raised to 100 ml by adding distilled water to the solution. KOH acts as a dissolving agent for the tissues. This method of using KOH to digest the tissues is known as Alkali-Digestion method of microplastic extraction (Karami et al., 2017, Dehaut et al., 2016). Edible tissues were incubated at 60°C for 24hrs (Dehaut et al., 2016) and inedible tissues at 40°C for 72 hrs with continuous shaking after every 24 hrs (Karami et al., 2017). After incubation, filtration step was done for each sample. Whatman filter paper grade 540 hardened ashless were used for filtration process. Gravity filtration was done in which filter paper cone were set and the samples were slowly poured into those cones. After complete filtration, filter papers were air dried. The filtrate was discarded and filter papers were air dried for further sampling which included light microscopy, FTIR and SEM.

Separation of Microplastics from organic matter

Microplastics were separated from other organic compounds under the microscope by looking at their surface, plastic particles are shiny and sharp in comparison to any organic matter and second confirmation was done by hot-needle test. Microplastics tend to change their shape by bending or shrinking when touches with a hot needle.

Result and Discussion

Various types of microplastic particles including fragments, films and fibers were found in both the edible and inedible tissues with an average 20.1 particles in muscle tissue/fish, 14.67 particles in skin tissue/fish and 16.5 particles in inedible tissues/fish. Out of the total microplastic particles being observed, 62% was microplastic fibers, 25% microplastic films/sheets and 13% microplastic fragments. While fibers were distinguish clear, fragments were separated from the plastic sheets/films by the shape and luster. Plastic films were more shiny and sharper than fragments. Color of the fibers varied from black, brown, red, green, blue and whitish. FTIR analysis of the samples produced peaks indicating polymer of polyethene standard. Polysiloxanes functional group was found in nearly half of the total samples of FTIR. SEM analysis of the selected samples showed microplastic fiber embedded in the mesh of filter paper.

Microscopic examination of the filter papers revealed a large number of microplastic fibers, fragments and films being spread all over the filter papers. In total extraction and filtration process a total of 1541 microplastic particles were found out of which 955 were microplastic fibers, 386 were microplastic films and 200 were microplastic fragments. We randomly chose 15 samples for FTIR analysis, 5 samples each from one type of tissue.

FTIR result indicated peaks which were similar to polymers like polyethene and other plastic polymers. Along with this, there were also present peaks of a distinct compound- polysiloxanes. Polysiloxanes are a class of rubber-like compounds which are identical to plastic in chemical composition and structure. They are often called liquid-plastic. Many scientists consider siloxanes as a distinguished class of plastics. Polysiloxanes or commonly silicone is widely used in pharmaceutical and medicals science. It's one of the most important application is in breast and butt implants.(Xu et al., 2015) But some studies showed that direct contact of body with silicone is harmful for the body in long-term and it may be carcinogenic (Wang et al., 2013). It may enter the respiratory system because of its volatility (Xu et al., 2015). Change in structure of lipid- bilayer (Mojsiewicz-Pieńkowska et al., 2015) or weakening of the cell membrane barrier (Glamowska et al., 2015) could be the possible effects if body is in direct contact with the silicone. But on the other hand there is another school of thought who emphasizes that medical grade silicone is not toxic or its toxicity is very low so it is not a threat of human system (Mojsiewicz-Pieńkowska et al., 2016). Either way, we cannot deny the fact that silicone is reaching inside human body with their diet.

Scanning Electron Microscopy is one of the most useful techniques for getting morphological details of the required sample. But because of its high cost and limited availability we could only process two of our samples by SEM. Both the samples were randomly selected without any biased choice. Fiber of microplastic particle was seen embedded in the filter paper mesh. It was distinct from its background and clearly visible

CONCLUSION :

We started our research with the hypothesis that microplastics could be present in the edible fishes of this area because of the under administered waste disposable in the area. We were able to extract the microplastic particles in each and every fish taken into process. Size of these contaminants marks their easy entry into our food. Practically no present technique is available to remove these contaminants from the fishes prior to eating. As a result microplastics are entering into human bodies with their food. Now when studies have shown presence of microplastic particles in human placenta (Ragusa et al., 2021 our food is also not safe anymore. Practically, we are eating and drinking plastic. Further studies are required to know the long term effects of consuming microplastics particles. Though worldwide study is going on in this area still the effects of microplastics in the living organisms are still ambiguous and this field requires a deep down approach for research and spreading awareness among the community.

