ISOLATION & CHARACTERIZATION OF MICROPLASTICS FROM PAKISTAN'S COASTAL REGION AND ASSESSMENT OF RELATED DEGRADATION ACTIVITIES

By

Abdul Saboor

Reg No. 29-FBAS/PHDES/S17

Department of Environmental Science

Faculty of Basic & Applied Sciences

International Islamic University, Islamabad, Pakistan

2025

ISOLATION & CHARACTERIZATION OF MICROPLASTICS FROM PAKISTAN'S COASTAL REGION AND ASSESSMENT OF RELATED DEGRADATION ACTIVITIES

By

Abdul Saboor

Reg No. 29-FBAS/PHDES/S17

Supervised By

Dr. Muhammad Asad Ghufran

Co-Supervised By

Dr. Nazneen Bangash (Comsats University, Islamabad)

Department of Environmental Science

Faculty of Basic & Applied Sciences

International Islamic University, Islamabad, Pakistan

2025

ISOLATION & CHARACTERIZATION OF MICROPLASTICS FROM PAKISTAN'S COASTAL REGION AND ASSESSMENT OF RELATED DEGRADATION ACTIVITIES

By

Abdul Saboor

Reg No. 29-FBAS/PHDES/S17

A thesis submitted to International Islamic University, Islamabad, in partial completion of the requirements for the degree of Doctor of Philosophy in Environmental Science

Supervised By

Dr. Muhammad Asad Ghufran

Co-Supervised By

Dr. Nazneen Bangash (Comsats University, Islamabad)

Department of Environmental Science

Faculty of Basic & Applied Sciences

International Islamic University, Islamabad, Pakistan

2025

DEDICATED TO MY PARENTS AND FAMILY FOR THEIR SACRIFICES, ENCOURAGEMENT AND SUPPORT DURING THE STUDY

ACKNOWLEDGEMENTS

First and foremost, I want to express my gratitude to Allah, the Almighty, the Most Merciful, and the Most Gracious, for the gifts he has showered upon me throughout my studies and the completion of this thesis. The blessings of Allah be upon Allah's final Prophet Muhammad (peace be upon him), his family, and associates. I'd like to convey my heartfelt gratitude to my dissertation supervisor, Prof. Dr. Muhammad Asad Ghufran, and co-supervisor, Dr. Nazneen Bangash, for their unwavering support, patience, inspiration, excitement, and vast expertise during my study. Their active monitoring and helpful criticism aided me in finishing my project on schedule. I want to express my gratitude to Mr. Mehboob Elahi who helped me out in the field area. I'm gateful to Higher Education Commission, Islamabad, Pakistan for their financial support for the project. I also want to thank my family for their support, encouragement, unending love, support, and prayers, which have always been a source of inspiration and guidance for me.

ABDUL SABOOR

LIST OF ABBREVIATIONS

ACRONYM	ABBREVIATION
PP	Polypropylene
MNP	Micro and Nano Plastics
MPs	Microplastics
PET	Polyethylene Terephthalate
PE	Polyethylene
PUR	Polyurethanes
PS	Polystyrene
PP	Polypropylene
PVC	Poly Venyl Chloride
PA	Polyamide
L-MPs	Large Microplastics
EPS	Expanded Polystyrene
S-Mps	Small Microplastics
FTIR	Fourier Transform Infrared Spectroscopy
MSM	Mineral Salt Medium

BLAST	Basic Local Alignment Search Tool
UV	Ultraviolet
CFU	Colony Forming Unit
LDPE	Low Density Polyethylene
MDPE	Medium Density Polyethylene
HDPE	High Density Polyethylene
PBT	Polybutylene Terephthalate
PMP	Primary Microplastics
SMP	Secondary Microplastics
CD	Chart Detum
SDZ	Sulfadiazine
AMX	Amoxicillin
TC	Tetracycline
CIP	Ciprofloxacin
TMP	Trimethoprim
PLA	Poly Lactic Acid

LIST OF FIGURES

LIST OF TABLES

TABLE OF CONTENTS

ABSTRACT

The continuous use of materials that interfere with the wellbeing of biosphere has pushed the modern world into an alarming situation. One of the freshly arising issues is the presence of microplastics in our environment. Microplastics, which refer to polymer particles lesser than 5mm, have been recognized as materials which interfere with the fresh water and marine ecosystems through affecting the lives of living organisms, adsorbing toxic materials and trophic transfer etc. Many research questions regarding the effect of microplastics, translocation and options for mitigation are demanding extensive research on one hand, certain areas including Pakistan have insufficient data for occurrence of these contaminants on the other hand. Keeping in view the current scenario, a study was planned to investigate the occurrence of microplastics in coastal region of Pakistan along with inquiring the options for mitigation through bacterial isolates. Entire coastal line of Pakistan was sampled at 15 locations through selective sampling of beach sediments. Following standardized methods, the enumeration and characterization of microplastics was done. Briefly, density separation of microplastics was carried out for purification of samples. Visual examination under microscope was done for enumeration and characterization of these particles. Isolation of bacterial species was done through serial dilution and identification was done through 16S rRNA sequencing. Potential species were identified and grown on 3 polymer types (PVC, PET, PE) in lab experiments. The weight loss in polymers was a criterion for degradation of microplastics by different species.

The results showed that all sites were found infested with microplastics, having different colors and shapes. However, locations showed natural variation in abundance of microplastics based on population, industry and tourism etc.

It was observed that urban areas of Karachi city showed maximum abundance of microplastics,

whereas western part of coastal line in Baluchistan province was less infested. Clifton (760 Particles/Kg) and Manora (607 Particles/Kg) areas of Karachi city were found most abundant in microplastics. Kund Malir (107 Particles/Kg) and Jiwani (167 Particles/Kg) in Baluchistan province were found to be least abundant in microplastics.

Black and Transparent were the most prevalent colors with a mean percentage of 23% and 22% respectively. Fibers and Fragments were most prevalent shapes of microplastics with a mean percentage of 47% and 23% respectively.

A total of 7 bacterial species from 5 genera (*Pseudomonas azotoformans, Pseudomonas aeruginosa, Micrococcus luteus, Bacillus subtilis, Vibrio alginolyticus, Halomonas campaniensis, Bacillus flexus)* were identified as plastic degraders. *Pseudomonas aeruginosa* showed the highest degradation potential for PET, recording mean percentage weight loss of 30.06%. *Bacillus flexus* demonstrated the highest average PVC degradation potential (27.53%), whereas *Pseudomonas azotoformans* exhibited the highest average potential for PE degradation at 37.97%.

Our results indicate that microplastics are present in moderate to High levels along the entire coastal line of Pakistan whereas bacterial isolates obtained from these locations have considerable potential to degrade different plastic types. Further research in the area may reveal more data and explore new possibilities for policy making and remediation measures.

CHAPTER 1

INTRODUCTION

The rate of hazardous compounds being release into the environment is escalating at an alarming rate. Every few years, contaminants that are either already in the ecosystem or have recently been released are identified as harmful to both humans and the environment. Rising concerns and awareness have led to many studies investigating the origins, fate and consequences of these compounds. Concerns about microplastics over the last decade have prompted scientists to study their existence and impacts.

1.1 PLASTIC WASTE

Plastics are increasingly recognized as most persistent and enduring pollutants in the environment. According to Shen et.al. (2020), plastics contribute significantly to long-term contamination, with an alarming 80% of waste found in agricultural field, landfills and water bodies composed of plastic materials (Rummel et.al., 2017). Studies have shown that between 110,000 and 730,000 tons of plastic waste are deposited into agricultural regions annually—this amount surpasses the plastic waste that enters the oceans. Household plastics, after being discarded, are washed away through wastewater systems and end up accumulating in the sludge of wastewater treatment plants. From there, these plastics are transported to agricultural soils, where they continue to build up over time, resulting in a concerning increase in plastic presence in our environment (Nizetto et.al., 2016). The widespread accumulation of these resilient polymers in the soil has serious consequences, including the potential spread of invasive species and the risk of harmful organisms being transported across different ecosystems.

Plastics are fundamentally solid polymers, which are materials that, when heated, can be transformed into a malleable state and shaped into various molds. These non-metallic, moldable substances can be crafted into nearly any form or size, depending on the requirements (Gao et.al. 2021). As versatile materials, plastics are used for a wide array of purposes, including packing, disposable diaper linings, agriculture and fishing nets. The broad application of plastics has become integral to nearly every sector of the economy. The infrastructure and manufacturing industries, such as agriculture, telecommunications, construction, packaging and healthcare, have all witnessed significant growth, which in turn drives an ongoing demand for plastic products. Plastics serve as the foundation for a multitude of everyday products that are indispensable in modern life. These include automobile parts, electrical appliances, plastic furniture, defense materials, agricultural pipes, packaging materials, sanitary products, plumbing fixtures, tiles and flooring, synthetic leathers, bottles, jars, PVC shoes and numerous household items. The omnipresence of plastic products underscores their central role in contemporary society, but it also highlights the growing environmental challenge posed by their persistence in ecosystems.

Plastics are widely utilized in the packaging of various products, including food, medicines, cosmetics, detergents and chemicals. Approximately 30% of the global plastic production is dedicated to packaging applications. The most commonly used plastics for packaging include polyethylene (in its different forms such as LDPE, MDPE, HDPE and LLDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and various types of nylon. At present, the packaging industry is divided into two sectors: the organized sector and the unorganized sector. The organized sector is capable of producing high-quality, standardized products, while the unorganized sector often struggles to meet these standards, manufacturing low-quality, in-expensive products using excessive amounts of plastic waste.

In most cases, organic polymers are made up of plastics. Many of these polymers are made up of chains of carbon atoms, either by themselves or in combination with sulfur, oxygen, or nitrogen. The backbone is the section of the chain that connects a high number of repeated units together on the primary "path." Different molecular groups "hang" from the backbone of a plastic in order to personalize its properties. These groups are normally "hung" as part of the monomers. Because of this property of the polymers, which is based on the molecular structure of the repeating unit, plastics have become an essential component of life in the twenty-first century.

1.2 CLASSIFICATION OF PLASTICS

Based on different criteria, plastics are classified into different groups. The classification is generally based on chemical composition, synthesis process, physical properties and origin.

Another widely used basis of classification of plastics is the chemical structure. Acrylics, silicones, polyesters, polyurethanes, halogenated polymers and other similar materials are significant groups in these classes.

Chemical processes involved in plastics manufacturing is another viable basis of classification. Based on this type of classification, there are two categories that can be applied to plastics: thermoplastics and thermosetting polymers. Thermoplastics are a type of plastic that, when heated, do not experience any chemical changes in their composition and can be manufactured multiple times due to their durability. Contrary to this, thermosets have an indefinite molecular weight. They are made up of multiple repeating molecular units that come from monomers. There will be thousands of repeating units in each polymer chain. Thermosets can be melted and shaped into different forms. Once they have become solid, they will stay that way. A chemical reaction that cannot be reversed takes place during the thermosetting process. Vulcanization is a process that makes rubber thermosetting. Before being heated with sulfur, poly-isoprene is a sticky, slightly liquid polymer. After vulcanization, however, the result becomes hard and non-tacky.

Another classification of plastics is on the basis of origin (Natural & Synthetic Plastics). Physical characteristics including density, high tensile strength and resistance to certain chemicals can also be used to categorize plastics.

1.3 TOXICITY OF PLASTICS

Because they do not dissolve in water and are relatively chemically inactive, pure plastics are typically not very hazardous. Certain plastic items can be harmful because they include certain compounds. For instance, plasticizers such as adipates and phthalates are frequently added to brittle plastics like polyvinyl chloride in order to make them more flexible. Traces of these substances can leach out of the product. It has been suggested that the chemicals that leach from polystyrene food containers may interfere with hormone activities and are suspected of being carcinogenic to humans. The resulting plastic is safe to use, but the monomers that are used to make the parent polymers might be harmful. However, in certain situations, small quantities of these compounds may remain trapped in the product unless appropriate processing is used.

The disintegration of larger plastic fragments into smaller ones produces microplastics, which is, by now a serious matter of concern among the scientific community due to its drastic and significant negative impacts. Since this study is primarily based on microplastics, a brief description of concepts, sources, fate and disposal solutions of microplastics is necessary.

