

# **ISOLATION AND IDENTIFICATION OF MICROPLASTIC AND HEAVY METAL FROM MARINE BIOTA AND SEDIMENTS**

Submitted in partial fulfillment for the award of  
Bachelor of Technology degree in Biotechnology

**By**

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**INSTITUTE OF SCIENCE AND TECHNOLOGY**

**(DEEMED TO BE UNIVERSITY)**

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## **DEPARTMENT OF BIOTECHNOLOGY**

### **BONAFIDE CERTIFICATE**

This is to certify that this Project Report is the Bonafide work of **Manikandan M (40230025)**, who carried out the project entitled **"ISOLATION AND IDENTIFICATION OF MICROPLASTIC AND HEAVY METAL FROM MARINE BIOTA AND SEDIMENTS"** Under our supervision from **September 2023 to April 2024**.

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## DECLARATION

I, Manikandan M (**40230025**), hereby declare that the Project Report entitled **“Isolation and Identification of Microplastic and heavy metal from Marine Biota and Sediments”** done by me under the guidance of **Dr. Indumathi S M** (Internal) and **Dr. T. Stalin Dhas** (External) at **Sathyabama Institute of Science and Technology, Chennai, Tamilnadu, India**, is submitted in partial fulfillment of the requirements for the award of **Bachelor of Technology degree in Biotechnology**.

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## **ABSTRACT**

The widespread distribution of microplastics in regularly ingested fish, soil, water, and sediments is a topic that requires further research due to the paucity of information regarding their presence in marine environments. According to studies, drainage carries typical plastic into the marine environment, where it breaks down into smaller pieces known as microplastics due to frigid temperatures and UV radiation. Microplastics are frequently composed of polystyrene (PS), nylon, polyethylene terephthalate (PET), polyvinyl chloride (PVC), and other materials. Here, we investigated the potential presence of MPs in the organs (gill, gut, skin, and muscle) of commonly consumed fish, crabs, and clams. We assess a variety of techniques, such as dissection, depuration, digestion, density separation, and filtration, for removing microplastics that have been consumed by biota. Microplastics have been characterized using stereo zoom microscopy and SEM in terms of size, morphology, and chemistry. Stereo Zoom microscopy made it possible to quantify the particle size variations and fiber lengths as well as classify the sorts of fragments or fibers present in the sample morphologically and Using SEM analysis, non-plastic particles were eliminated, and possible microplastics were screened out of the processed samples based on their surface features and element signatures. The MP chemical composition was then determined using Raman spectroscopy. These microplastics can also act as carriers of other contaminants within aquatic species' bodies, which then make their way up the food chain. Also, this study was to inquire about the presence of heavy metals in various aquatic environments. Heavy metal concentrations, including zinc, selenium, lead, mercury, cadmium, chromium, manganese, iron, and arsenic, have been measured using analytical methods. The heavy metal content has been measured using atomic absorption spectroscopy. Here, we recommend that future research measure the amounts of anthropogenic particles in fish tissues that are edible.

### **Key words**

Microplastics; Marine pollution; SEM; Raman spectroscopy;

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## LIST OF ABBREVIATIONS

|        |                                              |
|--------|----------------------------------------------|
| Zn     | Zinc                                         |
| Se     | Selenium                                     |
| Pb     | Lead                                         |
| Hg     | Mercury                                      |
| Cd     | Cadmium                                      |
| Cr     | Chromium                                     |
| As     | Arsenic                                      |
| Mg     | Magnesium                                    |
| Fe     | Iron                                         |
| g      | Gram                                         |
| mL     | Milliliter                                   |
| mm     | Millimeter                                   |
| µg     | Microgram                                    |
| min    | Minutes                                      |
| hrs    | Hours                                        |
| PCBs   | Polychlorinated biphenyl                     |
| XRD    | X-ray diffraction                            |
| AAS    | Atomic absorption spectrometry               |
| FAAS   | Flame atomic absorption spectrometry         |
| WHO    | World Health organization                    |
| PET    | Polyethylene terephthalate                   |
| FTIR   | Fourier-transform infrared spectroscopy      |
| ICP-MS | Inductively coupled plasma mass spectrometry |
| SEM    | Scanning Electron Microscope                 |

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## CHAPTER 1

### 1. INTRODUCTION

Plastics are versatile synthetic materials that are lightweight, affordable, and manufactured from a wide variety of polymers. Plastics deteriorate naturally over time in freshwater and oceanic ecosystems due to factors like sun exposure, abrasion, and contact with both inorganic and organic matter and other living things and break down into microscopic components known as microplastics. Microplastics (MPs) are fragments, threads, or beads with the longest dimension of less than 5 mm (about 0.2 in) that can be classified as primary or secondary microplastics. (Cole et al., 2014) Secondary microplastics are produced by breakage and mechanical, chemical, biological, and degrading processes, while primary microplastics are generated with sizes that fall within a reasonable range. (Frias & Nash, 2019; J. Li et al., 2018)



***Fig 1.1 Microplastics in marine environment***

A broad variety of aquatic biota, including those sold for consumption by people, like fish and mussels, have been polluted because of the extensive distribution of MPs in aquatic bodies (Karami et al., 2017). Our findings show that just 22.9 percent (44) of the world's 192 nations have conducted studies on microplastics. Studies on the impacts of microplastics on creatures have primarily focused on fish (38%), whereas those on other heavily impacted species like turtles (1%) are poorly documented (Ajith et al., 2020)

With our experimental setup We first aim to isolate and characterize the microplastics from marine biota in various feeding zones (Lehtiniemi et al., 2018). It is anticipated that during the coming years, the body of scientific literature regarding marine microplastics will continue to grow, considering the significance of the microplastics issue for the marine environment (Pauna et al., 2019)

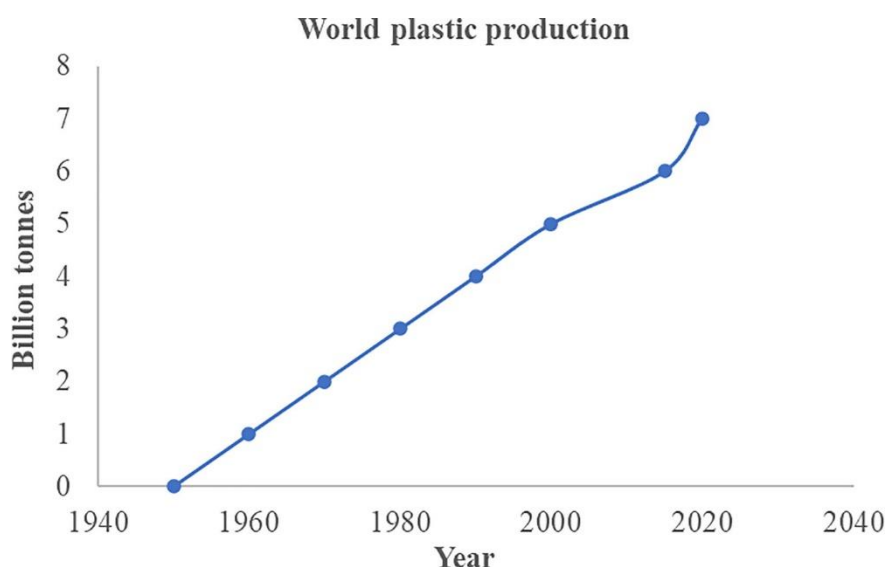
## **1.1 HEAVY METALS**

The toxicity, persistence, and bioaccumulation of heavy metals—a class of elements with high atomic weights—have made them regarded as serious environmental hazards. Fish provide a health concern to humans who eat them since they are known to accumulate and multiply heavy metals in their tissues, among other organisms. According to studies, several fish species can accumulate heavy metals like cadmium, lead, mercury, and arsenic, which can be extremely dangerous to human health. The primary cause of fish heavy metal buildup is prolonged contact to contaminated water, sediments, and food sources. Analysis was done on the buildup of heavy metals in fish samples taken from Pakistan's Sutlej River. The findings indicated that the amounts of heavy metals in the fish species under investigation—*Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*—were significantly higher than the World Health Organization's (WHO) acceptable limits. (Khan et al., 2019) Marine animals are seriously threatened by heavy metal pollution in their surroundings because these metals can build up to toxic amounts in their tissues. Atomic absorption spectroscopy (AAS) has become a potent analytical method for identifying and measuring heavy metals in marine life in order to address this problem. The capacity of AAS to identify heavy metals in a variety of marine species, such as fish, shrimp, and crab. Using AAS, the scientists examined the levels of several heavy metals, such as lead, cadmium, and mercury, in the various organs of these species. The findings demonstrated that the quantities of these metals differed considerably amongst the various species and tissues that were examined, underscoring the significance of carrying out thorough investigations to assess heavy metal pollution in marine environments.

## **1.2 GLOBAL SCENARIO**

There are warning signs of micro plastic pollution across the world's environment, especially in marine environments and freshwater (Peng et al., 2017). Comparatively

speaking to other regions, Asia has substantially lower per capita plastic material use. With 59.08 tons of plastics produced worldwide, China leads the field, followed by the USA (37.83 tons), the German nation (14.48 tons), Brazil (11.85 tons), Japan (7.99 tons), Pakistan (6.41 tons), Nigeria (5.96 tons), Russia (5.84 tons), Turkey (5.6 tons), and Egypt (5.46 tons)(devi et al., 2022)(Muniasamy et al., 2021). Because of the extremely high concentrations, the figures on micro-plastic dispersion in the world's marine environments are very alarming, which raises questions, particularly considering the potential effects on aquatic life of such a vast distribution (Issac & subramanian, 2021)



**Fig 1.2 World plastic production**

### 1.3 INGESTION AND IDENTIFICATION OF MICROPLASTICS

Many marine taxa belonging to different trophic levels have been shown to consume microplastics. This variety includes mammals, seabirds and fish (Besseling et al., 2015). Microplastic particles are also causing harm to zoobenthic creatures, including annelids, bivalve mollusks, sea cucumbers, crustaceans, daphnia and fish larvae (Steer et al., 2017) In another study, the consumption of plastic fragments was documented, and the potential impact of this pollution on the health factor of the Laguna estuary system's catfish, *Genidens genidens*, was noted (Dantas et al., 2019). Thus, as part of environmental monitoring, it's critical to report on the issue of plastic contamination in

fisheries resources. To enhance our understanding of how to address these issues brought about by the pollution of plastics, it was proposed that the trophic guild of the species affects the amount of microplastic that is consumed. (Elliott & Quintino, 2007)

For the evaluation of microplastics (MPs), fish tissues were digested using the method of (Rochman et al., 2015), with modifications. In brief, the samples were treated with potassium hydroxide and incubated with constant shaking at 40 °C for 48 h. The digested samples were then placed in beakers, NaCl was added up to the beaker limit and left to stand to allow MPs to float to the surface. Subsequently, it was subjected to filtration through cellulose nitrate membranes. Finally, the samples were oven dried for visualization and identification of MPs in the stereo microscope and examined by scanning electron microscopy (SEM). SEM is not commonly applied to determine the type of polymer of the suspected MPs particles, but it allows to determine the elemental composition of the suspect particles and finally the Microplastic chemical composition was then determined using Raman spectroscopy.

This study presents fresh perspectives on the various biological responses of marine organisms to microplastic pollution as well as on variables that may eventually endanger species that build habitats and feed on suspended matter.

## **CHAPTER 2**

### **2.REVIEW OF LITERATURE:**

#### **2.1 FATE OF MICROPLASTICS IN WATERBODIES:**

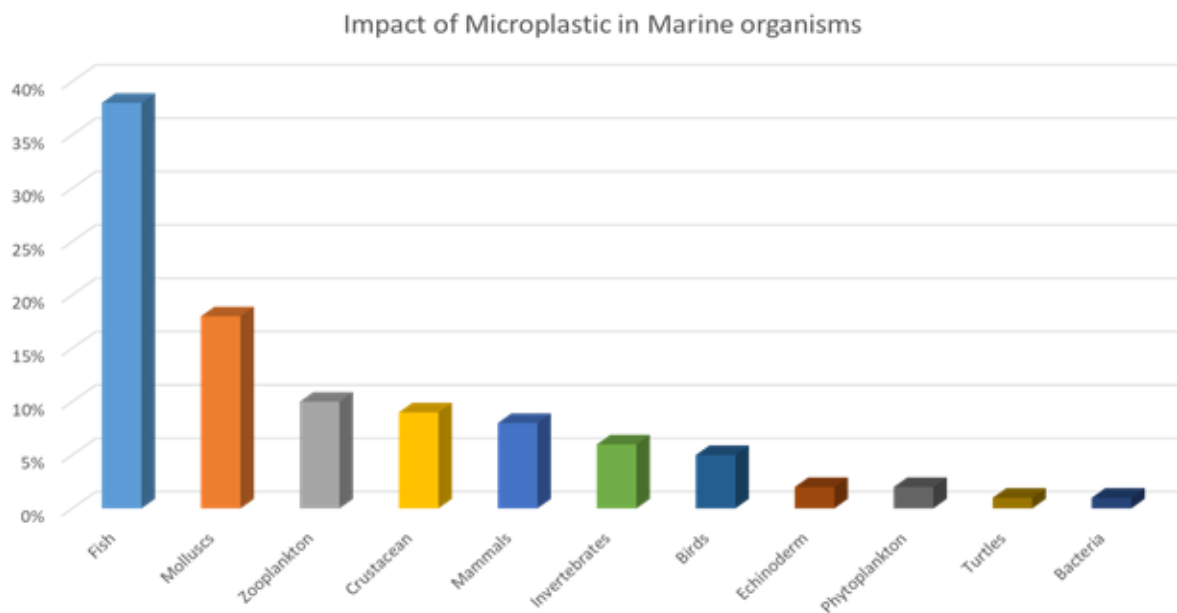
The prevalence of microplastics in marine ecosystems is caused by a variety of human activities, including tourism, municipal services, and industrial wastewater treatment systems (Dikareva & Simon, 2019). When examining the lifespan of microplastics, the marine environment serves as a major sink. Microplastics added to the ecosystem will eventually either build up along the coastline or sink to marine debris in the top layer of the ocean (Luo et al., 2019). Additionally, microplastics could bind and transport several types of ocean contaminants, acting as a vector that can cause the bioaccumulation of different hydrocarbons, dyes, heavy metals, etc. in the oceans (Amelia et al., 2021). With the knowledge of the facts and studies, it is possible to draw the conclusion that microplastics are one of the most serious emerging pollutants of the twenty-first century and pose a significant health danger to aquatic animals as well as other ecosystems related to aquatic organisms.

##### **2.1.1 FATE OF MICROPLASTICS INGESTED BY MARINE ORGANISM**

Ingestion was the primary uptake channel for microplastics in the initial research on this subject, which concentrated on the eco-toxicological consequences of all kinds of microplastics on animals. Researchers have investigated the distribution, excretion, bioaccumulation, and trophic transmission of ingested microplastics as well as their eco toxicological impacts (Franzellitti et al., 2019) (Guzzetti et al., 2018). Microplastics can be ingested by marine organisms and cause harm if they are absorbed. Ingestion of microplastics has been found to have negative effects in laboratory experiments (Fossi et al., 2016). Additionally, POPs and heavy metals can be absorbed by marine species and the environment using microplastics, which can similarly decrease invertebrate feeding activity (Brennecke et al., 2016; Chua et al., 2014). Finally, organisms that have consumed microplastics and still contain microplastics inside of them could eventually be consumed by animals at higher levels in the food chain, spreading the microplastics to additional trophic-level animals.

## 2.2 Impact of Microplastics on Marine life:

Numerous aspects related to marine organisms and the food chain that surrounds them will be impacted by marine microplastics. Microplastics may reduce food intake, delay growth, cause oxidative damage, and cause aberrant behavior in fish and other marine life (Y. Li et al., 2021)



***Fig 2.1 Impact of Microplastic in Marine Organisms***

### 2.2.1 EXPOSURE

For marine creatures, bathing contact and ingestion are the two main ways of MP exposure. The most frequent contact approach in MP biological analyses of natural marine settings is, of course, bathing, which makes it possible to analyze the many negative effects induced by microplastics, which on the marine life through touch (Wright et al., 2013). The effects of MP exposure on specific physical or biological processes have been explored. Most of the research carried out on MPs' effects on marine organisms has been conducted in laboratories. Much of the fish utilized in the MP being exposed testing were from the sea, albeit they came from a variety of settings. Following intake, MPs may accumulate in the fish's gastrointestinal system and cause blockages along the digestive

tract and restrict feeding due to hunger (Lusher et al., 2013) (Wright, Thompson, et al., 2013a). Additionally, due to their similarity in size and density to planktonic organisms and sediments, as well as their availability to and ease of exposure by larvae of a variety of marine animals, including shellfish and sea squirts, microplastics may be absorbed by low-nutrient organisms (Bergami et al., 2016; Browne et al., 2008; Mao et al., 2018; Murray & Cowie, 2011; Oliveira et al., 2013a; Setälä et al., 2016; Sleight et al., 2017; Wright, Thompson, et al., 2013b)

### **2.2.2 TRANSLOCATION**

Microplastics are frequently detected in the body tissues and circulatory systems of some marine organisms. Because they might penetrate cell membranes and epithelial tissues. This phenomenon was called "translocation." (Setälä et al., 2014) Microplastics can penetrate through cell membranes since they cannot be ingested or absorbed. After ingesting, go via the innermost layer of the epithelium of the intestines into the bloodstream and then into the tissue (Andrady, 2011). By exposing organisms to luminously marked microplastic fragments and then observing the results under a microscope, one of the most popular methods for researching translocation is now used. (for instance, confocal and fluorescent microscopy) to see MPs in the tissue and do the quantitative analysis using flow cytometry. (Collard et al., 2017; samy et al., 2018).

### **2.2.3 BIOACCUMULATION AND BIOAVAILABILITY**

MPs interact with species in the marine environment, and bioaccumulation and bioavailability are the two key indices to assess the effects of MPs on organisms (Collignon et al., 2012). Marine species can consume microplastics directly and it is transmitted and accumulated from lower trophic species to higher trophic organisms in the food web; the further up an organism's trophic level, the greater the potential for enrichment in microplastics inside the body (Welden et al., 2018). Additionally, hazardous contaminants might be moved around and gathered. Laboratory exposure tests have shown that microplastics are ingested by organisms along with other substances, including microplastics. It has been hypothesized that POPs could bioaccumulate extensively in the food chain through microplastics (Wardrop et al., 2016) (Bottari et al., 2019). The physiochemical characteristics of microplastics, including size, shape, and

density, substantially influence bioavailability (Wright, Thompson, et al., 2013c). The biggest effect, according to the conclusion, is the size of the microplastics. The likelihood of bioaccumulation and bioavailability rises as the size decreases (Law & Thompson, 2014) Scientists are becoming more interested in the possibility that microplastics could increase the bioavailability of contaminants that have been absorbed. It is unfortunately difficult to determine how the bioavailability of pollutants enhanced by microplastics due to the extremely high number of potential interacting factors that include physical (e.g., salinity, pH, and temperature), chemical (e.g., hydrolysis, which is oxidation, reduction, and enrichment), and biological (e.g., organisms' variables).

#### **2.2.4 IMPACT OF MICROPLASTICS ON CORALS**

Human influences on the tropical coral reef ecosystems around the world are growing. Their foundation species, Endo cellular algae and *hermatypic zooxanthellate* corals, coexist in a mutualistic relationship. Overfishing, pollution, coastal development, and other local stressors, as well as global stressors like rising temperatures and ocean acidification, have severely harmed coral reef ecosystems in recent decades. Because plastic waste is linked to an increased risk of disease, it has recently been recognized as a pollutant of growing concern for coral reefs (Heron et al., 2016; Hughes et al., 2018; Lamb et al., 2018)

### **2.3 TOXIC EFFECTS**

Marine species are poisoned by microplastics. When it comes to marine species, different types and sizes of microplastics have different harmful effects, which are eventually reflected in how organisms react physiologically (Besseling et al., 2013; Lee et al., 2013)

#### **2.3.1 FISH TOXICITY**

Several authors have documented the toxic effects of microplastics on fish species found in freshwater, the sea, and the oceans (Garcia et al., 2020)

## What Responses Do Fish Have to Microplastics Toxicants?

1) accumulation in the digestive tract, which causes physical injury including blockage and damage;

2) disperse as pseudo feces, impairing the transmission of energy between organisms.

3) transit into the body, making internal organs and tissues vulnerable to MPs (Ašmonaitė et al., 2018; Yong et al., 2020)

The presence of micro plastics in more than 100 different fish species, as well as the hard shards and fibers that were found in the stomach cells (Chan et al., 2019). Additionally, fish samples taken from a region near an urban population were shown to have a higher level of microplastic ingestion. Microplastics are made more harmful in nature by the addition of additional additives such as colors, metals, toxic chemical compounds, etc. for instance, under controlled conditions, researchers looked at the toxicity of pyrene microplastics on *Pomatoschistus* species. and found that it caused oxidative damage and decreased the activity of the enzyme isocitrate dehydrogenase. (Oliveira et al., 2013b) observed that the activity of the enzyme acetylcholinesterase decreased in *Pomatoschistus* species when heavy metals and microplastics were present. The harmful effects of microplastics can occasionally change the gene expression of aquatic fishes. For instance, the genes, choriogenin in males and vitellogenin and estrogen receptor in *O. lapites*, decreased gene expression.