ACKNOWLEDGEMENT: Funds for techniques and lab requirements for this project have been maintained by the JRF scholarship granted to the PhD researcher by the University Grant Commission, India.

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We are thankful to the Jaipur National University Campus. All the dissection and tissue storage work has been done in the labs of SIILAS Campus of Jaipur National University.

STATEMENT FOR CONFLICT OF INTEREST:

We hereby declare that there is no conflict of interest in this study.

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Figure 1: Microplastic fibers of different colors seen under the microscope at 10X (image A to F)

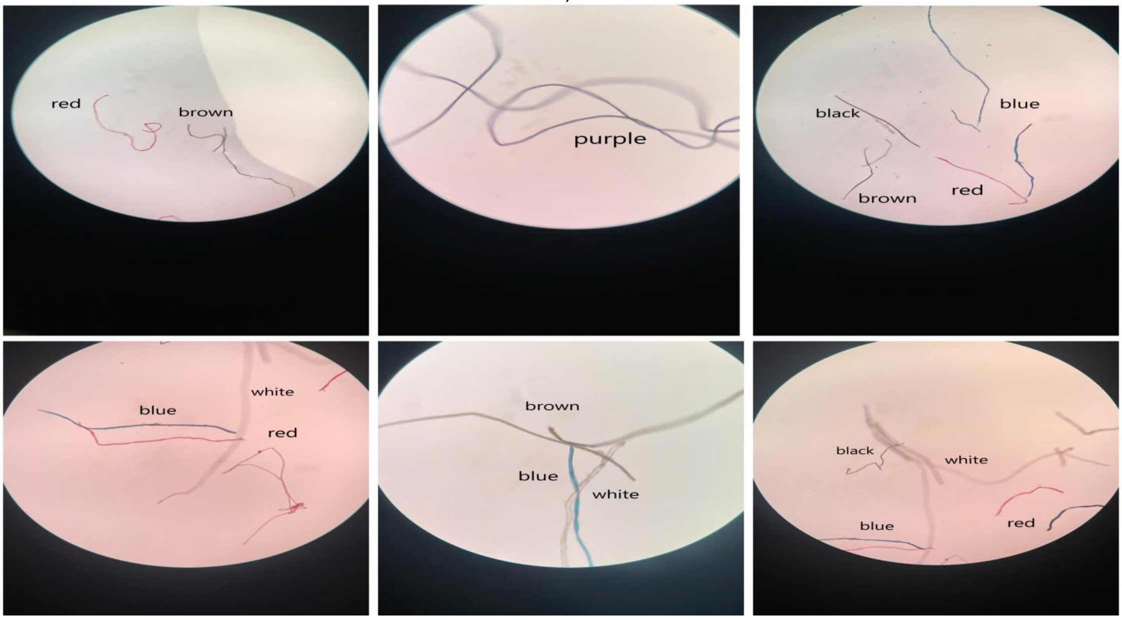

Figure 2: Microplastic fibers with characteristic colors seen at 40X magnification.

