

**A**  
**Project Report**  
**On**  
**“MICROPLASTICS ANALYSIS IN RIVER  
WATER”**

In the partial fulfillment of the requirement for Bachelor Degree in Civil Engineering

Submitted By

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**ENGINEERING, NEPTI, AHMEDNAGAR**  
**SAVITRIBAI PHULE PUNE UNIVERSITY, PUNE**

**2022-2023**

**AHMEDNAGAR JILHA MARATHA VIDYA PRASARAK SAMAJ'S**  
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## **CERTIFICATE**

*This is to certify that the following students have satisfactorily carried out BE project work entitled "**Microplastics Analysis in River Water**". This work is being submitted for the award of degree of Bachelor of Civil Engineering. It is submitted in the partial fulfillment of the prescribed syllabus of Savitribai Phule Pune University, Pune for the academic year 2022-2023.*

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# **PROJECT APPROVAL SHEET**

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**“MICROPLASTICS ANALYSIS IN RIVER WATER”**

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## Acknowledgement

We would like to express our sincere appreciation to all those who contributed to the successful completion of our project report. In particular, we would like to thank the following people.

We express our gratitude to our Guide **Prof. P. G. Nikam** for his guidance in completing the research work. His advice, encouragement during the preparation of this report, ideas about finding the research gap and motivation to do the work is highly appreciated. We also take this opportunity to express our sincere gratitude to **Prof. P. G. Nikam**, Head of Department of Civil Engineering and our respected Principal, **Dr. Y. R. Kharde** for their endless support and guidance.

We appreciate all backstage people for helping us by providing with the necessary materials to complete this project work. Lastly we are thankful to all those who directly or indirectly contributed to complete this project work.

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Your sincerely,

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## **ABSTRACT**

Microplastics are the newly emerged contaminants having presence almost everywhere. They are tiny plastic Particles Officially; they are defined as plastics less than five millimeters (0.2 inches) in diameter that result from both commercial product development and the breakdown of larger plastics. As a pollutant, microplastics can be harmful to the environment and animal health. A study on the effect of microplastics on aquatic organisms and human health is discussed. The studies show that the human person eats at least 50,000 microplastic particles every year because of the infiltrated food chain, drinking water, and breathing air.

We conducted a literature survey to review current identification methods and removal techniques for microplastics. In this work, we selected different location for analysis of microplastics on Sina river and also in Sina dam. For identification we use microscopy and FTIR instruments because they are widely used. This study can provide valuable reference for better understanding the microplastics pollution in wastewater of river and Dam area.

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## CHAPTER 1

### INTRODUCTION

The concentration of microplastics the concentration of microplastics (MPs) in water ecosystems increases as plastic production gradually increases every fiscal year. Currently, almost 71% of plastic waste is directly absorbed by the environment, and the remaining waste is reused in a different format, resulting in higher microplastic pollution. In 2016, approximately 335 million metric tons of plastic products were manufactured, whereas, in 2017, this amount increased up to 348 million metric tons. Thus, global plastic production increased by approximately 4% within only 1-year tons. There are more than 5.25 trillion macro and micro pieces of plastic in the oceans up to end of 2023. The first studies regarding microplastic contamination in oceans appeared in the 1970s and since then, Microplastics are defined directly in the literature, either as plastic particles smaller than 5 mm, or smaller than 1 mm. Due to the difficulty of monitoring microplastics in the environment, even decades later there are still not enough data to obtain a full picture of microplastic contamination. The difficulty in assessing microplastics in the environment lies in distinguishing microplastics from the complex mixture of natural organic and inorganic particles in any given environmental matrix. These can be, for example, inorganic particles like sand and silt, but also organic particles originating from biofilms, plant, and animal debris. Even with the advent of modern analytical methods such as Fourier-transform infrared spectroscopy (FTIR) and Raman spectroscopy, a natural matrix can still hinder the detection of microplastics or at least increase the error factor considerably. Therefore, appropriate sample preparation steps are necessary.

Plastics degrade slowly, MP's have a high probability of ingestion, incorporation into, and accumulation in the bodies and tissues of many organisms. The toxic chemicals that come from both the ocean and runoff can also bio magnify up the food chain. In terrestrial ecosystems, microplastics have been demonstrated to reduce the viability of soil ecosystems and reduce weight of earthworms. The cycle and movement of microplastics in the environment are not fully known, but research is currently underway to investigate the phenomenon.

## 1.1 Sources of Microplastics

The source of microplastics is generally thought to be well known: most plastic items are not recycled or incinerated when they are discarded. Plastic waste therefore ends up in landfill or in our rivers and oceans where it gradually breaks down into smaller and smaller pieces and particles.



*Fig 1.1.1: Plastic and Microplastic Fragments [3]*

The particles of microplastics are classified into two categories names as :

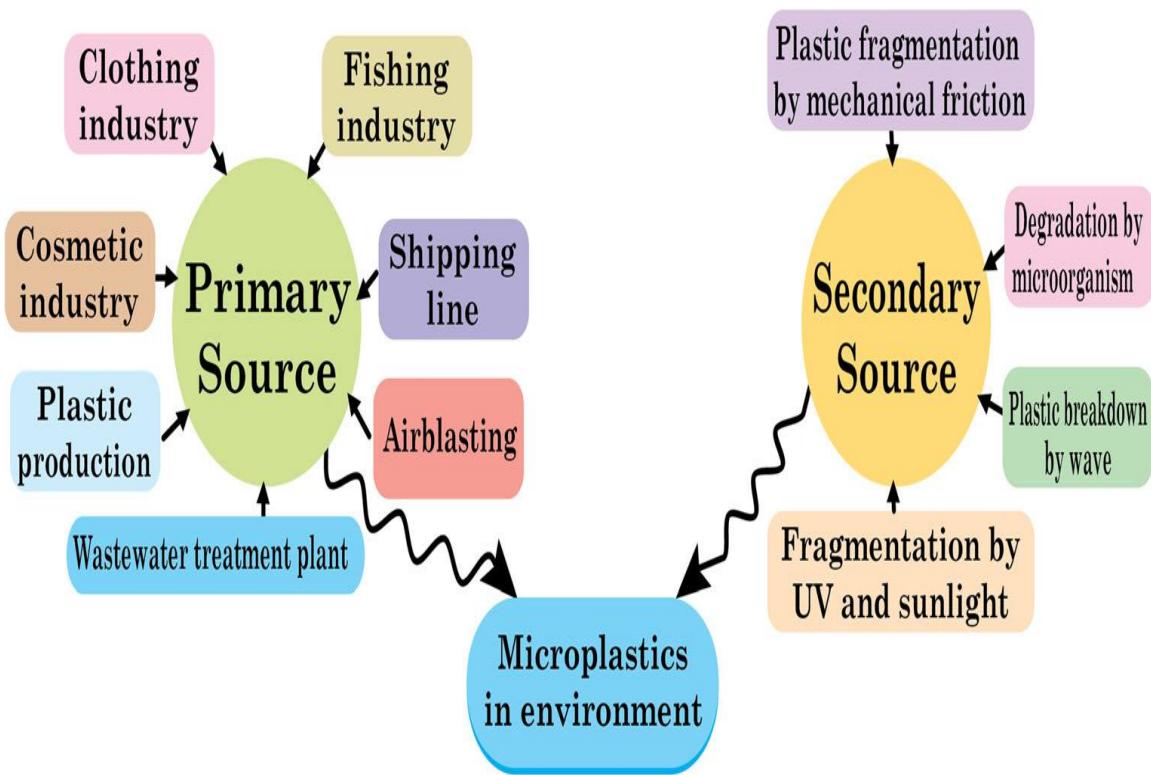
1. Primary Sources
2. Secondary Sources

### 1.1.1 Primary Sources

Primary microplastics are small pieces of plastic that are purposefully manufactured. They are usually used in facial cleansers and cosmetics, or in air blasting technology. In some cases, their use in medicine as vectors for drugs was reported. Microplastic "scrubbers", used in exfoliating hand cleansers and facial scrubs, have replaced traditionally used natural ingredients, including ground almond shells, oatmeal, and pumice.

### 1.1.2 Secondary Sources

Secondary plastics are small pieces of plastic derived from the breakdown of larger plastic debris, both at sea and on land. Over time, a culmination of physical, biological, and chem-photodegradation, including photo-oxidation caused by sunlight exposure, can reduce the structural integrity of plastic debris to a size that is eventually undetectable to the naked eye. From the breakdown of larger plastics; this typically happens when larger plastics undergo weathering, through exposure to, for example, wave action, wind abrasion, and ultraviolet radiation from sunlight.



*Fig 1.1.2.1 : Origin of Microplastics [2]*

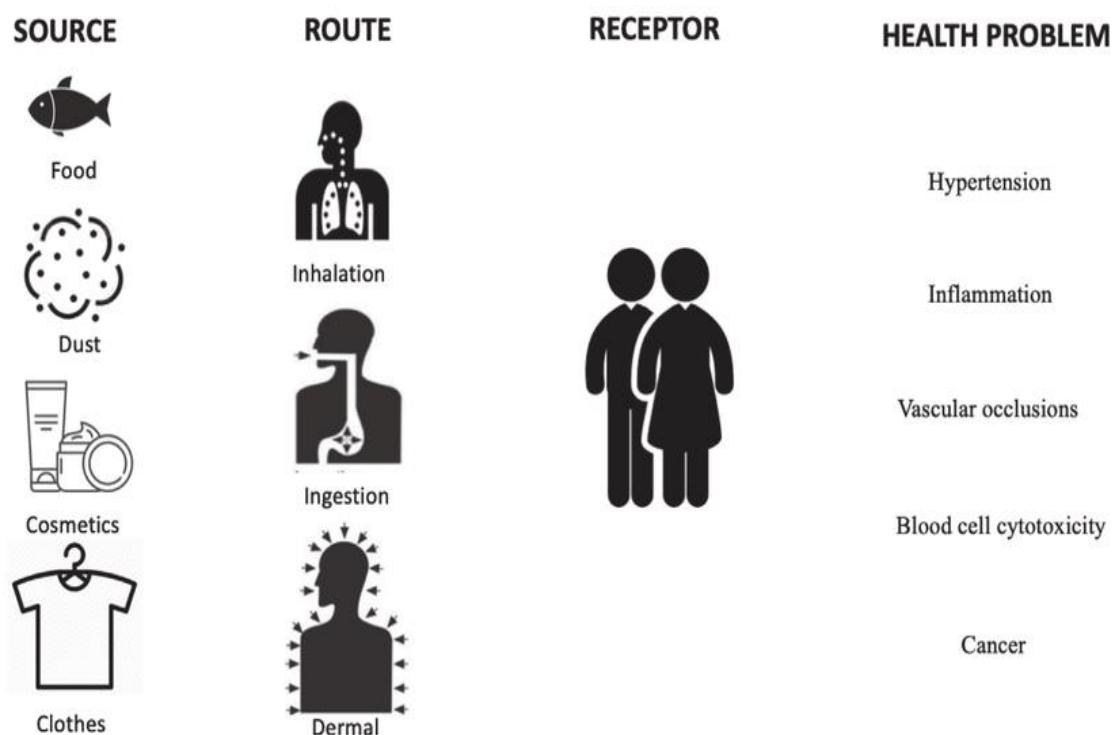
## 1.2 Effect of Microplastics

Microplastics are of concern because of their widespread presence in the rivers and the potential physical and toxicological risks they pose to organisms. They have hypothesized that human exposure to microplastics could lead to oxidative stress, DNA damage and inflammation, among other health problems. Microplastics can be ingested by a wide range of animals and have been found in organisms ranging in size from small invertebrates to large mammals.