In a few showed the existence of microplastic in the gills, gut, and liver of *Dainorerio*. The accumulation of these microplastics was generating many harmful effects, such as inflammation, an increase in the activity of the enzyme's superoxide dismutase and catalase, changed metabolic pathways, etc. Understanding toxic effects brought on by microplastics is vital to evaluate their environmental repercussions because the toxicity mechanism of microplastics is not fully understood. Due to the persistence of microplastics and the toxicity of the chemicals, there may be a long-lasting hazard to marine ecosystems. The research unequivocally shows that microplastics are present and may have deleterious effects on many fish species that live in marine habitats. Further

evidence of the contribution of human activities, particularly in urbanized areas, to microplastic pollution was provided by fish samples taken from water bodies close to urbanized regions that had greater microplastic levels than non-urbanized samples (Bergmann et al., 2015; Lusher et al., 2013b, 2014; Rochman et al., 2014)

### **2.3.2 BIOTA TOXICITY**

Micro-plastic is frequently found in marine cetaceans like whales and dolphins. The dolphin's gut contains microplastics. Numerous manmade polymers of various sizes and shapes, including nylon, polyvinyl chloride, and polystyrene, have been found in the intestine of Megaptera species (Hernandez-Gonzalez et al., 2018) consumption of these microplastics by many kinds of cetaceans can lead to multiple acute and chronic toxicities (Besseling et al., 2015b; Simmonds, 2012). According to reports, ingesting microplastic might affect digestion and clog the intestinal tract. Studies have also shown that micro-plastics could restrict or blockage the *Balaenoptera Physalus L.*, a marine organism's filtering system. There have also been reports of marine turtles consuming micro-plastic (Nelms et al., 2016).

A turtle's consumption of micro-plastic can have several harmful effects, including severe intestinal damage and obstruction, a decrease in stomach capacity, and even death. Others harmful consequences linked to marine life consuming micro-plastic turtles are changing their swimming habits and their immune systems reaction, a slowdown in growth, and a decline in the ability to prevent victims. Each of these elements is significantly contributing to the population decline. Following turtle entrapment in plastic waste, these plastics can cause numerous turtles to perish. Furthermore, plastic waste might have an impact on environmental elements like the habitat's temperature.

The turtle is endangered, and this change in ecological conditions has a detrimental effect on the sea turtle's ability to reproduce. Existence of Algal populations can potentially be impacted by microplastics in aquatic environments. That numerous other aquatic species feed on and use as an energy source. This seaweed Algae's uptake and retention of microplastics have been extensively documented in numerous species, including *Scenedesmus*, *Chlorella sp.*, Likewise, *Dunaliella sp.* decreases in

photosynthetic activity in addition to the Oxidative stress is one of the harmful impacts that microplastics have on algal.

Its absorption and buildup via algae are facilitated by the positive and negative charges on the microplastic and the algae itself. The overall food supply for other marine creatures that depend on algae is decreased when photosynthetic activity is reduced in the presence of microplastics, as this has a detrimental impact on the biomass of algae. The studies mentioned above made it abundantly evident that microplastics harm the marine biota. One important factor in assessing the condition of the maritime environment is the marine biota. Additionally, further research. To have a thorough understanding of the harmful consequences that microplastics have on marine life, it is necessary to indicate how the microbial community responds when microplastics are present. Subsequent studies have also demonstrated the potential of using microorganisms to clean up microplastic pollution in the water. Using algae, fungus, and bacterial species, microbial bioremediation is a cost-effective, environmentally beneficial, and natural method. Microplastics can be remedied using a variety of techniques, including bioaugmentation (adding pre-grown microorganisms), Bio stimulation (supplementing nutrients to drive the process), and Bio attenuation (natural breakdown) (Camedda et al., 2014; Padervand et al., 2020; Urbanek et al., 2018; Wilcox et al., 2013).

### **2.3.3 INVERTEBRATES TOXICITY**

As microplastics become available in water bodies, aquatic invertebrates are exposed to them and consume them. When consumed, this artificial polymer performs has no enzyme available to break it down into a simpler, non-toxic molecule; therefore, it doesn't go through any enzymatic breakdown via a metabolic route mixture. In addition, the absorption of several organic and inorganic chemicals, including heavy metals, dyes, and hydrocarbons, among others increase the toxicity of microplastics because these pollutants also have their own poisoning. As these pollutants are absorbed, the microplastic becomes based on many microplastic characteristics, including chemical dimensions, form, and so forth. looked into how *Ostrea edulis* was affected by biodegradable microplastics such polylactic acid and high-density polyethylene. The study was conducted at various microplastic concentration levels, and it was found that

at increasing microplastic concentrations, *Ostrea edulis* respiration rate rises (Green, 2016). It was also noted that increased concentrations of polylactic acid, high-density polyethylene, and polyvinyl chloride caused *Arenicola marina* L. to exhibit an increase in its rate of transpiration. The buildup of microplastic in *Arenicola marina* L.'s digestive system or the increased inflammation brought on by the harmful effects of micro-plastic could be the source of the rise in respiration rate. Researchers found that in one study, polyvinyl chloride caused *Arenicola marina* L. to accumulate substances including triclosan and nonylphenol (Browne et al., 2013). Furthermore, within 72 hours (about 3 days) of exposure, accumulated microplastic is transported by the circulatory system from the digestive system to hemolymphs and hemocytes. A reduction in the stability of the lysosomal membrane and inflammation are two of the harmful effects of the microplastic in *Mytilus edulis*. Furthermore, the seafood industry makes extensive use of *Mytilus edulis*, and contaminated *Mytilus edulis* can serve as a conduit for the biomagnification of microplastics from marine ecosystems to human life.

## **CHAPTER 3**

### **3. AIM AND OBJECTIVE**

- To analyze the presence of Microplastics in the Fishes from marine biota, water, soil sediments along the East coast of Tamilnadu (Kovalam and Rameswaram)
- To study the morphological characterization of the Microplastics from marine biota in various feeding zones
- To analyze of polymer types in the collected samples
- To analyze the concentration of Heavy metals from marine biota along the East coast of Tamilnadu (Kovalam and Rameswaram)

PO's covered: PO2, PO4

## CHAPTER 4

### 4. MATERIALS AND METHODS

#### 4.1 SAMPLE COLLECTION

Samples are collected from Kovalam and Rameswaram for identification of microplastic and heavy metal because microplastic and heavy metal pollution can vary significantly across different regions and even within the same region. Fish samples (*Namatulosa nasus* (Bloch's Gizzard shad, *Scylla serrata* (crab), *Mugil Cephalus* (mullet), *Plotosus canius*(catfish), *Meretrix casta* (clams)) were collected from both the Kovalam (latitude 13.0604° and longitude 80.2496°) and Rameswaram (latitude 9.136724° and longitude 78.586649°). The fish samples that were gathered represent various feeding organisms.

- ***Meretrix casta* (clams)**

A filter feeder

- ***Nematulosa nasus* (Bloch's Gizzard shad)**

A filter feeder

- ***Scylla serrata* (crab)**

Omnivorous, Mollusca, crustaceans and worms, occasionally algae

- ***Mugil Cephalous* (mullet)**

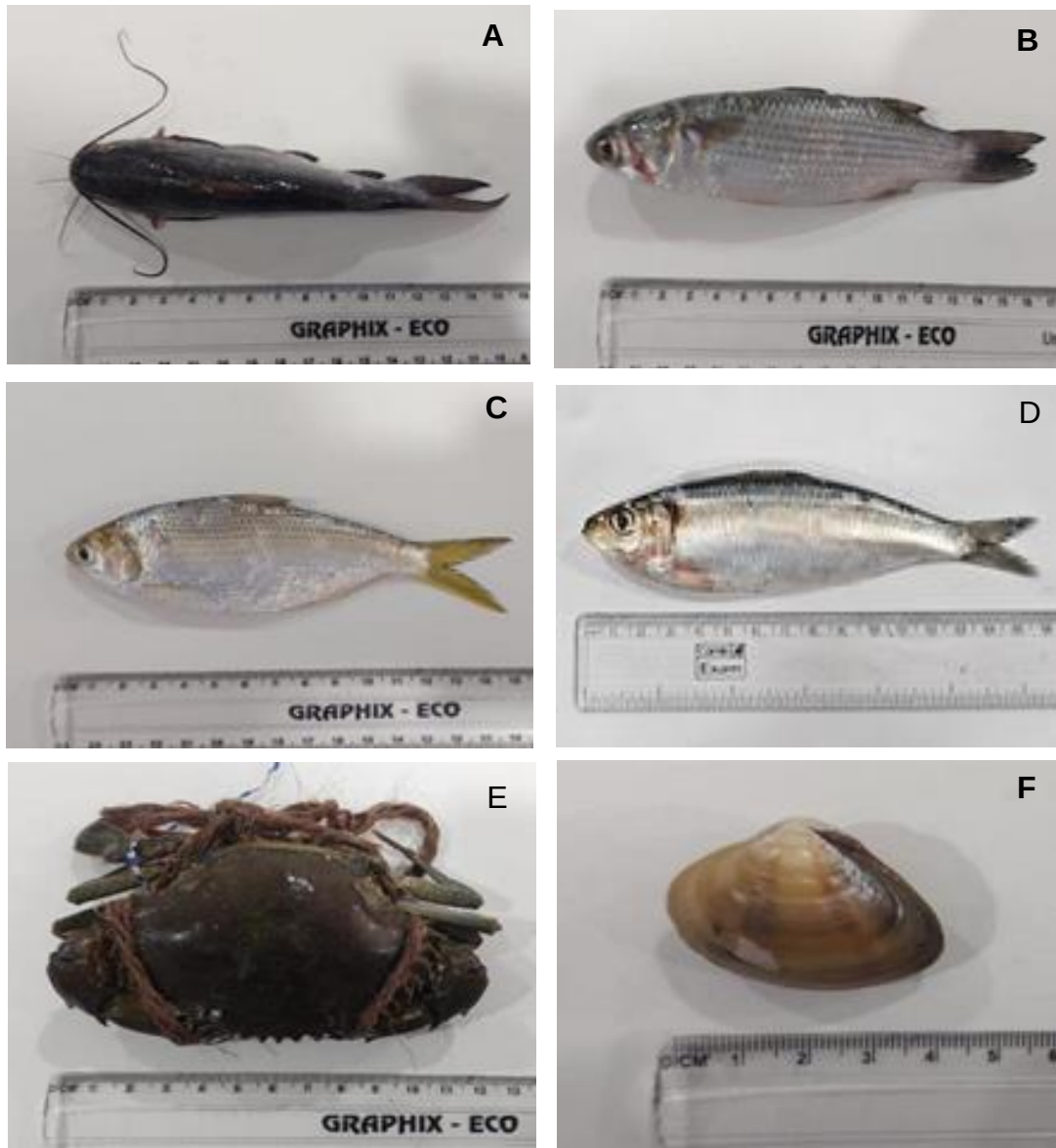
Consuming mainly zooplankton, dead plant matter, and detritus. Mulletts have thick-walled gizzard-like segments in their stomach along with a long gastrointestinal tract that enables them to feed on detritus.

- ***Plotosus canius* (catfish)**

Feed on crustaceans, Mollusca and fishes

Collected samples were stored in -80°C Freezer

### Samples collected from Kovalam



**Fig 4.1 A- *Plotosus canius* (catfish), B- *Mugil Cephalous* (mullet), C- *Nematulosa nasus* (Bloch's Gizzard shad), D- *Sardinella gibbosa* (Sardine), E- *Scylla serrata* (crab), F- *Meretrix casta* (clams)**

**Samples collected from Rameswaram**



**Fig 4.2 A- *Plotosus canius* (catfish), B- *Mugil Cephalous* (mullet), C- *Nematulosa nasus* (Bloch's Gizzard shad), D - *Scylla serrata* (crab)**

## 4.2 FISH DISSECTION

Anus was located, which was the solitary aperture on the underside, and began by making a shallow cut that extended to the gills. And made a small incision along the side of the fish to allow me to see the inside of the guts while they were still in place. Followed the digestive system, beginning at the mouth or the anus and taking out the fish's intestines, which were frequently very lengthy. Proceeded to the fish's head, removed its gills, and gently cut the skin and muscle. The collected organs were stored in the container and kept in an  $-80^{\circ}\text{C}$  freezer.



***Fig 4.3 Images shows the Fish dissection***

### 4.3 LYOPHILIZATION

Lyophilization, also known as freeze-drying, is a process used to remove water from a sample while preserving its structure and integrity. This technique involves three main steps: freezing, primary drying, and secondary drying. Using lyophilizer all the samples were lyophilized. As a result, the water is removed from the sample without causing significant damage to its structure and then the samples are stored back in an -80°C freezer.

### 4.4 ALKALI DIGESTION

Alkali digestion is a chemical process used to break down complicated organic structures into more easily soluble forms. The alkaline solution hydrolyzes the bonds that hold molecules together. Here, the digestion of fish tissues (gills, stomach, skin, and muscle) was accomplished using potassium hydroxide (KOH). Each 100ml conical flask should include 0.3g of tissue from each organ (gill, gut, skin, and muscle). The samples should then be treated with 20ml of potassium hydroxide (20% KOH) and maintained in the environmental shaker for 48 hours (about 2 days) to aid digestion.



***Fig 4.4 Environmental shaker***

As illustrated in Figure 4.4 tissues that have been treated with 20 ml of 20 % KOH are continuously shaken in an environmental shaker.

#### 4.4.1 DENSITY SEPARATION

Density separation is the method used to extract microplastics from digested tissues by using Sodium chloride (NaCl) salt. A separating funnel is used to extract microplastics from digested tissues. The sodium chloride (33.3%) solution needs to be prepared, followed by setting up the separating funnel on the stand. The digested tissue samples are then added inside, and three times the volume of the digested tissue samples is subsequently added to the separating funnel with NaCl. In comparison to most microplastics, sodium chloride salt solution has a higher density, which causes less dense microplastics ( $0.9\text{--}1.5\text{ g cm}^{-3}$ ) to float and gather in the conical flask as the denser objects settle and drain.



***Fig 4.5 Separating unit***

Figure 4.5 illustrates the separation of microplastics using density gradient separation techniques.

#### 4.4.2 SUCTION (VACUUM) FILTRATION

Vacuum filtration, or suction filtration, is the process of separating a solid from a liquid through a 0.45-micron nitrocellulose membrane on the Whatman filter. In this instance, The Buchner filter flask is taken, topped with the Whatman filter paper, and then topped with a 0.45-micron nitrocellulose membrane. Next, the Buchner funnel is set on top, the vacuum tube is attached to the motor, and the motor is allowed to run. Now, the 0.45-micron nitrocellulose membrane has successfully trapped the microplastics. Carefully transfer the membrane to a petri dish and then place it in a desiccator to dry.



***Fig 4.6 Vacuum filtration unit***

Figure 4.6 illustrates the vacuum filtration method's process for filtering microplastics

## 4.5 CHARACTERIZATION

### 4.5.1 STEREO ZOOM MICROSCOPIC ANALYSIS

The dried samples were screened under the stereo zoom microscope (Leica M205A, Germany) with LES 4.0 software to determine the shape, size, and color of microplastics. In Stereo Zoom Microscopic Analysis, samples were examined in three dimensions at varied angles to a magnitude of 100  $\mu\text{m}$  (Resolving power: up to 476nm). During the analysis a lot of microplastic fibers and pellets were observed in the samples.



***Fig 4.7 Stereo Zoom Microscope (Leica M205A, Germany)***

### 4.5.2 HIGH RESOLUTION SCANNING ELECTRON MICROSCOPIC ANALYSIS

The dried samples were analyzed using High Resolution Scanning Electron Microscope Analysis (Thermoscientific Apreo S) to study the morphological surface structure of Microplastics. The samples different morphologies such as: microfiber strands, pellets and foam like structures. Ranging from: 500  $\mu\text{m}$  to 1  $\mu\text{m}$  were observed. This method is viable for such structural analysis studies over the Stereo Zoom Microscopic Analysis due to the HR-SEM's high magnification range and imaging resolution.

#### **4.5.3 RAMAN SPECTROSCOPIC ANALYSIS**

The presence of microplastics were identified using Raman spectroscopy (inViaTM, Reinshaw, UK). This method was used to identify the chemical composition of microplastics. In this method, the samples were incident with a laser of wavelength: 785nm, to determine the type of molecular bond present within the polymers. The polymer's peaks were then compared and studied using the Raman spectrum library.

#### **4.6 ACID DIGESTION**

An approach that is frequently used to analyze heavy metals in a variety of samples, including biological tissues, water, soil, and sediment, is acid digestion. For heavy metal analysis, a typical acid digestion process is described in the protocol that follows.

1. Weigh the sample (about 0.3 g dry sample) into a glass vessel.
2. Add 15 mL of concentrated HNO<sub>3</sub> to the sample and place the digestion vessel onto an electromagnetic stirrer and heat gradually to 100 °C for 15- 20 min.
3. Remove the digestion vessel from the stirrer and allow it to cool to room temperature.
4. Transfer the solution to a clean plastic or glass container and send for atomic absorption spectroscopy (AAS) to find the concentration of heavy metal.

## CHAPTER 5

### 5. RESULTS AND DISCUSSION

#### 5.1 MICROPLASTIC ANALYSIS

First, the shape, color, and dimensions of the microplastics were identified using stereo zoom microscopic analysis. Next, the microplastics were analyzed using High Resolution Scanning Electron Microscope Analysis to study the morphological surface structure of the microplastics. Finally, the polymer type was confirmed by Raman spectroscopy measurements of MPs on filter membrane using the inVia™, Reinshaw, UK, which was equipped with an argon-ion laser at an excitation wavelength of 785 nm.

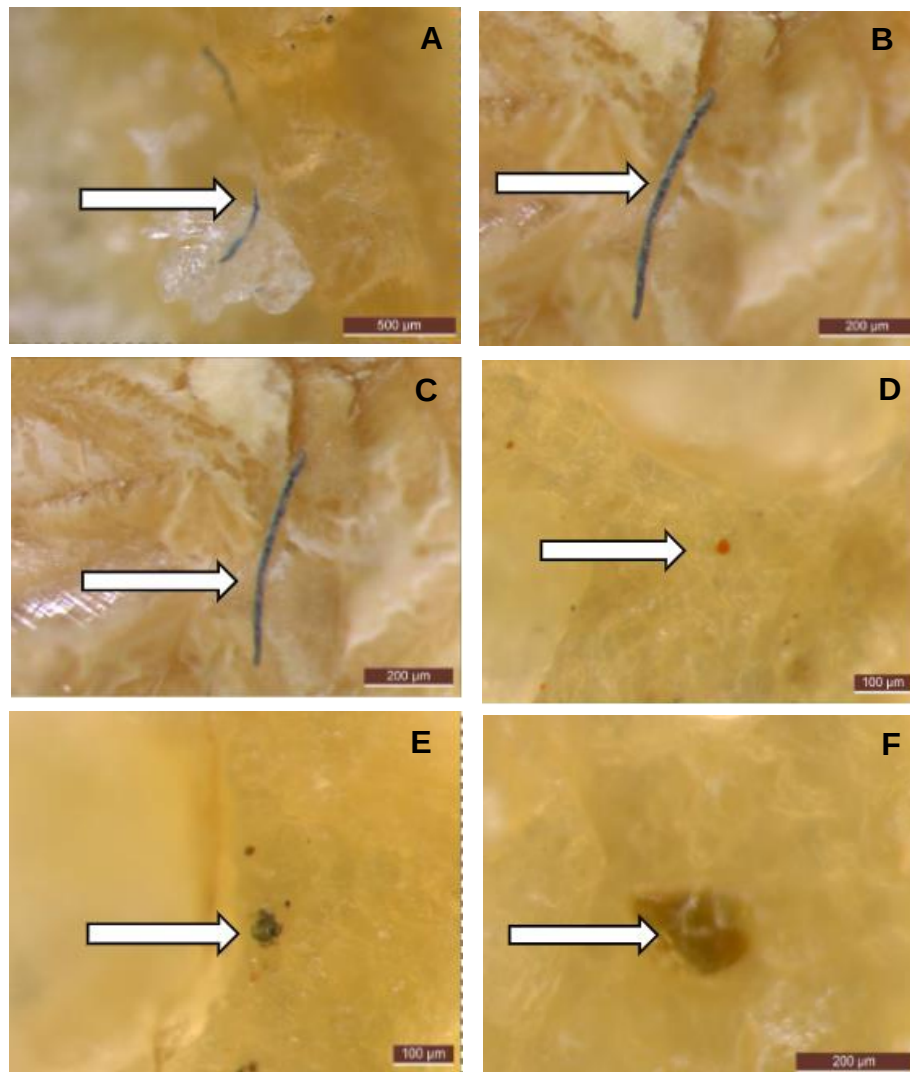
##### 5.1.1 STEREO ZOOM MICROSCOPIC ANALYSIS

To identify the shape, size, and color of microplastics, the dried samples were screened using LES 4.0 software under a stereo zoom microscope (Leica M205A, Germany). Samples were analyzed using stereo zoom microscopic analysis in three dimensions and at different angles up to a magnitude of 100  $\mu\text{m}$  (resolving power: up to 476nm). Numerous microplastic pellets and fibers were found in large quantities in the samples during the study.

Here, we detected numerous microplastics up to 100  $\mu\text{m}$  in size, including fibers, pellets, beads, foams, and films, from fish samples (*Nematulosa nasus*, *Plotosus canius*, *Mugil cephalus*, *Scylla serrata*, and *Meretrix casta*), water, and soil sediments found in the kovalam and Rameswaram areas. Furthermore, we noticed that there were greater quantities of microplastics in Kovalam and fewer in Rameswaram. Here, we may conclude that, in comparison to Rameswaram, the Kovalam region was more contaminated.