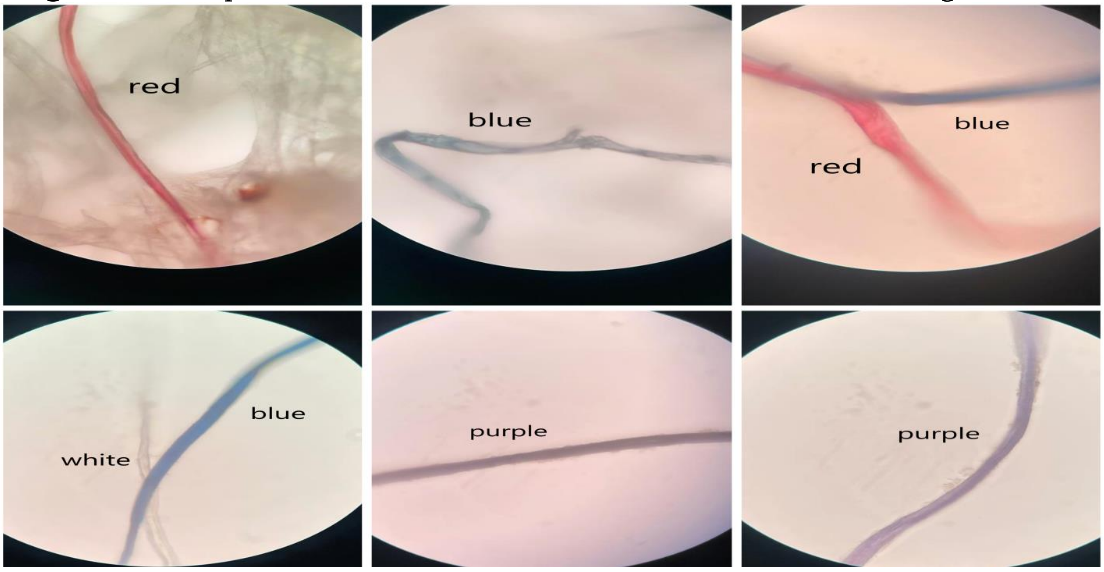

Figure 3: Types of microplastic particles extracted from the sample fishes: films (a to c), fragments (d and e) and fibers (f and g)

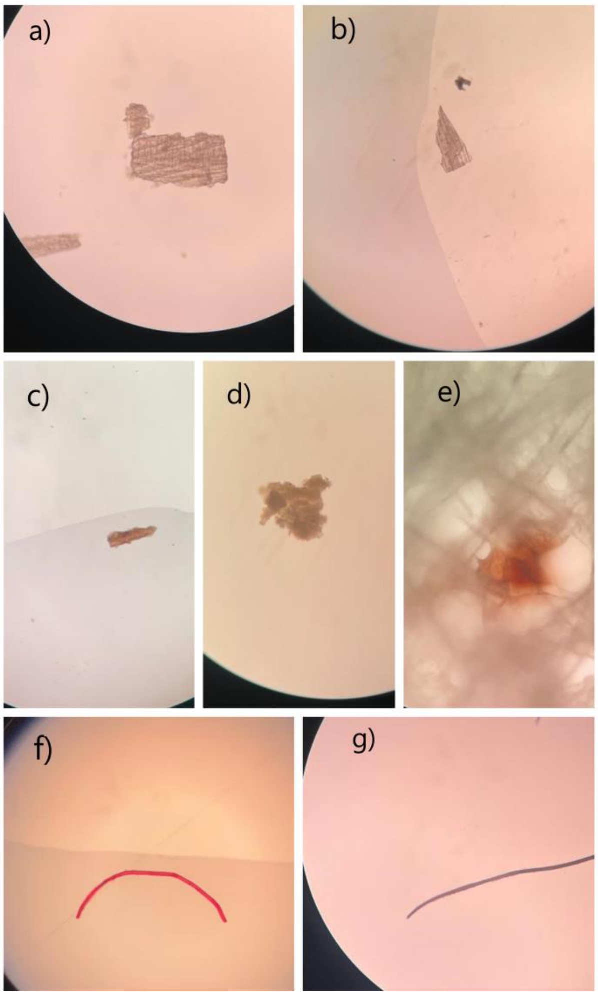

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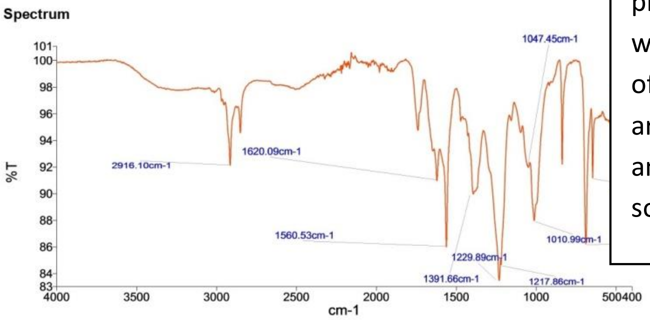
Figure 4: FTIR plots of various samples being analyzed to see the presence of particular polymers and respective functional group analysis:

Figure 4a) Following is the FTIR plot of skin tissue and functional groups present in the given sample. Along with other distinct functional groups of polymers, silanols and siloxanes are also present. Functional group analysis is done using Lab Cognition software.

PerkinElmer Spectrum
Saturday, May 2



Functional Group Analysis of 'SKIN.asc' 9:12:00 PM 7/21/2022

Operator: delight
Department:

LabC0gnitian
Analytical Software GmbH & Co. KG

SKIN.asc

Matching Functional Groups

Quality	Functional Group	Chemical Sub Class	**Chemical Class**
100	Aliphatic Ester, Acetate		**Aliphatic Ester**
100	Linear Bromo Compound	Aliphatic Brominated Compound	Aliphatic Halogen Compound
95	Aliphatic Ester - Carbonyl Compound		Aliphatic Ester
92	Aliphatic Amine Salt, Primary, Hydrochloride		Aliphatic Amine
91	Aromatic Sulfoxide or Ether	Aromatic Sulfoxy Compound	Aromatic Sulfur Compound
91	**Silanol**	**Silanols**	Aliphatic Silicon Compound
91	Unsaturated Hydrocarbon, Cis Alkene		Alkene
91	Unsaturated Hydrocarbon, Cyclic,> C5		Alkene
90	Aliphatic Sulphoxide	Aliphatic Sulfoxy-compound	Aliphatic Sulfur Compound
87	CF3 group	Fluorine compounds	Organic Halogen Compounds
86	Primary Alcohol		Aliphatic Alcohol
85	Polysiloxanes	Siloxanes	Aliphatic Silicon Compound
82	Aliphatic Mercaptan, Branched, Methyl	Aliphatic Thiol	Aliphatic Thiocompound
81	Hydroxy, Possibly 1,2-Diol		Aliphatic Alcohol
80	Alkoxy Substituent	Substituent Group	Side Chain or Substituent
80	N-MethylAmino Substituent	Substituent Group	Side Chain or Substituent
80	Tertiary Alcohol, Possibly 2-OH and Methyl		Aliphatic Alcohol
79	Aliphatic Amino Acid - Carbonyl Compound		Aliphatic Amino Acid
77	Aromatic Thiol or Mercaptan, General		Aromatic Thiol
77	Aryl Subs. Possibly Phosphine	Aryl Group	Aromatic Compound
77	Aryl Substituted Aliphatic Ether	Aryl Substituted Ether	Aromatic Ether
75	Aliphatic Amine, Primary, Branched		Aliphatic Amine
75	Unsaturated Hydrocarbon, Ether Conjugated		Alkene
74	Aliphatic Ether or Sulfoxide	Substituent Group	Side Chain or Substituent
74	Hydroxy Compound	Substituent Group	Side Chain or Substituent
74	Possibly Primary Hydroxy	Substituent Group	Side Chain or Substituent

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PerkinElmer Spectrum Version 10.4.00
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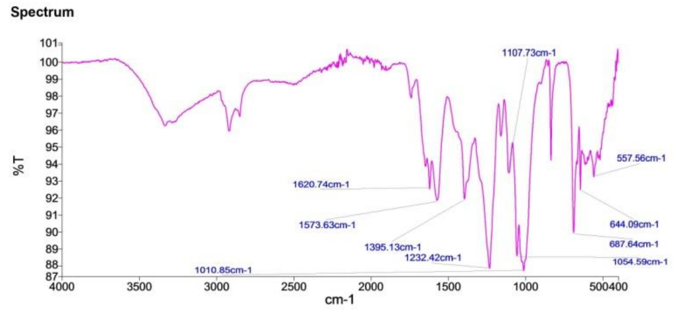

Figure 4b): Following is the FTIR plot and functional group analysis of the muscle tissue sample taken randomly. This sample also showed the presence of polysiloxanes and silanols as characteristic functional groups.