1.4 MICROPLASTICS POLLUTION IN THE ENVIRONMENT

Microplastics are smaller plastic fragments (<5mm), which are by now part of our ecosystems

(Arthur et.al., 2009). Microplastics are part of our daily lives with diversified sources and serious impacts. These tiny particles have huge impacts on the wellbeing of fish, invertebrates and other life forms. In a broader sense, major sources of microplastics include industrial operations, clothing and cosmetics.

1.5 CLASSIFICATION OF MICROPLASTICS

The general classification of plastics has already been discussed earlier, which is definitely applicable to microplastics. However, since microplastics are a separate and unique class of plastics with different impacts, we are bound to discuss their classification separately. Generally, microplastics are divided into two categories based on their origin, viz. Primary and Secondary microplastics. Primary microplastics are directly man-made plastics that are used in various industries, whereas secondary microplastics are produced as a result of weathering and breakdown of larger plastic particles. Both types are expected to have equivalent persistence and effects once released into the environment. Another basis of classification of microplastics is their shape. Microplastics may exist as beads, fibers, films, fragments, pallets etc. It is not surprising that regular-shaped microplastics like beads and fibers are generally primary microplastics, while irregular shapes like fragments, films and pellets are secondary microplastics. Yet another classification of microplastics is by their chemical structure, already discussed earlier. Research suggests that the common types of microplastics found in water include polyethylene (Fok et.al., 2017).

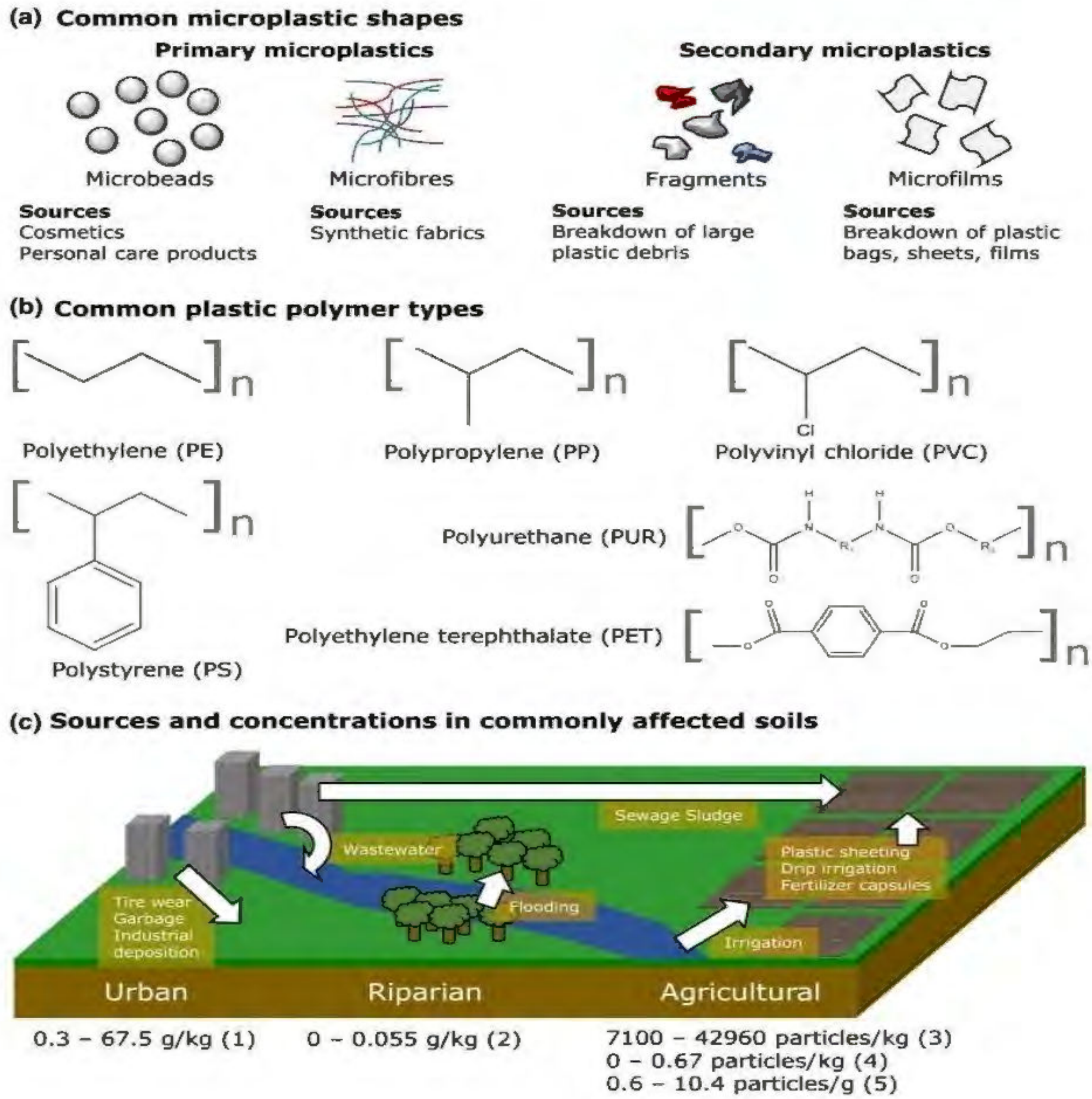


Fig. 1.1 Microplastic Types and Sources

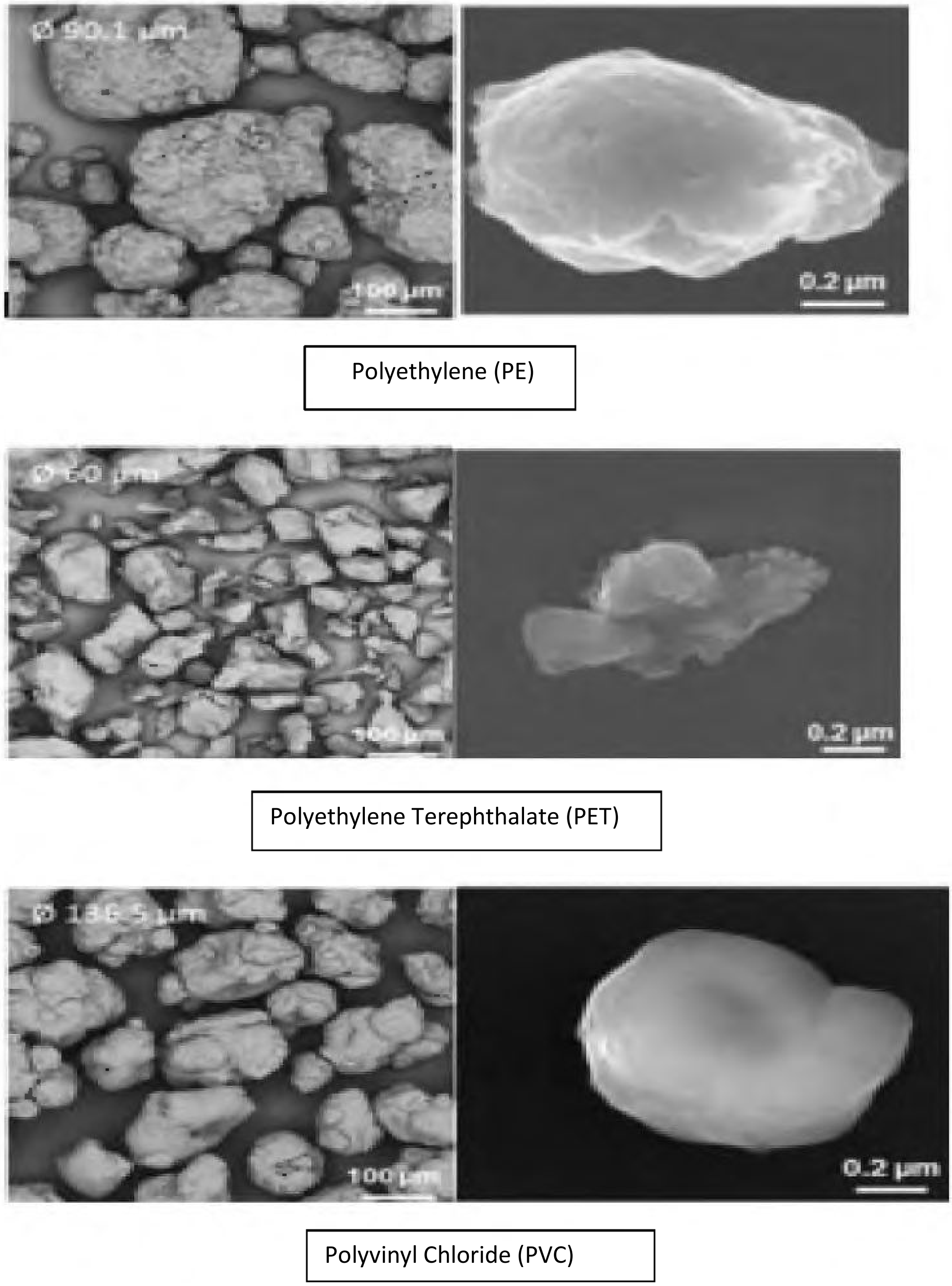


Fig. 1.2 Standard Images of plastic types, PE, PET and PVC under a microscope

1.6 SOURCES OF MICROPLASTICS

Microplastics are obtained from a variety of different sources. The production of cosmetics, exfoliating scrubs, hand cleansers, toothpaste, textiles and drilling fluids all employ primary microplastics, which are created at a tiny scale in industrial operations (Duisand Coors, 2016). Larger plastic waste, for example plastic bags, pipes, cutlery, bottles and other items, can break down into secondary microplastics (Ballent et.al. 2016).

1.7 DISTRIBUTION OF MICRO PLASTICS

The entire globe is infiltrated by microplastic pollution. As reported by Dris et.al. (2015) and Van Cauwenberghe et.al., (2015), freshwater and even some saltwater bodies have microplastics. Nevertheless, some terrestrial and oceanic activities enable the microplastics to integrate into the ecosystems. Murphy et.al (2016), McCormick et.al. (2016) suggest that the freshwater bodies are saturated with marine activities micro-sphere for marketing purposes, along with the flora and fauna. Noted by Alomar et..al. (2016), microplastics might also find their way to the ocean due to the breakdown of macro plastics from landfills and neglected waste sites. The likelihood of these particles being encountered and exposed to life under soil and water increases as bigger plastic waste is broken down into tiny fragments. Since water is the primary carrier of toxins naturally and oceans are the final sink for all water-based ecosystems, the presence of practically all contaminants in the oceans is not surprising. In the maritime environment, microplastics are pervasive (Shimand Thompson, 2015).