***Nematalosa nasus*** (Bloch's Gizzard shad) gut sample

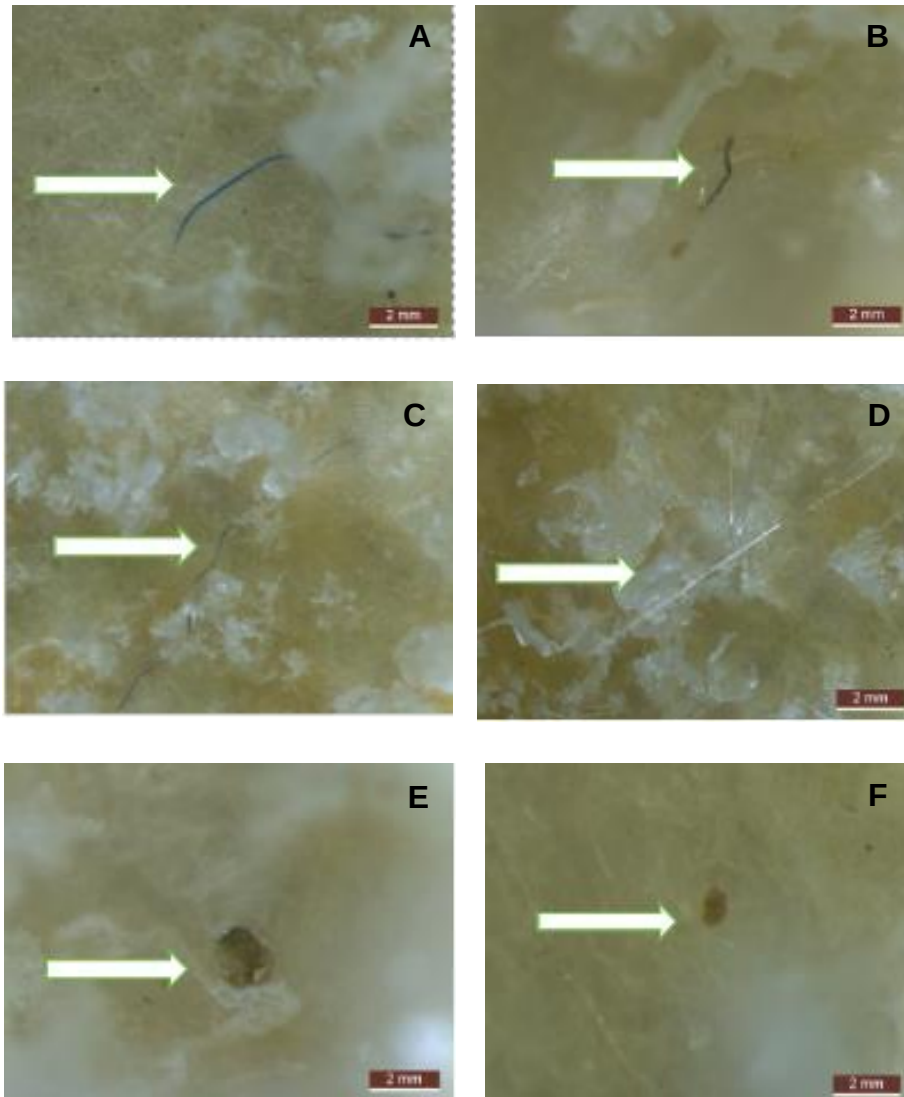
The given samples are *Nematalosa nasus* gut sample under Stereo Zoom Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



**Fig 5.1 The Microplastic fiber (A, B, C - blue) and pellets (D - red, E, F - black) are found of varying colors and size in the gut sample.**

***Nematalosa nasus*** (Bloch's Gizzard shad) Gill sample

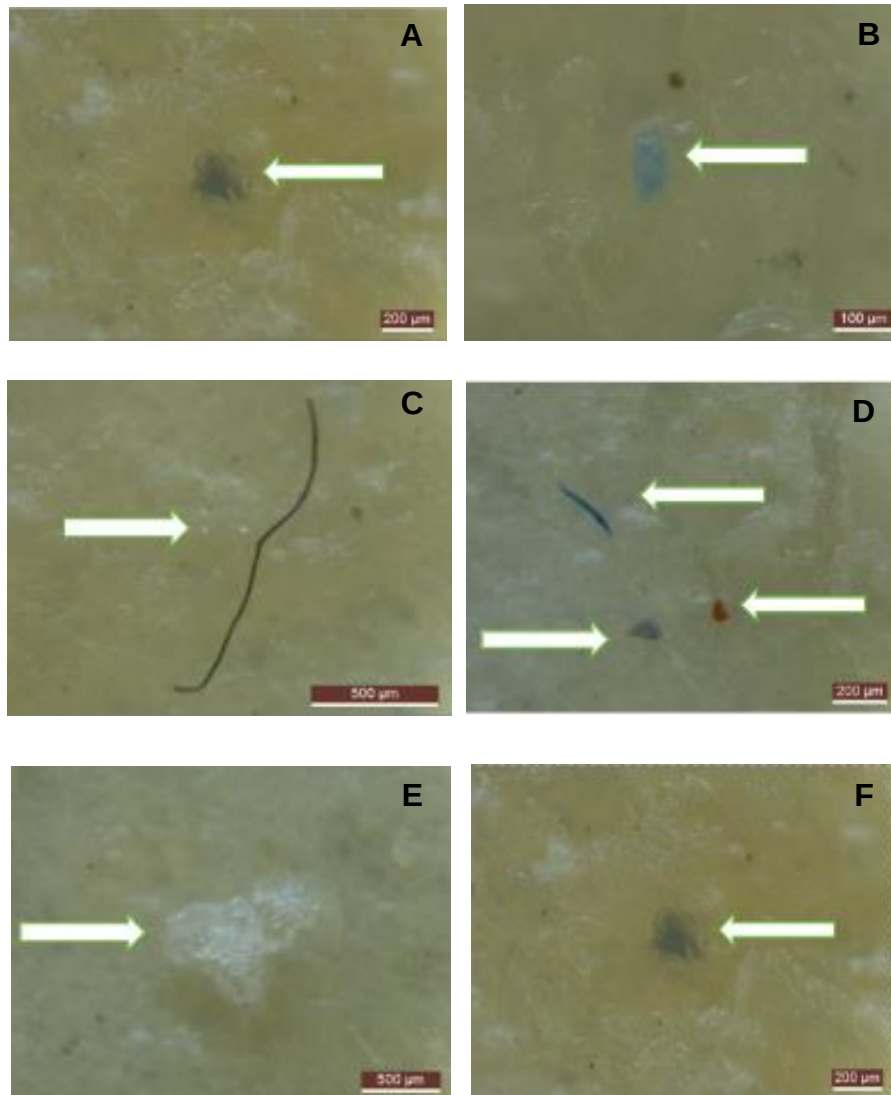
The given samples are *Nematalosa nasus* gill sample under Stereo Zoom Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



**Fig 5.2 The Microplastic fibers (A, C - blue, B – black, D - Transparent) and pellet (E, F black) are found of varying colors and size in the gill sample.**

***Nematalosa nasus*** (Bloch's Gizzard shad) skin sample

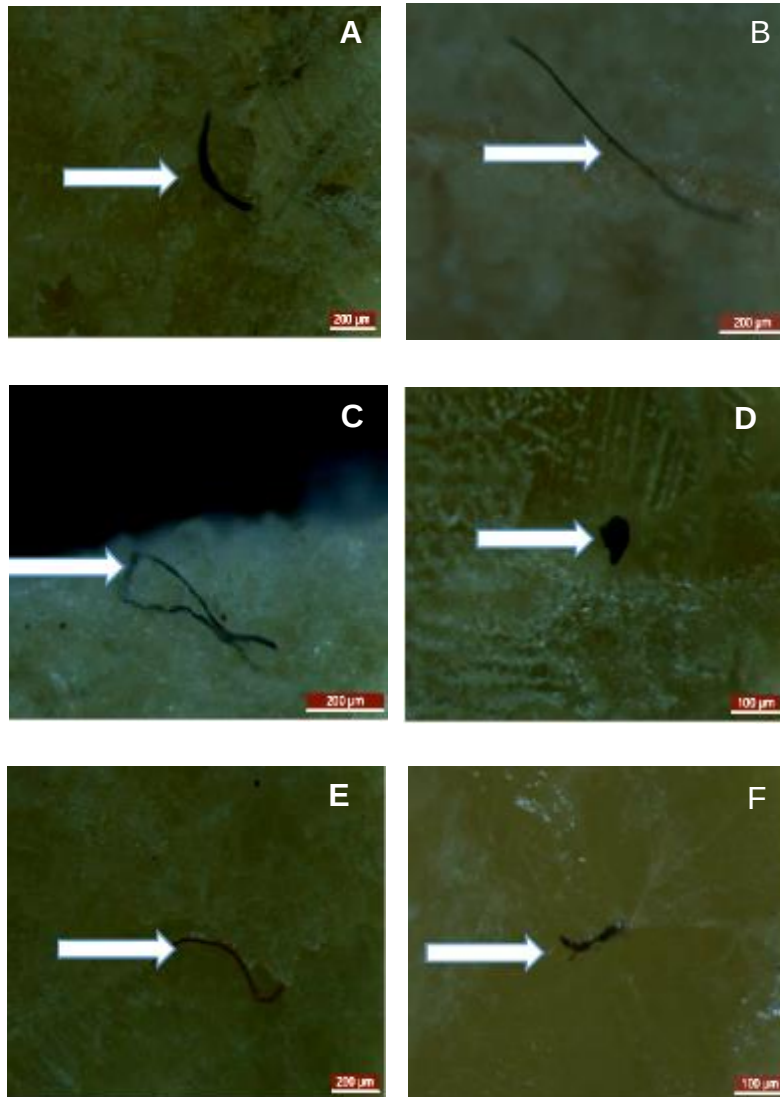
The samples given are *Nematalosa nasus* skin sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



**Fig 5.3 The Microplastic fibers (D - blue and C - black), pellet (A, F - black, B - blue, D - orange), E - foam are found of varying colors and size in the skin sample.**

***Nematalosa nasus*** (Bloch's Gizzard shad) muscle sample

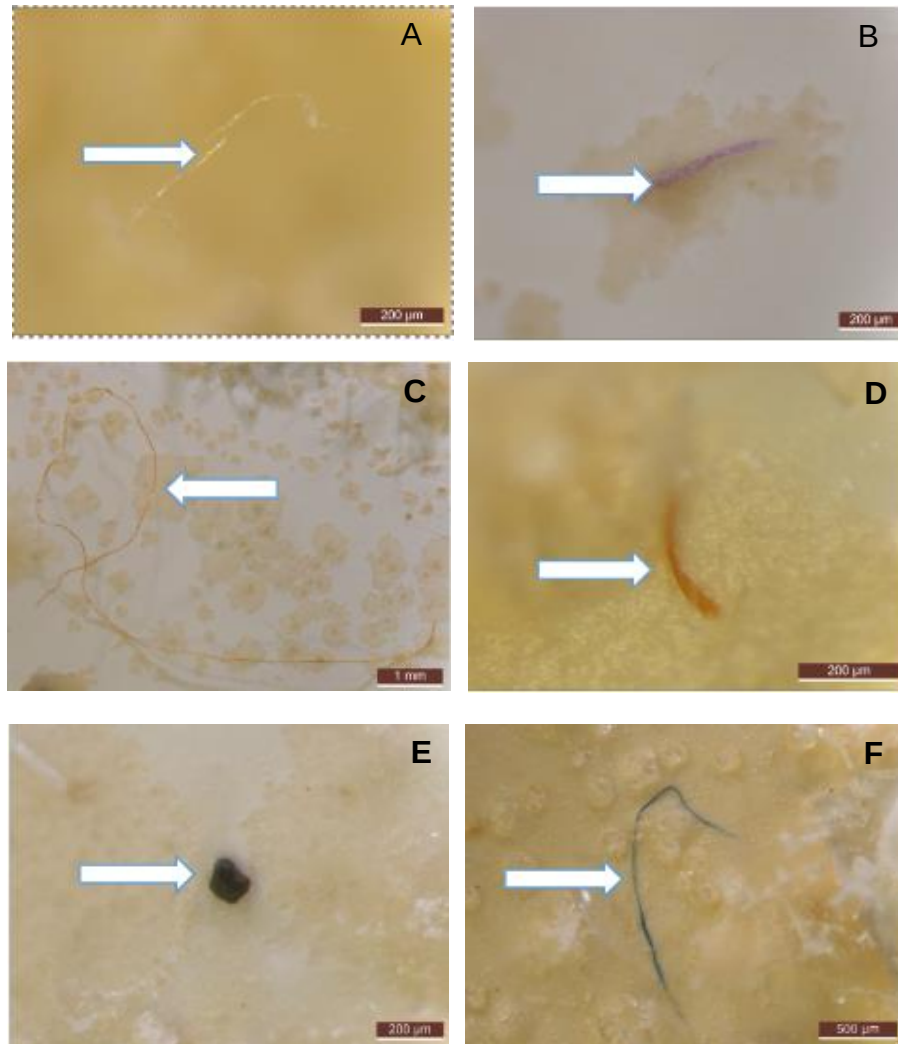
The samples given are *Nematalosa nasus* muscle sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



**Fig 5.4 The Microplastic fibers (E - red, A, B, C, F - black) pellet (D - black) are found of varying colors and size in the muscle sample.**

***Plotosus canius*** (catfish) gut sample

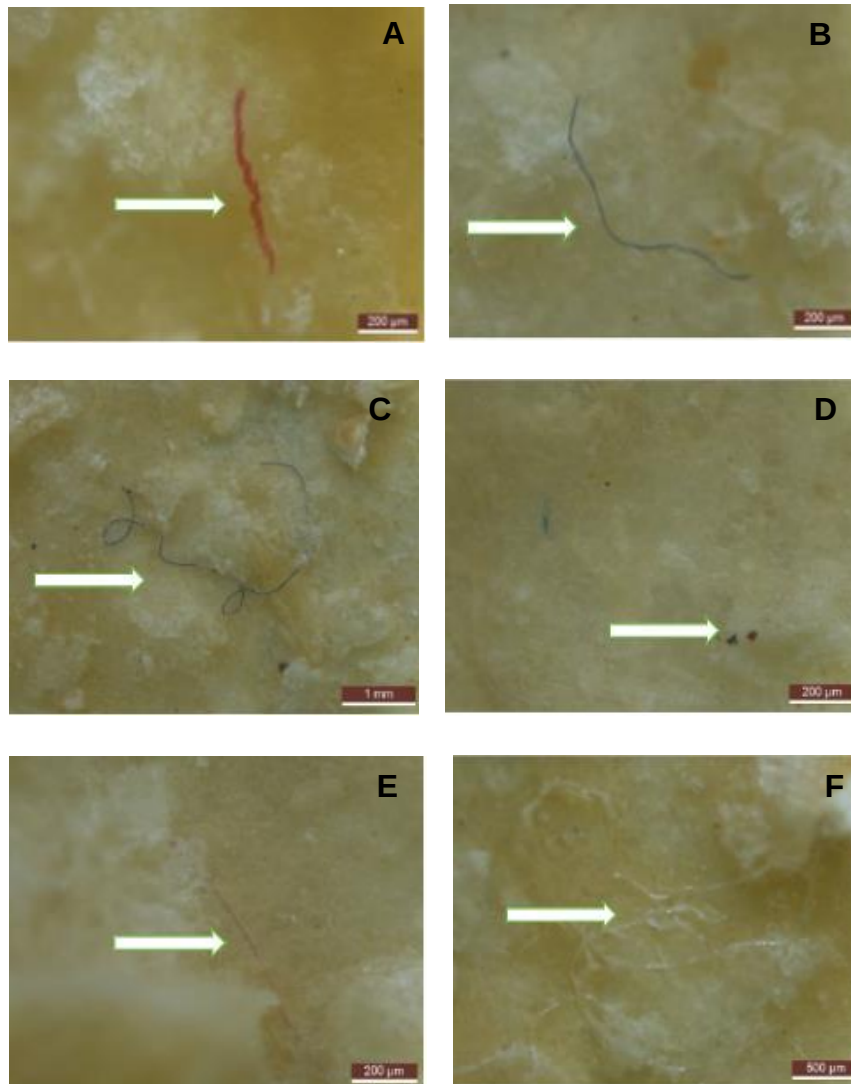
The samples given are ***Plotosus canius*** (catfish) gut sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



**Fig 5.5 The Microplastic fibers (F - blue, B - violet, C, D - golden, and A - transparent) pellet (E - black) is found of varying colors and size in the gut sample.**

***Plotosus canius*** (catfish) gill sample

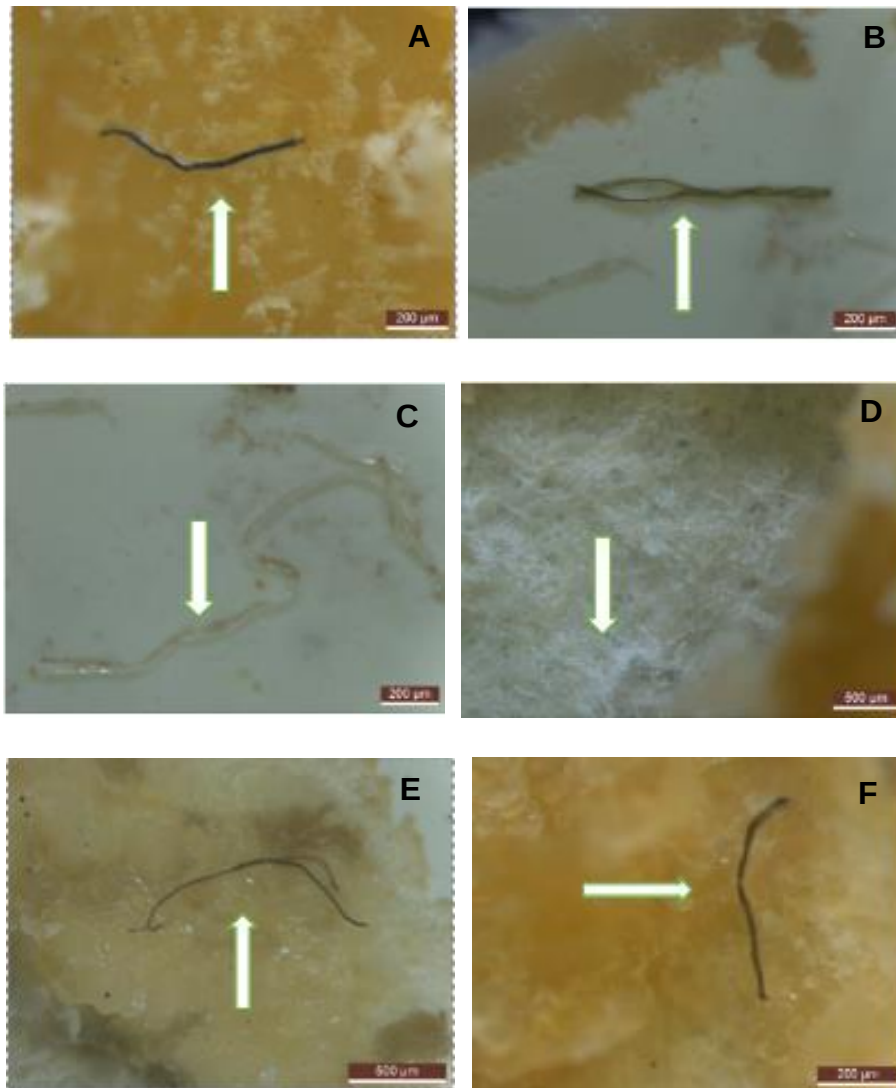
The samples given are ***Plotosus canius*** (catfish) gill sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.6 The Microplastic fibers (B, C - blue, A - red), pellet (D - black), foam (E, F) is found of varying colors and size in the gill sample.***

***Plotosus canius*** (catfish) muscle sample

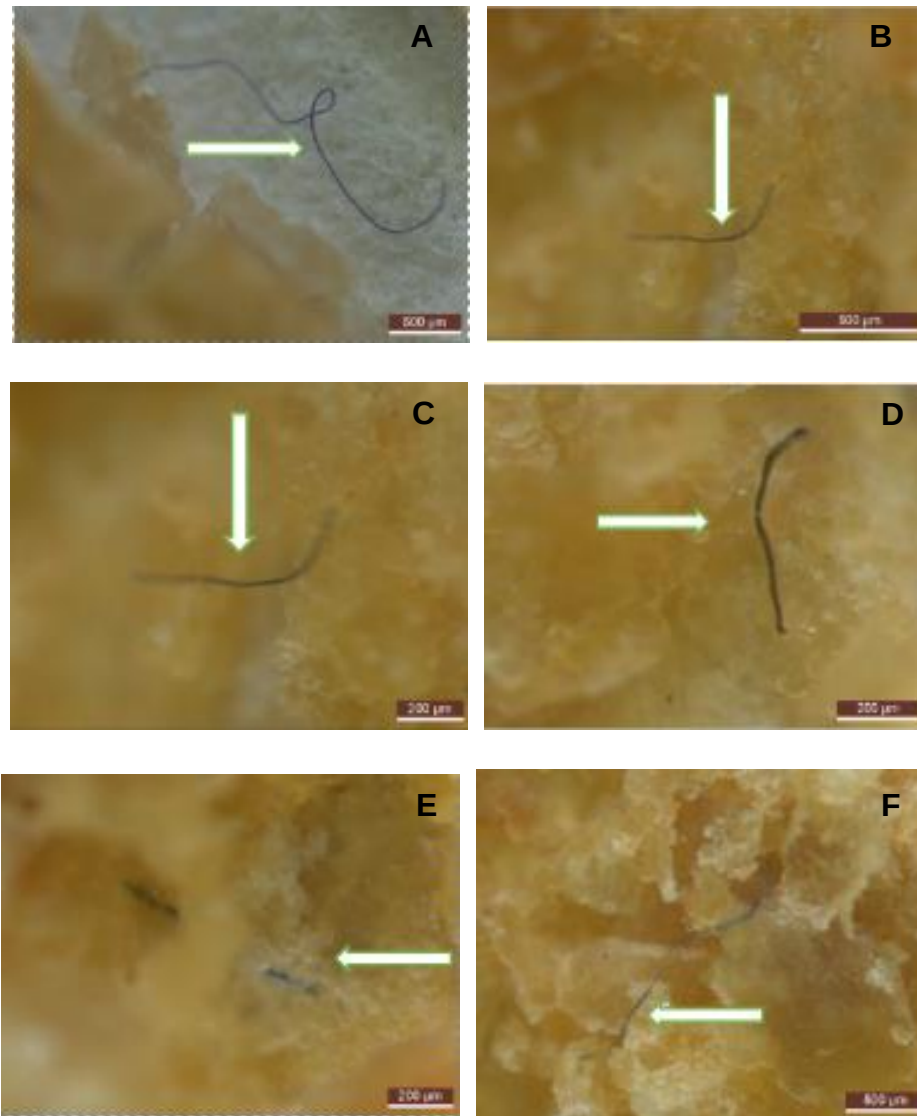
The samples given are ***Plotosus canius*** (catfish) muscle sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.7 The Microplastic fibers (A - blue, B - gold, E, F - black, C - transparent), D - foam is found of varying colors and size in the muscle sample.***