### 1.2.1 On Human Health

Microplastics can enter the human body through ingestion and inhalation where they may be taken up in various organs and might affect health, for example, by damaging cells or inducing inflammatory and immune reactions. Microplastics can build up and damage the air sacs in our lungs. This can increase your risk of developing emphysema and lung cancer. Some of the smallest microplastics can also get into your bloodstream.

Human consumption of microplastics can result in an increased exposure to these chemicals and might lead to poisonous effects. For humans, fish is considered to be a promising source of protein. Thus, at the end of the food chain, people consumed a wide variety of fish and crustaceans and, at the same time, ingested microplastics (MPs) through this species. As a result, this type of consumption poses a potential threat to human health and has become an emerging concern.



*Fig 1.2.1.1: Effect of Microplastics on Human Health [6]*

Among the different plastic polymers, PET is mainly used for plastic products such as drinking water bottles, building insulation materials, pipes, and food packaging materials. However, frequent exposure to this plastic polymer could pose a potential threat to human health due to its carcinogenic effect. Some plastics, such as PVC and PS, are responsible for discharging hazardous monomers that can cause cancer in humans.

### 1.2.2 On Aquatic Organisms

The reduced size of microplastic makes it easier for intake by aquatic organisms resulting in amassing of noxious wastes, thereby disturbing their physiological functions. microplastics are laden with harmful chemicals, which make the river water toxic. In other cases, harmful bacteria may grow on them, posing a biohazard. As river water recharges groundwater, microplastics smaller than what the soil can filter, escape into groundwater. microplastics are taken up and mostly excreted rapidly by numerous marine species, and so conclusive proof on biomagnification is not obtained. However, effects of MP uptakes result in reduced food intake, developmental disorders, and behavioural changes.

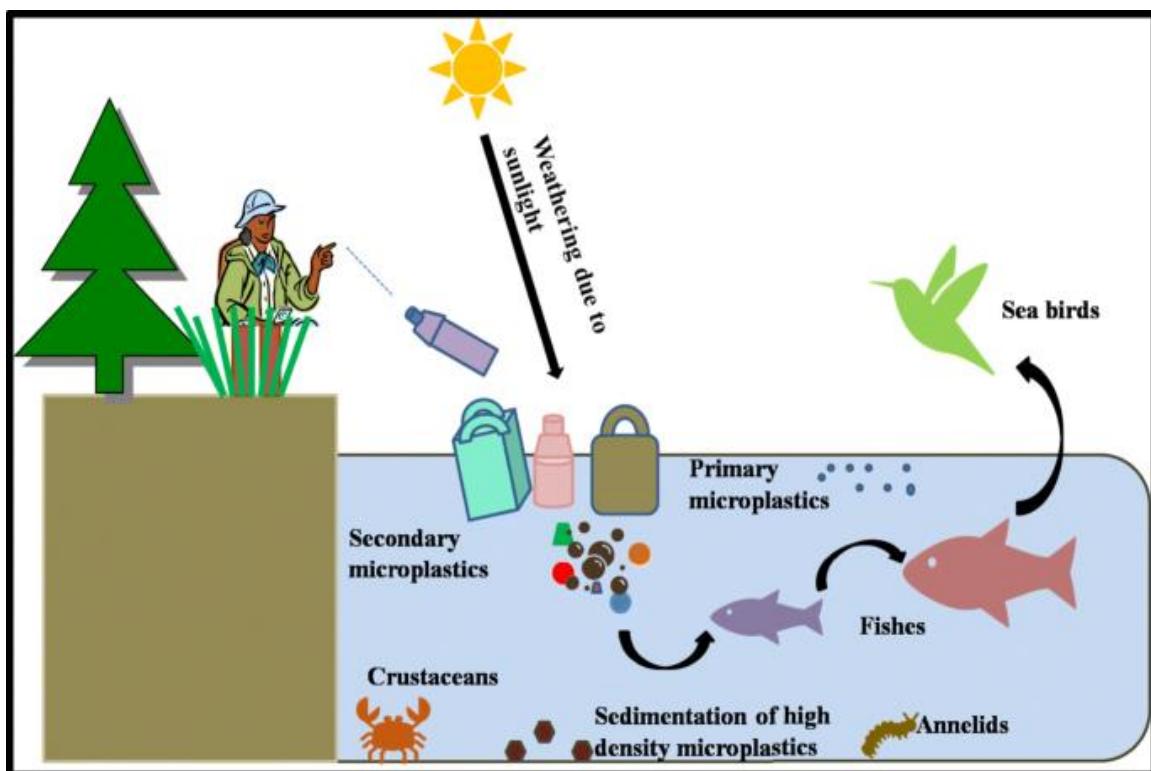


Fig 1.2.2.1: Effect of Microplastics on Aquatic Organisms [3]

Additive-free microplastics are not chemically hazardous to aquatic organisms, but they create problems in physical conditions such as bowel obstructions. Depending on the demand of products, certain additives are added to the virgin microplastics resulting in additional property of adsorption of pollutants present in water and thereby impersonate as vectors. Researches reveal the harmful threats plastic poses to human health at any point of the plastic lifecycle, from the extraction of fossil fuels to consumer use to disposal and further more.

Aquatic animals including plankton passively ingest MPs due to their inability to differentiate MPs and food. The minute size, buoyancy and attractive colour of MPs particles make them ideal candidates as food for fish. In fish, after ingestion, the MP's may translocate across the gastrointestinal tract and gills and enter the circulatory system.

### **1.3 Objectives**

1. Identification of microplastics content in river water.
2. Identification of type of microplastics.
3. Identification of impacts of MPs on human health.
4. Suggestion of best possible method to treat microplastic in water.

## CHAPTER 2

### LITERATURE REVIEW

#### **2.1 "Removal of microplastics from wastewater available techniques and way forward" by Surya Singh Madhanraj Kalyanasundaram and Vishal Diwan (2021).**

In this paper we have discussed the existing and upcoming treatment technologies for the removal of microplastics from wastewater and also tried to present an overview for the future approaches also Discussed the existing and upcoming treatment technologies for the removal of microplastics from wastewater.

- Efficient removal of microplastics from wastewater is inevitable considering its human health aspects.
- Conventional wastewater treatment plants are not fully capable to remove microplastics.
- The need is to have efficient, simple, and low-cost technology for microplastics' removal.
- Combination of technologies may provide a suitable option.

Microplastic particles have proved to be harmful for plants, animals, humans, and for the ecosystem in general. Water is one of the most important routes through which it transfers from one place to another. Moreover, water is also an important route for the ingestion of microplastics in human, which results in various health issues, such as cancer, mutagenic and teratogenic abnormalities, etc. Thus, microplastics in water is an emerging public health issue which needs attention and hence, it is important to get acquaintance with the removal techniques of microplastics in wastewater.

#### **2.2 “Microplastic types in the wastewater system—a comparison of material flow-based source estimates and the measurement-based load to a wastewater treatment plant” By Kristina Borg Olesen & Stefan Anderberg (2021)**

The aim of this paper is to contribute to increased understanding of microplastic sources in wastewater and test the possibilities of source tracking. Previous research has focused either on estimating microplastic contribution from various sources or on quantifying occurrence based on measurements. In this paper, these two approaches are

compared. Microplastic types detected in the influent to a WWTP in Sweden are compared with estimations of sources in the WWTP catchment area. A Comparison of Material Flow-Based Source Estimates and the Measurement-Based Load to a Wastewater Treatment Plant. An exception was cellulose acetate, which was not found at the inlet despite a large theoretical contribution. Many uncertainties remain, which lead to large intervals for the source estimates. The comparison can give an indication into which part of the intervals is most likely. Investigating more WWTPs with different characteristics and including particle morphology will further increase the understanding of sources that contribute to the presence of microplastics in wastewater. Traditional Min/Maximization problems.

### **2.3 “Detection and removal of microplastics in wastewater: evolution and impact”**

**By Thuhin K. Dey & Md. Elias Uddin & Mamun Jamal (2021)**

The aim of this paper study on the effect of microplastics on aquatic organisms and human health is also discussed. Thus, this analysis provides a complete understanding of entire strategies for detecting and removing microplastics and their associated issues to ensure a waste discharge standard to minimize the ultimate potential impact in aquatic.

Evidence shows that microplastics act as a potential vector by adsorbing different heavy metals, pathogens, and other chemical additives widely used in different raw plastic production. Microplastics are ingested by aquatic creatures such as fish and different crustaceans, and finally, people ingest them at the tertiary level of the food chain. This phenomenon is responsible for blocking the digestion tracts, disturbing the digestive behavior, finally decreasing the reproductive growth of entire living organisms. Because of these consequences, microplastics have become an increasing concern as a newly emerging potential threat, and therefore, the control of microplastics in aquatic media is required. This paper provides a critical analysis of existing and newly developed methods for detecting and separating microplastics from discharged wastewater, which are the ultimate challenges in the microplastic treatment system.

## **2.4 “Effect of microplastics in water and aquatic systems” By Merlin N Issac & Balasubramanian Kandasubramanian (2020).**

The aim of this paper study the effects of plastic debris in the water and aquatic systems from various literature and on how COVID-19 has become a reason for microplastic pollution. The reduced size of microplastic makes it easier for intake by aquatic organisms resulting in amassing of noxious wastes, thereby disturbing their physiological functions. microplastics are abundantly available and exhibit high propensity for interrelating with the ecosystem thereby disrupting the biogenic flora and fauna.

About 71% of the earth surface is occupied by oceans, which holds 97% of the earth’s water. The remaining 3% is present as water in ponds, streams, glaciers, ice caps, and as water vapor in the atmosphere. Microplastics can accumulate harmful pollutants from the surroundings thereby acting as transport vectors; and simultaneously can leach out chemicals (additives). Plastics in marine undergo splintering and shriveling to form micro/nanoparticles owing to the mechanical and photochemical processes accelerated by waves and sunlight, respectively. Microplastics differ in color and density, considering the type of polymers, and are generally classified according to their origins, i.e., primary and secondary.

## **2.5 “Validation of sample preparation methods for microplastic analysis in wastewater matrices—reproducibility and standardization.” By Mohammed S. M. Al-Azzawi, Simone Kefer, Julia Reichel, (2020).**

In this paper this study suggest that both  $H_2O_2$  and Fenton reactions are most effective in terms of organic matter removal from microplastic samples while not affecting the tested polymers, where as KOH dissolved most PLA and PET particles. Monitoring microplastics in the environment is difficult due to the complex matrices that can prevent reliable analysis if samples are not properly prepared first. Unfortunately, sample preparation methods are not yet standardized, and the various parts to validate them overlook key aspects. The goal of this study was to develop a sample preparation method for wastewater samples, which removes natural organic matter without altering

the properties of microplastics. Three protocols, based on KOH, H<sub>2</sub>O<sub>2</sub>, and Fenton reactions, were chosen out of ten protocols after a literature review and pre-experiments.