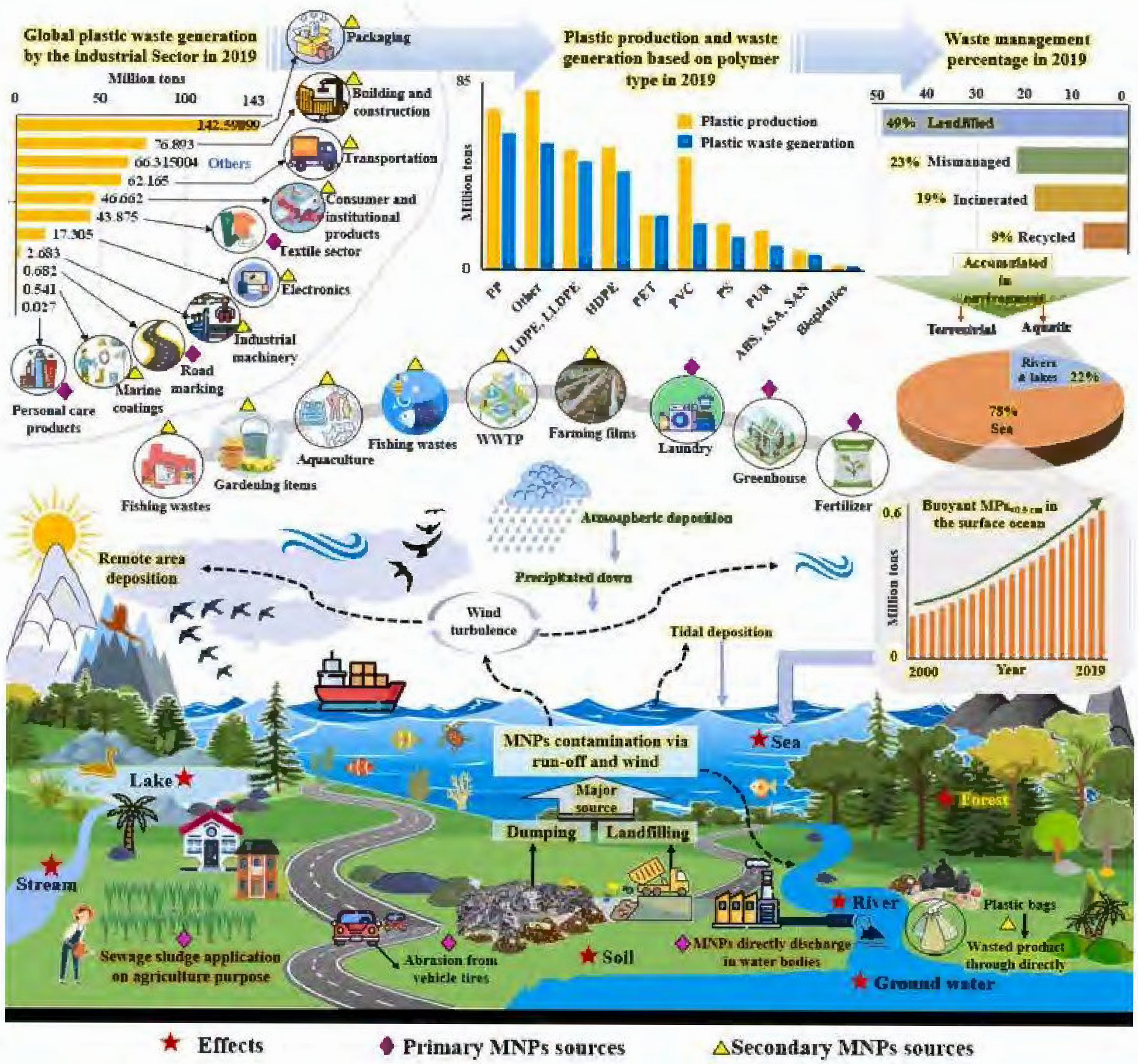


Fig. 1.3 **A comprehensive flow chart showing statistics, sources, types and effects of Microplastics.**

1.8 EFFECTS OF MICROPLASTICS ON LIFE FORMS

Microplastics have devastating direct and indirect effects on life forms, especially marine life, as marine ecosystems are the final sinks of microplastics. However, recent studies have also highlighted the effects of these tiny particles on humans. The direct and direct effects of microplastics on marine life and humans will be discussed in detail in next chapter. However, a brief overview of these effects is necessary at this stage to highlight the research problem.

Being minute particles in terms of size and weight, microplastics are easily carried away with ingested food of invertebrates, fish, algae and other species. Diverse types of creatures, including invertebrates, fish, seabirds, turtles and mammals have been found to swallow plastic (Peters and Bratton, 2016; Welden and Cowie, 2016). Ingestion of plastic may cause internal obstructions and damage to fish's digestive tract, according to earlier field investigations (Cannon et.al., 2016; Nadal et.al., 2016). Additionally, it has been demonstrated in the lab that fish suffer detrimental effects from plastic exposure (Peda et.al., 2016).

Different studies have revealed that microplastics pose significant health issues to humans in terms of physiological setbacks. These effects include damage to DNA, malfunctioning of organs, oxidative stress, poor immunity, disorders in metabolism and other issues. (Yue et.al., 2023; Wei et.al, 2023).

The indirect impacts of microplastics include, trophic transfer and adsorption to heavy metals, causes organic pollutants, antibiotics and other harmful pollutants. Microplastics have a high potential to collect, spread contaminants across the food chain and destroy marine life. Consequently, they also reach the human body through consumption of fish and other sea food. This trophic transfer worsens and widens the impacts of microplastics, beyond marine ecosystems. Furthermore, the adsorption of microplastics to poisons and harmful pollutants as mentioned above, increases the spread of microplastics across the ecosystems by long distance transmission.

1.9 DEGRADATION OF MICROPLASTICS

Owing to their drastic effects on ecosystems and life, the possible avoidance, degradation and remediation of microplastics is naturally, a subject of interest for the scientific community. Generally, the degradation of microplastics is by two methods, viz. Physical degradation or weathering and biodegradation through microbes. Physical degradation is by means of physical factors such as light, heat, moisture, etc. whereas biodegradation involves degradation by the action of living organisms, mostly microbes. Enzymatic activities that result in a chain cleavage of the polymer into monomers are the root cause of the degradation of plastics that occurs as a result of microbe attacks. Polythene film is one of the sources of carbon that microorganisms use, which leads to the partial decomposition of plastics. A biofilm is formed as a result of their colonization on the surface of the polyethylene. The hydrophobicity of the cell surface of these organisms was discovered to be a significant element in the creation of biofilm on the surface of the polythene, which therefore leads to an increase in the biodegradation of the polymers. Once the organisms have attached themselves to the surface, they will begin to proliferate by utilizing the polymer as their source of carbon supply. Oligomers, dimers and monomers are the products of the primary degradation process, which involves the cleavage of the main chain and subsequently results in the production of low-molecular-weight fragments. The extracellular enzyme that is secreted by the organism is the cause of the degradation. These molecules with a low molecular weight are consumed by the microorganisms as sources of carbon and energy in the future. Microorganisms are required to make full use of the fragments that are produced as a result of the breakdown process.

1.10 PROBLEM STATEMENT

Microplastics are predicted to have negative effects on a range of living forms and ecosystems once they are introduced into the environment. Because of their tiny size, microplastics are easily digested or absorbed by both small and big animal species and there is an inverse relationship between the particle size of microplastics and the likelihood that marine creatures would consume those (Wright et.al, 2013). It is recognized that many marine creatures, including mammals and filter organisms, absorb microplastics. Microplastics therefore have a high potential to disrupt the food chain. There have also been reports of the trophic transmission of microplastics at different stages of the food chain. (Batel et.al, 2017). Finally, biologists are quite concerned about how microplastics may affect these species. There have been several studies reporting the effects of various pollutants.

It is essential to have a complete understanding about quantity, composition and geographic distribution of microplastics on international, national and regional scale in order to provide assistance in the management of marine litter made of plastic. On the other hand, there is a paucity of information regarding the microplastic contamination of shorelines at national level in some areas. It is necessary for the governments to have knowledge about the geographical distribution of microplastics on a national basis, taking into account their size, shape and the type of polymer they are made of.

Once released into the environment, the recovery of microplastics is impossible as they are tiny particles and are dispersed at a larger scale. However, the degradation of these tiny particles through physical factors and through microbial metabolism is viable and possible. Therefore, to mitigate the microplastic pollution, microbial species with substantial capacity to degrade microplastics need to be identified for their subsequent introduction in the most infested areas.

1.11 NOVELTY AND SIGNIFICANCE OF STUDY

Pakistan, being a developed country has limited technology and resources for scientific research. Research on microplastic abundance, specifically along the coastal line of Pakistan was not done till the year 2022, when a study to evaluate the abundance of microplastics along the coastal line of Pakistan was done (Ahmed, et, al., 2022) However, a confirmative study to this effect is still missing. Further, the study does not cover the locations in extreme western portion of Pakistan's coastal line. Yet, the potential of microflora in marine sediments for microplastic degradation remains completely untapped.

A region-specific literature review and assessment of baseline information regarding the emergence and effects of microplastics within Pakistan needs to be be conducted. This includes the identification, collection and mapping of additional locations that were not previously studied or were understudied. To begin, the research will target the gaps in the previously available data and later extend its focus towards regions that have barely been explored and hold great potential for new information on microplastics and their range. In addition, measures that can be taken to reduce microplastic pollution will also be a part of this study as this issue continues to raise more concerns. In particular, research will consider the use of local microflora of these locations for the breakdown of microplastics. The research seeks to provide deep rooted answers to the issue of microplastics pollution and provide Pakistan with practical and environmentally friendly eco-efficient options for managing plastic waste.

Definitely, the study will provide a major contribution towards better management of microplastic pollution in Pakistan's coastal region. The inclusion of microbial remediation of microplastics in Pakistan's coastal area can be proposed based on this study.

1.12 OBJECTIVES OF STUDY

Keeping in view the lack of information available, especially at regional level, for microplastics and the role of microbes in their remediation, the present study is planned with the following objectives:

- To investigate the abundance, characteristics and spatial distribution of microplastics along the coastal region of Pakistan
- To isolate and screen out microplastics degrading bacteria from marine environment.
- To investigate the potential of bacteria isolated from marine environment for degradation of microplastics.

CHAPTER 2

REVIEW OF LITERATURE

Ecological and health-related consequences are posed by microplastics. Their sources are different such as the breakdown of bigger pieces of plastic, exfoliation from artificial fibers, tire wear and, most importantly, the intentional use of microplastics in cosmetics and industrial products. There is an urgent and growing need to address the environmental pollution caused by microplastics, as well as to thoroughly investigate the potential for microbial degradation of these pollutants. Microplastics, which are small plastic particles that have resulted from the breakdown of larger plastic objects, are rapidly accumulating in ecosystems worldwide, posing serious threats to environmental health. This paper seeks to provide a quantity analysis of the various types and compositions of microplastics, alongside an examination of their advantages and disadvantages. It also explores the significant impact microplastics have on microbiomes and microbial communities, focusing on the complex and often detrimental interactions between microplastics and microbial ecosystems. The study delves deeply into the microbial degradation of microplastics, discussing the processes involved in identifying, characterizing and culturing microorganisms capable of breaking down these persistent pollutants. Furthermore, the paper highlights the mechanisms behind microplastic degradation, including the crucial role of microbial enzymes, which offer exciting potential for biotechnological applications aimed at mitigating microplastic pollution. In addition, the study presents various strategies for reducing microplastic pollution, including policy recommendations and the implementation of improved waste management practices. The paper also outlines the challenges and opportunities that lie ahead, stressing the importance of international cooperation, advancements in research and active public engagement in addressing this global environmental crisis. Ultimately, this work underscores the critical need for concerted and coordinated efforts to combat microplastic pollution for researchers, policymakers and stakeholders dedicated to environmental preservation (Panel et.al., 2023).

The rising levels of micro- and nano-plastics (MNPs) in ecosystems are of growing concern, as they pose significant risks to various environmental components. Microplastics result from the fragmentation of major plastic items, as well as from their widespread use in commercial products, particularly in packaging materials. These small plastic particles have infiltrated ecosystems to the extent that they now permeate the food chain, agricultural systems and even the human body. As exposure to MNPs continues to spread, it is becoming clear that traditional methods for monitoring and characterizing these particles are still in development. This makes the exploration of innovative remediation techniques especially important. Among the many available remediation strategies, microbial remediation has shown considerable promise as an effective and sustainable method for degrading and removing MNPs from the environment. Microorganisms have evolved specialized genes that enable them to break down plastic pollutants, adapting to eco-physiological processes influenced by both biotic and abiotic factors. Through these mechanisms, microorganisms can utilize the carbon content in plastics as an energy source, leading to the destruction or elimination of MNPs. This review explores the sources of MNPs, their degradation processes and highlights the need for continued research to support ongoing global efforts in environmental protection. Identifying further research gaps will be essential to advancing the current understanding of MNPs and enhancing global initiatives aimed at reducing environmental plastic pollution (Panel et.al., 2024).

The term 'plastic' is derived from the Greek word “Plastikos,” meaning “able to be molded into various shapes and sizes” (Sandra, 2022). Plastics are synthetic materials made from a combination of organic and inorganic raw materials, including elements like carbon, silicon, hydrogen, nitrogen and oxygen. These basic components are derived from natural resources such as oil, coal and natural gas, which are essential for the production of plastics (Kunwar et.al., 2016). One of the challenges associated with plastics is their resistance to microbial degradation. Due to the relatively short time that plastics have been in existence compared to the length of natural evolutionary processes, microorganisms have not developed the necessary enzymes to effectively degrade synthetic polymers (Mueller, 2006). Today, the global production of synthetic plastics has reached approximately 140 million tons annually and a significant portion of these plastics is introduced into ecosystems as industrial waste products (Geyer, 2020).