***Plotosus canius*** (catfish) skin sample

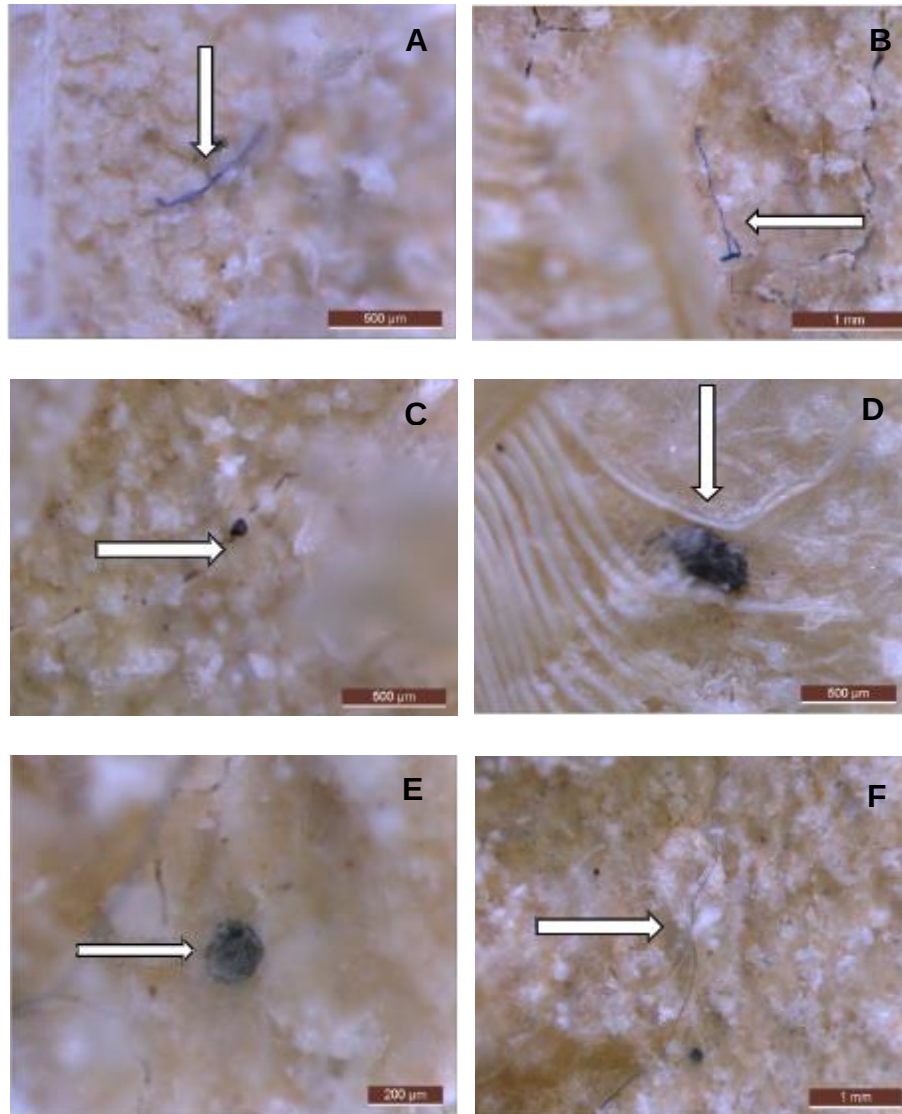
The samples given are ***Plotosus canius*** (catfish) skin sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.8 Microplastic fibers (A, E, F - blue, B, C, D - black) are found of varying colors and size in the skin sample.***

***Scylla serrata*** (crab) gill sample

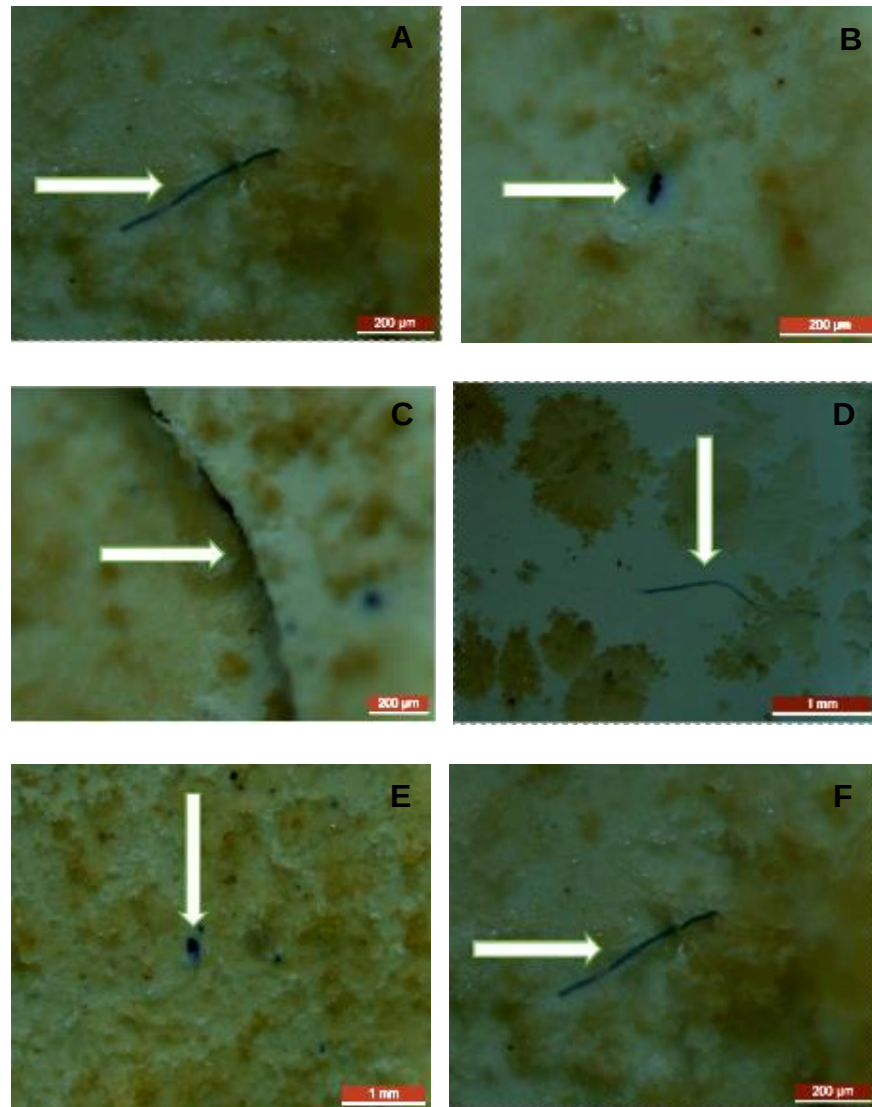
The samples given are ***Scylla serrata*** (crab) gill sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.9 Microplastic fibers (A, B, F - blue) and pellet (C, D, E black) are found of varying colors and size in the gill sample.***

***Scylla serrata*** (crab) hepatopancreas sample

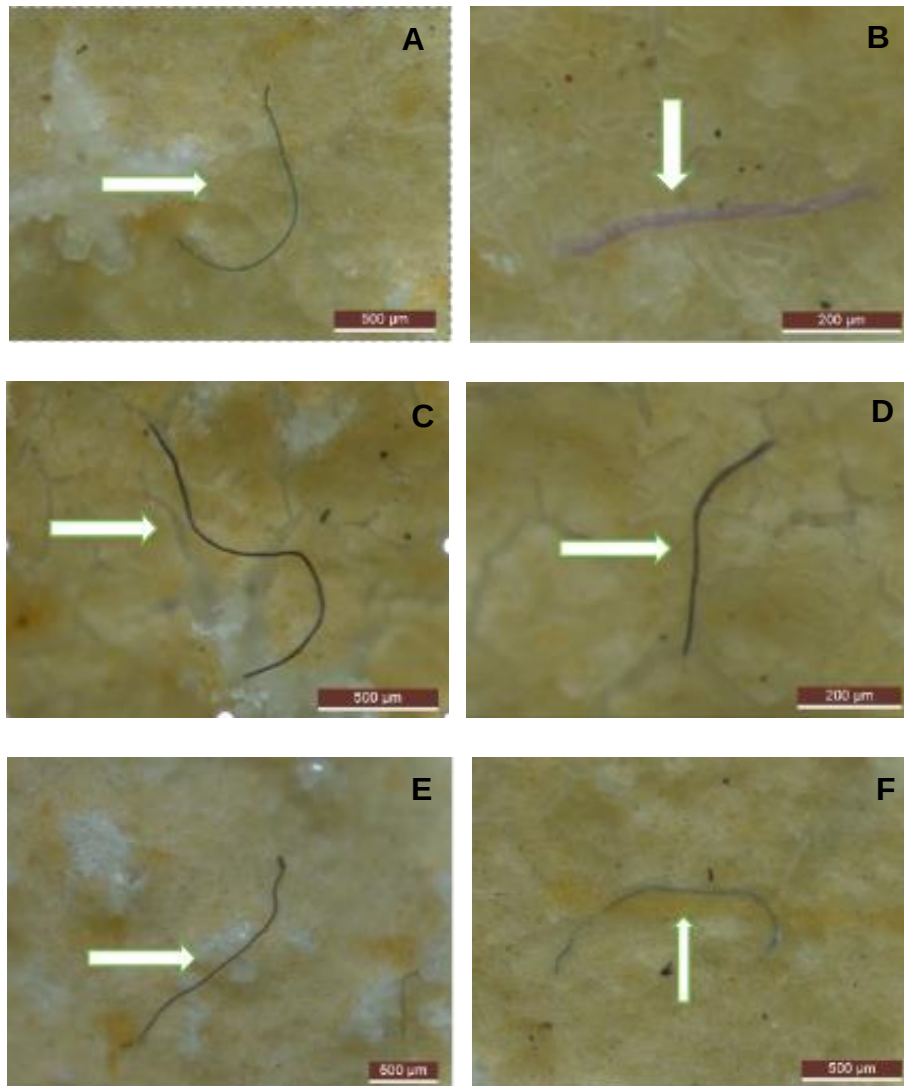
The samples given are ***Scylla serrata*** (crab) hepatopancreas sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.10 Microplastic fibers (A, C, D, F - blue) and pellet (B, E - blue) are found of varying colors and size in the hepatopancreas sample.***

***Scylla serrata*** (crab) tissue sample

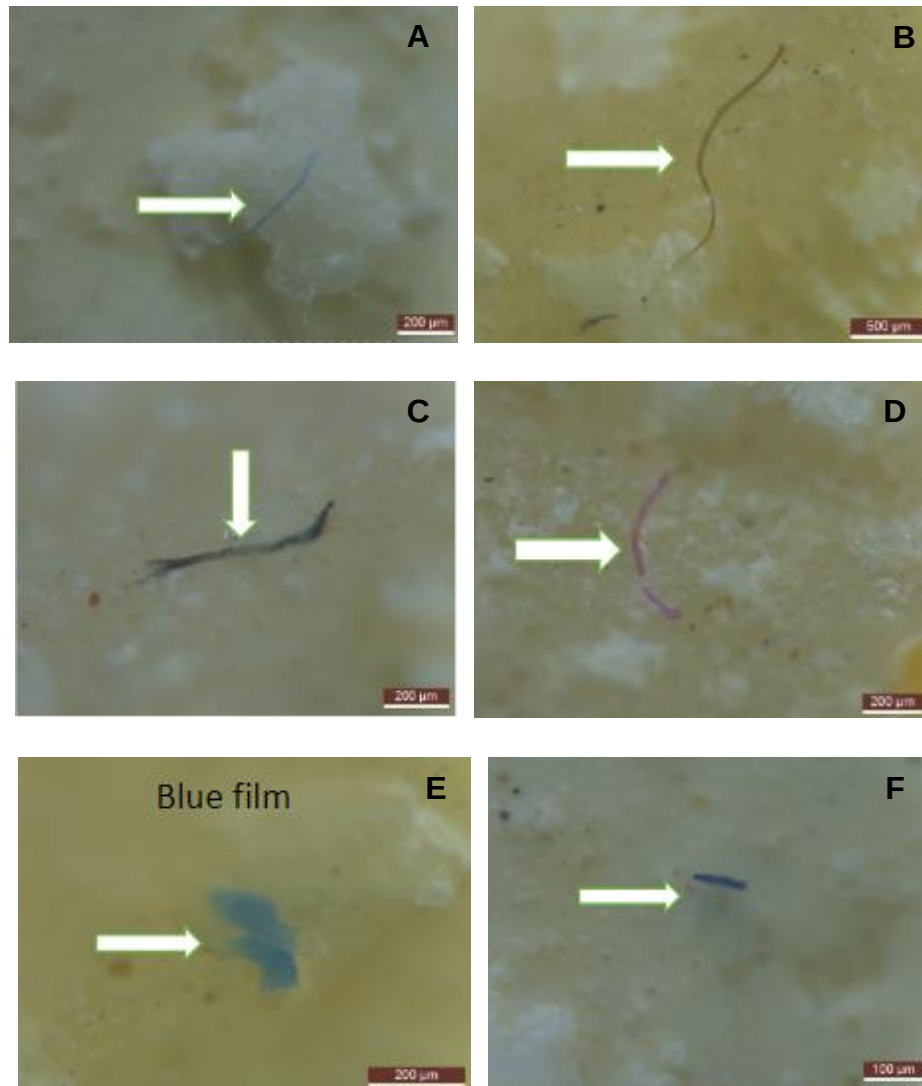
The samples given are ***Scylla serrata*** (crab) tissue sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.11 Microplastic fibers (A, F - blue, C, D, E - black, B - violet) and pellet (C - blue) are found of varying colors and size in the tissue sample.***

***Mugil Cephalus*** (mullet) gill sample

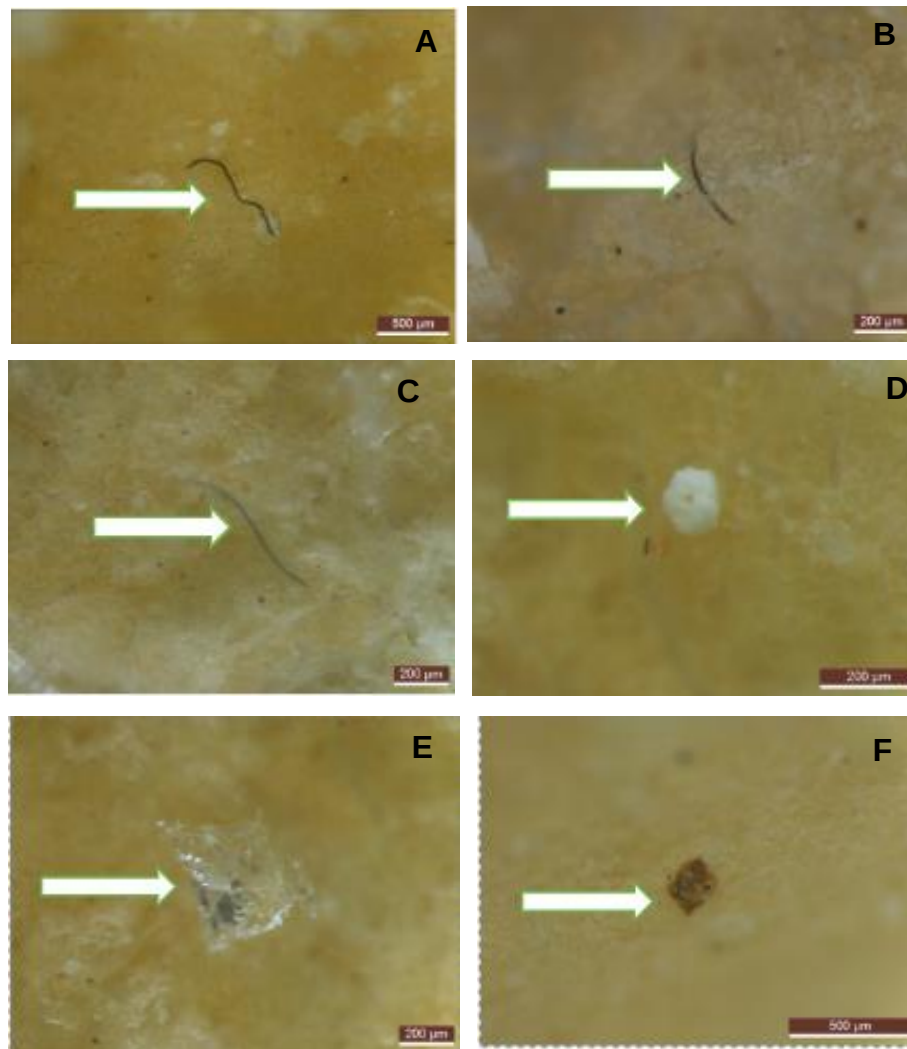
The samples given are ***Mugil Cephalus*** (mullet) gill sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.12 Microplastic fibers (A - blue, C - black, D - violet, B - golden) and pellet (F blue) E – blue films are found of varying colors and size in the gill sample.***

***Mugil Cephalus*** (mullet) gut sample

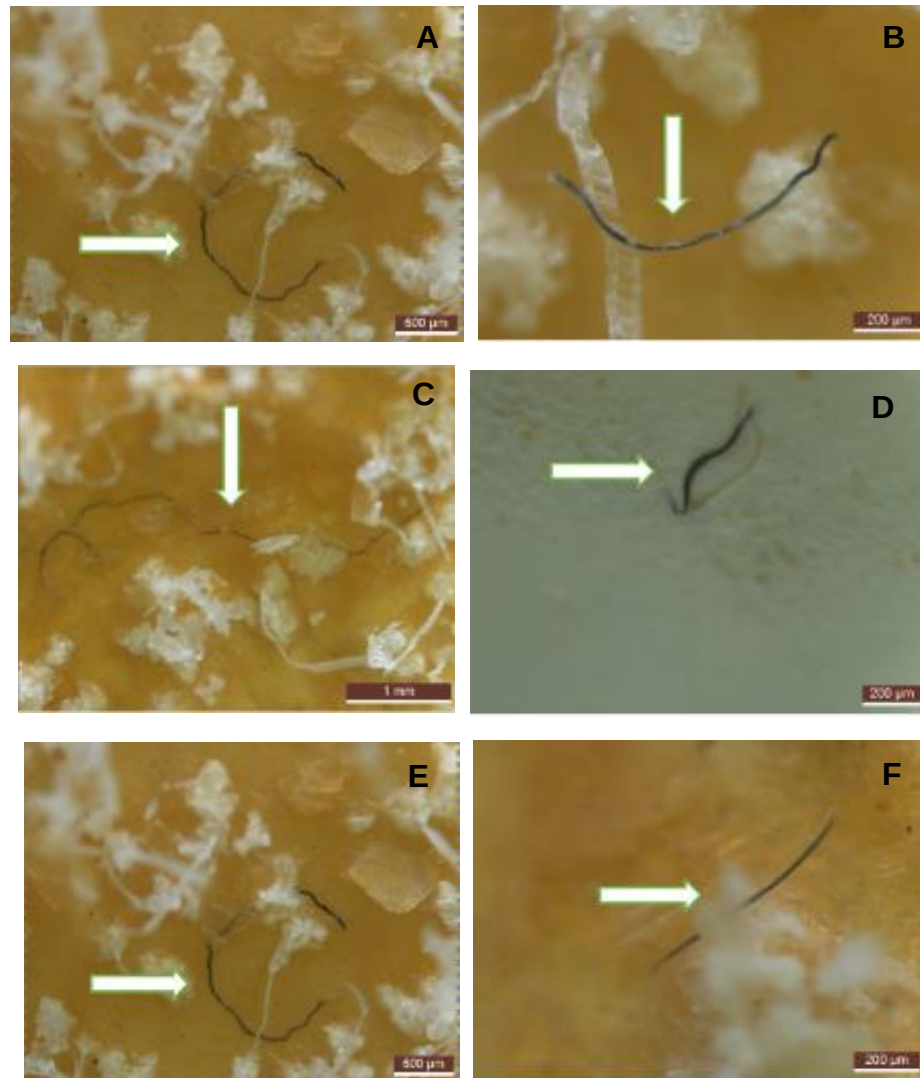
The samples given are ***Mugil Cephalus*** (mullet) gut sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.13 Microplastic fibers (C - blue, A, B - black) and pellet (F - brown), E - transparent film and D - foam are found of varying colors and size in the gut sample.***