In order to investigate the effects of these reagents on seven polymers (PS, PE, PET, PP, PA, PVC, and PLA), this study employed FTIR, laser direction-based particle size analysis, as well as TD-Pyr-GC/MS. Furthermore, the study discussed issues and inconsistencies with the Fenton reactions reported in the literature in previous validationoneorts..

## **2.6 "Microplastics in wastewater treatment plants: Detection, occurrence and removal" By Jing Sun, Xiaohu Dai, Qilin Wang, Mark C.M. van (2018).**

In this review, the up-to-date status on the detection, occurrence 24 and removal of microplastics in WWTPs were comprehensively reviewed. Specifically, the different techniques used for collecting microplastics from both wastewater and sewage sludge, and their pretreatment and characterization methods were reviewed and analyzed. The key aspects regarding microplastics occurrence in WWTPs, such as concentrations, total discharges, materials, shapes and sizes were summarized and compared.

Microplastics removal in different treatment stages and their retention in sewage sludge were explored. The development of potential microplastics-targeted treatment technologies is also presented. Although previous researches in microplastics have undoubtedly improved our level of understanding, it is clear that much remains to be learned about microplastics in WWTPs, as many unanswered questions and thereby concerns still remain; some of these important future research areas are outlined. The key challenges appear to be to harmonize detection methods as well as microplastics mitigation from wastewater and sewage sludge.

## **2.7 "Analysis of microplastics and their removal from water." By Abiola Oladejo (2016)**

In this paper removal of microplastics from water was studied using extraction with oil. The aim of the thesis was to remove microplastics from water using an organic medium, and to analyses the amount of microplastics in the media involved. The separation of different microplastic types was done by conducting experiments in the

laboratory. The microplastics were made by grinding and sieving plastics with a grinding machine before adding them to water and oil, which serves as the organic medium.

**2.8 " Impact of microplastics on aquatic organisms and human health: a review " By Sanjay Kumar1, Mridula Rajesh, Rajesh KM, Suyani NK (2020).**

In this research especially regarding the potential exposure and associated human health Two experiments were done: in Experiment 1, the microplastic solution was not mixed in the oil solution, and in Experiment 2, the solution was mixed vigorously in oil. The resulting solution then underwent separating funnel method for separation into two phases. The water phase was filtered in order to know the amount of microplastics in the water medium. The analysis of the amount of microplastics in the water medium was done by weighing the mass of the filter paper containing the microplastics substrate after drying. Replicate experiments and measurements were done. risk to micro and nano-sized plastics. plastics have become an increasingly important packaging option worldwide. Unfortunately, these materials are increasingly under environmental scrutiny.. Thus, massive efforts are required to investigate the distribution and abundance of microplastics in the ocean, and in considering strategies to reduce the problem.

**2.9 "Human health concerns regarding microplastics in the aquatic environment - From marine to food systems" By Zhihao Yuan, Rajat Nag, Enda Cummins(2022).**

In this paper, aimed to conduct a traditional review of the current state of the art regarding microplastics (MPs) definition and characterization, including an assessment of MPs detected in marine and food systems. The review revealed that plastic waste is not biodegraded and can only be broken down, predominantly by physical processes, into small particles of micron to nanometer size. Particles ( $<150 \mu\text{m}$ ) can be ingested by living organisms, migrate through the intestinal wall and reach lymph nodes and other body organs.

The primary pathway of human exposure to MPs has been identified as gastrointestinal ingestion, pulmonary inhalation, and dermal infiltration. Marine plastic waste pollution is one of the most urgent global marine environmental problems worldwide. It has attracted worldwide attention from governments, the public, the

scientific community, media and nongovernmental organizations and has become a hot issue in current marine ecology and environmental research.

**2.10 "Analysis and prevention of microplastics pollution in water: current perspectives and future directions" by Yolanda Pico, and Damia Barcelo (2017).**

The analysis, prevention, and removal of microplastics (MPs) pollution in water is identified as one major problem the world is currently facing. MPs can be directly released to water or formed by the degradation of bigger plastics. Nowadays, it is estimated that annually between 4 and 12 million tons of plastic go into the seas and oceans with a forecast for them to outweigh the amount of fish in 2050. Based on the existing studies, the characterization of MPs in waters is still one of the remaining challenges because they can be easily confused with organic or other types of matter.

Consequently, there is an urgent necessity to establish pathways for the chemical identification of the MP nature. In this perspective, the recent techniques and instrumentation for MP characterization (Raman and Fourier-transform infrared spectroscopies and microscopies, pyrolysis and thermal desorption gas chromatography, imaging techniques, etc.) are discussed including considerations to the multidimensionality of the problem. This perspective also summarizes and provides updated data on the sources and occurrence, transport and fate of MPs in aquatic ecosystems, as well as influencing conditions and factors affecting dispersal. Additionally, how engineering and biotechnological tools, such as advanced water treatments, would help to control, reduce, or even eliminate MP pollution in the near future is outlined.

**2.11 "Microplastic pollution of lakeshore sediments from remote lakes in Tibet plateau, China" By Kai Zhang, Jing Su, Xiong Xiong (2016).**

In this work, lakeshore sediments from four lakes within the Siling Co basin in northern Tibet were sampled and examined for microplastics (<5 mm). Microplastics were detected in six out of seven sampling sites with abundances ranging from  $8 \pm 14$  to  $563 \pm 1219$  items/m<sup>2</sup>. Riverine input might have contributed to the high abundance of microplastics observed in this remote area. Morphological features suggest that microplastics are derived from the breakdown of daily used plastic products.

Polyethylene, polypropylene, polystyrene, polyethylene terephthalate, and polyvinyl chloride were identified from the microplastic samples using laser Raman spectroscopy, and oxidative and mechanical weathering textures were observed on the surface of microplastics using scanning electron microscope.

**2.12 "Plastic driven pollution in Pakistan: the first evidence of environmental exposure to microplastic in sediments and water of Rawal Lake" By Tahira Irfan, Sofia Khalid (2020).**

The study was conducted with the objective of investigating the microplastic presence and concentration in the surface water and sediments of the Rawal Lake, in the capital city of Pakistan. The average microplastic abundance for water and sediments was 0.142 items/0.1 L and 1.04 items/0.01 kg, respectively. Results indicated that the fibers and fragments were the most dominant types of microplastics.

The dominant colors were blue, red, black, and transparent. FTIR analysis of visible microplastic particles displayed a greater similarity with polyethylene, polypropylene, polyesters, polyethylene terephthalate, and polyvinyl chloride because of the appearance of characteristic peaks of these polymers. The study also revealed greater concentration of microplastics in the sediments as compared with water of the Rawal Lake. High population density surrounding lake, improper waste disposal, tourism, and recreational activities may be the major reasons for the microplastic contamination of the lake.

**2.13 "Microplastic pollution in Vembanad lake, Kerala, India: the first report of microplastics in lake and estuarine sediments in India" By Yolanda Pico, and Damia Barcelo (2017).**

This study, being the first report from India on MPs in lake sediments, provide impetus for further research on the distribution and impact of this emerging pollutant on the biota of many aquatic systems spread across India. The impact of microplastics pollution on the environment and biota is not well known. Vast data exist in the literature on marine microplastics while reports on freshwater ecosystems are scarce.

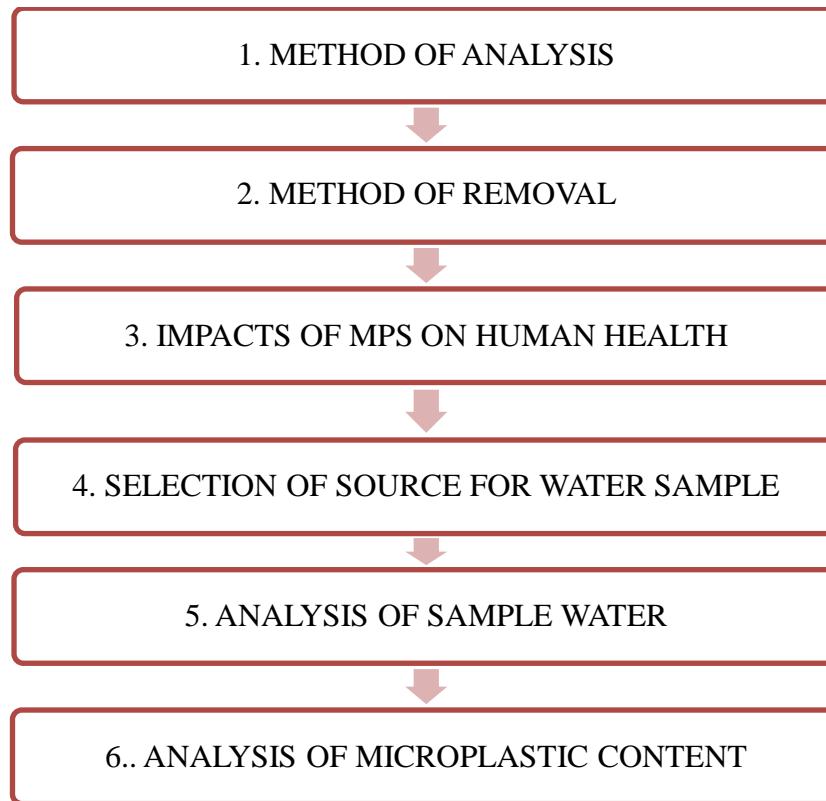
In this context, to examine the occurrence of microplastic particles (MPs) in the Vembanad Lake, samples were collected from ten sites and processed for microplastic extraction through density separation. Identification of the polymer components of MPs was done using micro Raman spectroscopy. MPs were recovered from all sediment samples, indicating their extensive distribution in the lake. The abundance of MPs recorded from the sediment samples is in the range of 96-e496 particles m<sup>2</sup> with a mean abundance of  $252.80 \pm 25.76$  particles. Low density polyethylene has been identified as the dominant type of polymer component of the MPs. As clams and fishes are the major source of protein to the local population, the presence of MPs in the lake becomes critically important, posing a severe threat of contaminating the food web of this lake.

**2.14 "Microplastics in surface waters of Dongting lake and Hong lake, China" By Wenfeng Wang, and Wenke Yuan (2018).**

This study investigated the occurrence and properties of microplastics in surface waters of two important lakes in the middle reaches of the Yangtze River. The concentration ranges of microplastics in Dongting Lake and Hong Lake were 900–2800 and 1250–4650 n/m<sup>3</sup>, respectively. Fiber was the dominant shape. Colored items occupied the majority. Particles with a size of 330 µm comprised N20% of total microplastics collected in both lakes. Most of the selected particles were identified as plastics, with polyethylene (PE) and polypropylene (PP) being the major components. This study can provide valuable reference for better understanding the microplastics pollution in inland fresh water ecosystems.