Plastics are widely utilized in the packaging of products including food, drugs, cosmetics, detergents and chemicals. It is believed that approximately 30% of worldwide plastic production is used for packaging purposes, with this usage continuing to expand at an annual rate of 12%. Plastics have largely replaced traditional packaging materials like paper and cellulose-based products due to their superior physical and chemical properties, including strength, lightness, resistance to water and durability against most water-borne microorganisms. Some of the most commonly used plastics in packaging include polyethylene, polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), poly(ethylene terephthalate) (PET), poly(butylene terephthalate) (PBT) and various types of nylon. The widespread adoption of plastics can be attributed not only to their favorable mechanical and thermal properties but also to their remarkable stability and durability (Farah et.al., 2016). Given their long-lasting nature, plastics have garnered significant attention from both the public and the media, particularly because of their visibility in litter and their persistence in the environment. The global demand for plastics was estimated to exceed 107 million tons in 1993 and increased to around 146 million tons by the year 2000. This rapid growth in plastic production and usage continues to raise concerns about the long-term environmental impacts of plastic waste.

The escalating production of commodity plastics used in food packaging, as well as in agriculture and industry, has attracted public concern for the potential environmental accumulation and pollution issues which may last for centuries (Mishra et.al 2023). Plastic waste is dealt with through landfilling and recycling. Many communities are now more aware of the negative consequences that discarded plastic can cause to the environment, ranging from concern for wildlife to the visual beauty of forests and cities. Mismanaged plastic waste is a great environmental pollution threat to life. Besides, the incineration of polyvinylchloride (PVC) plastics is associated with the generation of persistent organic pollutants (POPs) such as dioxins and furans (Jayasekara et.al 2005).

At present, most of the industrially used plastics, such as place polyesters polyurethanes and starch blended polyethylenes, are resistant to microbial degradation. However, these would not persist in the environment for long. The negative attitude of these groups has also caused concern about these products, which will inflame these and stimulate research activity to modify existing products or come up with more biodegradable polymers (Dewil et.al 2020).

According to Boucher et.al. (2016), about 4.8 million tonnes of plastic garbage from land are dumped into the ocean. Particularly, microplastics (5 mm in diameter) are a major source of anthropogenic litter in aquatic habitats and are found in large quantities throughout the world's marine settings (Eriksen et.al., 2014). Polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), Nylons and polyethylene terephthalate (PET) are the primary types of microplastics that make up 92.4% of plastic trash (Santana et.al., 2016). Microplastics can be destroyed by certain bacteria even though they are resistant to degradation and persistent in the environment (Paco et.al., 2017).

Due to their opportunistic nature, microorganisms may naturally adapt to practically any environment (Aujoulat et.al., 2012). Additionally, microorganisms have the ability to change a wide range of substances, including polymeric polymers. According to Luigi et.al. (2007), this adaptive characteristic helps bacteria metabolize considerably in the presence of contaminants and, in some situations, enhances degradation and biotransformation. Abioye et.al. (2010), lubricating oil (Emenike et.al. 2016), crude oil (Auta et.al. 2014), benzo[a]pyrene (Aziz et.al. 2017) and a few examples of environmental pollutants for which studies have shown the viability of bacterial isolates for remediation. In their 2016 study, Yoshida et.al. looked into the breakdown of PET by the bacteria Ideaonellasakaiensis201-F6, which can thrive only on PET as a source of carbon and energy. According to Mohan et.al. (2016), high-impact PS that has been brominated can be degraded by Pseudomonas sp. and Bacillus sp. The reaction of the fungus *Zalerion maritimum* to various PE pellet incubation durations was assessed by Paco et.al. (2017). The outcomes showed that the fungus can use PE under the studied circumstances and that doing so reduced the quantity and size of the pellets. These results suggested that fungi that exist naturally may break down microplastics. In their 2014 study, Sowmya et.al. described how Bacillus cereus breaks down PE. According to Harshvardhan and Jha (2013), marine bacteria (*Kocuria palustris* M16, Bacillus pumilus M27 and Bacillus subtilis H1584) degrade PE. After 30 days of incubation, these bacterial species showed weight losses of 1%, 1.5% and 1.75%, respectively. Pseudomonas stutzeri, Alcaligenes faecalis, Pseudomonas putida, Brevi bacillus borstelensis, Streptomyces sp. and Staphylococcus sp. are some other bacteria can degrade polymers (Ghosh et.al., 2013; Caruso, 2015).

Natural plastics are biodegradable materials derived from renewable sources such as plants, animals and algae. These plastics are capable of breaking down completely in their natural environment and are produced by various bacteria and archaea. PHA presents a promising alternative to traditional petrochemical plastics due to its biodegradable, eco-friendly and biocompatible properties. As the world faces the challenge of depleting natural resources, non-petroleum-based biological polyesters like PHA are increasingly being considered as vital candidates for the next generation of sustainable polymers. PHA shares several physical and chemical properties with widely used synthetic plastics such as polyethylene (PE) and polypropylene (PP), making it a viable option for many applications (Kim and Lenz, 2001; Rehm, 2003). Many microorganisms naturally accumulate PHA as an intracellular energy reserve, particularly when carbon is available in excess relative to other nutrients such as nitrogen, sulfur, phosphorus and oxygen (Grey, 2020; Reddy et.al., 2003). Although approximately 250 different organisms are known to produce PHA, only a few species are capable of synthesizing it in high concentrations. Examples include Alcaligenes latus, Pseudomonas oleovorans and Cupriavidus necator (formerly Ralstonia eutropha) (Kourmentza et.al., 2017; Kim et.al., 1994).

PHA can be classified based on the length of the repeating units in the polymer. Short-chain-length PHA (scl-PHA), which consists of monomers with C3 to C5 hydroxyl fatty acids (e.g., polyhydroxybutyrate or PHB and hydroxyvalerate or PHV) and medium-chain-length PHA (mcl-PHA), which consists of C6 to C16 hydroxyl fatty acids, are the two primary types (Kumari et.al., 2022). The widespread use of antibiotics in agriculture, hospitals, animal husbandry and industry, along with the heavy contamination of environments, has introduced significant challenges to the production of PHA at an industrial scale. Antibiotics and heavy metals can directly or indirectly contaminate microbial cultures, leading to potential disruptions in the microbial processes required for PHA production. To mitigate these challenges, researchers are exploring strains of bacteria that are resistant to specific antibiotics, allowing for better control over contamination in PHA production systems (Sobti et.al., 2022). PHA serves as an important response to environmental stress, with many bacteria capable of accumulating PHA as an energy source in contaminated environments.

One of the most prevalent and non-degradable solid wastes in the environment today is polythene, which cause major threat to marine life. Polythene can cause severe blockages in the intestines of fish, birds and marine mammals, leading to their injury or death (Ryan, 2019). The degradation of polythene presents a significant challenge due to the increasing amount of this material being produced and disposed of worldwide. An estimated 57 million tons of plastic waste are generated globally each year, contributing to serious waste management issues, especially in coastal megacities (Bollag et.al., 2000). Low-density polyethylene (LDPE), one of the most widely used plastics, is a major contributor to environmental pollution. Polyethylene, a polymer made from long-chain ethylene monomers, is growing in usage at a rate of approximately 12% per year. Approximately 140 million tons of synthetic polymers, including polyethylene, are produced annually across the globe (Shimao, M., 2001). The large-scale accumulation of polyethylene in the environment has created a significant ecological dilemma, as it can take thousands of years for polyethylene to degrade efficiently. This highlights the urgent need for biodegradable alternatives to plastics.

Biodegradable polymers are specifically designed to break down after disposal through the action of living organisms. These polymers decompose in various environmental media through a process called depolymerization, which is influenced by both physical and biological forces.

In contrast, microbial biodegradation is widely accepted as a key method for enhancing the breakdown of plastics. Recent studies have identified several microorganisms that produce enzymes capable of breaking down plastics. These enzymes lead to the chain cleavage of polymers into smaller oligomers and monomers, which are water-soluble and can be absorbed by microbial cells. Once absorbed, these products are metabolized through aerobic or anaerobic processes. In aerobic metabolism, the end products are carbon dioxide and water, while in anaerobic conditions, carbon dioxide, water and methane are produced (Pathak, 2017; Gu et.al., 2000). The microbial degradation process breaks down plastics into simpler monomers, which can be easily accumulated and further degraded by microbial cells.

Plastics, due to their unique combination of properties such as affordability, light weight and impact resistance, have become highly popular materials across industries, leading to a dramatic increase in global production. From 1.5 million tons in 1950, global plastic output surged to 322 million tons by 2015 (Plastic Europe, 2016). While the widespread use of plastics benefits society in many ways, their persistence in the environment, due to their resistance to degradation, poses a serious environmental threat (Barnes et.al., 2009; Roy et.al., 2011). According to estimates by Jambeck et.al. (2015), between 4.8 and 12.7 million tons of plastic waste are dumped into the ocean annually, primarily originating from coastal regions. As plastics continue to break down into smaller particles, their potential for ingestion by marine species, particularly those at the base of the food chain like filter feeders, increases significantly (Browne et.al., 2008; von Moos et.al., 2012). This highlights the urgent need for global efforts to address plastic waste, especially in marine ecosystems and to develop sustainable alternatives that can reduce environmental harm (Ambrosini et.al., 2019).

Rezaei et.al. (2019) reported that in Iran commonly distributed MNPs in the environment are $\sim$1.42 g/cm^3 of polyformaldehyde, $\sim$1.38 g/cm^3 of PVC, $\sim$1.2 g/cm^3 of PC, $\sim$1.03 g/cm^3 of polyamide, $\sim$1.37 g/cm^3 of polyester, $\sim$0.94 g/cm^3 of HDPE, $\sim$0.92 g/cm^3 of LDPE and $\sim$0.84 g/cm^3 of PS. Consequently, a recent study (Abbasi et.al., 2019) reported that a youngster consumes more than 900 MNP particles per year under typical exposure conditions.

Groundwater, freshwater, marine water, ice, snow, sediment, air, soil and even re-suspension from sea spray have MNPs pollutants (Allen et.al., 2022).

Investigations of MNPs in freshwater ecosystems (such as ponds, lakes, wetlands, rivers, springs, streams and bogs) are fewer compared to those of marine habitats. Freshwater bodies are inadvertently found closer to many urban and rural settlements, suggesting that they may collect enormous amounts of MNPs from surface runoffs (coupled with storm water overflow events, farmland and atmospheric deposition), sewer overflows, fishing activities (mainly from fishing net deterioration), tourism and direct discharge of industrial effluents (van den Berg et.al., 2020). Mason et.al. estimated that the average discharge of MNPs from municipal WWTPs in the USA rises to 13 billion particles/day after analyzing effluent samples from 17 WWTP locations (Mason et.al., 2016). Among the freshwater bodies, rivers serve as the main transport pathways for dispersing MNPs into marine ecosystems (Allen et.al., 2022, Sharma et.al., 2023) and also serve as a long-period (especially in the dry season) sink for MNPs deposition (van Emmerik et.al., 2022). Fischer et.al. (2016) found an abundance of MPs ranging from 112 to 234 particles/kilogram dry weight in Lake Bolsena, Italy (Lake Chiusi). Similarly, another recent study reported about the MNP's abundance in sewage water, which is released into the Lake Tana and Blue Nile rivers every day in Ethiopia and the finding showed that the presence of 30.4% of PE, followed by 21.74% of PP, 13.04%of PET, 13.04% of PS, 13.04% of PA and 10.87% of PVC (Mhiret Gela and Aragaw, 2022). Likewise, Wibuloutai et.al. (2023) found MNPs in Chi River, Thailand, that contain 336 μg/L (<20–450 nm) where the dominant shape was fibres and fragments and the majority of MNPs are composed of PET, PP and PE. Freshwater ecosystems support vast biodiversity and render several ecological services that profit human populations. Their use as a sustainable source of drinking, irrigation, domestic and industrial waters may potentially facilitate the dispersal of

MNPs and increase their risks of causing environmental and health problems (Chen et.al., 2021, Strungaru et.al., 2019).