***Mugil Cephalus*** (mullet) skin sample

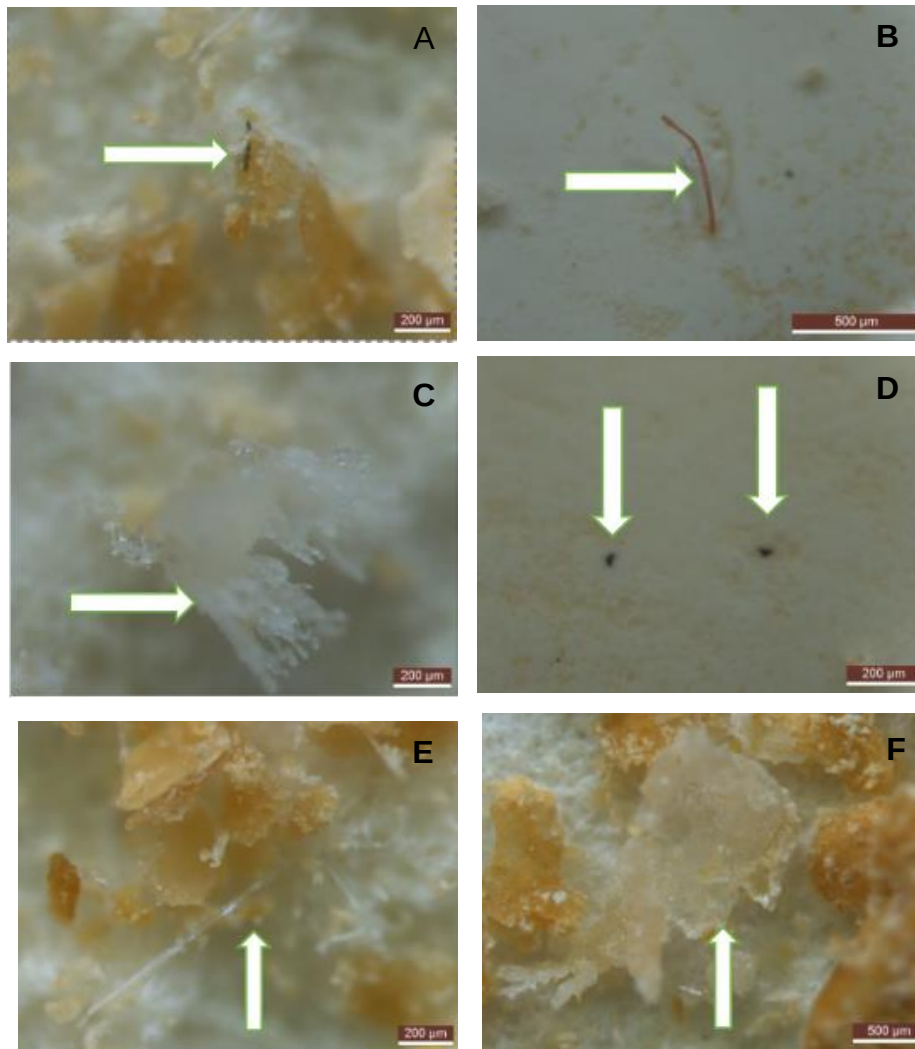
The samples given are ***Mugil Cephalus*** (mullet) skin sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.14 Microplastic fibers (B, F - blue, A, C, D, E - black) are found of varying colors and size in the Skin sample.***

***Mugil Cephalus*** (mullet) muscle sample

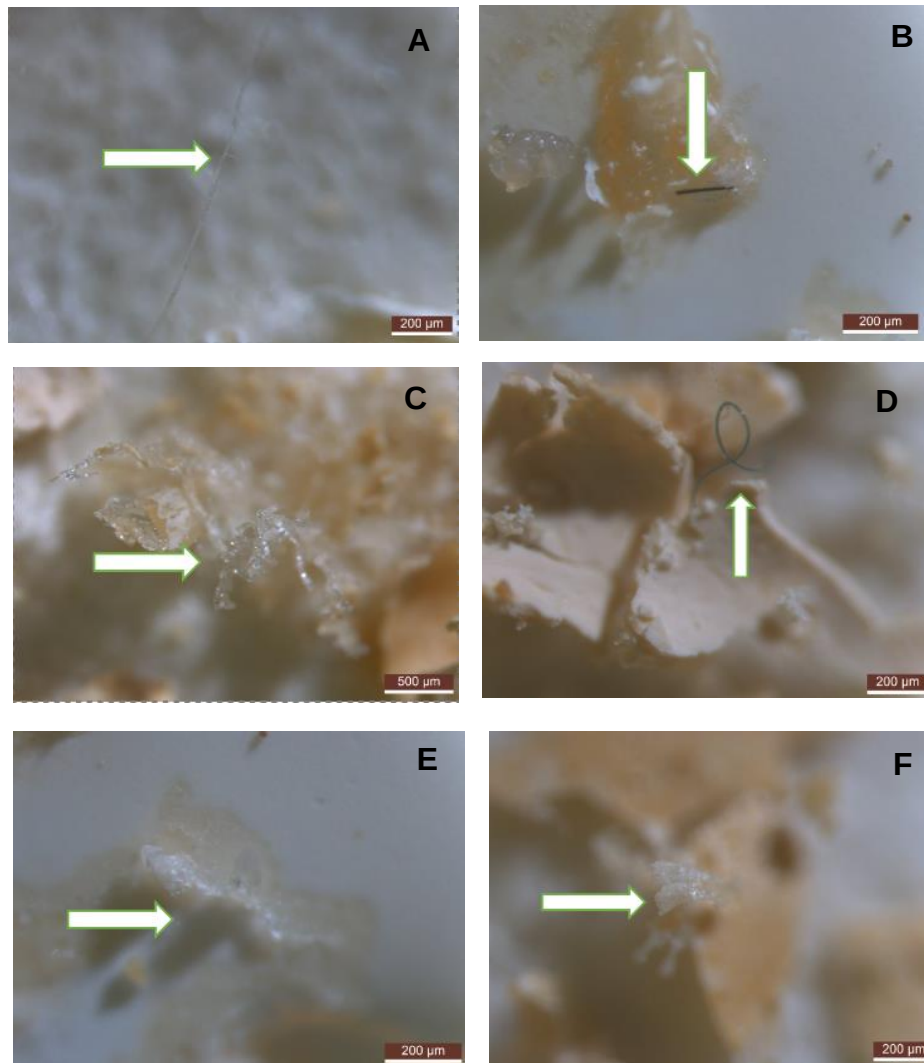
The samples given are ***Mugil Cephalus*** (mullet) muscle sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.15 Microplastic fibers (B - red, A - black, E - transparent), C - film, D - pellet and F - foam are found of varying colors and size in the muscle sample.***

***Meretrix casta*** (clams) foot sample

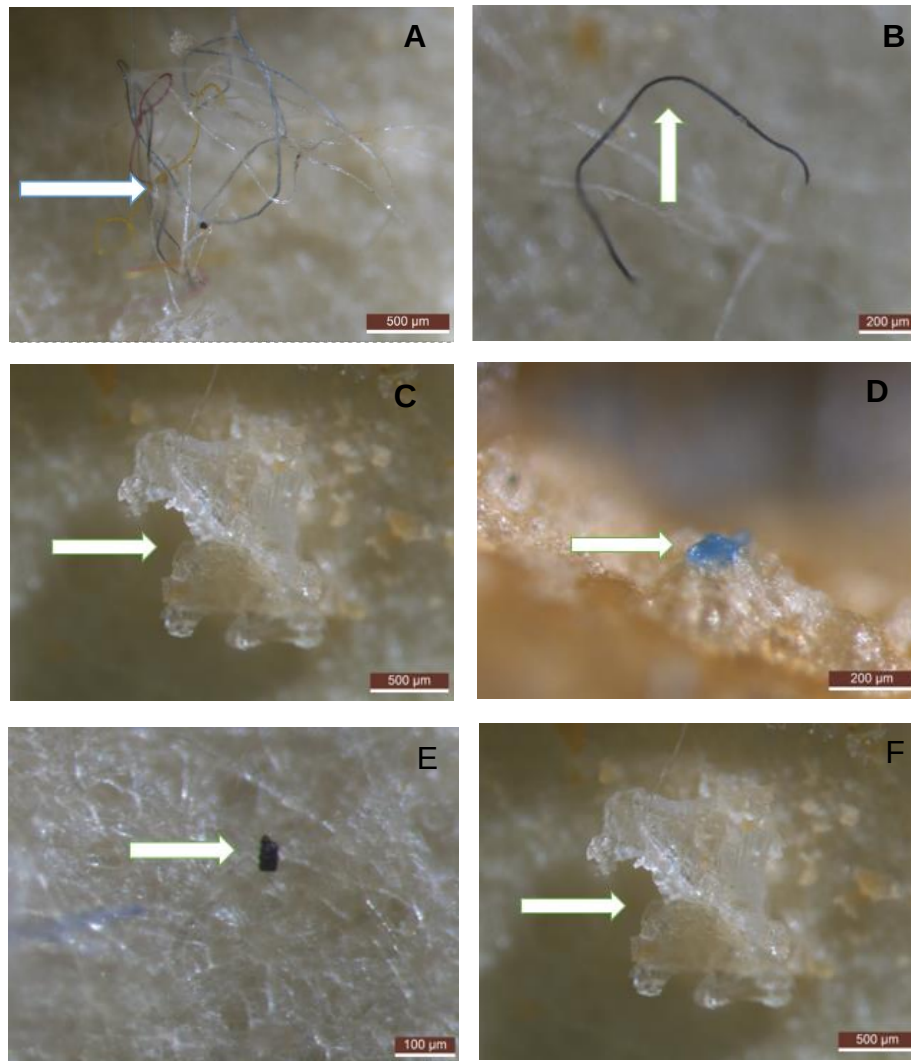
The samples given are ***Meretrix casta*** (clams) foot sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.16 Microplastic fibers (D - blue, B - black, A - transparent), C, E - film, and F -foam are found of varying colors and size in the foot sample.***

***Meretrix casta*** (clams) gill sample

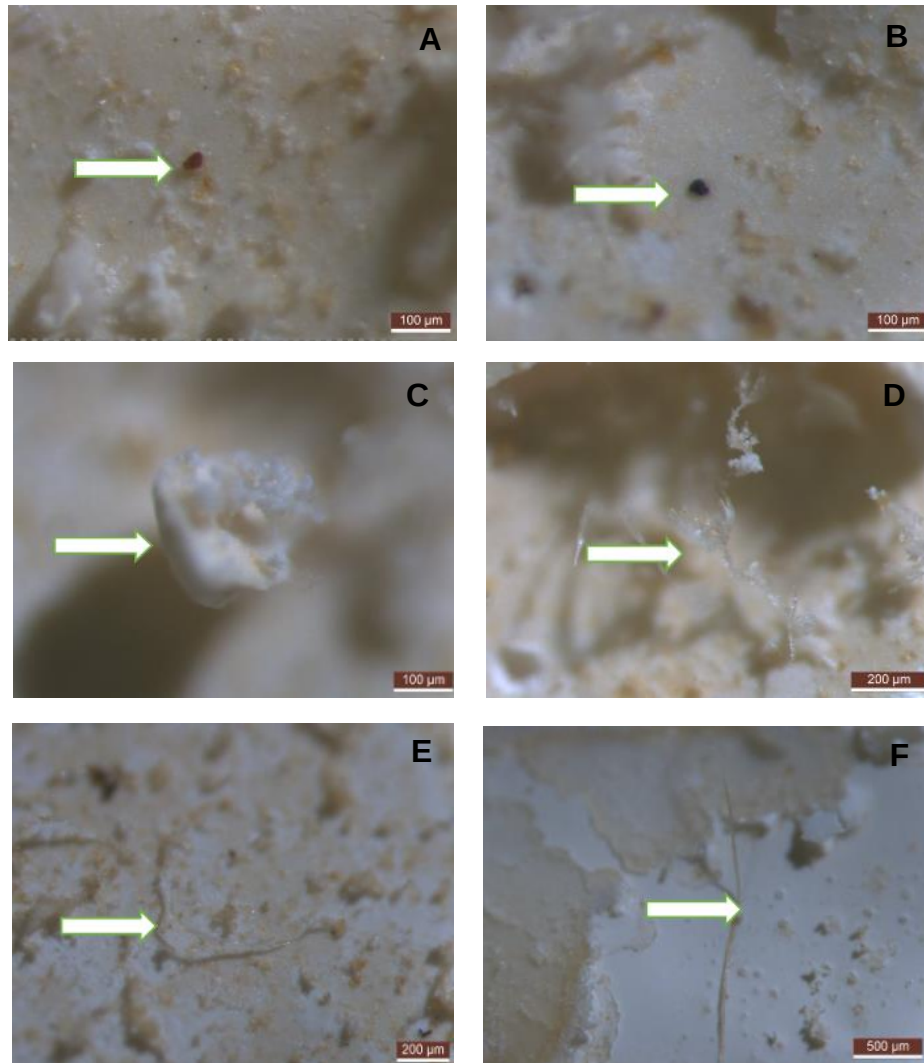
The samples given are ***Meretrix casta*** (clams) gill sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.17 Microplastic fibers (A - blue, B - black, A - transparent, A - violet, A - gold), pellets (E - black, D - blue) and C, F - film are found of varying colors and sizes in the gill sample.***

***Meretrix casta*** (clams) muscle sample

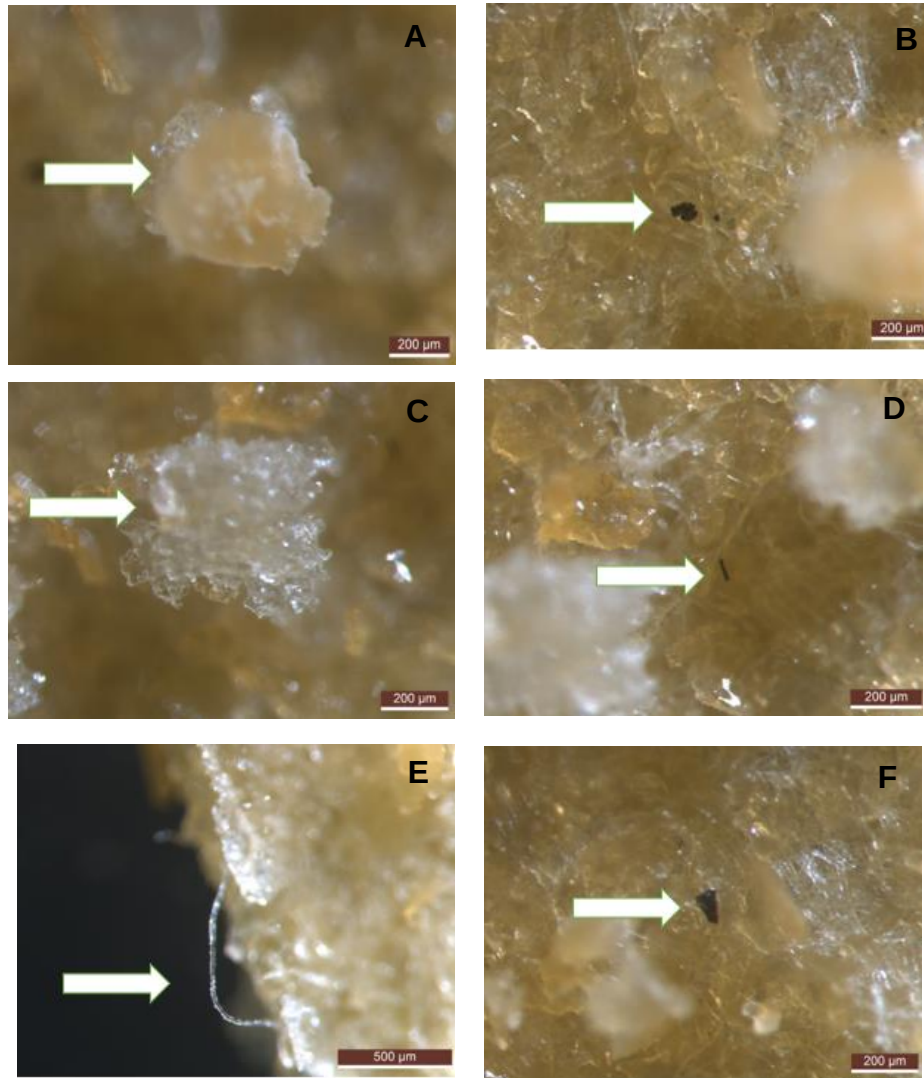
The samples given are ***Meretrix casta*** (clams) muscle sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.18 Microplastic fibers (D, E - transparent, F - gold), pellets (B - black, A - red) and C - foam are found of varying colors and sizes in the muscle sample.***

***Meretrix casta*** (clams) Adductor muscle sample

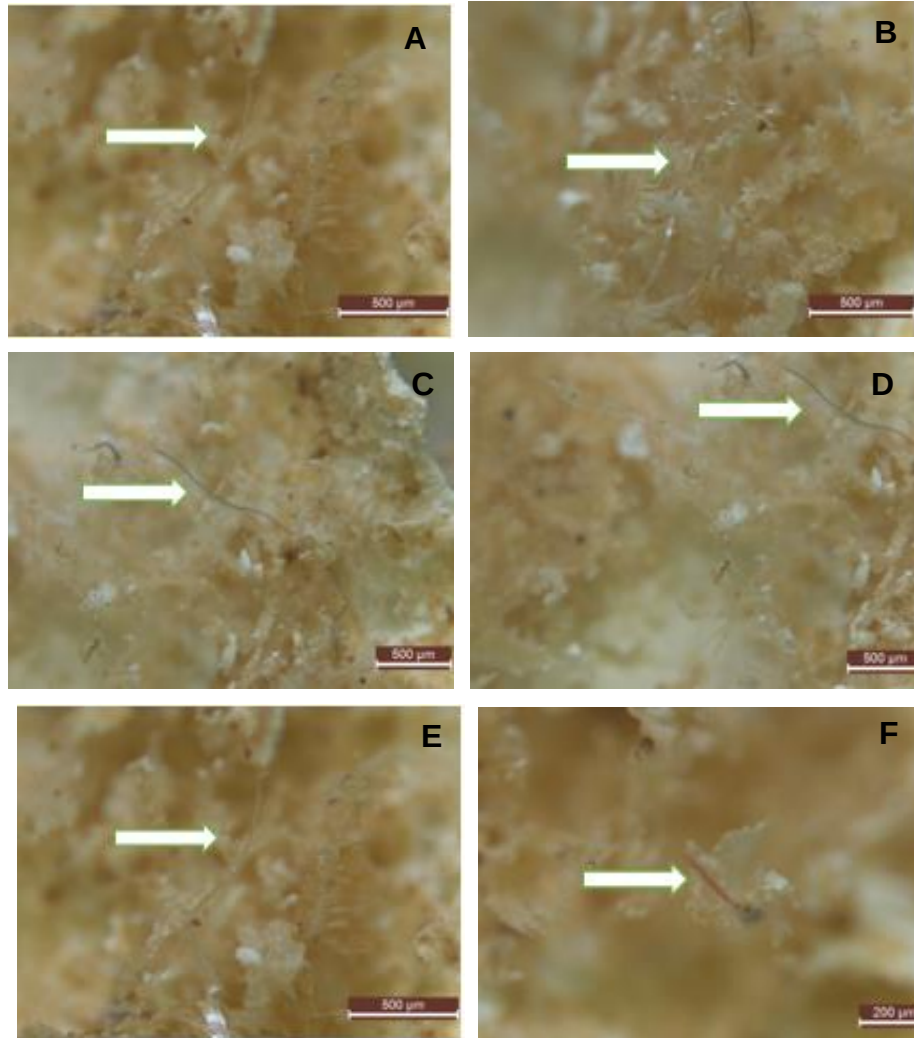
The samples given are ***Meretrix casta*** (clams) Adductor muscle sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.19 Microplastic fiber (E - transparent), pellets (B, C, F - black), D - film and A - foam are found of varying colors and sizes in the Adductor muscle sample.***

### Blue flag beach soil sample

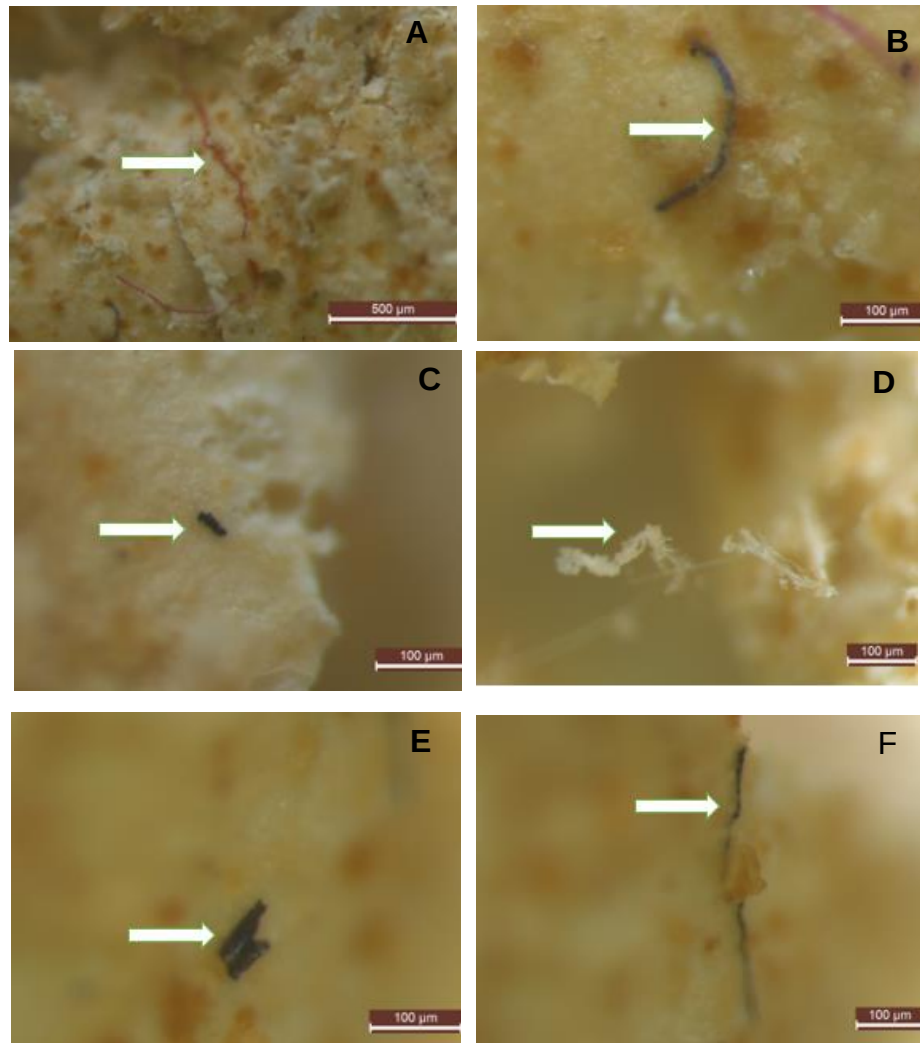
The samples given are **soil** sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.20 Microplastic fiber (A, B – transparent, C, D – blue, E, F – red fiber) are found of varying colors and sizes in the soil sample.***

### Blue flag beach water sample

The samples given are **water** sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.