Functional Group Analysis of 'MUSCLE2.asc' 9:08:46 PM 7/21/2022

Operator: delight
Department:

LabC0gnitian
Analytical Software GmbH & Co. KG

MUSCLE2.asc

Matching Functional Groups

Quality	Functional Group	Chemical Sub Class	Chemical Class
100	Polysiloxanes	**Siloxanes**	**Aliphatic Silicon Compound**
100	Primary Alcohol		Aliphatic Alcohol
99	**CHF2**	Fluorine compounds	Organic Halogen Compounds
99	Linear Bromo Compound	Aliphatic Brominated Compound	Aliphatic Halogen Compound
98	Aliphatic Ester, Acetate		Aliphatic Ester
98	Aliphatic Mercapto Group	Substituent Group	Side Chain or Substituent
98	Hydroxy, Possibly 1,2-Diol		Aliphatic Alcohol
94	Aliphatic Carboxylate, Possibly Acetate		Aliphatic Carboxylate
91	Unsaturated Hydrocarbon, Cis Alkene		Alkene
91	Unsaturated Hydrocarbon, Cyclic,> C5		Alkene
90	Aliphatic Ester - Carbonyl Compound		Aliphatic Ester
89	Aliphatic Tertiary		Aliphatic Amide
89	**Silanol**	Silanols	Aliphatic Silicon Compound
83	Aliphatic Amino Acid - Carbonyl Compound		Aliphatic Amino Acid
83	Aliphatic Mercaptan, Branched, **Methyl**	**Aliphatic Thiol**	Aliphatic Thiocompound
78	Hydroxy Compound	Substituent Group	Side Chain or Substituent
77	CF3 group	Fluorine compounds	Organic Halogen Compounds
75	Aliphatic Primary Amide		Aliphatic Amide
74	Unsaturated Hydrocarbon, Ether Conjugated		Alkene
73	Unsaturated Hydrocarbon, Trans Conjugated		Alkene
70	**Aliphatic Alkoxy, Methoxy**		Aliphatic Ether

Corresponding Chemical Classification Rules

Functional Group: 'Polysiloxanes', Chemical Class: 'Aliphatic Silicon Compound', Chemical Sub Class: 'Siloxanes'

Vibration	Start WN	EndWN	Threshold	Priority
Si-C Stretch	845	780	Strong	Mandatory
Si-O Stretch	1100	1000	Very Strong	Mandatory
C-H Stretch	2975	2900	Variable	High

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PerkinElmer Spectrum Version 10.4.00
Saturday, May 21, 2022 5:00 PM

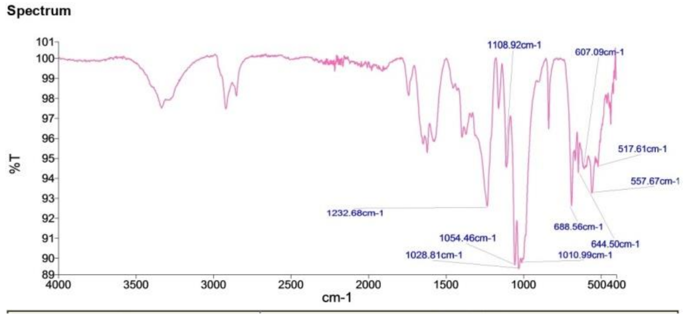

Figure 4c) Following is the FTIR data and functional group analysis of inedible tissue sample taken randomly. Here also characteristic siloxanes have been highlighted.