## CHAPTER 3

### METHODOLOGY



#### 3.1 Methods of Analysis.

It is difficult to identify microplastics of various sizes, shapes, and polymer types fully and reliably from complex environmental matrices using a single analytical method. Therefore, the combination of more than two analytical techniques has been widely used. In general, microplastic analysis consists of two steps: physical characterization of potential plastics followed by chemical characterization for confirmation of plastics.

##### 3.1.1 Visual identification

Before the term ‘microplastics’ became popular, the occurrence and distribution of large microplastics (1–5 mm) was reported mostly on beaches and, to a lesser extent, in surface waters. Due to the relatively large size range of microplastics, sorting and identification were usually performed simultaneously in a tray with forceps and the naked eye.



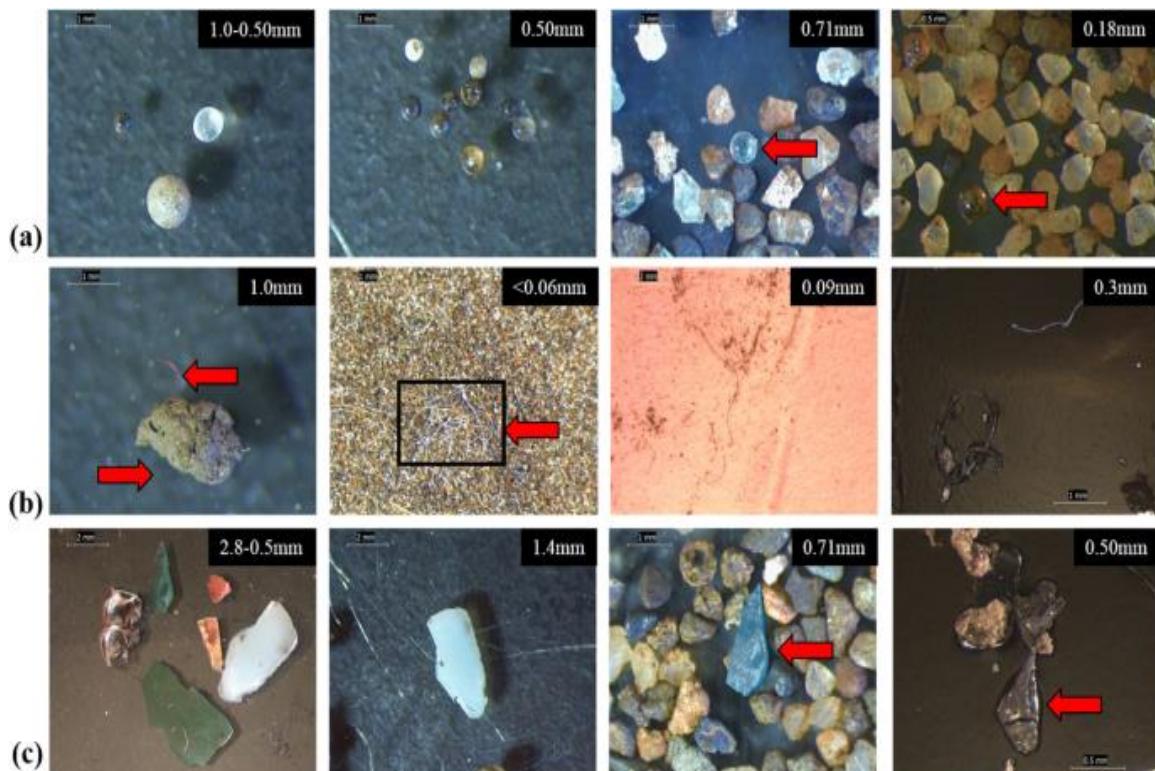
*Fig 3.1.1.1: Microplastics [5]*

Some small plastics can be identified using this visual method, and colorful plastic fragments and pre-production resin pellets that have a size range of 2–5 mm can be identified with the naked eye. samples on beaches with high levels of interfering inorganic and organic materials around 1 mm in size whose colors are similar to the interfering particles, there is a higher probability of missing small plastic particles by sorting. However, visual sorting and identification of large microplastics offers an easy, simple and fast method for both experts and the non-professional volunteers who have received brief training.

### **3.1.2 Microscopy**

Microscopy is the technical field of using microscopes to view samples & objects that cannot be seen with the unaided eye (objects that are not within the resolution range of the normal eye). Stereo- (or dissecting) microscopy is a widely-used identification method for microplastics whose size falls in the hundreds of micron range (e.g., neuston net samples). Magnified images using microscopy provide detailed surface texture and structural information of objects, which is essential for identifying ambiguous, plastic-like

particles. Although most particles of this size range are usually identifiable by microscopy, particles of the sub-hundred micron size range (< 100 mm) with no color or typical shape are difficult to characterize with confidence as plastics. Sediment samples for which light sediment particles are poorly separated by density can interfere with microscopic identification of microplastics on filter paper.



*Fig 3.1.2.1: Microplastics images under Microscopy [6]*

In addition, biogenic materials from sediment and neuston net samples that have not been completely eliminated by chemical digestion also make microscopic observation difficult. Previous studies have shown that false identification of plastic-like particles using microscopy was often over 20%, and over 70% for transparent particles; these results were confirmed with subsequent spectroscopic analysis. Only 1.4% of the particles visually resembling microplastics were of synthetic polymer origin. Synthetic (e.g., polyester) and natural (e.g., colored cotton) fibers were difficult to distinguish by microscopy alone. Numerous field monitoring studies reported that fibers comprised the dominant or predominant microplastics in water, sediment and biota. Scanning electron microscopy (SEM) can provide extremely clear and high-magnification images of plastic-

like particles. High-resolution images of the surface texture of the particles facilitate the discrimination of microplastics from organic particles. Further analysis with energy-dispersive X-ray spectroscopy (EDS) provides the elemental composition of the same object. The elemental composition of particles is useful for identifying carbon-dominant plastics from inorganic particles. A SEM-EDS is expensive, and requires substantial time and effort for sample preparation and examination, which limits the number of samples that can be handled. The colors of plastics cannot be used as identifiers in SEM. The method is recommended for further surface characterization and elemental analysis of specific plastic particles. Other advanced microscopy techniques have been used to identify plastic particles in specific cases. Polarized optical microscopy was successfully used to identify polyethylene (PE) particles in laboratory accumulation and toxicity experiments. Crystallinity varies among polymer types and even within the same polymers depending on the manufacturing process. The crystal structure within plastics can influence the transmission of polarized light, which can then be measured. This method is not applicable to microplastic samples on opaque filter paper, however. The microplastics should be thin to allow sufficient polarized light to pass through.

### **3.1.3 Fourier Transform Infrared (FTIR) spectroscopy**

FTIR spectroscopy provides information on the specific chemical bonds of particles. Carbon-based polymers are easily identified with this method, and different bond compositions produce unique spectra that discriminate plastics from other morganic and inorganic particles. A well-established polymer spectrum library enables not only confirmation of plastics, but also identification of specific polymer types. Small microplastics require the use of micro-FTIR ( $\mu$ -FTIR), which is used to perform microscopic observation of micro-sized plastic-like particles prior to spectroscopic confirmation on a single platform by switching between the object lens and IR probe. Transmission and attenuated total reflectance (ATR) modes are available in FTIR analysis for microplastics.

Unlike the transmission mode, the reflectance and ATR mode does not require the sample preparation step for thick and opaque microplastics. In addition, the ATR mode produces stable spectra from irregular microplastic surfaces, which produce unstable

spectra in the reflectance mode. In theory, microplastics as small as the diameter of the IR beam aperture (e.g., 10 mm) of the ATR probe are detectable. Currently,  $\mu$ -ATR-FTIR offers a useful method to identify microplastics in environmental samples, with identification of plastic-like particles by microscopy and subsequent chemical confirmation by spectroscopy.



*Fig 3.1.3.1: FTIR Device [5]*

However, microplastics with a maximum length  $< 50 \mu\text{m}$  require many trials or it can be difficult to obtain clear spectra that enable accurate identification. ATR-FTIR measurement is a form of surface contact analysis. The pressure produced by the ATR probe may damage highly-weathered or fragile microplastics, and tiny plastic particles can be pulled from the filter paper by adhesion to or electrostatic interaction with the probe tip. An ATR probe made of germanium can be easily damaged by hard and sharp inorganic particle remnants on a filter paper from sand samples in contact analysis. Fingerprinting of each plastic-like particle using the IR spectrum avoids false-positive quantification of non-plastic.

### 3.1.4 Raman Spectroscopy

Raman spectroscopy has also been used to identify microplastics. The laser beam falling on an object results in different frequencies of back-scattered light depending on the molecular structure and atoms present, which produce a unique spectrum for each polymer. Raman analysis not only identifies plastics, but also provides profiles of the polymer composition of each sample similar to FTIR.



*Fig 3.1.4.1: Raman microscope [2]*

In terms of the combination of non-destructive chemical analysis with microscopy, Raman spectroscopy is comparable to the FTIR method, including the requirement for expensive instrumentation. The different responses and spectra between FTIR and Raman spectroscopy from a microplastic can compromise each other in complex microplastic identification. The smaller diameter of the laser beam in Raman spectroscopy relative to FTIR allows the identification of microplastics as small as a few  $\mu\text{m}$  in size. The non-contact analysis of Raman spectroscopy offers the benefit that the microplastic samples remain intact for possible further analysis.

Confocal microscopy with Raman spectroscopy can be used to identify microplastics in zooplankton samples. However, Raman spectroscopy is sensitive to the additive and pigment chemicals in microplastics, which interfere with the identification of polymer types.

### 3.1.5 Novel methods

The development of new analytical instruments and their coupling to one another or to existing conventional instruments could address current difficulties in microplastic identification. One of the challenges in microplastic analysis that remains to be addressed is the limitation of detectable size. The current analytical methods have a minimum detectable size limit of a few micrometers. Although the size dependent adverse biological effects of microplastics vary with the toxicological end-points, smaller plastic particles tend to cause greater toxic effects.