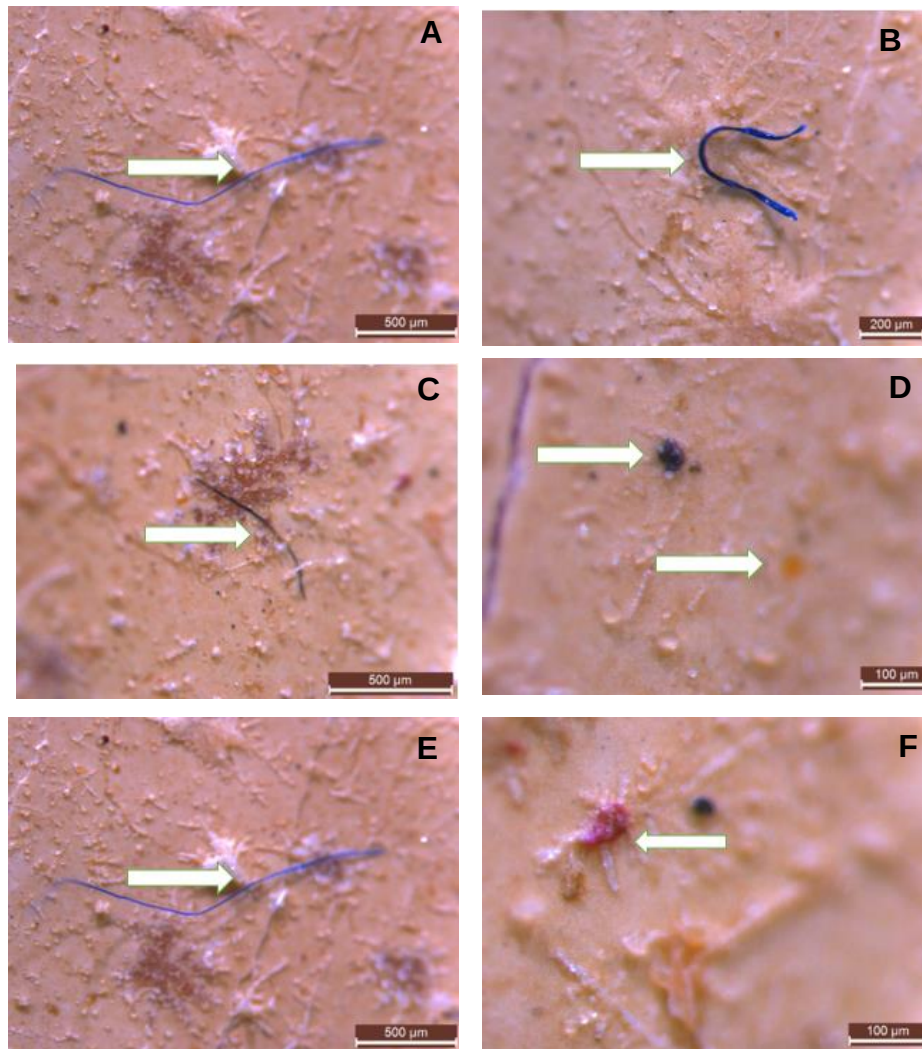


***Fig 5.21 Microplastic fiber (A - red, B – blue, D – transparent, F – black fiber), pellets (C, E – black) are found of varying colors and sizes in the water sample.***

## Rameswaram sample

### *Plotosus Canius* gill sample

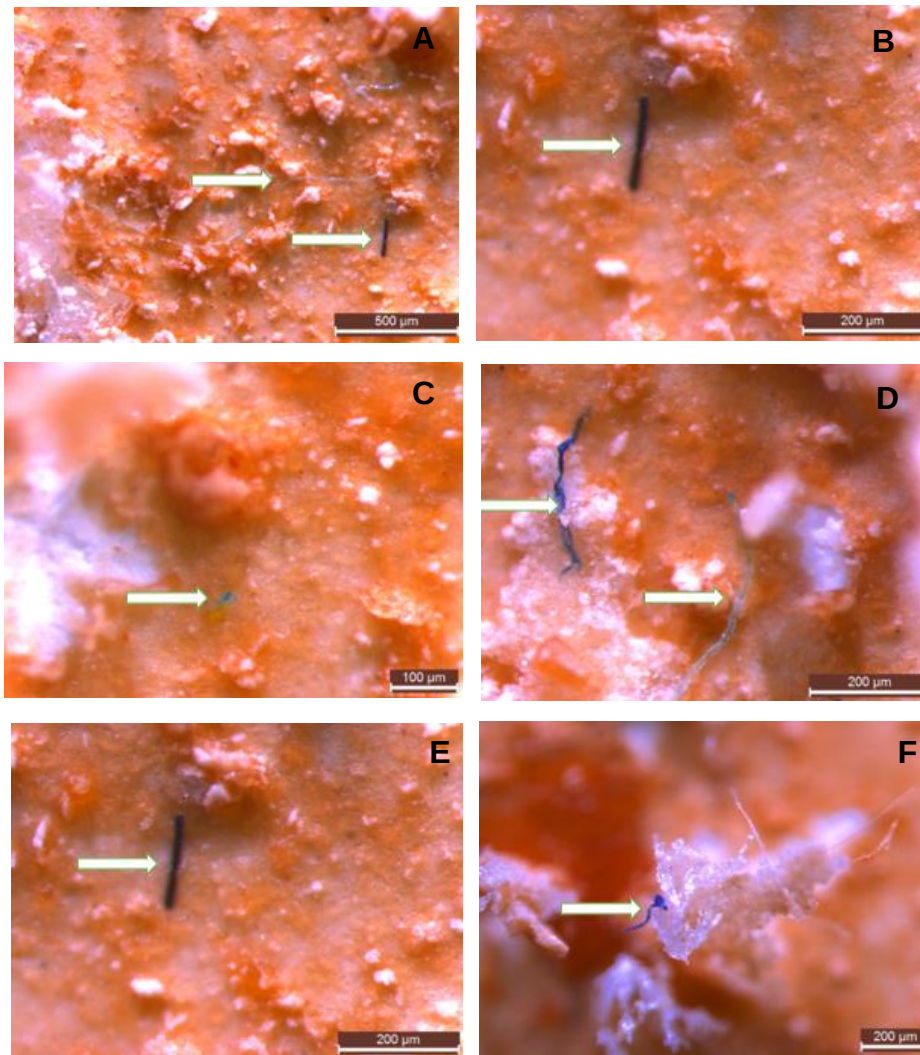
The samples given are *Plotosus canius* (*cat fish*) gill sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



**Fig 5.22 Microplastic fibre (A, B, E – blue fibre, C - black fiber), pellets (D, F – black, red pellet) are found of varying colors and sizes in the gill sample.**

### ***Plotosus canius* gut sample**

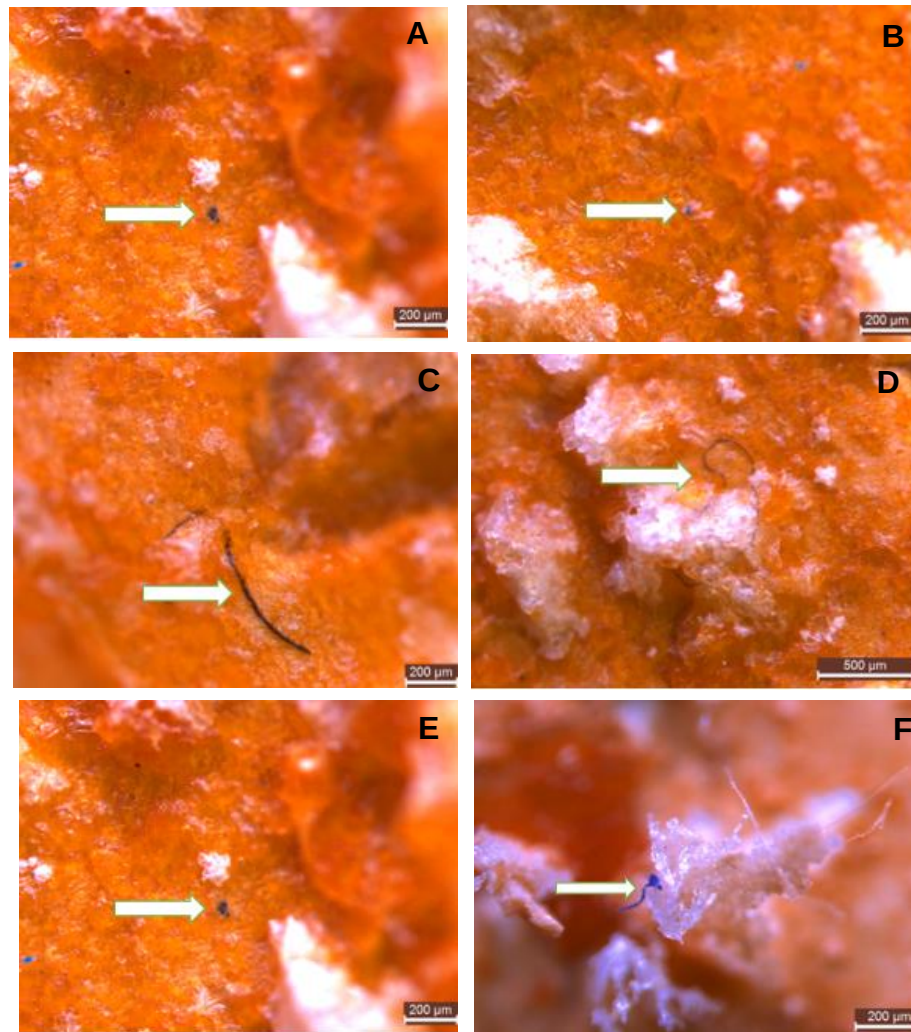
The samples given are ***Plotosus canius* (cat fish)** gut sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.23 Microplastic fibre (A, B, E – black fibre, D, F - blue, transparent fibre), pellets (C – blue pellet) are found of varying colors and sizes in the gill sample.***

### ***Plotosus canius* skin sample**

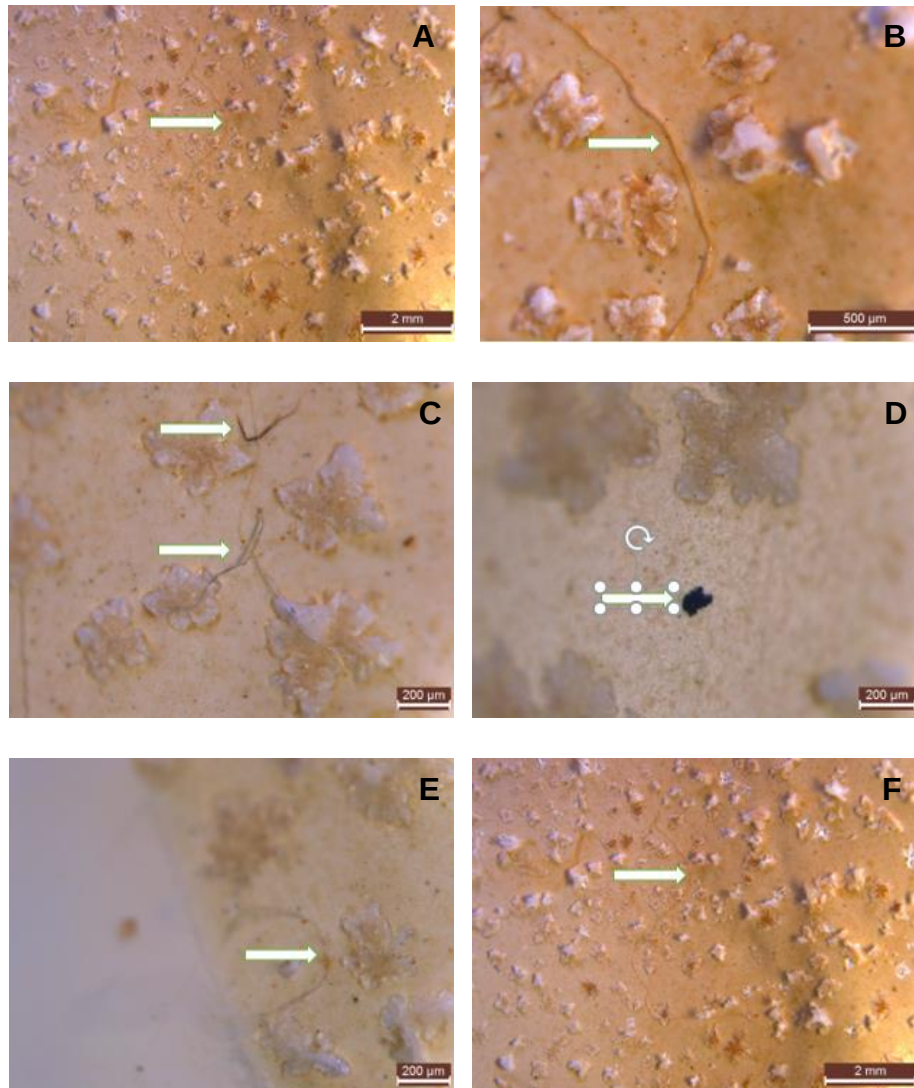
The samples given are ***Plotosus canius* (cat fish)** skin sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.24 Microplastic fibre (C, D – black fibre, F - blue) pellets (A – black pellet, B – blue pellet) are found of varying colors and sizes in the gill sample.***

### ***Plotosus canius* muscle sample**

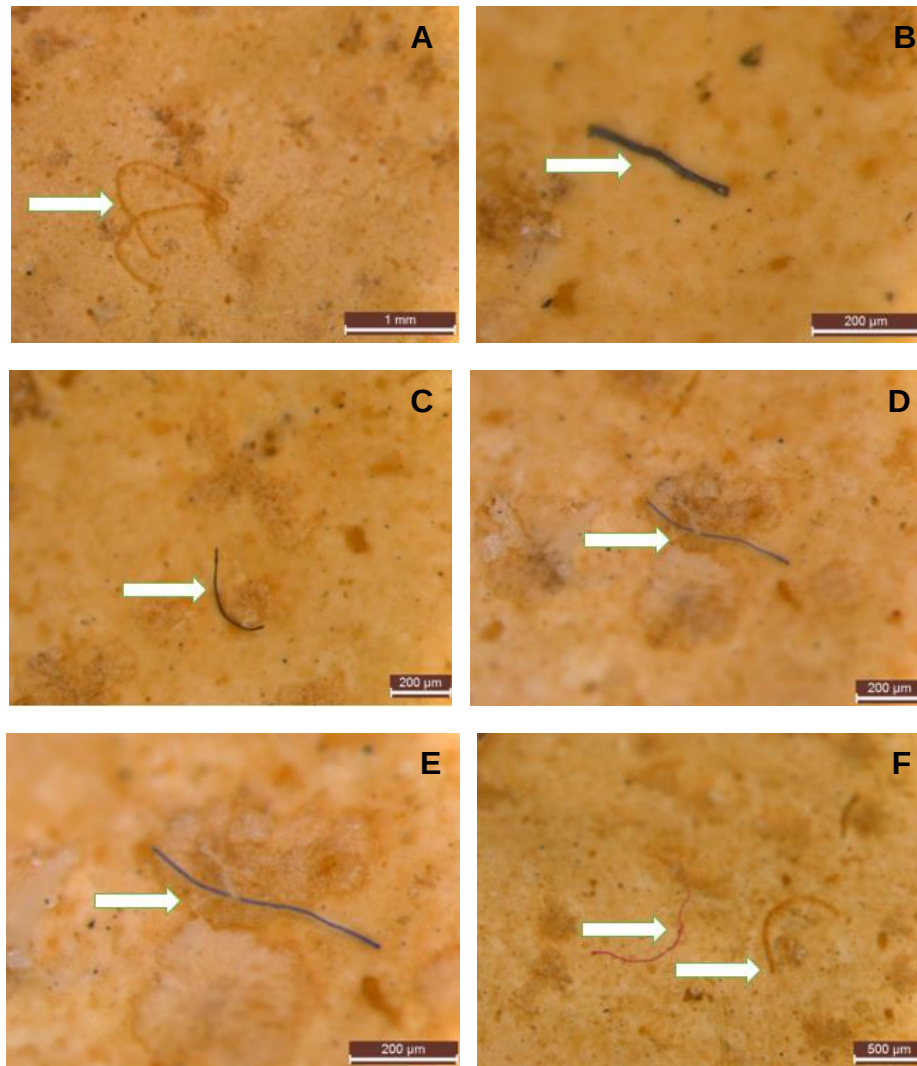
The samples given are ***Plotosus canius* (cat fish)** muscle sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.25 Microplastic fibre (A, B, F – golden fibre, C – black fibre, E – transparent fibre) pellet (black pellet) are found of varying colors and sizes in the muscle sample.***

### ***Scylla serrata* gill sample**

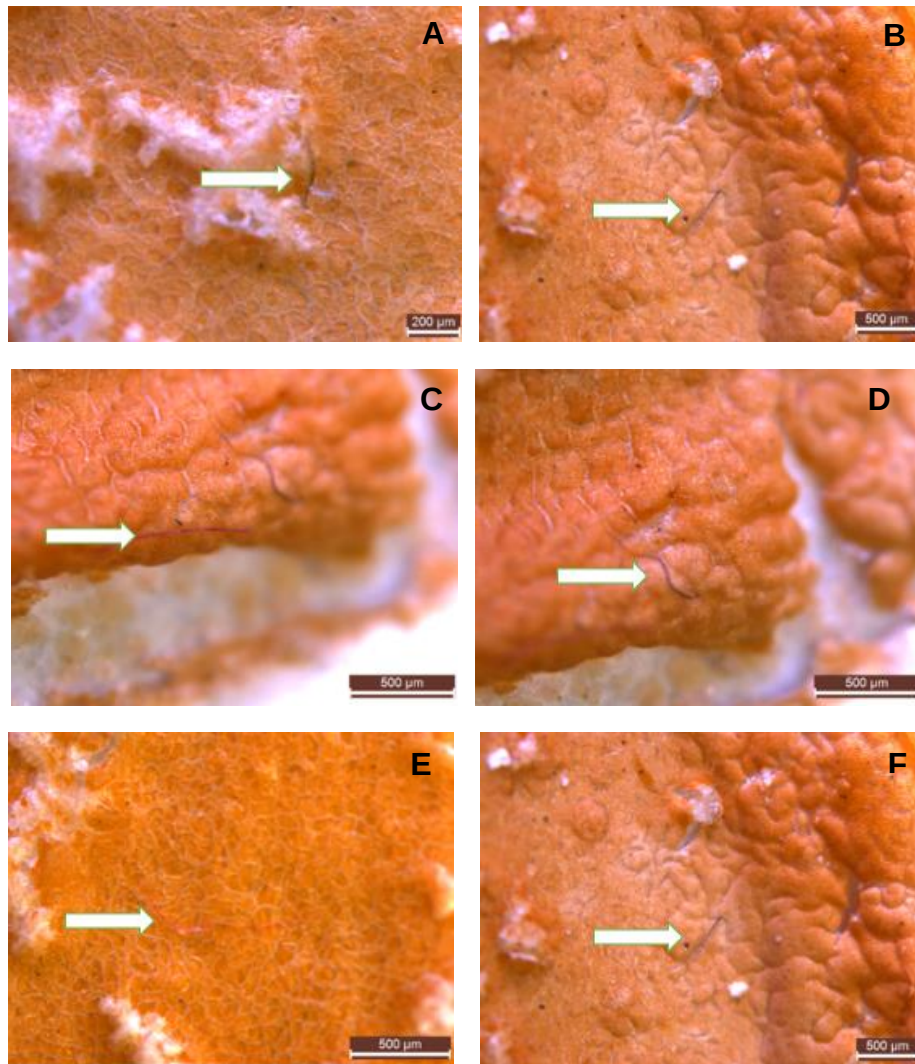
The samples given are ***Scylla serrata* gill** sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



**Fig 5.26 Microplastic fibre (A – golden fibre, D, E – blue fibre, B, C – black fibre, F – red, golden fibre) are found of varying colors and sizes in the gill sample.**

***Scylla serrata* Hepatopancreas sample:**

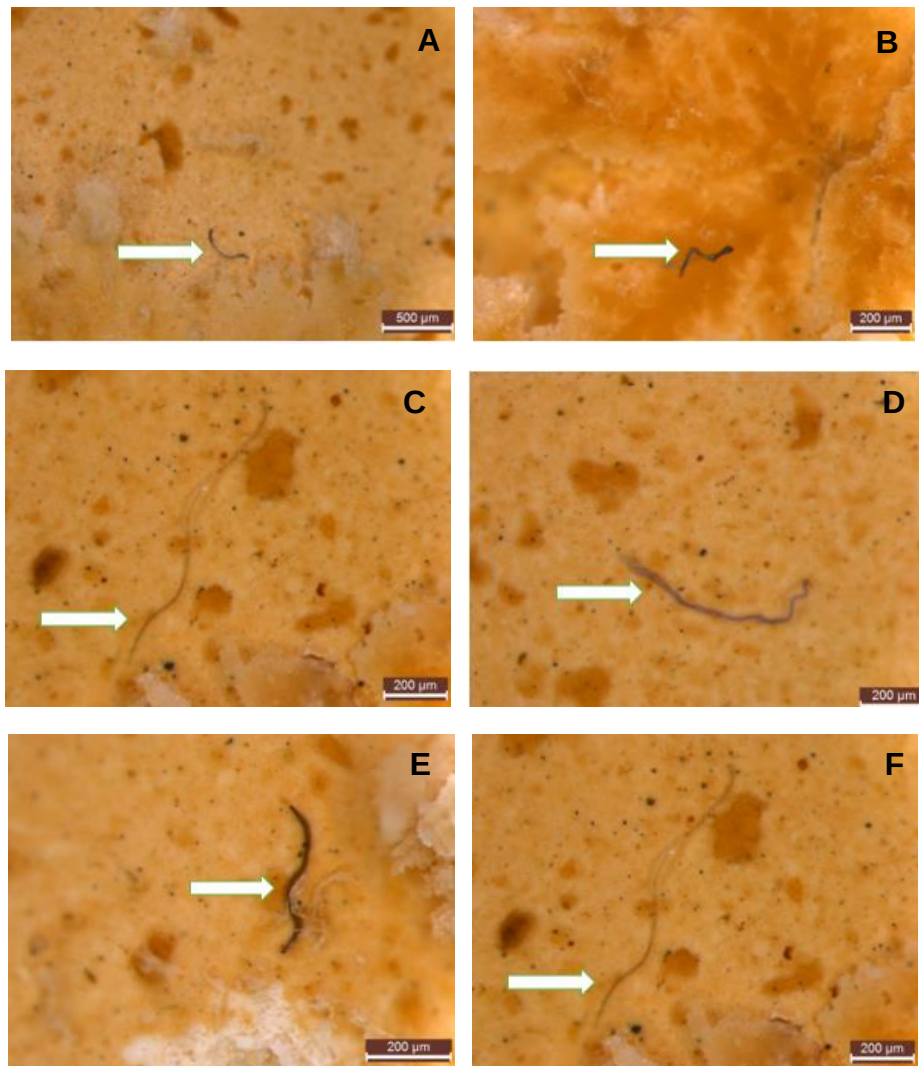
The samples given are ***Scylla serrata* Hepatopancreas** sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.27 Microplastic fibre (A – black fibre, B, F, D – blue fibre, C, E – red fibre) are found of varying colors and sizes in the hepatopancreas sample.***