Marine habitats have long been recognized as significant sinks for plastic trash from the coast to the deep Ocean. Borrelle and her colleagues projected that 19–23 MMT amounting to 11% of the plastic garbage produced worldwide in 2016, ultimately reaching into the aquatic ecosystems (freshwater and marine; Borrelle et.al., 2020). While these figures may be regarded as conservative, around 75–90% of marine plastic is recognized to come from terrestrial sources, with a lesser quantity (10–25%) contributed from marine sources via fishing and boating, aquaculture, oil and gas development, agricultural activities, atmospheric transport and wind erosion (Allen et.al., 2019, Allen et.al., 2021a, Allen et.al., 2021b, Allen et.al., 2022). Gies et.al. (2018) reported Vancouver WWTP produced about 1.76 trillion MNPs that get into the environment annually, of which 0.3 trillion particles of MNP were washed into the marine ecosystem. MNPs contaminate the Arabian Bay and the North-western Pacific Ocean (Xu et.al., 2019) with concentrations of 4.38×10^4 items/km^2 and 640–42,000 items/km^2, respectively. Likewise, Naik et.al. (2019) reported that oceans contain ∼15–51 trillion MNP fragments (that ∼ is 93–236 thousand metric tonnes).

MNP particles tend to gather at the bottom because they combine more quickly when coated with biofilm (Pierdomenico et.al., 2019), which lowers their buoyant efficiency and favours their sedimentation and accumulation on the seabed; after that, they may either be buried by sediments or get adrift in ocean currents to be redeposited elsewhere (Kane et.al., 2020). These are the reasons behind the presence of significant quantities of MNPs in Oceanic ice in the Arctic and Antarctica (Allen et.al., 2022). As an example, field studies conducted on the Antarctic sea ice showed the presence of MNPs; the average concentration was 52.3 μg/L in size <200 nm with 19.8–38.0 μg/L of PE, 9.0–20.7 μg/L of PP, 8.3–8.9 μg/L of PET identified as the abundant type (Materić et.al., 2022a, Materić et.al., 2022b).

In agricultural land, MNPs mainly come from irrigation, fertilisers, sewage sludge/biosolids, plastic film mulch and greenhouse materials (Azeem et.al., 2021, Zhou et.al., 2020). A study stated that about 4.4 million tons of plastic were introduced into the soil in 2012 from agricultural areas where plastic mulching is used (Sintim and Flury, 2017, Yongming et.al., 2018). It is challenging to efficiently recover plastic mulch film from the soil, especially very thin films (8–50 μm). According to Peccia and Westerhoff, WWTPs contribute one of the highest levels of plastic pollutants on agricultural land, about 7.76 million tons (Peccia and Westerhoff, 2015).

MPs concentration in wastewater sludge varies from 1500 to 56,400 particles kg^{-1} (Mintenig et.al., 2017, Li et.al., 2018). A recent study noted that the use of sewage water and sludge contaminates farmlands in North America (44,000 - 300,000 tons yearly) and Europe (63,000 - 430,000 tons yearly) (Rai et.al., 2021). Correspondingly, Weithmann et.al. (2018) revealed that organic fertilizer could contain up to 895 MP particles kg^{-1}, further lending support to the concept that continual use of organic fertilizers and sludge increases MP contaminants in agricultural soil and food produce (Weithmann et.al., 2018).

MP abundances were 78.00 and 62.50 N/kg in the 0–3 and 3–6 cm soil stratum in Shanghai (Eastern China) and suburban vegetable farms (n=20), correspondingly (Liu et.al., 2018). In an aquaculture system in Shanghai, the MP concentrations identified in rice and aquaculture soil were 16.1 and 4.5 items/kilogram, correspondingly (Lv et.al., 2019). Likewise, another study reported that MP abundance in farming soil varied from 420 to 1290 items/kilogram in Nanjing and Wuxi and was 571 and 263 items/kg in mulched and non-mulched land, correspondingly, in the bay area of

Hangzhou, Southeast China (Li et.al., 2019, Zhou et.al., 2019a). Wuhan (central China) suburban farming soil has an MP abundance of 320 and 12,560 particles/ kilogram (Chen et.al., 2020, Zhou et.al., 2019b).

Non-agricultural soil (such as forest soil) is the MNPs storage hub in the global plastic cycles hub which are received from two different pollution sources (Horton et.al., 2017): the point and the non-point. Sewage sludge treatment can cause point source contamination, reaching the soil environment through sewage discharge (Afrin et.al., 2020). The primary non-point source pollution includes landfills, agricultural practices, tyre abrasion, atmospheric deposition and waste disposal facilities (Afrin et.al., 2020, Mandal et.al., 2024). Worldwide plastic pollution in the natural environment is predicted to rise to almost 12,000 MMT by 2050 due to its extended persistence in nature (Zhang et.al., 2022). Landfills may grip between 21 and 42 percent of global plastic waste (Afrin et.al., 2020). The mass and particle concentrations of MNP in untreated leachate were 11.4 g/L and 235.4 items/L with particle sizes <50 μm (Sun et.al., 2021). Among them, PE and PP were the most common polymers in solid municipal waste sent to landfills (He et.al., 2019), with diameters that varied from 100 to 1000 μm. Particles produced by landfills and other surface deposits could become airborne due to atmospheric resonances/displacement (Rillig et.al., 2017). According to Klein and Fischer (2019) and Allen et.al. (2019), atmospheric MNP deposition rates in rural or near-urban forests vary between 331 and 512 particles per square meter daily. Northern hemisphere forest streams and lakes have an average content of 563 μg/L, which can be attributed to atmospheric NPs deposits (Materić et.al., 2022a). Leonard et.al. (2023) conducted the first direct evaluation of MPs at up to 25 particles/cm on urban phyllosphere and soils that participate in the transferring of particulate matter (PM). Hence, these tremendously hamper the soil's physiochemical properties (Zhou et.al., 2022) and microbial activity (Guo et.al., 2020).

Advancements in atmospheric MNP research have witnessed a boost throughout the last decade. MNPs are discharged into the atmosphere as a result of operations in synthetic textile industries, mechanical activities, severe wind events, sea surface spray and wave breaking (Chang et.al., 2020), which frequently entrain them (Brahney et.al., 2021). Seaspray emits MNPs from the water's surface into the air, with a worldwide estimated value of 136,000 tonnes blowing ashore yearly (Allen et.al., 2020). Every year, a large content of MPs are released from tyres wear (10×10^4 metric tonnes) and brakes (4×10^4 tonnes) that are carried via the air (Evangeliou et.al., 2020). A research by Hale et.al. (2020) found that each individual in the US contributes 4.7 kg of tyre wear MNPs every year, which equates to 1.8 MMT. In the European cities, the mean MPs abundance from dry and wet deposition is 118, 275 and 1008 particles m^{-2} every day in Paris, Hamburg and London, respectively (Dris et.al., 2015, Klein and Fischer, 2019, Wright et.al., 2020). While in China cities, the average MPs abundance is between 313 (Dongguan) and 602 (Yantai) particles m^{-2} per day in the atmospheric deposition (Cai et.al., 2017, Zhou et.al., 2017). Similarly, Edo et.al. (2023) reported that in Spain, the atmospheric deposition rate of MNPs ranged from 5.6 to 78.6 m^{-2} per day (size ranging from 22 to 398 μm) and most abundant was by polyester, representing over 70% of the synthetic polymers identified, with the majority (> 90%) in the form of fibres. In the remote area of the Pyrenees Mountains in France, the result illustrates an average MPs deposition of 365 particles m^{-2} d^{-1} dominated by PS and PE (Allen et.al., 2019). Ambrosini et.al. (2019) analyzed atmospheric deposited samples collected from the Forni Glacier, Alps, in 2018, where sediment content of 74.4 items/kg of MPs concentration, which contained 39% of polyester, 9% of polyamide, 9% of PE and 4% of PP. Fibres accounted for 65.2%, with fragments accounting.

According to a certain research (Lusher et.al., 2015), efforts have been made to document the levels of microplastic contamination across various marine habitats worldwide, but comprehensive data is still lacking in many areas. To improve the management and reduction of marine plastic pollution, it is necessary to conduct detailed research on the presence, quantity and composition of microplastics at national, regional and international scales. Despite these efforts, there is still limited knowledge regarding the extent of microplastic contamination along different shorelines (Ng and Obbard, 2006), particularly in more remote or less studied regions.

Microplastic contamination has been observed in various marine environments globally and extensive documentation has highlighted the widespread presence of these particles across different marine compartments (Lusher et.al., 2015). According to Lee et.al. (2015), sandy beaches are especially prone to accumulating both macroplastics and microplastics. The build-up of plastic waste on these shorelines is largely a result of two main factors: waste originating from land-based sources, as well as floating plastic debris that enters the ocean. Once plastics make their way to the beach, they often degrade due to exposure to the elements, including UV radiation, heat and saltwater. This process of weathering leads to the fragmentation of larger plastic items into smaller pieces, eventually contributing to the formation of microplastics. These fragments, which can be as small as a few millimeters or even microns in size, pose a significant threat to marine life and ecosystems, as they are easily ingested by various marine organisms, including fish, seabirds and invertebrates. Furthermore, as microplastics continue to accumulate on beaches and in coastal waters, they become a persistent environmental problem that requires urgent attention and further research (Corcoran et.al., 2009). In terms of the high availability of UV radiation, oxygen, mechanical forces and high temperatures, beach habitats offer comparatively favourable natural weathering conditions for plastic debris compared to the sea surface and seabed (Song et.al., 2017).

In addition, compared to the collection of sea surface and bottom samples, beach sampling efforts and expenses are comparatively insignificant. Large microplastics (L-MPs, 1 to 5 mm) and macroplastic debris greater than 5 mm have been the subject of many beach investigations (Lee et.al., 2013; Baztan et.al., 2014).

Small microplastics (S-MPs, 1 mm) need more time and effort to analyse than larger microplastics (L-MPs), therefore the amount of information on them is restricted (Browne et.al., 2010; Costa et.al., 2010; Carson et.al., 2011; Martins and Sobral, 2011; Hidalgo-Ruz et.al., 2012; Kim et.al., 2015). With a decrease in microplastic size, marine creatures have a greater chance of ingesting it (Wright et.al., 2013a). Although there is a high positive correlation between the abundances of mesoplastics (5e25 mm) and L-MPs on beaches (Lee et.al., 2013), the link between L-MPs and SMPs in beach settings is still unclear. By weathering, stranded plastic waste may be broken down into smaller microplastics and tiny microplastics can be dumped into the ocean (Browne et.al., 2011). In the marine environment, rivers constitute a significant source of microplastics (Zhao et.al., 2014; Barnes et.al., 2009).

There are several environmental (such as wind and current direction) and source-related (such as population and proximity to a river mouth) variables that might affect the spatial distribution of microplastics along shorelines. For instance, Yonkos et.al. (2014) found a positive correlation between microplastic abundances and population density in the Chesapeake Bay and globally (Browne et.al., 2011). According to another study (Rech et.al., 2014), the amount of plastic trash typically decreased with increasing distance from the river mouth. Additionally, wind, water circulation, tide, meteorological conditions and proximity to urban sources have an impact on the dispersal of microplastics (Sadri and Thompson, 2014). Uncertainty persists regarding the key variables affecting pollution levels and the geographic distribution pattern of microplastics on beaches.

The bacteria initially attach to the polymer surface during polymer breakdown, exposing it to microbial colonisation. Extracellular enzymes are secreted during polymer colonisation and these enzymes attach to the polymer and trigger hydrolytic cleavage (Shah et.al., 2008). The microorganism uses the carbon dioxide (CO2) and water (H2O) produced by the mineralization of the polymer to provide energy (Tokiwa et.al., 2009). The polymer is then decomposed into low-weight polymers. The organism's microplastic particles travel past the cellular membrane, where cellular enzymes break them down within the organism's cells (Gewert et.al., 2015).

Numerous environmental and source-related (e.g., population and proximity to a river mouth) variables can influence the spatial distribution of microplastics along shorelines. For instance, Yonkos et.al. (2014) found a positive correlation between microplastic abundances and population density in the Chesapeake Bay and globally (Browne et.al., 2011). According to another study (Rech et.al., 2014), the amount of plastic trash typically decreased with increasing distance from the river mouth. Additionally, wind, water circulation, tide, meteorological events and proximity to urban sources all have an impact on the dispersal of microplastics (Kukulka et.al., 2012; Sadri and Thompson, 2014; Yonkos et.al., 2014; Nel and Froneman, 2015). The main elements, however, that affect pollution levels and the dispersion of microplastics in space are remained unidentified.