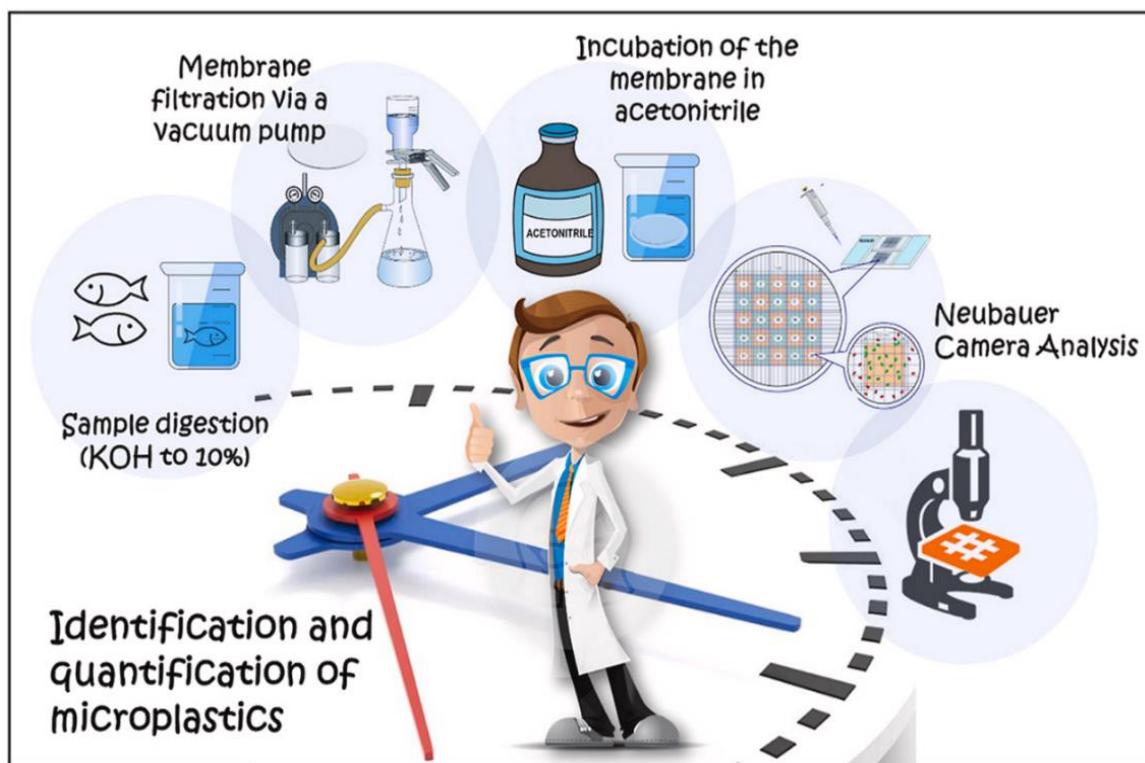


Fig 3.1.5.1: Novel Method Process [2]

There is a growing need to determine the presence, distribution, fate and toxicity of nanosized plastics (hereafter, nano plastics) in the environment. Clearly, sampling, extraction, clean-up and concentration techniques for nano plastics should be developed

concurrently with new identification methods. Detection and identification of nano plastics currently represents a major challenge in microplastic research. Atomic force microscopy (AFM) combined with either IR or Raman spectroscopy is a potential candidate for nano plastic analysis. AFM can provide images at nanometer resolutions, and AFM probes can be operated in both contact and noncontact mode with objects. IR or Raman spectroscopy combined with AFM can determine the chemical composition of the object. Raman analysis is a simple combination of two instruments using the simultaneous or independent scanning of the same object, while AFM-IR is the actual merging of two instruments into one. Thermal expansion of the sample by infrared absorption gives rise to oscillations of the AFM cantilever whose ring-down pattern is analyzed by Fourier transform, which allows extraction of the frequencies and amplitudes of the oscillations. AFM-IR can acquire IR absorption spectra and absorption images with spatial resolutions of 50–100 nm. AFM and IR spectra of 100-nm polystyrene beads have been successfully acquired. It is difficult and time consuming to find a nano-sized single particle to focus by AFM-IR in an unknown sample. Another possible analytical combination that may be applicable to microplastic identification is that of automated particle tracking, image analysis, and Raman spectroscopy. Particles volumetrically measured and nebulized by high air pressure as a single layer onto a glass slide are automatically tracked one by one, and microscopic image and Raman spectroscopic analyses are also automatically conducted. Fully automated particle analysis and subsequent spectroscopy can save time and effort. The characteristics of each particle and the particle size distribution (PSD), together with their polymer types identified using spectroscopy, can provide more information relating to the distribution, fate and source of microplastics in the sample.

One of the more time-consuming analytical steps in conventional microplastic analysis is the manual spotting of plastic particles from other interfering particles. The time required for this process depends on the removal efficiency of inorganic and organic particles during the separation and clean-up steps. In addition, manual identification may miss plastic particles, especially small and transparent ones that are difficult to recognize. Automated FTIR/Raman mapping or particle tracking with simultaneous Raman spectroscopy can resolve these problems, but requires expensive instruments, which not every microplastic research laboratory can afford.

### 3.2 Methods to Remove Microplastic.

#### 3.2.1 Vacuum filtration

In this method showed that with the how the microplastic is removed or extracted by using the vacuum filtration, The different amount of water could be filtered through a 20  $\mu\text{m}$  sieve. Currently 20-50  $\mu\text{m}$  sieves have been widely used as the final filtration step for the recovery of MPs. However, it should be noted that there is still lack of effective standard methods to isolate nano plastic particles from wastewater and sludge forth characterization of sub-micron-sized plastic particles.

The Vacuum filtration can result in the solubilization of non-MP particulate organics and the dissolved organics can, then, be removed by the filtration using 20  $\mu\text{m}$  sieves. The Vacuum filtration step will reduce the organic load for the subsequent chemical treatment, which will not only improve the chemical treatment efficiency but will also reduce the chemical consumption and the potential aggressive extent of the chemical reaction.

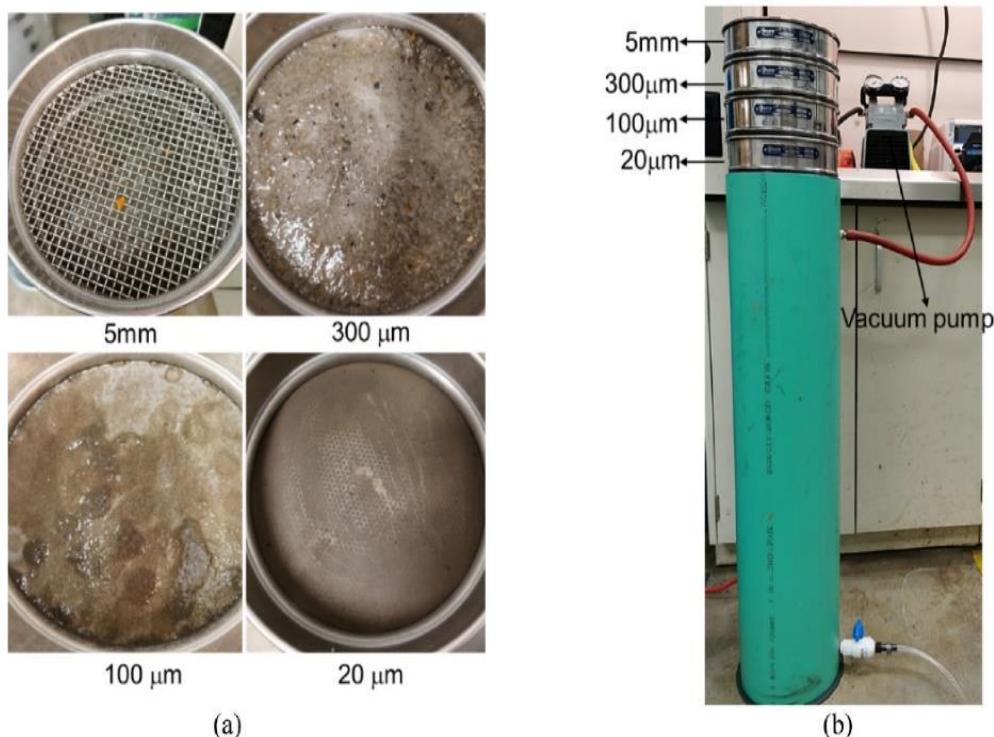


Fig 3.2.1.1: Microplastics Removal By using Vacuum Filtration.[2]

In this Removal technique,

- a) Solids and MPs recovered from water by stainless-steel sieves with pore sizes of 300 µm, 100 µm and 20 µm
- b) Vacuum apparatus used to accelerate MP collection.

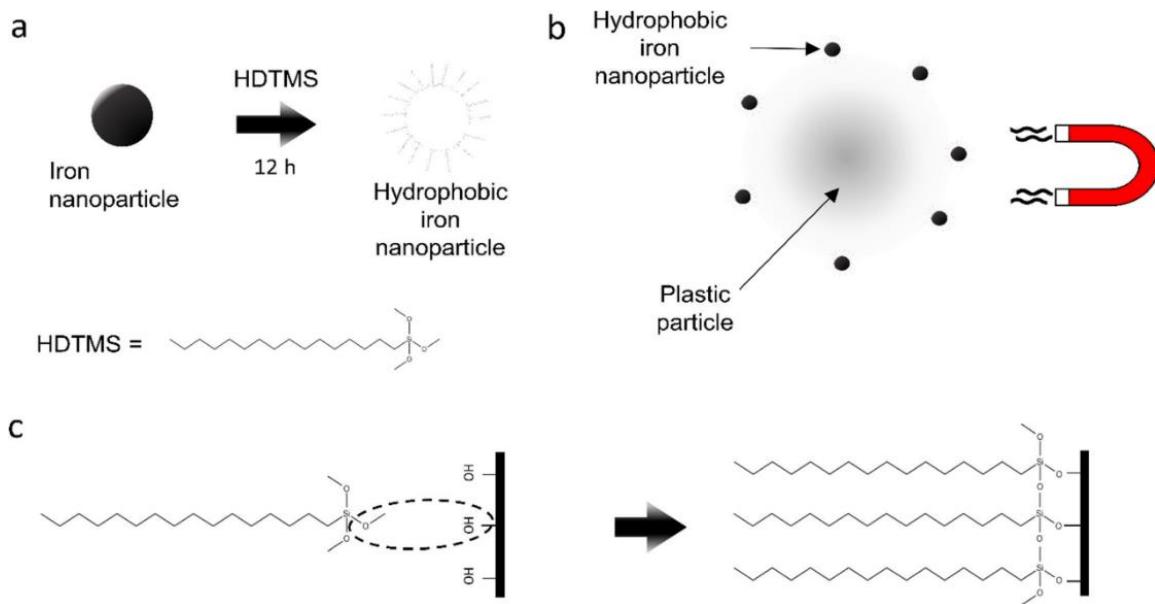
### **3.2.2 Algal masses**

Microalgae may offer a possibility for the removal of microplastics, as it has been seen that microalgae colonize the microplastic particles thus altering the buoyancy of the aggregates. It results in differential sedimentation rate as compared to the un-aggregated particles. This property may be utilized for the removal of microplastics by incorporating the microalgae. Sundbaek et al. employed a marine seaweed namely, *Fucus vesiculosus*, for the possible removal of microplastics through translocation in the algal tissues. Due to narrow channels in the algal cells, the movement of microplastics was restricted and thus the plastic particles were captured. Efficiency of as high as ~94.5% was observed, esp. in the dissected areas of the algae. As the dissected areas ooze out anionic polysaccharide substance, the adherence of plastic particles gets enhanced. Sorption of microplastic particles has also been reported by the other researchers. The major mechanism for the sorption of microplastic particles onto the algal surface is the electrostatic charge. The positively charged microplastic particles tend to sorb more onto the algae owing to the presence of anionic polysaccharide substance in the algal cell wall.