### ***Scylla serrata* tissue sample**

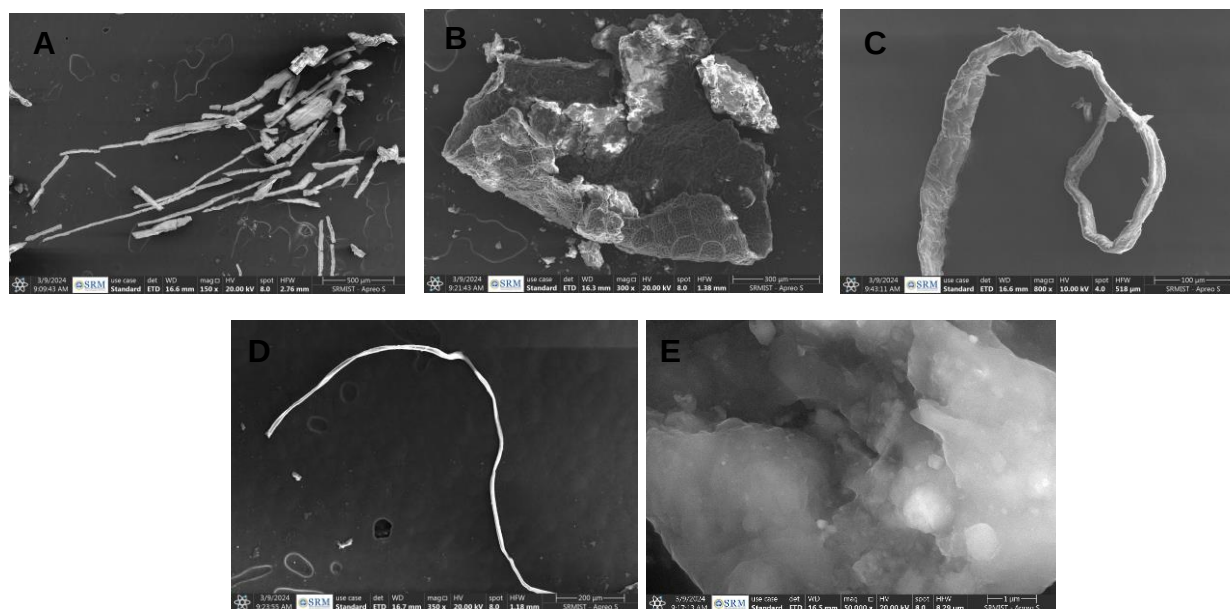
The samples given are ***Scylla serrata* tissue** sample under Stereo Microscope and it was here that we found a large number of microplastics in the form of fibers, pellets, foams, and films.



***Fig 5.28 Microplastic fibre (A, B, E – black fibre, C, F - transparent fibre, D – blue fibre) are found of varying colors and sizes in the tissue sample.***

### 5.1.2 High Resolution Scanning Electron Microscopic Analysis:

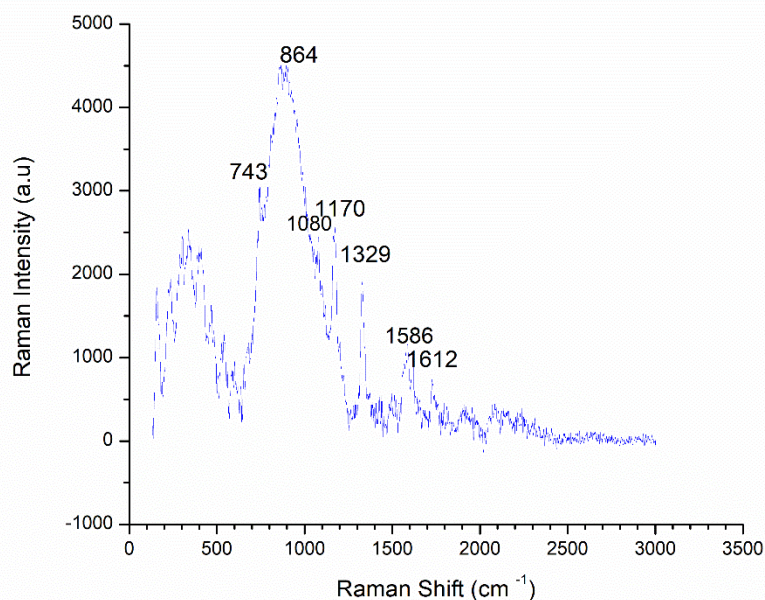
The presence of microplastics were identified by the variety of elastic and in-elastic scattering between the electrons. Specifically, by analyzing the back-scattered electrons passing through the microplastics, that produce an increasing intensity providing the necessary information about the topography and contrast of the microplastic, while the secondary electrons provide a detailed image that aids in understanding the morphology of microplastics. In this analysis microplastic structures such as: Transparent fibers, pellets, foams, were observed. The sources of these microplastic structures were found to be Polypropylene, Polyethylene and Polyolefin upon co-relating with various other analytical studies.



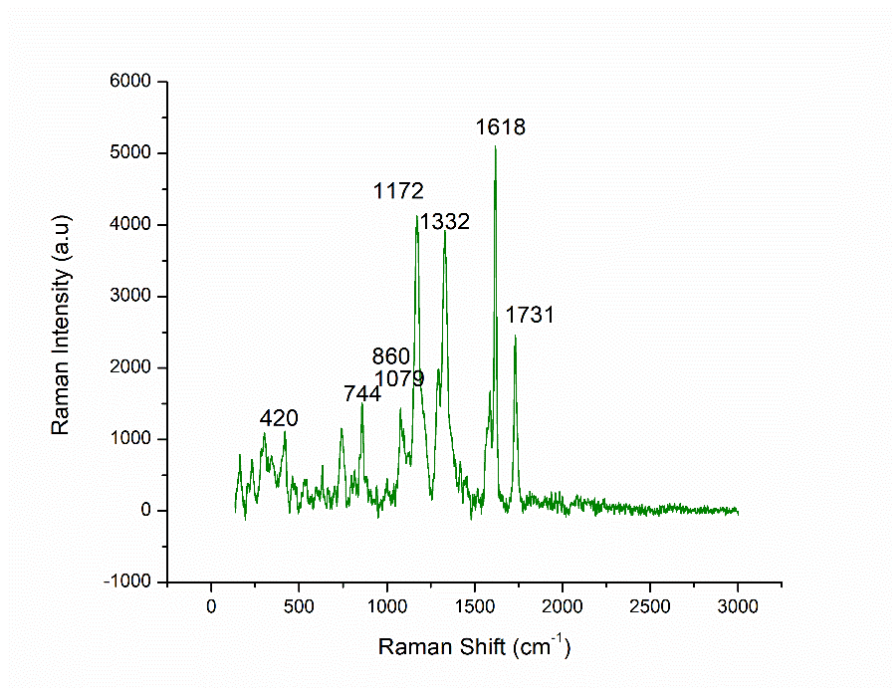
**Fig 5.29 Images of microplastics identified by SEM (A) Transparent fiber 150x (working distance (WD) 16.6mm and horizontal field width (HFW) 2.76 mm) (B) Brown pellet 300x (working distance (WD) 16.3 mm and horizontal field width (HFW) 1.38 mm) (C) Red fiber 800x (working distance (WD) 16.6 mm and horizontal field width (HFW) 518  $\mu$ m) (D) Blue fiber 350x (working distance (WD) 16.7 mm and horizontal field width (HFW) 1.18 mm) (E) Foam 50000x (working distance (WD) 16.5 mm and horizontal field width (HFW) 8.29  $\mu$ m)**

### 5.1.3 RAMAN SPECTROSCOPIC ANALYSIS

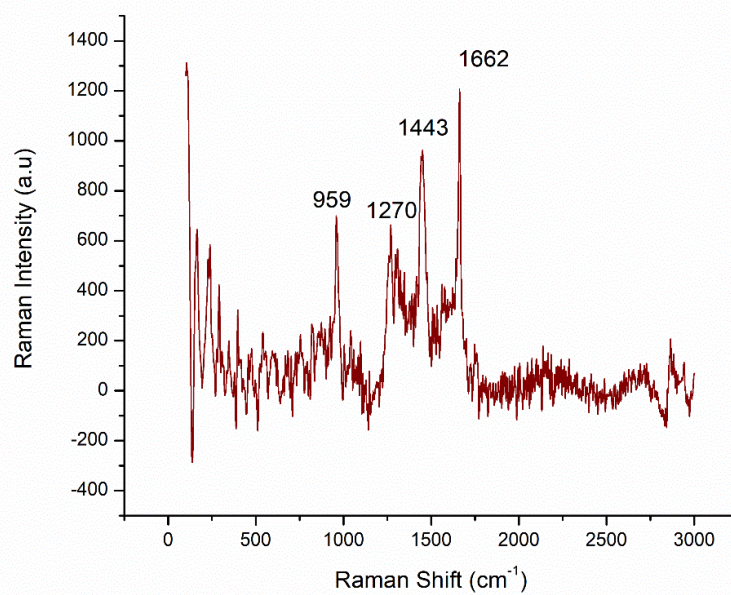
Raman Spectroscopic measurements were done for MPs on filter membrane using the inViaTM, Reinshaw, UK equipped with an argon-ion laser at an excitation wavelength of 785nm. The WIRE Spectral acquisition wizard was used to capture the spectrum of the MPs. Presence of biofilm on the polymer particles often resulted in a broad fluorescent band around 800-1800  $\text{cm}^{-1}$  region, which overshadowed the underlying polymer peaks. This resulted in poor intensity Raman bands. (Ghosal et. al., 2017). The main characteristic peaks of the individual MP such as 1080  $\text{cm}^{-1}$  for Polystyrene (PS) in *Plotosus canius* Gut, 1618  $\text{cm}^{-1}$  and 1713  $\text{cm}^{-1}$  for PET in *Scylla serrata* Tissue, 1662  $\text{cm}^{-1}$  for PET in *Nematalosa nasus* Gill, (Sobhani et. al., 2019). The Raman analysis was done for gill, gut and tissue samples were done and good quality spectra identified based on intensity bands were plotted as Raman shift ( $\text{cm}^{-1}$ ) vs Raman intensity (a.u) using the Origin Pro software version 8.5.



**Fig 5.30 Raman Spectrum of Fiber from *Plotosus canius* Gut**



**Fig 5.31 Raman Spectrum of Fiber from *Scylla serrata* Tissue**



**Fig 5.32 Raman Spectrum of Fiber from *Nematulosa nausus* Gill**

## 5.2 HEAVY METAL CONCENTRATION

### 5.2.1 Atomic Absorption Spectroscopic Analysis

The heavy metal concentrations in the samples were analyzed using Atomic Absorption Spectrometer (AAS), including zinc, selenium, lead, mercury, cadmium, chromium, arsenic, magnesium and iron. Here, we investigated fish samples (*Nematalosa nasus*, *Mugil cephalus*, *Plotosus canius*, *Meretrix casta*, *Scylla serrata*), water, and soil sediments in the Kovalam and Rameswaram area for the content of heavy metals such zinc, selenium, lead, mercury, cadmium, chromium, arsenic, magnesium, and iron.

In Kovalam and Rameswaram, we investigated the effects of heavy metals on the marine biota, and Zinc, selenium, and manganese were found in high concentrations in kovalam samples, while iron was found in significant concentrations in Rameswaram samples.

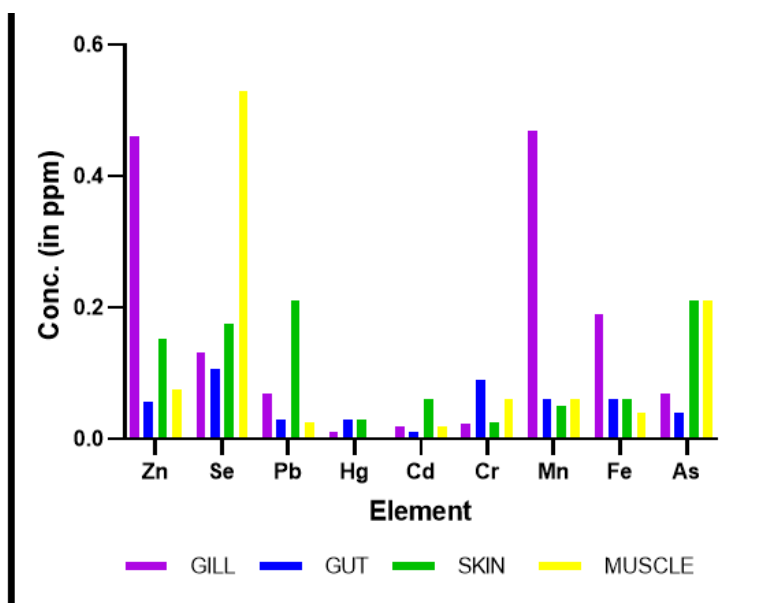
This study examined the impact of heavy metal contamination on marine samples, and the potential outcomes are listed below. Here, we have listed the concentrations of heavy metals found in fish (*Nematalosa nasus*, *Mugil cephalus*, *Scylla serrata*, *Plotosus canius*, *Meretrix casta*) in their gut, gills, skin, and muscles. A graph has also been included for easy comprehension.

***Namatulosa nausus*** (Bloch's Gizzard shad)

The concentration of heavy metals, including zinc, selenium, lead, mercury, cadmium, chromium, arsenic, manganese, and iron, was analyzed in the gills, gut, skin, and muscle of *Nematulosa nausus* fish.

**Table 5.1 Heavy metal concentration of *Nematulosa nausus***

| S.NO | Elements | Gill  | Gut   | Skin  | Muscle |
|------|----------|-------|-------|-------|--------|
| 1    | Zn       | 0.46  | 0.057 | 0.153 | 0.076  |
| 2    | Se       | 0.132 | 0.106 | 0.176 | 0.53   |
| 3    | Pb       | 0.07  | 0.03  | 0.21  | 0.026  |
| 4    | Hg       | 0.01  | 0.03  | 0.03  | 0.003  |
| 5    | Cd       | 0.02  | 0.01  | 0.06  | 0.02   |
| 6    | Cr       | 0.023 | 0.09  | 0.026 | 0.06   |
| 7    | Mn       | 0.47  | 0.06  | 0.05  | 0.06   |
| 8    | Fe       | 0.19  | 0.06  | 0.06  | 0.04   |
| 9    | As       | 0.07  | 0.04  | 0.21  | 0.21   |



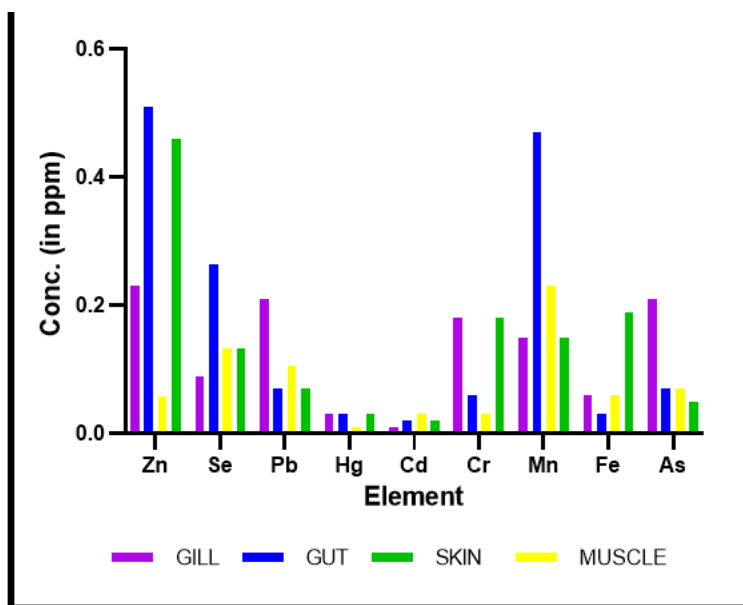
**Fig 5.33 AAS of *Nematulosa nausus***

***Plotosus canius*** (catfish)

The concentration of heavy metals, including zinc, selenium, lead, mercury, cadmium, chromium, arsenic, manganese, and iron, was analyzed in the gills, gut, skin, and muscle of *Plotosus canius* fish.

**Table 5.2 Heavy metal concentration of *Plotosus Canius***

| S.NO | Elements | Gill  | Gut   | Muscle | Skin  |
|------|----------|-------|-------|--------|-------|
| 1    | Zn       | 0.23  | 0.51  | 0.057  | 0.46  |
| 2    | Se       | 0.088 | 0.265 | 0.132  | 0.132 |
| 3    | Pb       | 0.21  | 0.07  | 0.105  | 0.07  |
| 4    | Hg       | 0.03  | 0.03  | 0.01   | 0.03  |
| 5    | Cd       | 0.01  | 0.02  | 0.03   | 0.02  |
| 6    | Cr       | 0.18  | 0.06  | 0.03   | 0.18  |
| 7    | Mn       | 0.15  | 0.47  | 0.23   | 0.15  |
| 8    | Fe       | 0.06  | 0.03  | 0.06   | 0.19  |
| 9    | As       | 0.21  | 0.07  | 0.07   | 0.05  |



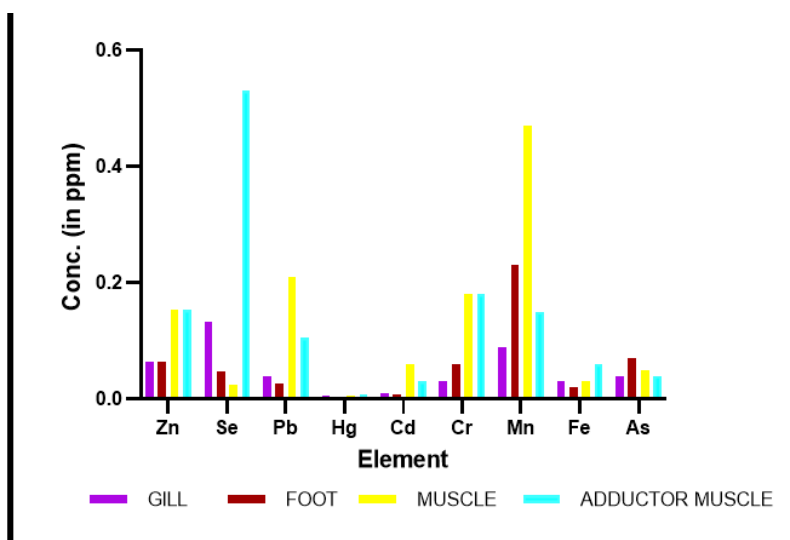
**Fig 5.34 AAS of *Plotosus canius***

### ***Meretrix casta*** (clams)

The concentration of heavy metals, including zinc, selenium, lead, mercury, cadmium, chromium, arsenic, manganese, and iron, was analyzed in the gills, foot, adductor muscle, and muscle of *Meretrix casta* fish.

**Table 5.3 Heavy metal concentration of *Meretrix casta***

| S.NO | Elements | Gill  | Foot  | Muscle | Adductor Muscle |
|------|----------|-------|-------|--------|-----------------|
| 1    | Zn       | 0.065 | 0.065 | 0.153  | 0.153           |
| 2    | Se       | 0.132 | 0.048 | 0.025  | 0.53            |
| 3    | Pb       | 0.04  | 0.026 | 0.21   | 0.105           |
| 4    | Hg       | 0.006 | 0.003 | 0.006  | 0.007           |
| 5    | Cd       | 0.01  | 0.007 | 0.06   | 0.03            |
| 6    | Cr       | 0.03  | 0.06  | 0.18   | 0.18            |
| 7    | Mn       | 0.09  | 0.23  | 0.47   | 0.15            |
| 8    | Fe       | 0.03  | 0.02  | 0.03   | 0.06            |
| 9    | As       | 0.04  | 0.07  | 0.05   | 0.04            |



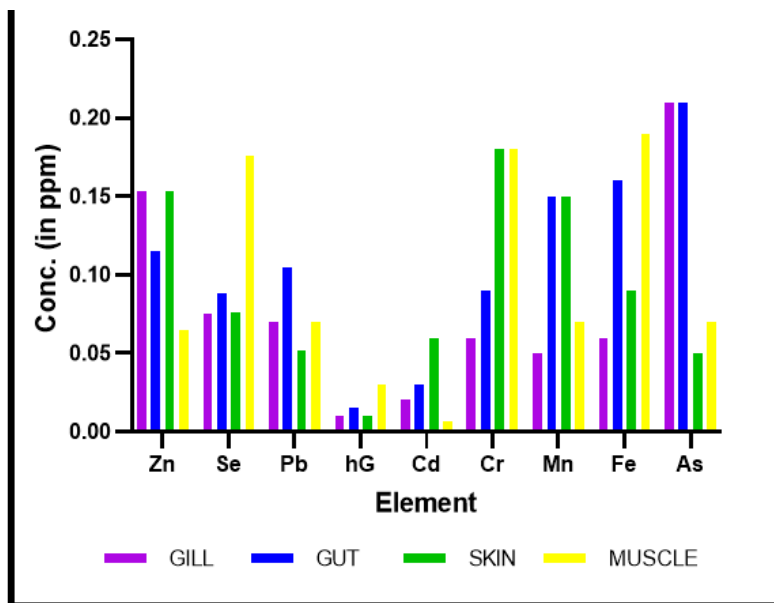
**Fig 5.35 AAS of *Meretrix casta* (clams)**

### ***Mugil Cephalus*** (mullet)

The concentration of heavy metals, including zinc, selenium, lead, mercury, cadmium, chromium, arsenic, manganese, and iron, was analyzed in the gills, gut, skin, and muscle of *Mugil Cephalus* fish.