Coastal and marine habitats are among of the sources of the most productivity on Earth. Studies show that microplastics have harmful effects on specific algae or zooplankton species. which also have an impact on both primary and secondary production. Based on published laboratory results, a modelling technique was utilised in this work to quantify the effects on ecosystem production. This study made use of the Delft 3D GEM biogeochemical model of the North Sea. The geographical patterns of secondary production demonstrating local variations of 10%, despite the model's prediction that microplastics do not impact the total primary or secondary output of the North Sea as a whole. Strong assumptions were needed to account for the plastic concentrations and their effects under field settings because there aren't many pertinent field data on microplastics. The key information gaps that must be filled in order to enhance the initial estimate are revealed by these assumptions (Liu et.al. 2023)

To enable MP management, a basic understanding of the quantity, make-up and geographic distribution of microplastics on a national scale is required. This study was carried out to determine the baseline level of microplastic contamination at 20 sandy South Korean coastline beaches. Using Fourier transform infrared spectroscopy, all extracted microplastic particles were detected down to a size of 20 mm. Small microplastics (S-MPs; 0.02e1 mm) and large microplastics (L-MPs; 1e5 mm) were both abundant, with abundances ranging from 0 to 2088 and 140 to 62800 n/m2, respectively. Maximum microplastic abundance was found in the 100–150 mm size range and 81% of the overall abundance was found in particles smaller than 300 mm. 95% of L-MPs were made of expanded polystyrene (EPS), whilst polyethylene (49%) and polypropylene (38%%) made up the majority of S-MPs. With the exception of EPS, population, precipitation, proximity to a river mouth and the quantity of macroplastic trash on beaches were all strongly correlated with the regional distribution of L-MPs. However, other than macroplastic debris and L-MPs excluding EPS, there were no associations between S-MPs and other environmental or source-related parameters. According to these findings, L-MPs other than EPS are mostly imported from land-based sources and are also partially created on beaches, whereas S-MPs are primarily formed on beaches by weathering (Chubarenko et.al., 2020)

Antibiotics and microplastics are two developing pollutant groups with potentially dangerous effects on aquatic environments. Antibiotics that have been adsorbed onto microplastics may be transported over great distances and may have compound-combination effects. In this study, we examined the adsorption of 5 antibiotics on 5 different types of microplastics in freshwater and saltwater systems. The antibiotics were sulfadiazine (SDZ), amoxicillin (AMX), tetracycline (TC), ciprofloxacin (CIP) and trimethoprim (TMP). Microplastics exhibit distinct surface characteristics and varying levels of crystalline structure, according to SEM and X-ray diffractometer (XRD) examination. This is because of its porous structure and hydrogen bonding. The adsorption capability of the other four microplastics was found to be quite low. The quantities of five antibiotics that were adsorbed on PS, PE, PP and PVC declined in the following order: CIP > AMX > TMP > SDZ > TC, with Kf showing a positive correlation with the octanol-water partition coefficients (Log Kow). When compared to a freshwater system, the saltwater system's adsorption capability dramatically dropped and neither CIP nor AMX showed any signs of adsorption. Our findings suggested that frequently found polyamide particles might transport antibiotics in aquatic environments (Dong et.al., 2021)

Microplastics age and interact with other materials, such as organic pollutants. Therefore, in order to assess the environmental impact of microplastics, it is crucial to comprehend the sorption interactions between aged microplastics and organic pollutants. Less is known about how microplastics' sorption behaviour and other characteristics change as they age. The effects of an accelerated UV-aging technique on polystyrene microplastics, which are found in items like skin cleansers and foams, were thus the subject of a study that was planned. Ageing resulted in substantial surface oxidation and limited localised microcrack development, according to particle characterizations. After ageing, polystyrene microplastics' sorption coefficients for organic compounds were up to an order of magnitude lower than they were for unaltered particles. Internal cross-validation was used to validate the ppLFER models, confirming its robustness. As a result, our method produces better estimates of the interactions between contaminated organic materials and aged polystyrene microplastics (Ding et.al., 2020)

Little attention has been paid to measuring the amount of microplastic contamination in the Antarctic Treaty region (south of latitude 60°S). The quantities of microplastic particles in sediment samples from 20 sites up to 7 km from Rothera Research Station were studied in this study. Sediment that was collected close to the station's sewage treatment plant outfall had the greatest levels of microplastic (5 particles 10 ml1). The amounts were comparable to those seen in shallow- and deep-water marine sediments outside of Antarctica and the microplastics resembled those made by washing garments. For the purpose of informing policy debates and the creation of effective management measures, more study on microplastics around Antarctic stations is advised (Bargagli & Rota, 2022)

On the beaches of four Lesser Antilles islands—Anguilla, St. Barthélemy, St. Eustatius and St. Martin/Maarten—microplastic pollution was examined. The North Atlantic subtropical gyre, which has a high concentration of microplastics, lies nearby these islands. On Saint Martin's Grandes Cayes, a high of 620 96 microplastics were discovered, with an average of 261 6 microplastics/kg of dry sand. More than 95% of these microplastics were fibres. The amount of microplastics varied amongst the islands, with St. Eustatius having substantially lower amounts than the other Islands.

There were no differences in microplastic concentrations between leeward and windward beaches. Our work offers a thorough analysis of the microplastics found on beaches in the Lesser Antilles and contributes to a better knowledge of the scale of the microplastic problem in the Caribbean, a hotspot for biodiversity (Cruz-Salas et.al., 2022)

Concerns over the contamination of inland and coastal waterways by plastic litter are growing on a global scale. This assessment compiles the data on microplastic contamination in China's inland water systems that is currently accessible. China is both the world's greatest producer of plastic and the largest developing country. The findings demonstrate the pervasiveness of microplastics in the examined inland water systems and substantial microplastic abundances were found in populated regions. Although identical sampling and analytical techniques were employed for the study of microplastics in both inland and coastal water systems, it is advised that methods of examination be standardized going forward. The properties of the microplastics found point to secondary sources as their principal origins. Microplastics have been shown to have biological and ecological consequences, but it is currently impossible to assess their hazards due to differences between microplastics collected in the field and those employed in Eco toxicological investigations. Although China has several rules and regulations in place to manage and reduce plastic trash, these laws and regulations have proven difficult to apply and ineffectual when they have been. With the goal of addressing plastic pollution issues to safeguard the environment and uphold international obligations, this research helped identify a number of research topics (Zhang et.al., 2022)

There are several studies on the effects of microplastic contamination on coastal ecosystems, particularly high energy beaches. Mudflats, on the other hand, are thought to retain more microplastics because of their lower energy levels and more biological diversity. From 10 mudflats and 10 sandy beaches in Hong Kong, extending from the eastern to western waterways, microplastics were counted and described. At the strandline, 1.0 and 1.5 metres above the chart datum (CD), sediment samples were taken. Microplastics were present in sediment in quantities ranging from 0.58 to 2116 pieces kg_1, with mudflats having ten times the amount of microplastics as beaches. The three most prevalent plastics were polyethylene (46.9%), polypropylene (13.8%) and polyethylene terephthalate (13.5%). The most prevalent material in the strandline samples was expanded polystyrene, but not at 1.0m and 1.5m above CD. Although earlier research identified the Pearl River as a significant source of microplastics on Hong Kong's coastlines, this study has shown that local pollution sources, such as sewage treatment plant outflow, also contribute to microplastic contamination and should not be disregarded (Ronda et.al., 2023)

Investigations were done into the amount of microplastic particles in freshwater and drinking water. In particular, the raw and processed water from three water treatment plants (WTPs) that were chosen to receive water from various types of water bodies were examined for the presence of microplastics (MPs). All water samples included microplastics and depending on the WTP, the average abundance of these particles varied from 1473,34 to 360,497 particles L-1 in raw water and from 338,76 to 628,28 particles L-1 in treated water. This study is one of the few to measure microplastics as tiny as 1 m and it found that MPs smaller than 10 m made up up to 95% of the MPs in both raw and treated water samples. Additionally, MPs were separated into three groups based on their form. At two of the WTPs, fragments were obviously in the lead, whereas in one case, fragments and fibres co-predominated. Despite there being 12 distinct substances that may be used to make microplastics, PET (polyethylene terephthalate), PP (polypropylene) and PE (polyethylene) made up the bulk of MPs (N70%) Shen et.al. (2021).

Widely present in the environment, microplastics (MP; 5 mm) have the potential to be hazardous to living things. Fewer studies have examined the effects of MPs on freshwater ecosystems, with the majority of study concentrating on the effects of MPs on marine and estuarine animals. In the current study, the susceptibility to primary (PMP) and secondary (weathered) microplastics (SMP) was evaluated in two temperate Cladoceran species, Daphnia magna and Daphnia pulex, as well as a smaller tropical species, Ceriodaphnia dubia. To assess the impact of temperature as an additional stressor, a prolonged acute toxicity experiment (up to 72 or 96 h) was conducted at 18 °C, 22 °C and 26 °C. Survival data were evaluated using the toxicokinetic-toxicodynamic (TK-TD) model. Temperature significantly affected the acute sensitivity of D. magna and D. pulex to PMP and SMP, but C. dubia's remained mostly unchanged. At 18 _C, C. dubia was the species that was the most sensitive, followed by similarly sensitive species D. pulex and D. magna. However, the No Effect Concentration (NEC) estimations of the TK-TD model showed that this ranking was inverted at 26 _C. SMP and PMP both affected D. magna and D. pulex similarly, although PMP was more harmful to C. dubia. After the typical 48 h test period, effects on survival were significantly worse and were strongly time-dependent. Our findings suggest that, even at high exposure concentrations, temperature may have a significant impact on the susceptibility of various species to different forms of microplastics (Al Mamun et.al., 2023)

It was determined if fish and shellfish sold for human consumption included anthropogenic detritus. We took samples from marketplaces in California, the USA and Makassar, Indonesia. Wherever it was practicable, every fish and shellfish was recognised by species. A 10% KOH solution was used to remove anthropogenic waste from the digestive systems of entire fish and shellfish and the waste was then measured under a dissecting microscope. Anthropogenic waste was discovered in 55% of all species and in 28% of individual fish in Indonesia. Similar findings were observed in the USA, where 67% of all species and 25% of individual fish included anthropogenic detritus. Additionally, 33% of the examined shellfish included anthropogenic detritus. Plastic made up the whole of the anthropogenic waste found in fish in Indonesia, whereas fibres made up the majority of the waste found in fish in the USA. Different sources and methods of waste management between nations are believed to be the cause of variations in debris kinds. We present some of the earliest discoveries of plastic debris in fish that are sold specifically for human consumption, raising questions about human health.

The constant rise in the manufacture of synthetic plastic and the careless handling of plastic trash have a negative impact on the aquatic environment. As a result, microplastics (5mm) are created and persist in freshwater and ocean environments. This brand-new kind of developing pollutant is becoming a major source of worry. There are several sources of microplastics in freshwater systems, with wastewater treatment plants being the majority of those sources. Location-specific microplastic concentrations range from more than 1 million pieces per cubic meter to less than one piece every 100 cubic meters. Through entanglement and ingestion, they cause a number of negative impacts on people and other living things. Fresh water is first collected for microplastics in research on microplastics using nets with a typical mesh size of 330 mm. Following the process of volume reduction, the materials underwent a purification step that included density separation using inorganic salts such sodium chloride and digestion using oxidizing agents or enzymes. The order in which these two procedures (purification and digestion) are carried out depends on the kind of material. Numerous analytical techniques may be used to examine the purified materials. For the qualification investigations, FTIR, Raman, pyrolysis-GC/MS and liquid chromatography are the most frequently employed techniques. The quantification research might make use of a tagging technique. According to our examination of the literature, there are still no instruments that can be used to measure and categorize microplastics in freshwater that are universally approved (Dey et.al., 2024)

Seven intertidal mangrove ecosystems in Singapore were investigated for the presence of microplastics. Using a floating technique, microplastics from mangrove sediments were removed, counted and grouped according to particle size and form. For polymer identification, representative microplastics from Lim Chu Kang. All seven ecosystems tested positive for microplastics, with Lim Chu Kang sediments in Singapore's northwest having the highest concentration. Most microplastics were fibrous and less than 20 lm in size. Four different kinds of polymers, including polyethylene, polypropylene, nylon and polyvinyl chloride, were found. A link between the quantity of microplastics and the size of the sediment grains was also explored, although none was found. Microplastics are probably present because of the breakdown of marine plastic waste that has accumulated in the mangroves. (Nor & Obbard, 2014)