### **3.2.3 By magnetic extraction**

Magnetic extraction is more likely to separate MPs from wastewater, according to the investigation. This method included magnetic seeds (Fe nanoparticles), oxalic acid (as Fe di-sorbent), and external magnetic attraction to separate MPs from seeds. Iron-based nanoparticles have been used due to their ferromagnetic properties, low-cost availability, and more available specific surface area. Hydrophobicity of nanoparticles was ensured by the deposit of hexadecyltrimethoxysilane on the surface of nanoparticles, and this modification allowed the bonding of plastic particles. Almost 92% of PS and PE beads with a range of 10 to 20 µm can be removed. On the contrary, MPs (PET, PVC, and PP) of a smaller size (less than 1 mm) have been removed up to 93%. Besides, 78 and 84% of medium-sized (200 µm–1 mm).

MPs were removed from the sediment and fresh water, respectively. This method is, therefore, useful for small plastic particles (less than 10  $\mu\text{m}$ ). However, these nanoparticles are not biodegradable and cannot be reused, leading to secondary pollution. Also, the presence of soil particles and lipophilic substances may reduce the plastic removal percentage by damaging nanoparticles. Recently, TiO<sub>2</sub>-based photocatalytic micromotors are introduced by Wang et al. to remove MPs in aqueous media.



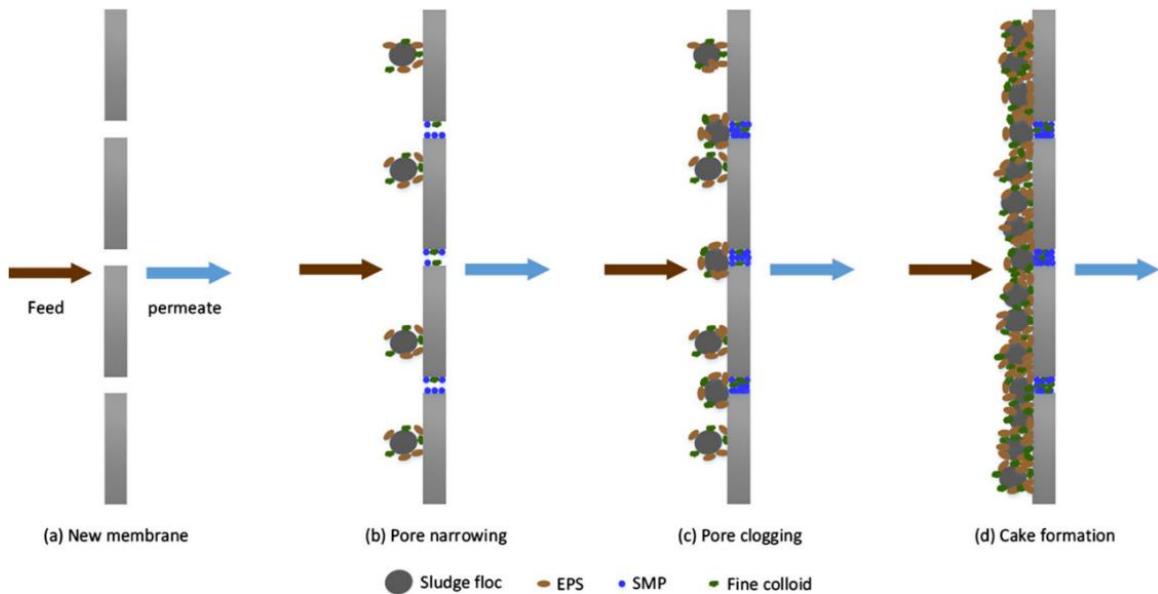
*Fig 3.2.3.1: Magnetic extraction [3]*

Authors proposed two new processes to extract microplastics while phoretic interaction is incorporated to remove primary microplastics (personal care products) and chains of magnetic micromotor (via shoveling effect) are used to ensure the motility of microplastics under magnetic field for higher segregation. But still, a challenge remained to confirm.

### 3.2.4 By membrane filtration

Various treatment processes are available to remove MPs, including sedimentation, skimming, and a variety of advanced tertiary level filtration systems. But none of them is dedicated solely to the removal of MPs. As a matter of principle, the development of microplastic removal technology is still at the preliminary stage of research. Although many water treatment plants are used to separate MPs, many plastic

particles are still entering the aquatic ecosystem through sludge management and effluent discharge. Various researchers investigated the efficacy of the microplastic removal using different membranes as a tertiary level of the treatment system. At this level, membrane filtration is a physical barrier to extract plastic particles from water, although not intended for plastic removal.



*Fig 3.2.4.1: Membrane filtration [3]*

Above all, membranes can remove MPs from the water system with higher efficiency and some advantages, such as stable effluent quality and ease of treatment. Comparing with different tertiary level treatment efficacy such as rapid sand filter, disk filter, and dissolved air flotation refining secondary effluent with 97, 98.5, and 95% removal efficiency, respectively, the membrane bioreactor encounters primary effluent with 99.9% efficiency. MPs can also be removed by reverse osmosis as well as by ultrafiltration. On the contrary, advanced granular separation systems and active biological filters have not deduced plastic concentrations in water treatment plants.

### 3.2.5 Bacterial Degradation

Researchers are investigating the potential of different bacteria to degrade MPs into environmentally friendly monomers and could be an emerging alternative to remove plastic debris from the ecosystem. Experiments have been conducted to detect PET degrading whole-cell biocatalysts (*Comamonas testosterone*) for the removal of MPs.

Three types of media were considered, including bacteria in neutral pH media (pH 7), bacteria in alkaline pH media (pH 12), and alkaline media without bacteria. Degradation of PET with bacteria was performed for 48 h, including a temperature of 37°C and a stirring rate of 140 rpm. The mean PET particle diameter before treatment was 7.3 µm. After treatment, the particle size was 7.3, 2.63, and 1.58 µm for bacteria with no media, neutral pH media, and alkaline pH media, respectively.

PET degradation rate with biocatalyst in higher pH is better than neutral media (Gong et al. 2018). In 2016, research work was also carried out by Shusuke Yoshida and his team members on the isolation of bacteria capable of PET degradation into environmentally friendly monomers, TA (terephthalic acid), and ethylene glycol. This bacterium can secrete two enzymes (PETase and Mhetase) to hydrolyze PET and use plastic waste as the primary source of carbon nutrients. The PET film degradation rate was 0.13 mg cm<sup>-2</sup> per day at a temperature of 30°C, while 75% of the decomposed PET film was converted to carbon dioxide at 28°C. At the same time, another research paper on PE film degradation via bacterium (*Bacillus subtilis*) was published, which showed that the biosurfactant secretion from this bacterium was responsible for degradation.

Low-density polyethylene (LDPE) pretreatment with ultraviolet therapy increased degradation for 72 h due to increased plastic intake of isolated bacteria (*Bacillus subtilis*). In these experiments, a weight loss of up to 9.26% was noted in the presence of biosurfactants within 30 days of incubation of 180 rpm at 32°C . Here, the point to be noted is that microplastic degradation via bacteria is more rapid than fungal activities. Moreover, enzymes and biosurfactants are playing vital role for plastic breakdown. So, pretreatment can also be added here, including photo-induced degradation (photolysis) and chemical degradation before MP exposures under bacteria to minimize degradation time and ensure commercial feasibility.

### 3.3 Identification of impacts of MPs on human health

Microplastics have been found in different foods such as fish and seafood, table salt, beer, honey and sugar, and tap water. On the other hand, it is found in soil as a result of contamination from items such as discarded packaging or plastic agricultural equipment

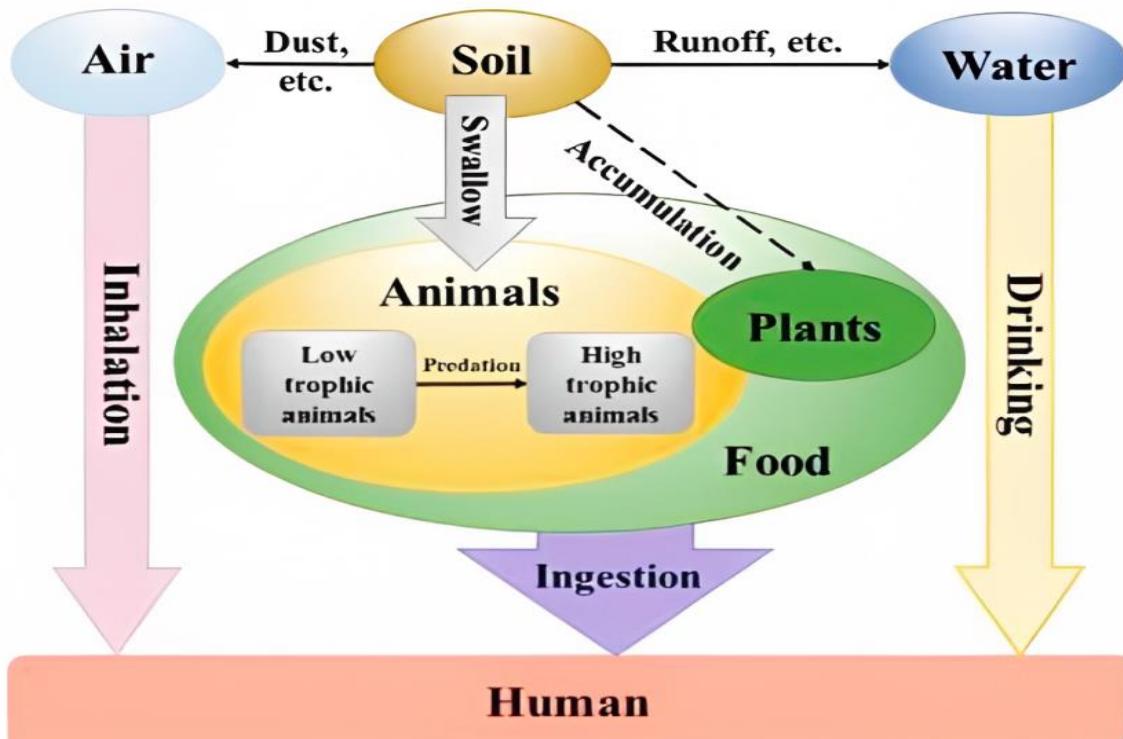


Fig 3.3.1: Pathways of concentration microplastics in human body [12]

The studies show that the human person eats at least 50,000 microplastic particles every year because of the infiltrated food chain, drinking water, and breathing air. There are seven types of sources can be considered as food sources of microplastics that includes bottled water, beer, sea food (Shellfish and fish), salt, tea Bag, canned Food, and ready meals. Water in plastic bottles that is used for drinking is one of the worst sources of microplastic, which results in ingesting around 130,000 fragments of microplastic in the human body yearly. The infiltration only gets worse when the bottle is exposed to direct sunlight. The tap water contains tiny plastic bits but the level in bottled water is double than tap water.