Functional Group Analysis of 'IT 2.asc' 9:10:58 PM 7/21/2022

Operator: delight
Department:

LabC0gnitian
Analytical Software GmbH & Co. KG

IT 2.asc

Matching Functional Groups

Quality	**Functional Group**	**Chemical Sub Class**	**Chemical Class**
100	**Aliphatic Hydroxy, 2-Amino**		**Aliphatic Amine**
100	Hydroxy, Possibly 1,2-Diol		Aliphatic Alcohol
100	**Polysiloxanes**	**Siloxanes**	Aliphatic Silicon Compound
100	Primary Alcohol		Aliphatic Alcohol
98	Methyl Mercapto	Aliphatic Thioether	Aliphatic Thiocompound
97	Aliphatic Tertiary		Aliphatic Amide
86	Aliphatic Ester, Acetate		Aliphatic Ester
81	Linear Bromo Compound	Aliphatic Brominated Compound	Aliphatic Halogen Compound
79	Aromatic Amino Acid		Aromatic Amino Acid
78	Alkoxy Substituent	Substituent Group	Side Chain or Substituent
78	Linear Chiaro Compound	Aliphatic Chlorinated Compound	Aliphatic Halogen Compound
77	Hydroxy Compound	Substituent Group	Side Chain or Substituent
77	**Silanol**	**Silanols**	Aliphatic Silicon Compound
75	Unsaturated Hydrocarbon, Cyclic,> C5		Alkene
74	Aliphatic Amido, Possibly Subs. Urea		Aliphatic Amide
74	Aliphatic Ester - Carbonyl Compound		Aliphatic Ester
73	Aliphatic Amine, Primary, Branched		Aliphatic Amine
71	Methoxy Substituent	Substituent Group	Side Chain or Substituent
70	Aliphatic Alkoxy, Methoxy		Aliphatic Ether
66	CF3 group	Fluorine compounds	Organic Halogen Compounds
58	Aliphatic Primary Amide		Aliphatic Amide
58	Unsaturated Hydrocarbon, Ether Conjugated		Alkene

Corresponding Chemical Classification Rules

Functional Group: 'Aliphatic Hydroxy, 2-Amino', Chemical Class: 'Aliphatic Amine'

Vibration	**Start WN**	**End WN**	**Threshold**	Priority
O-H/N-H Deformation	855	820	Variable	Mandatory
C-O Stretch	1075	1025	Very Strong	Mandatory
O-H Deformation	1400	1300	Strong	Mandatory
C-H Bend, CH2/CH3	1475	1450	Variable	Mandatory
N-H Bend	1605	1580	Variable	Mandatory

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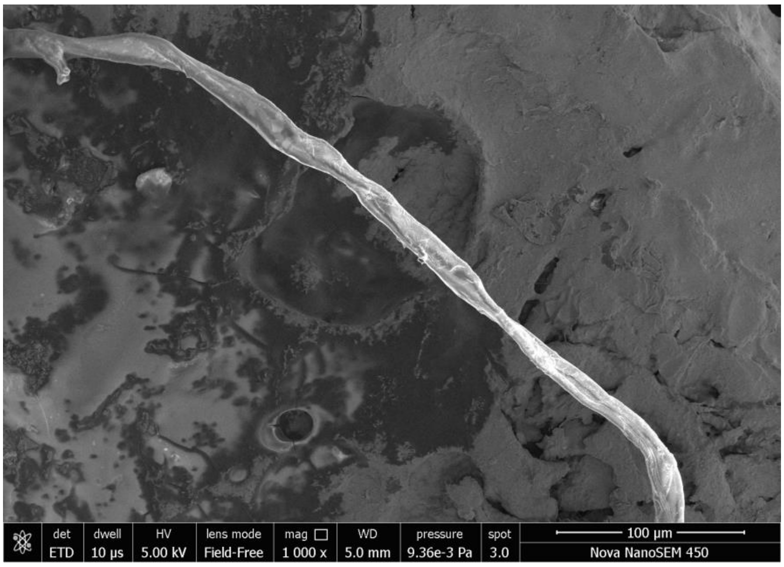
Figure 5: SEM image of the microplastic fiber sample taken at 2500X (a) and 1000X (b) magification respectively. Microplastic fiber is seen embedded over the filter paper membrane.

a)



b)

Figure 6: Pie chart for the percentage of different types of microplastic particle.