**Table 5.4 Heavy metal concentration of *Mugil Cephalus* (mullet)**

| S.NO | Elements | Gill  | Gut   | Skin  | Muscle |
|------|----------|-------|-------|-------|--------|
| 1    | Zn       | 0.153 | 0.115 | 0.153 | 0.065  |
| 2    | Se       | 0.075 | 0.088 | 0.076 | 0.176  |
| 3    | Pb       | 0.07  | 0.105 | 0.052 | 0.07   |
| 4    | Hg       | 0.01  | 0.015 | 0.01  | 0.03   |
| 5    | Cd       | 0.02  | 0.03  | 0.06  | 0.007  |
| 6    | Cr       | 0.06  | 0.09  | 0.18  | 0.18   |
| 7    | Mn       | 0.05  | 0.15  | 0.15  | 0.07   |
| 8    | Fe       | 0.06  | 0.16  | 0.09  | 0.19   |
| 9    | As       | 0.21  | 0.21  | 0.05  | 0.07   |



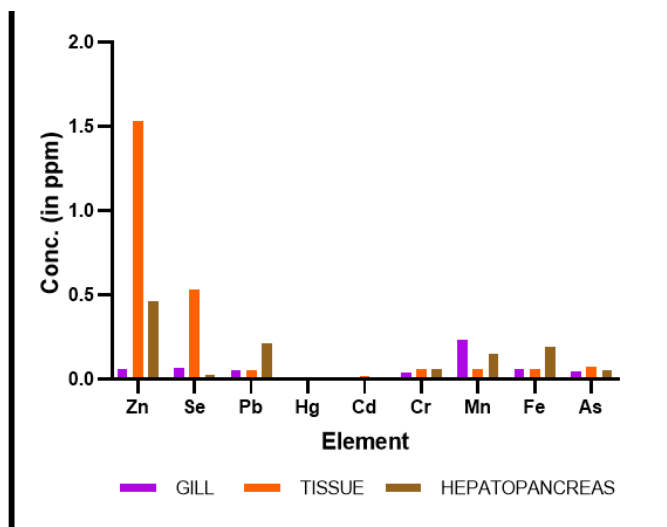
**Fig 5.36 AAS of *Mugil Cephalus* (mullet)**

### ***Scylla serrata*** (crab)

The concentration of heavy metals, including zinc, selenium, lead, mercury, cadmium, chromium, arsenic, manganese, and iron, was analyzed in the gills, hepatopancreas and muscle of *Scylla serrata* fish.

**Table 5.5 Heavy metal concentration of *Scylla serrata***

| S.NO | Elements | Gill  | Tissue | Hepatopancreas |
|------|----------|-------|--------|----------------|
| 1    | Zn       | 0.057 | 1.53   | 0.46           |
| 2    | Se       | 0.066 | 0.53   | 0.025          |
| 3    | Pb       | 0.05  | 0.052  | 0.21           |
| 4    | Hg       | 0.007 | 0.01   | 0.015          |
| 5    | Cd       | 0.007 | 0.02   | 0.002          |
| 6    | Cr       | 0.04  | 0.06   | 0.06           |
| 7    | Mn       | 0.23  | 0.059  | 0.15           |
| 8    | Fe       | 0.06  | 0.06   | 0.19           |
| 9    | As       | 0.042 | 0.07   | 0.05           |



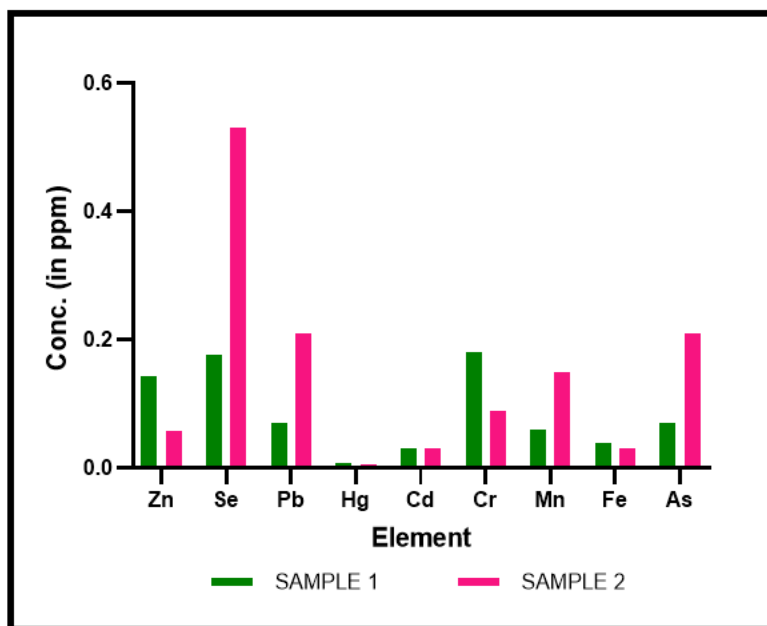
**Fig 5.37 AAS of *Scylla serrata***

## WATER SAMPLE

The concentration of heavy metals, including zinc, selenium, lead, mercury, cadmium, chromium, arsenic, manganese, and iron, was analyzed in the *Water sample*

**Table 5.6 Heavy metal concentration of water samples collected from kovalam**

| S.NO | Elements | Sample 1 | Sample 2 |
|------|----------|----------|----------|
| 1    | Zn       | 0.143    | 0.057    |
| 2    | Se       | 0.176    | 0.53     |
| 3    | Pb       | 0.07     | 0.21     |
| 4    | Hg       | 0.007    | 0.006    |
| 5    | Cd       | 0.03     | 0.03     |
| 6    | Cr       | 0.18     | 0.09     |
| 7    | Mn       | 0.06     | 0.15     |
| 8    | Fe       | 0.04     | 0.03     |
| 9    | As       | 0.07     | 0.21     |



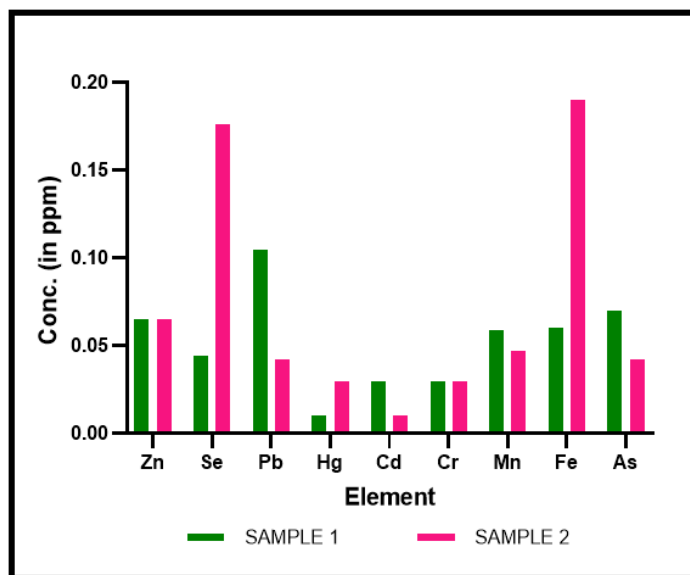
**Fig 5.38 AAS of Water samples collected from Kovalam**

## SOIL SAMPLE

The concentration of heavy metals, including zinc, selenium, lead, mercury, cadmium, chromium, arsenic, manganese, and iron, was analyzed in the Soil sample

***Table 5.7 Heavy metal concentration of soil samples collected from kovalam***

| S.NO | Elements | Sample 1 | Sample 2 |
|------|----------|----------|----------|
| 1    | Zn       | 0.065    | 0.065    |
| 2    | Se       | 0.044    | 0.176    |
| 3    | Pb       | 0.105    | 0.042    |
| 4    | Hg       | 0.01     | 0.03     |
| 5    | Cd       | 0.03     | 0.01     |
| 6    | Cr       | 0.03     | 0.03     |
| 7    | Mn       | 0.059    | 0.047    |
| 8    | Fe       | 0.06     | 0.19     |
| 9    | As       | 0.07     | 0.042    |



***Fig 5.39 AAS of soil collected from kovalam***

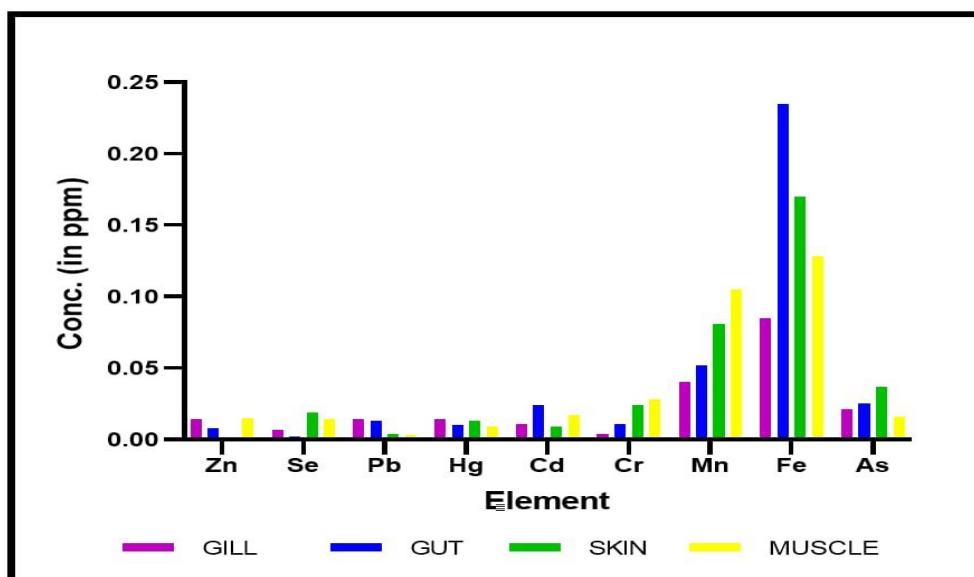
## Rameswaram samples

### *Plotosus canius* (cat fish)

The concentration of heavy metals, including zinc, selenium, lead, mercury, cadmium, chromium, arsenic, manganese, and iron, was analyzed in the gills, gut, skin, and muscle of *Plotosus canius* fish.

**Table 5.8 Heavy metal concentration of *Plotosus canius***

| S.NO | ELEMENTS | GILL  | GUT   | SKIN  | MUSCLE |
|------|----------|-------|-------|-------|--------|
| 1    | Zn       | 0.014 | 0.008 | 0.001 | 0.015  |
| 2    | Se       | 0.007 | 0.002 | 0.019 | 0.014  |
| 3    | Pb       | 0.014 | 0.013 | 0.004 | 0.003  |
| 4    | Hg       | 0.014 | 0.010 | 0.013 | 0.009  |
| 5    | Cd       | 0.011 | 0.024 | 0.009 | 0.017  |
| 6    | Cr       | 0.004 | 0.011 | 0.024 | 0.028  |
| 7    | Mn       | 0.040 | 0.052 | 0.081 | 0.105  |
| 8    | Fe       | 0.085 | 0.235 | 0.170 | 0.128  |
| 9    | As       | 0.021 | 0.025 | 0.037 | 0.016  |



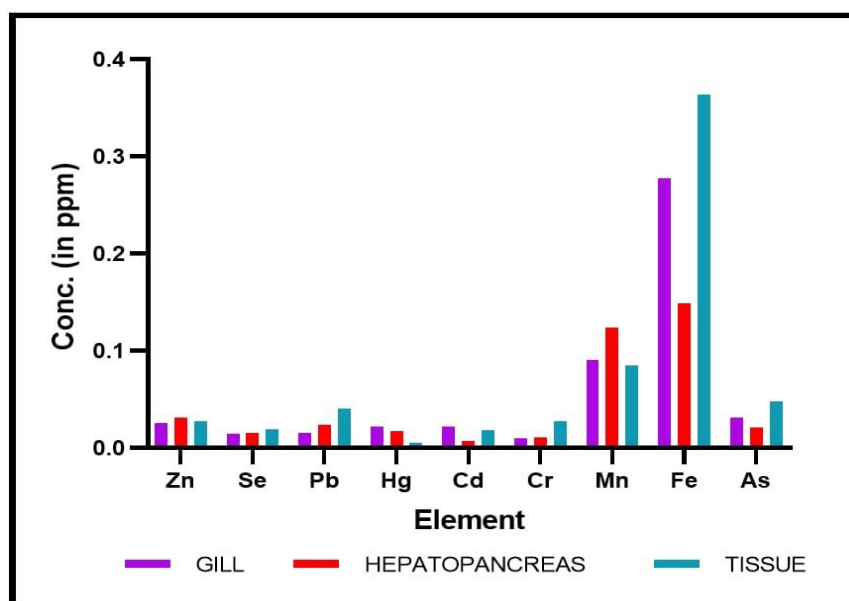
**Fig 5.40 AAS of *Plotosus canius* collected from Rameswaram**

### ***Scylla serrata* (crab)**

The concentration of heavy metals, including zinc, selenium, lead, mercury, cadmium, chromium, arsenic, manganese, and iron, was analyzed in the gills, gut, hepatopancreas, and muscle of *Scylla serrata* fish.

**Table 5.9 Heavy metal concentration of scylla serrata**

| S.NO | ELEMENTS | GILL  | HEPATOPANCREAS | TISSUE |
|------|----------|-------|----------------|--------|
| 1    | Zn       | 0.025 | 0.031          | 0.028  |
| 2    | Se       | 0.015 | 0.016          | 0.019  |
| 3    | Pb       | 0.016 | 0.024          | 0.041  |
| 4    | Hg       | 0.022 | 0.017          | 0.005  |
| 5    | Cd       | 0.022 | 0.007          | 0.018  |
| 6    | Cr       | 0.010 | 0.011          | 0.028  |
| 7    | Mn       | 0.091 | 0.124          | 0.085  |
| 8    | Fe       | 0.277 | 0.149          | 0.364  |
| 9    | As       | 0.031 | 0.021          | 0.048  |



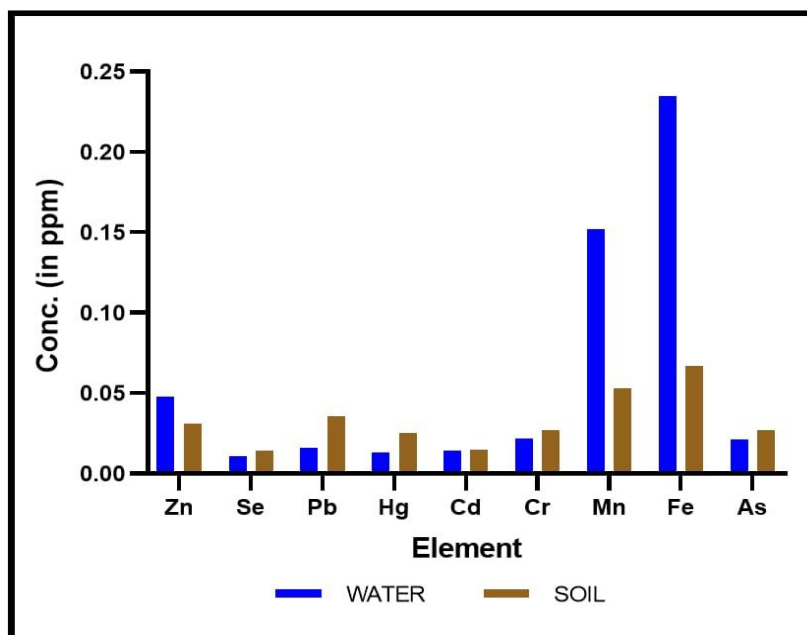
**Fig 5.41 AAS of *Scylla serrata* collected from Rameswaram**

### Water and Soil samples collected from Rameswaram

The concentration of heavy metals, including zinc, selenium, lead, mercury, cadmium, chromium, arsenic, manganese, and iron, was analyzed in the Soil and water sample

**Table 5.10 Heavy metal concentration of Water and Soil**

| S.NO | ELEMENTS | Water | soil  |
|------|----------|-------|-------|
| 1    | Zn       | 0.048 | 0.031 |
| 2    | Se       | 0.011 | 0.014 |
| 3    | Pb       | 0.016 | 0.036 |
| 4    | Hg       | 0.013 | 0.025 |
| 5    | Cd       | 0.014 | 0.015 |
| 6    | Cr       | 0.022 | 0.027 |
| 7    | Mn       | 0.152 | 0.053 |
| 8    | Fe       | 0.235 | 0.067 |
| 9    | As       | 0.021 | 0.027 |



**Fig 5.42 AAS of Water and Soil samples collected from Rameswaram**

## **CHAPTER 6**

### **SUMMARY AND CONCLUSION**

Understanding how human activity affects marine ecosystems requires analyzing problems on microplastic contamination and heavy metal levels in marine creatures. Hazards to human health and the environment can arise from the accumulation of heavy metals, including lead, cadmium, and mercury, in marine species.

On the other hand, Investigation of tiny plastic particles known as microplastics have the potential to harm marine life by releasing harmful compounds and causing physical harm. The buildup of microplastics in the food chain may be detrimental to human health as well.

Research has indicated that a range of marine creatures, such as fish, crustaceans, and mollusca, are contaminated with heavy metals and microplastics. The species, geographic location, and ambient factors can all affect the amounts of these contaminants.

To reduce the buildup of heavy metals and microplastics in marine animals, human activities that contribute to environmental pollution must be effectively managed and regulated. To fully understand the long-term impacts of these pollutants on the marine ecology and public health, more investigation is also required.

My goal is to contribute to research aptitude, long-term learning, investigative method, and multidisciplinary thinking through my project.

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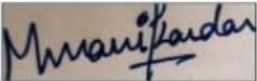
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## ANNEXURE

### Gate Score

Your GATE 2024 Result [BT]

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|                                   |                                                                                                                                                                                                                     |                                                                                                   |                                 |                                   |         |             |           |  |
|-----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|---------------------------------|-----------------------------------|---------|-------------|-----------|--|
| Name                              | <input type="text" value="MANIKANDAN M"/>                                                                                                                                                                           | <br>Photograph |                                 |                                   |         |             |           |  |
| Registration Number               | <input type="text" value="BT24S57402018"/>                                                                                                                                                                          |                                                                                                   |                                 |                                   |         |             |           |  |
| Gender                            | <input type="text" value="Male"/>                                                                                                                                                                                   |                                                                                                   |                                 |                                   |         |             |           |  |
| Parent's/Guardian's name          | <input type="text" value="MUNIYASAMY"/>                                                                                                                                                                             |                                                                                                   |                                 |                                   |         |             |           |  |
| Date of Birth (YYYY-MM-DD)        | <input type="text" value="2002-10-30"/>                                                                                                                                                                             |                                                                                                   |                                 |                                   |         |             |           |  |
| Examination Paper                 | <input type="text" value="Biotechnology (BT)"/>                                                                                                                                                                     | <br>Signature |                                 |                                   |         |             |           |  |
| Marks out of 100 <sup>#</sup>     | <input type="text" value="8.67"/>                                                                                                                                                                                   |                                                                                                   |                                 |                                   |         |             |           |  |
| Qualifying Marks <sup>##</sup>    | <table><tr><td><input type="text" value="38.9"/></td><td><input type="text" value="35"/></td><td><input type="text" value="25.9"/></td></tr><tr><td>General</td><td>OBC-NCL/EWS</td><td>SC/ST/PwD</td></tr></table> | <input type="text" value="38.9"/>                                                                 | <input type="text" value="35"/> | <input type="text" value="25.9"/> | General | OBC-NCL/EWS | SC/ST/PwD |  |
| <input type="text" value="38.9"/> | <input type="text" value="35"/>                                                                                                                                                                                     | <input type="text" value="25.9"/>                                                                 |                                 |                                   |         |             |           |  |
| General                           | OBC-NCL/EWS                                                                                                                                                                                                         | SC/ST/PwD                                                                                         |                                 |                                   |         |             |           |  |

<sup>#</sup>Normalized marks in case of multisession papers (CE and CS).

<sup>##</sup>A candidate is considered qualified if the marks secured are greater than or equal to the qualifying marks mentioned for the category, for which a valid category certificate, if applicable, must be produced along with the Score Card.

[FAQ for GATE Score](#)

CLOSE

## Plagiarism Report



# SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited "A++" Grade by NAAC | 12B Status by UGC | Approved by AICTE

[www.sathyabama.ac.in](http://www.sathyabama.ac.in)

Name of the student : Manikandan M

Register Number : 40230025

Title of the Thesis : ISOLATION AND IDENTIFICATION OF MICROPLASTIC  
AND HEAVY METAL FROM MARINE BIOTA AND SEDIMENTS

Internal Guide Name : Dr.Indumathi S M

External Guide Name : Dr. Stalin Dhas T

| PO / PSO /<br>PEO / SDG | Justification                                                                                                          |
|-------------------------|------------------------------------------------------------------------------------------------------------------------|
| PO2                     | Identify and analyze the amount of microplastic and heavy metals present in the marine environment to create awareness |
| SDG14                   | To create sustainable living in marine environment                                                                     |