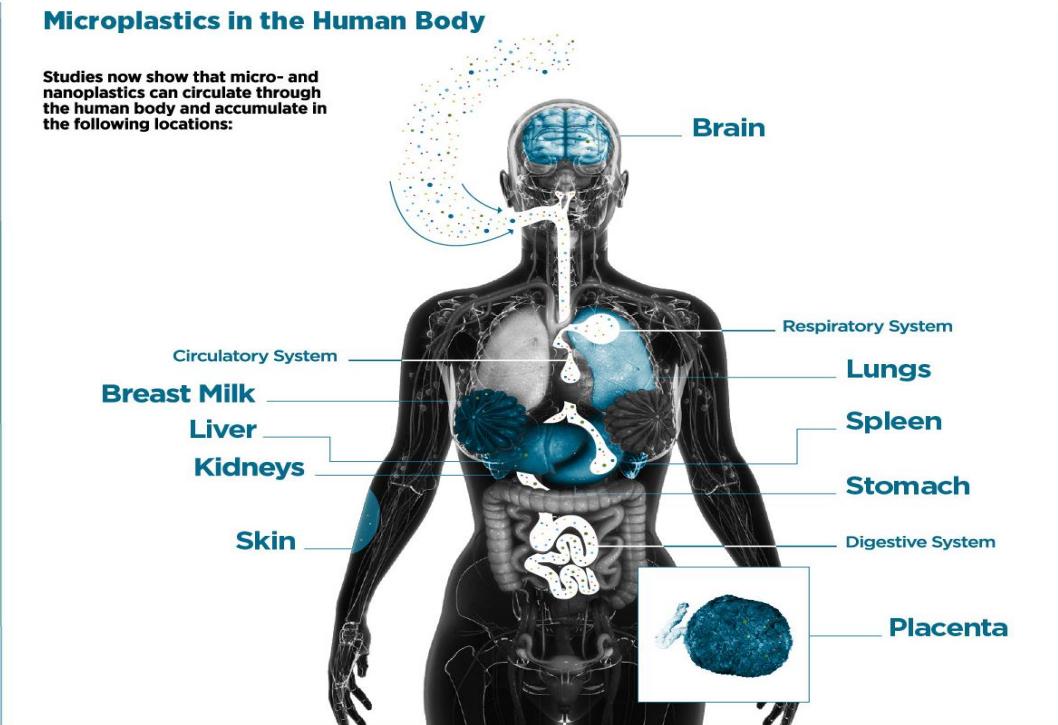


Fig 3.3.2: Accumulation of microplastics in human body [12]

Researchers show that human exposure to microplastics could lead to

1. Oxidative stress
2. DNA damage
3. Inflammation
4. Neurotoxicity
5. Reproductive toxicity
6. Disruption of immunity

Many sources of food and landfill have microplastic waste that goes directly or indirectly to the human body by eating food or breathing air, which affect human health. It is very important to reduce throwing of plastic waste on the ocean and landfill to avoid the effect of microplastic on the human health.

### 3.4 Selection of source for water sample.

For analysis, samples are collected from different location of Sina River and Sina Dam.

#### 3.4.1 Sampling site

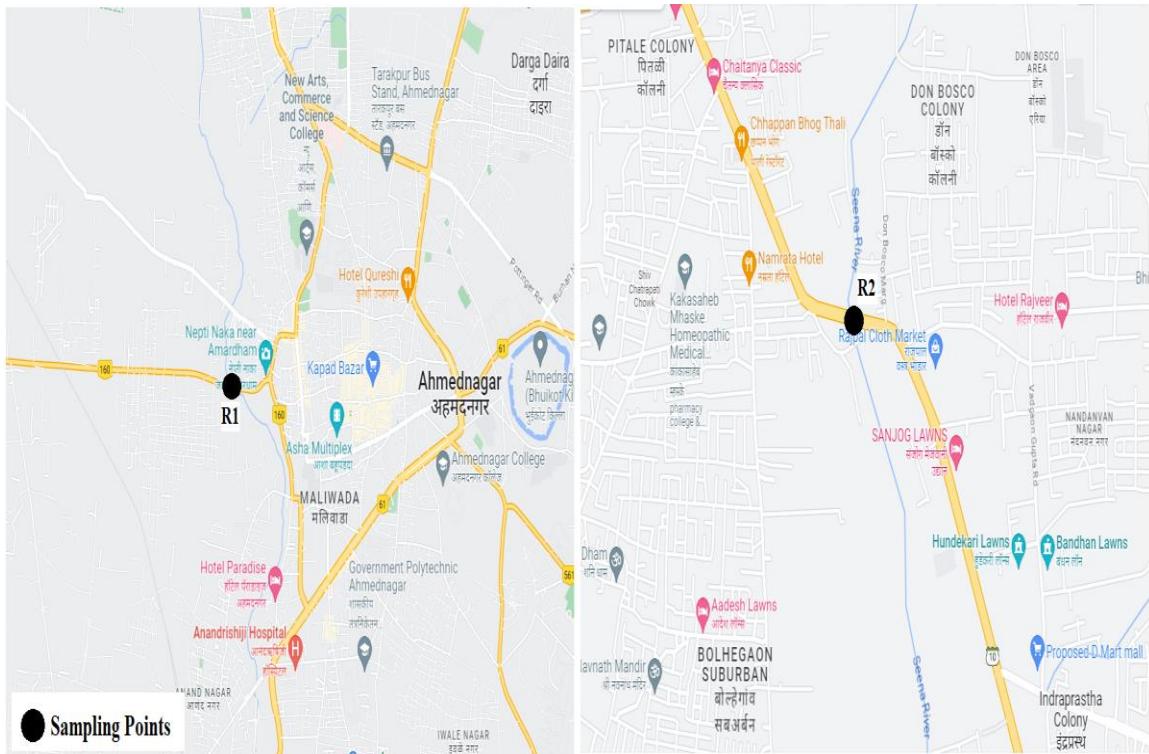
The sampling was performed in between September and October 2022. The Sina River was selected as a sampling site. Two sampling points at Sina River were selected. Each of the sampling points was coordinated and illustrated as shown in Table 1.

**Table 3.4.1.1.** Information of each sampling points water

Points	Place	Latitude	Longitude
<b>R1</b>	Sina River (Near Nepti Naka, Ahmednagar)	19°05'42.6"N	74°43'34.8"E
<b>R2</b>	Sina River (Near Rajpal)	19°08'22.7"N	74°43'03.7"E
<b>D1</b>	Sina Dam	18°49'38.7"N	74°56'48.9"E
<b>D2</b>	Sina Dam	18°49'21.0"N	74°56'16.7"E

#### Location R1 :

- **Sina River**



*Fig 3.4.1.1: Sample Location (Source: Google Map)*



Fig 3.4.1.2: Sina River Sampling

### Location 2 :

- Sina Dam



Fig 3.4.1.3: Sina Dam (Source: Google Map)



Fig 3.4.1.4: Sina Dam Sampling

### 3.5 Analysis of Sample Water.

For analysis samples are collected from different location of Sina River. Various initial tests are taken to identify the different physical and chemical properties of sample. In tests, pH Value, Dissolved Oxygen, Turbidity tests are taken.

#### 3.5.1 pH Test :

pH is really a measure of the relative amount of free hydrogen and hydroxyl ions in the water. Water that has more free hydrogen ions is acidic, whereas water that has more free hydroxyl ions is basic.

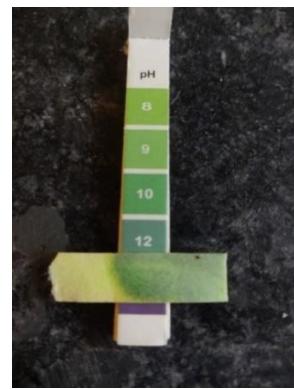


Fig 3.5.1.1: pH Test

### 3.5.2 DO Test :

DO is considered an important measure of water quality as it is a direct indicator of an aquatic resource's ability to support aquatic life.



*Fig 3.5.2.1: DO Meter*

### 3.5.3 Turbidity:

Turbidity is caused by particles suspended or dissolved in water that scatter light making the water appear cloudy or murky. Particulate matter can include sediment - especially clay and silt, fine organic and inorganic matter, soluble colored organic compounds, algae, and other microscopic organisms.



*Fig 3.5.3.1 : Turbidity Test*

### 3.5.4 Initial Test Outcomes :

**Table 3.5.4.1. Test Results with Standards**

<b>Parameters</b>	<b>Standard</b>	<b>Outcomes</b>				<b>Remark</b>
		<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>	<b>D<sub>1</sub></b>	<b>D<sub>2</sub></b>	
<b>pH</b>	7.5-8.5	10	9	8	8	Alkaline
<b>DO</b>	3-7.5 ppm	2	2	7.5	7.4	Flooding ,Even Death
<b>Turbidity</b>	<5 NTU	10	9	2	2	Difficult to Breathe

### 3.6 Analysis of microplastics content.

It is difficult to identify microplastics of various sizes, shapes, and polymer types analysis provides a complete understanding of entire strategies for detecting microplastics.

#### Steps Followed for Analysis

- Provides samples were sieved through 5-mm sieve to eliminate larger particles and to retain particles of < 5 mm size.



*Fig 3.6.1: 5mm Sieve*

- The samples were treated with 5 ml of 30% H<sub>2</sub>O<sub>2</sub> in order to degrade the organic matter.

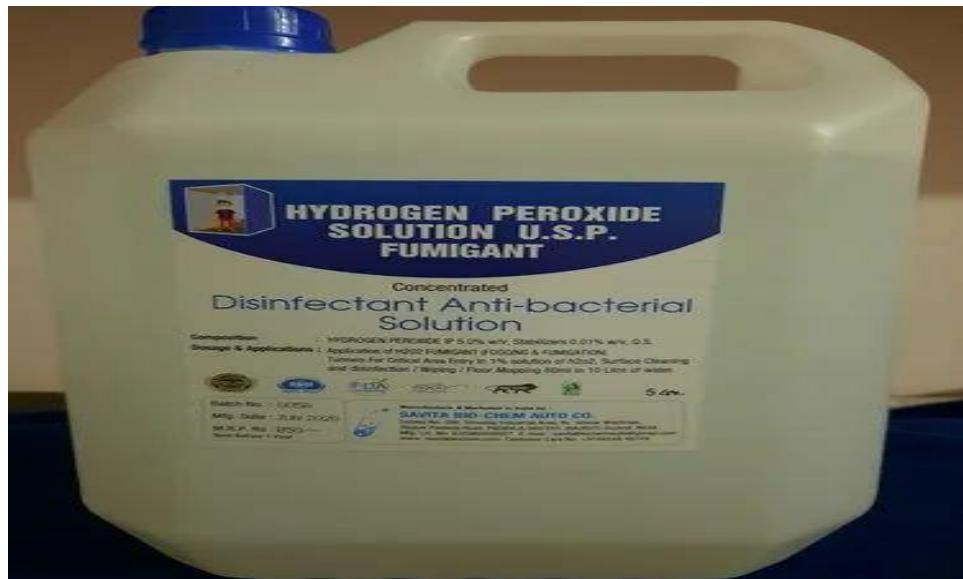


Fig 3.6.2 : H<sub>2</sub>O<sub>2</sub> (30% Cons.)