Microplastics are frequently bioavailable to marine creatures, either directly through ingestion or indirectly through contaminated prey. In a lab setting, the latter has been seen in low-trophic level species, but there isn't enough proof for high trophic level taxa. It is difficult to analyse pollution in nature and distinguish between microplastics that are consumed directly and indirectly. The ethical restrictions on using huge creatures in laboratory research make it difficult to resolve these restrictions. In this work, these problems were answered by studying full digestive tracts of the wild-caught Atlantic mackerel (Scomber scombrus), which they consume, as well as sub-samples of scat from captivity-raised grey seals (Halichoerus grypus). To get rid of extra organic material and make it easier to see synthetic particles without harming them, an enzymatic digestion technique was used. Fourier-Transform Infrared (FTIR) spectroscopy was used to determine the kind of polymer. The entire time, extensive contamination control procedures were used. Fish samples (32%; n 14 10) and scat subsamples (48%; n 14 15) both contained 1-4 microplastics. The predominant colours of the particles were black, clear, red and blue. Scats and fish had mean lengths of 1.5 mm and 2 mm, respectively. The most often found polymer type in both was ethylene propylene. Our research indicates that trophic transfer provides an indirect but potentially significant channel of microplastic ingestion for any creature whose feeding ecology involves the intake of entire prey, such as humans (Nelms et.al. 2018)

Global concern over plastic pollution is on the rise. In a study, researcher examined plastic contamination in 6 species of freshwater fish from China and 21 species of marine fish. It was discovered that all of the species consumed micro- or mesoplastics. Microplastics were present in average abundances of 1.1 to 7.2 items per person and 0.2 to 17.2 items per gram. Mesoplastics ranged in abundance from 0.1 to 3.9 items per gram and from 0.2 to 3.0 items per person. Microplastics were widespread across 26 species, making up 55.9 - 92.3% of all plastic products in each species. The most microplastics were found in Thamnaconus septentrionalis (7.2 items/individual). In comparison to freshwater benthopelagic fish, the average abundance of plastics in marine benthopelagic fish was much greater. Fibers in shape, transparency in colour and cellophane in substances dominated the plastics. The greatest concentration of plastics, measured in items/individual, was found in the stomach of Harpodon nehereus and the intestines of Pampus cinereus, at 3.3 and 2.7 respectively. The results imply that plastic pollution was pervasive in the fish species under investigation and that its abundance was higher than in other studies conducted globally. The environment and gastrointestinal tract anatomy have a direct impact on the consumption of plastic by fish. Future studies of plastic contamination in fish should strongly consider using the entire gastrointestinal system and digestive process. (Jabeen et.al., 2017).

CHAPTER 3

MATERIALS AND METHODS

Microplastic contamination is widespread, but this particular study focuses exclusively on the coastline of Pakistan, which spans approximately 850 kilometers. The research explores four key aspects related to microplastics: their occurrence, characterization, bacterial isolation and the efficacy of bacterial degradation. Each of these aspects is examined using specific methodologies, which are outlined in the following sections.

3.1 STUDY AREA & SITE SELECTION

The primary focus of this study is the coastline of Pakistan, stretching across roughly 850 kilometers. A strategic selection of sites was made to ensure representation of the entire coastal region, taking into account factors such as accessibility, population density, tourism value and proximity to industrial zones. The chosen sites reflect a broad spectrum of coastal environments. During field visits android applications and GPS devices were used to track and record the precise coordinates of each site. Ultimately, 15 locations along the coastline were identified as ideal for sample collection and assessment, based on their suitability for capturing a comprehensive view of microplastic contamination along Pakistan's coast.

3.2 SAMPLE COLLECTION

Sample collection was carried out using a standardized method described by Besley, et.al. (2017). The high tide zones were identified dynamically by observing the areas where wet sand was most prevalent, as well as focusing on other key features such as seashells and various types of debris deposited by the sea during high tide. To ensure the reliability of the data, samples were collected in triplicates following a Completely Randomized Block Design. A total of 5 samples were collected separately in each replication, with a minimum distance of 20 meters maintained between sampling points. A metallic quadrate measuring $0.25m^2$ was used to define the sampling area at each location along the coastline. From each selected site, the top 5 cm layer of sand sediment was carefully extracted using a metal spoon. Before being packed into non-plastic containers, the sediment samples were sieved using a 5mm sieve to eliminate larger debris. To maintain consistency and prevent cross-contamination, the metal spoon was thoroughly rinsed with seawater after each sample collection.

3.3 EXTRACTION

The extraction of microplastics from the collected sand samples was performed using a density separation method, as described by Besley et.al. (2017). Initially, 100 grams of wet sand was oven-dried at 60°C for two days to remove moisture. After drying, 50 grams of the sand was mixed with 200 mL of a fully saturated NaCl solution (358.9 g salt per liter) in a conical flask. Prior to mixing and agitated at 900 rpm for 2 minutes to ensure proper mixing. Following the stirring process, the sand was allowed to settle for approximately 8 hours. After settling, the supernatant was collected via vacuum filtration using a 0.45 μm filter and vacuum pump. To prevent the floating material from sticking to the walls of the flask or the surface of the filter paper, the conical flask was gently rotated during filtration and about 100 mL of supernatant was carefully poured into the vacuum pump. Once filtration was complete, the filter paper containing the microplastics was carefully stored in a clean petri dish for further processing. This extraction process was repeated three times for each sample to ensure the maximum recovery of microplastics. Special precautions were taken to prevent contamination throughout the procedure. All equipment was thoroughly rinsed with distilled water before and after use and Petri dishes and other containers were kept clean and covered when not in use.

3.4 IDENTIFICATION & ENUMERATION

The identification and enumeration of microplastics followed three key guiding principles to ensure accuracy: 1) the microplastics should exhibit a clear and consistent color, 2) they should be free of organic matter and 3) the particles should have a uniform width. The filter papers were examined under a stereo-microscope at up to 40× magnification and the microplastics present were counted, following the methods outlined by Besley et.al. (2017) and Lots et.al. (2017). This approach was effective for identifying microplastics within the size range of 0.3 to 5mm. During the identification process, the filter paper was divided into four quarters, with each quarter being inspected sequentially in a clockwise direction. To minimize the risk of human error, at least two independent researchers conducted the quantification process for each sample. This ensured greater accuracy and reliability in the counting and identification of microplastics in the collected samples

3.5 SAMPLE CHARACTERIZATION AND ANALYSIS

Followed by enumeration, the characterization of microplastics in terms of color and shape was performed. Instead of separate observations, a single detailed observation was performed by each observer to quantify and characterize the microplastics to save time and effort. After obtaining data, sorting of data for quantification of microplastics by color and shape was done for data presentation.

3.6 BACTERIAL ISOLATION

Isolation of bacteria was done by mixing 1 g sediment sample with 0.9% NaCl Solution. The resulted mixture was vortexed for 3 hours at an RPM of 180 making in orbital shaker. A suspension was therefore formed which was serially diltued, plated on nutrient agar (NA) and incubated at 37°C for 24 h (Emenike et.al., 2016). Finally, to obtain distinct pure cultures, single colonies were sub-cultured on freshly prepared NA. All experiments were be carried out in triplicates as mentioned earlier.

3.7 BACTERIAL IDENTIFICATION

Based on high level microplastic degradation, the potential microbes were selected for identification only. Bacterial Identification was carried out by observing the 16S rRNA gene traits. The 16S rRNA gene sequencing was performed by the services of MACROGEN, Korea. Sequences of closely related strains were obtained from suitable databases by analyzing the BLAST searches and phylogenetic tree was constructed. Mega 12 version was used to construct the phylogenetic tree of obtained sequences as explained by Naeem et.al, 2019.

3.8 SCREENING OF BACTERIA FOR MICROPLASTIC DEGRADATION

To assess the potential of bacterial species in degrading microplastics, a method described by Kannahi and Sudha (2013) was applied. This approach utilized Mineral Salt Media (MSM), a medium that provides all the essential nutrients required for bacterial growth, with the exception of a carbon source. The bacteria were then tested for their ability to utilize specific polymers—polyethylene (PE), polyethylene terephthalate (PET) and polyvinyl chloride (PVC) as their sole source of carbon and energy. Each bacterial isolate was cultured individually in MSM, supplemented with 0.5 grams of one of the selected polymers and incubated for a period of four weeks at standard room temperature. In parallel, a control experiment was conducted, where MSM was inoculated without the addition of any polymer to observe the bacterial growth under these conditions. To ensure the reliability of the results and to account for any potential laboratory errors, each experiment was repeated three times with independent replicates.

3.9 PREPARATION OF MICROBIAL INOCULUM AND BIODEGRADATION

Once the bacterial species with significant microplastic degradation potential were identified, they were grown on Nutrient Agar (NA) plates at a temperature of 33°C for up to 24 hours to obtain pure cultures. To further optimize their growth, the same bacteria were cultured in a stationary phase in an orbital shaker at 29°C and 150 rpm, ensuring consistent conditions for the experiment. The bacterial suspensions were collected at the same physiological phase, characterized by an absorbance of 1.09 at 600 nm, to standardize the inoculum for the biodegradation experiments. Equal volumes of these bacterial suspensions were prepared for inoculation in biodegradation trials.

Pure cultures of the plastic-degrading bacteria, with a cell concentration of approximately 3.8 × 10^8 CFU/mL, were then inoculated into 270mL conical flasks containing MSM broth and 0.5 grams of microplastics (PE, PET, or PVC). Control flasks were also prepared, containing MSM with polymers but without bacterial inoculation, to evaluate the natural degradation process in the absence of microbial activity. Similar to the screening procedure, all experiments were performed in triplicate to ensure accuracy and minimize experimental variability. The flasks were placed on a shaker set at 150 rpm and the samples were regularly monitored at 10-day intervals for up to 40 days. Key parameters, such as optical density (OD), pH and microbial counts, were recorded to assess the bacterial growth and the degradation of microplastics over the study period.

3.10 DETERMINATION OF WEIGHT LOSS IN MICROPLASTIC PARTICLES

After six week of incubation, the microplastics inoculated and non-inoculated broths were extracted using filtration. To ensure that any contaminants were removed, the microplastics were washed with 70% ethanol. Following the washing procedure, the microplastic particles were dried in a hot air oven set to 50°C overnight. After drying, the residual weight of the polymers was measured to quantify the extent of degradation that had occurred over the experimental period. The degree of plastic degradation was determined by calculating the weight loss of the polymers using the formula outlined by Mor and Sivan (2008) and Mohan et.al. (2016). This method provides an accurate measure of the microbial degradation efficiency and offers insights into the potential of bacterial species to break down harmful microplastics in the environment. Formula is appended below:

$$\text{Percentage weight loss} = \frac{\text{Initial weight of polymer - Final weight of polymer}}{\text{Initial weight of polymer}} \times 100$$

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 RESEARCH AREA AND SITE SELECTION

Various areas throughout Pakistan's entire coastline were investigated for this study. There was, however, a significant amount of sampling done around the eastern end of the coastal line, which had a greater population and industry. It was decided to choose samples from fifteen important areas along the entire coastline line. These locations included sandy beaches, deltas, creeks, mangroves and other types of environment. For the purpose of mapping and marking the sampling sites, the smartphone application UTM Geo Map was utilized. In terms of urbanization, recreation and industry, these locations have had a significant amount of intrusion from humans.