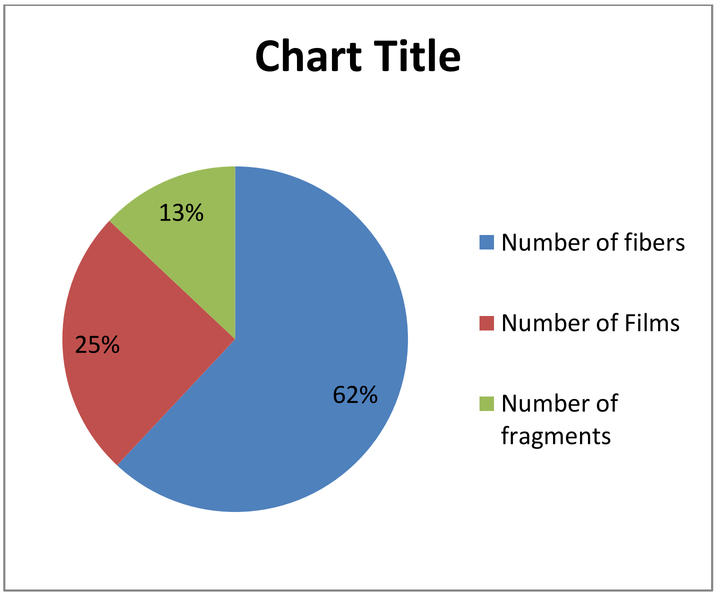


Figure 7: Pie chart for the percentage abundance of different colors of microplastic fibers in the sample.

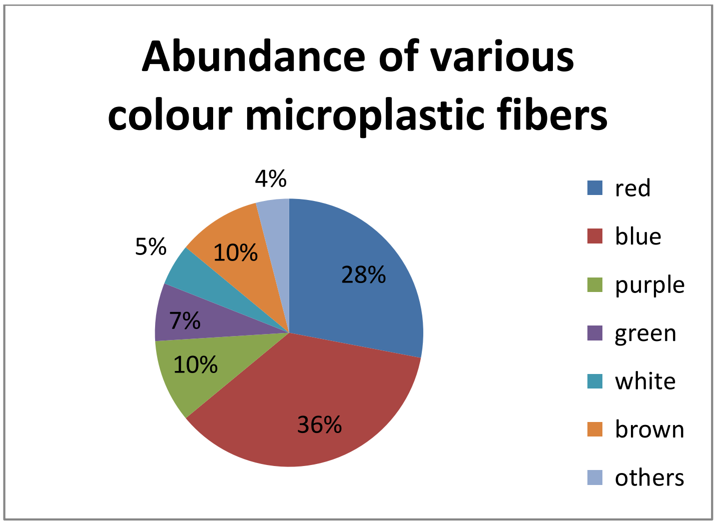


Table 1: Data of total number of microplastic particles in the fishes serial wise.

Fish No.	Number of Microplastics		
	Muscles	Skin	Inedible Tissue
1	15	10	6
2	20	12	23
3	12	16	19
4	16	11	16
5	18	9	11
6	31	6	19
7	13	22	23
8	19	15	20
9	16	21	15
10	28	17	13
11	22	14	18
12	17	13	11
13	20	18	9
14	10	19	22
15	16	15	14
16	18	13	17
17	21	16	20
18	33	11	14
19	28	16	22
20	13	20	18
21	10	6	15
22	27	18	19
23	31	21	20
24	22	13	11
25	26	6	5
26	22	12	11
27	17	23	15
28	19	22	32
29	21	12	15
30	22	16	22
Total	603	443	495
Average	20.1	14.76667	16.5

Table 2: Data depicting the percentage abundance of different types of microplastics.

Total Number of Microplastic particles	Number of fibers	Number of Films	Number of fragments
1541	955	386	200
Percentage	62%	25%	13%