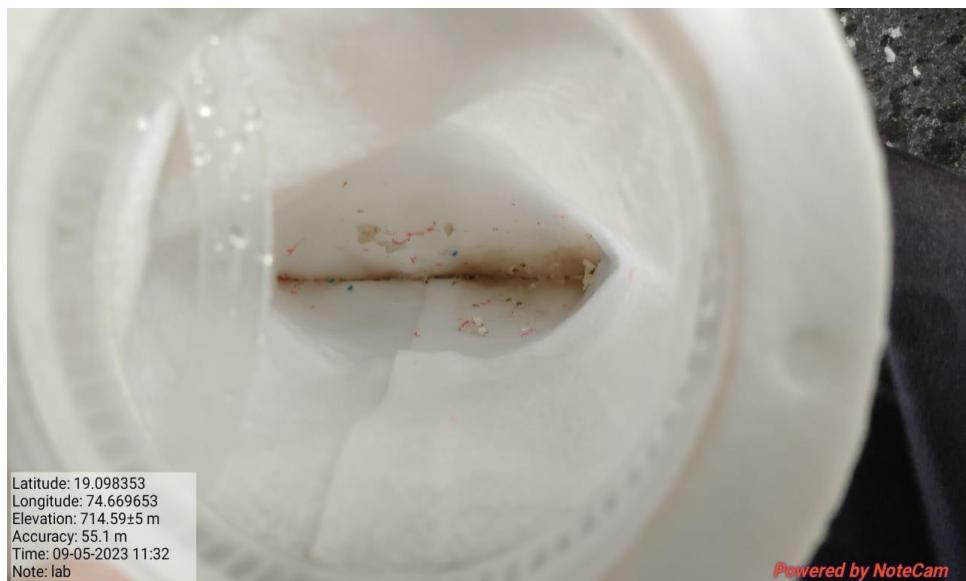
Fig 3.6.3 : 5 Micron Filter Bag

- The samples were allowed to settle for 24 hrs to achieve density separation. The clear supernatant was then filtered through 5 Micron Filter.



*Fig 3.6.4: Filtration*

- The filter were dried for 15 min before further examination.
- The Particle were trapped in filter which are Collected by Using Distilled Water



*Fig 3.6.5: Trapped Microplastics*

- Then this Sample is Placed in Sunlight Until sample not get dry. Proper Arrangement is taken during evaporation for getting sample intact.
- Collected Samples are Separate Out by their size, shape, color Under Microscope
- The Collected Sample is Analysis under Fourier transform infrared (FTIR) spectroscopy for Identify and characterize unknown materials.

### 3.7 Analysis using FTIR

FTIR offers a useful method to identify microplastics in environmental samples, with identification of plastic-like particles by microscopy and subsequent chemical confirmation by spectroscopy.



Fig 3.7.1 :FTIR Analysis at SPPU Lab

### 3.8 Best Possible Method to Treat Microplastic in Water

There are different methods which are useful for remove microplastics. The following methods which are mostly used to treat microplastics for river water. The methods are Vacuum filtration, Algal masses, Magnetic extraction, Membrane filtration. In below table there is a detailed comparison of this methods which is useful for find out the best possible method to treat microplastic.

*Table 3.8.1. Comparison of different methods for the Removal of MPs*

Sr No	Parameters	Methods of Removal			
		Vacuum filtration	Algal masses	Magnetic extraction	Membrane filtration
1	Efficiency(%)	98	94.5	93	95
2	Lowest size of microplastic particle removed( $\mu\text{m}$ )	5	20	10-20	20-190
3	Speed of Removal	High	Low	Moderate	Medium
4	Maintenance	Less	NA	More	More
5	Installation cost	Moderate	less	low	Moderate
6	Lifespan	More	Less	Less	Moderate
7	Area Required	less	More	Moderate	More
8	Advantages	Separate out the microplastic with different sizes	No chemical, electrical,& mechanical operations	Remove small microplastic	Low filtration resistance

After above study of different methods which are useful for removal of microplastic for river water, the vacuum filtration is one of the most effective and efficient method for treat Microplastic.

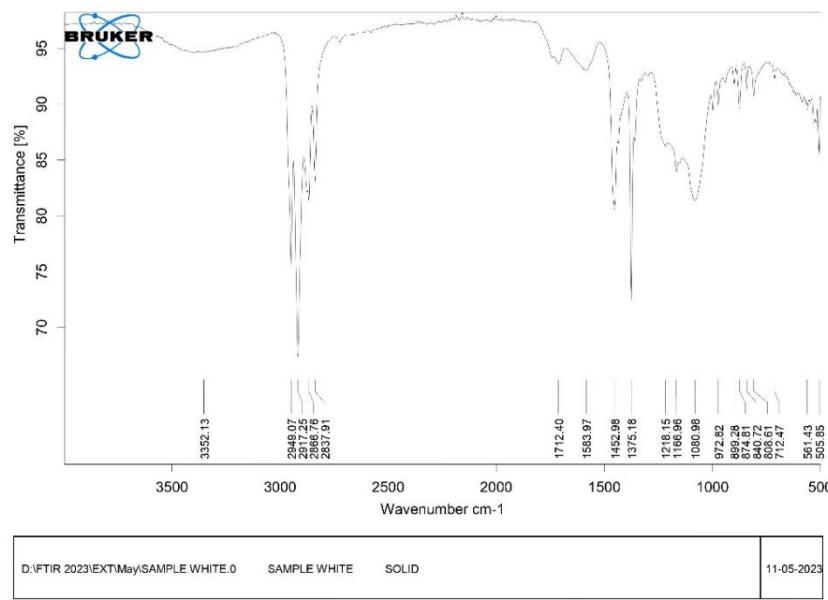
## CHAPTER 4

### RESULTS AND DISCUSSIONS

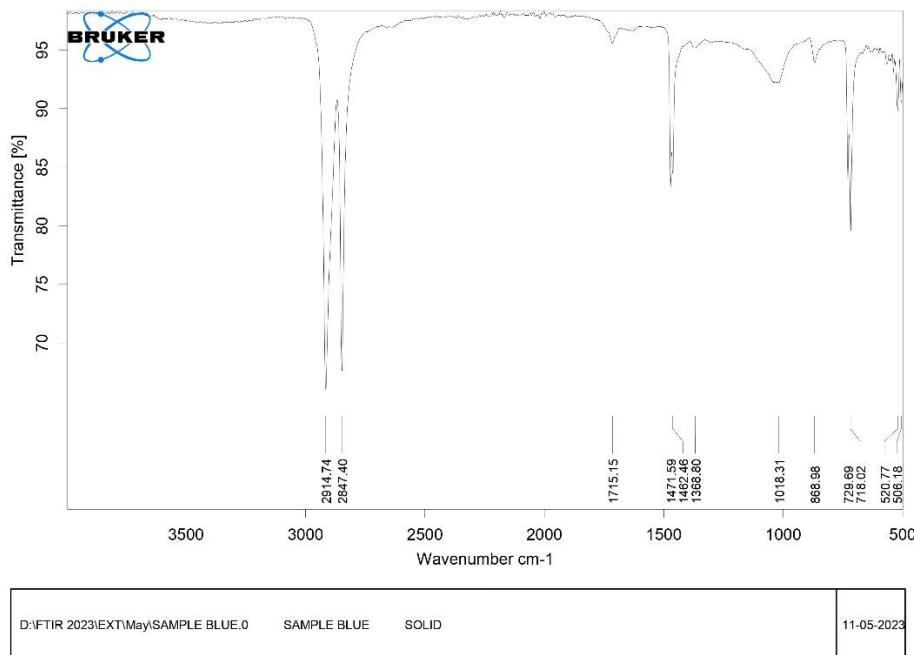
Floating microplastics were present in all surface water of river and dam also present in surrounding of them. The average number and concentration of the microplastics for 4 stations as in average was 34.5 pieces per stations. The highest concentration of microplastics were found at R<sub>1</sub> with 43 pieces. The lowest concentration of microplastics found was as D<sub>2</sub> with 23 pieces.

**Table 4.1 .FTIR identification of selected microplastics (5 mm-300um).**

Sr. No	Locations	Microplastic numbers			
		5mm – 300um	300um – 100um	100um – 20um	Total number
1	R <sub>1</sub>	15	20	8	43
2	R <sub>2</sub>	17	13	11	41
3	D <sub>1</sub>	5	17	9	31
4	D <sub>2</sub>	9	11	3	23



**Fig 4.1 FTIR spectroscopy spectra of the microplastics (5 mm–300 um) collected in the river**



*Fig 4.2 FTIR spectroscopy spectra of the microplastics (5 mm-300 um) collected in the dam*

**Table 4.1** FTIR identification of selected microplastics (5 mm-300um).

PE = Polyethylene, LDPE = low density PE, PP = Polypropylene, PS = Polystyrene.

Station	Polymer (% match)	Characteristic band (cm <sup>-1</sup> )
R <sub>1</sub>	PP (96), PE (97), LDPE (97)	PP: 2952, 2844, 2911, 1458, 1372, PE, LDPE: 2911, 2847, 1467, 720
R <sub>2</sub>	PP (96), PS (96), PE (97), LDPE (97)	PS: 3022, 2911, 2844, 1647, 1502, 1439, 1024
D <sub>1</sub>	PP (96), PE (97), LDPE (97)	
D <sub>2</sub>	PS (96), PP (96), PE (97), LDPE (97)	

We limited identification of plastic polymers to the microplastics classified as the 5 mm- 300um. From 46 pieces observed, most polymers found in the study area were Polypropylene (PP) and Polyethylene (PE), low density PE (LDPE), and Polystyrene (PS) were shared with equal percentage. Other minor polymers was LDPE. PP is indicated by the prominent presence of the  $2949.07\text{ cm}^{-1}$ ,  $2837\text{ cm}^{-1}$ , and  $2917\text{ cm}^{-1}$  absorbing groups. The spectral assignments correspond to bands  $1458\text{ cm}^{-1}$  and  $1372\text{ cm}^{-1}$ , with asymmetrical types of vibration (C-CH<sub>2</sub>) and asymmetrical deformation vibrations (C-CH<sub>3</sub>), respectively.

LDPE was characterized by high CH<sub>3</sub> methyl groups, shown by an intense  $1375\text{ cm}^{-1}$  peak. LDPE might be indicated by the presence of  $1713\text{ cm}^{-1}$ ,  $1178\text{ cm}^{-1}$  and  $1631\text{ cm}^{-1}$  bands that correspond to the carbonyl group.

## **CHAPTER 5**

### **CONCLUSION**

1. After the analysis of different water samples we found that there is presence of microplastic in Sina River as well as in Sina Dam.
2. After analysis of water some major types of microplastics are found in both Sina River as well as Sina Dam like Polypropylene (PP) and Polyethylene (PE), low density Polyethylene (LDPE), and Polystyrene (PS).
3. The occurrence of microplastics in river and their impacts on humans are important issues to be addressed. It was found from the literature survey that the human person eats at least 50,000 microplastic particles every year. Still, there is not enough data that help to know how microplastics affect humans. However, there are some solutions to eliminate the effect of microplastics on the humans and animals.
4. After the overall study of removal microplastic we conclude the till the date the vacuum filtration is best, easy and effective method to remove microplastic through water.

## CHAPTER 6

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## PHOTO GALLERY



*Photo 1: Sina Dam*



*Photo 2: Wide view Sina Dam*



Photo 3: Row Fishing Net



Photo 4: Decomposition of Plastic waste



Photo 5:FTIR Device



Photo 6 :At SPPU Lab