For convenience, each site was labeled with a site code comprising of 3 alphabetic letters, as explained in Table 4.1

Table 4.1: List of Sites with Site Code

S.No.	Site Code	Location Coordinates	Site Name	Province
1	CLF	24° 47' 16.02" N | 67° 2' 29.40" E	Clifton	Sindh
2	GDN	25° 07' 08" N | 66° 43' 17.15" E	Gadani	Baluchistan
3	GHR	24° 44' 25.96" N | 67° 35' 28.36" E	Gharo	Sindh
4	GWD	25° 07' 06" N | 62°19'47.05"E	Gawadar	Baluchistan
5	HKB	24° 51' 21.93" N | 66° 52' 24.53" E	Hawkes Bay	Sindh
6	IBH	24° 47' 25" N | 67° 08' 46.13 "E	Ibrahim Haideri	Sindh
7	JWN	25° 02' 48" N | 61° 44' 15.14" E	Jewani	Baluchistan
8	KDM	25° 23' 28.35" N | 65° 27' 32.81" E	Kund Malir	Baluchistan
9	KTB	24° 08' 33" N | 67° 26' 56.21" E	Keti Bandar	Sindh
10	MNR	24° 47' 36.58" N | 66° 58' 29.45" E	Manora	Sindh
11	ORM	25° 15' 34" N | 64° 38' 42.21" E	Ormara	Baluchistan
12	PDP	24° 50' 34.5" N | 66° 48' 24.21" E	Paradise Point	Sindh
13	PSN	25° 15' 38" N| 63° 28' 44.25" E	Pasni	Baluchistan
14	PTQ	24° 47' 23.79" N | 67° 24' 33.05" E	Port Qasim	Sindh
15	SMN	25° 26' 58.87" N | 66° 33' 35.89" E	Sonmiani	Baluchistan

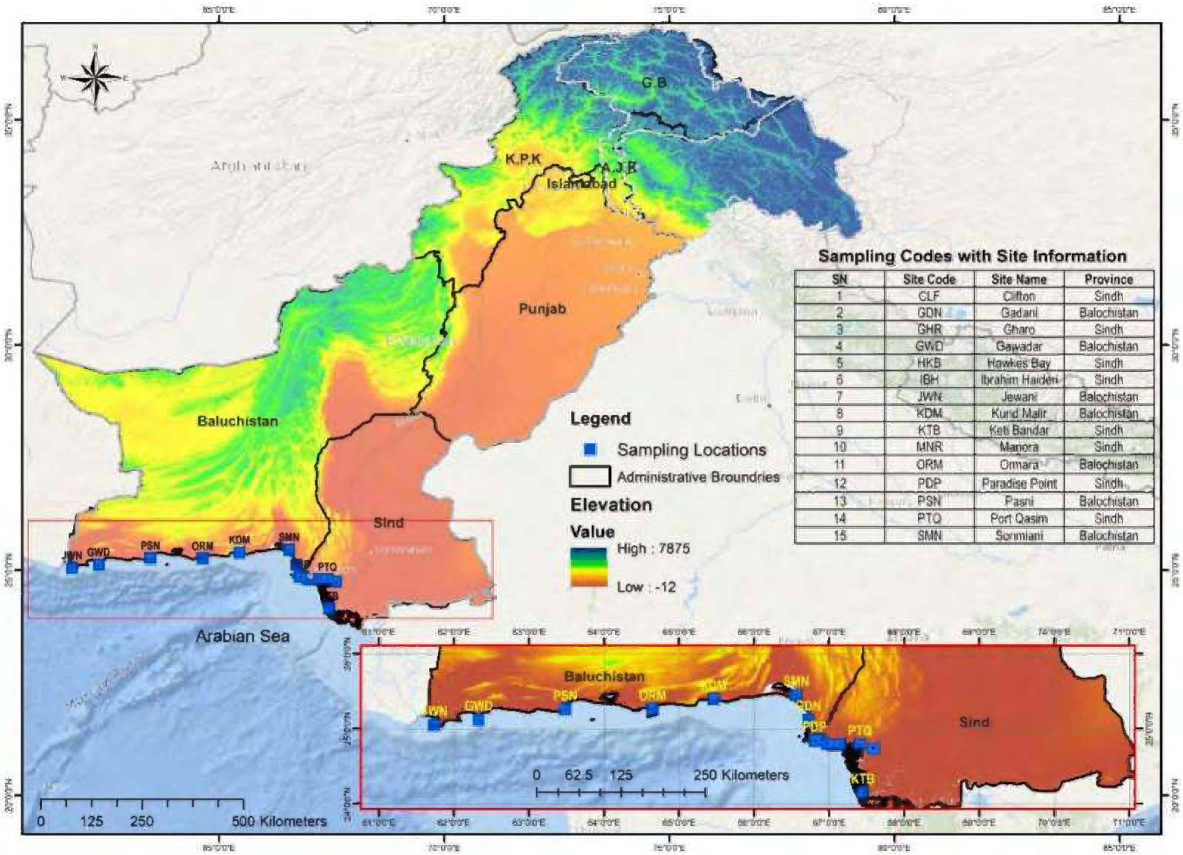


Fig 4.1 Map Showing Sampling Sites

4.2 ENUMERATION OF MPs

In accordance with the preceding explanation, the filter papers that contained microplastics that were removed using density separation were analyzed using a stereo-microscope at a magnification of up to 40 times and the number of microplastics was counted (Besley et.al., 2017; Lots et.al., 2017). The efficacy of this method for counting microplastics was found to be in the range of 0.3 to 5 millimeters. In the course of this identification procedure, four quarters of filter paper were marked and then examination of each quarter was carried out in a clockwise direction, one by one. Each and every sample was repeated three times. Microplastics were quantified in each sample by a minimum of two researchers or observers in order to decrease the possibility of human error. Results of enumeration are tabulated in Table 4.2

Table 4.2: Enumeration Of MPs in Terms of Particles Per 50 Gram Dry Weight of Sediment Sample

Site Name	R1	R2	R3	Sum	Mean	SD
KTB	8	10	12	30	10	2.00
GHR	17	19	21	57	19	2.00
PTQ	21	19	18	58	19	1.53
KDM	8	5	3	16	5	2.52
SMN	10	11	13	34	11	1.53
GDN	12	15	14	41	14	1.53
MNR	32	31	28	91	30	2.08
PDP	20	16	18	54	18	2.00
HKB	19	24	25	68	23	3.21
CLF	35	38	41	114	38	3.00
IBH	28	25	23	76	25	2.52
PSN	12	14	18	44	15	3.06
GWD	16	17	16	49	16	0.58
JWN	8	10	7	25	8	1.53
ORM	10	14	9	33	11	2.65

4.3 IMPACT OF ANTHROPOGENIC ACTIVITIES ON MPs ABUNDANCE

Depending on population, industry and tourism potential, all sites were divided into low, moderate and high potential areas. A brief summarization of maximum and minimum values and Mean abundance per Kg of sediment is appended in Table 4.3

Table 4.3: Mean Abundance & Ranges of microplastics per Kg of sediment Sample at different locations

Site Code	Landscape Type	Population, Industry & Tourism	Mean MP Abundance Particles per Kg	Minimum Value (per Kg)	Maximum Value (Per Kg)
KTB	Delta Area	High	200	160	240
GHR	Delta Area	Moderate	380	340	420
PTQ	Mangrove Area	High	387	360	420
KDM	Sandy Beach	Low	107	60	160
SMN	Creek Area	High	227	200	260
GDN	Rocky & Sandy Beach	Moderate	273	240	280
MNR	Rocky & Sandy Beach	High	607	560	640
PDP	Rocky & Sandy Beach	Moderate	360	320	400
HKB	Sandy Beach	Moderate	453	380	500
CLF	Sandy Beach	High	760	700	760
IBH	Creek Area	High	507	407	560
PSN	Sandy Beach	Moderate	293	240	360
GWD	Sandy Beach	Moderate	327	320	340
JWN	Sandy Beach	Moderate	167	140	200
ORM	Sandy Beach	Moderate	220	180	220

4.4 CHARACTERIZATION OF EXTRACTED MPs

Physical characterization of microplastics was done in terms of Colour and shape. A general trend of occurrence of various colours and shapes of MPs was also studied and compared with similar studies. Detailed data and discussion is discussed as under:

4.4.1 Characterization by Colour

MPs have considerable colour variation which represents a wide range of sources for plastics (Li et.al., 2019). Our results revealed black, transparent, brown, red, black, white, green and blue colors in micro plastics. Compared to others, the black coloured micro plastics were most frequently observed and indicated the highest presence of 23%. Our results showed that Transparent micro plastics (22%) were the second most abundant plastic followed by Green (16%) while rest of microplastics were Red, Blue, Yellow and Brown containing 9%, 11%, 3% and 5% respectively (Fig 4.2). Hence, our results displayed a strong variation in colors. Some of our sampling sites are close to commercial, recreational and fishing metropolis that supports many industries that use plastics. That can explain the high color variation we observed in this area.

Table 4.4: Tabulated Mean data of colors (Particles/50 g) observed in MPs for all 15 locations

Site Name	Black	White	Red	Yellow	Green	Transparent	Blue	Brown
KTB	2	1	1	0	1	3	1	1
GHR	5	2	2	0	3	5	2	0
PTQ	5	2	1	1	3	4	2	1
KDM	2	1	0	1	1	1	0	0
SMN	2	1	1	0	2	2	1	1
GDN	4	1	1	1	2	3	2	1
MNR	7	4	3	1	5	6	4	0
PDP	5	2	2	1	2	3	2	0
HKB	4	4	2	1	4	6	3	0
CLF	10	4	3	1	6	9	3	2
IBH	6	2	1	1	3	5	4	3
PSN	4	2	1	0	3	3	1	0
GWD	4	2	2	1	3	3	2	0
JWN	1	1	0	0	2	2	1	1
ORM	2	1	1	0	2	2	1	1

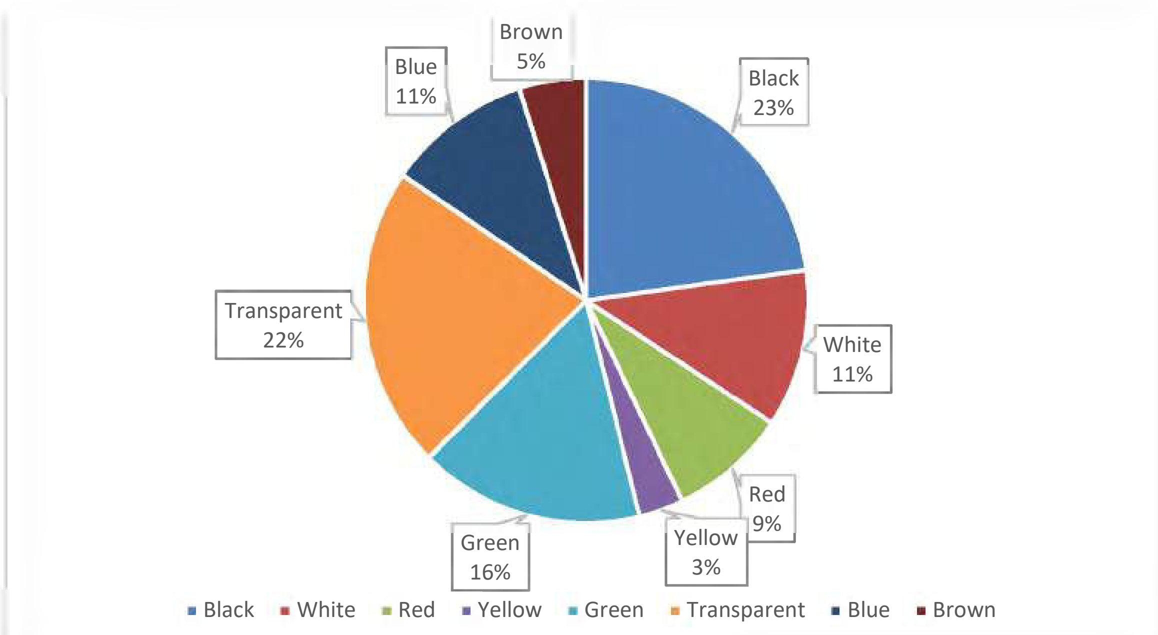


Fig 4.2 Classification of MPs by colour

4.4.2 Characterization by Shape

Classification of MPs based on the shape of particles was observed in the samples. It was observed during analysis, that 47% of micro plastic were comprising of fibers and indicated the highest incidence among other type of samples. Fragments were the second leading type of micro plastic comprising 23% and were followed by pellets and films consisting of 19% and 11% respectively. Figure 4.3 displays the percentage of MPs in terms of shape. Corresponding mean values for each site in terms of particles per Kg are tabulated in Table 4.5

Table 4.5: Tabulated Mean data (particles/50g) of shapes observed in MPs for all 15 locations

Site Code	Fiber	Film	Fragment	Pellet
KTB	5	1	3	2
GHR	10	2	5	3
PTQ	9	2	4	4
KDM	3	1	1	0
SMN	6	2	2	1
GDN	6	2	4	3
MNR	15	3	7	5
PDP	9	2	4	3
HKB	12	2	5	4
CLF	17	5	7	9
IBH	13	3	6	3
PSN	6	1	4	3
GWD	7	1	4	4
JWN	3	0	2	2
ORM	5	1	2	3

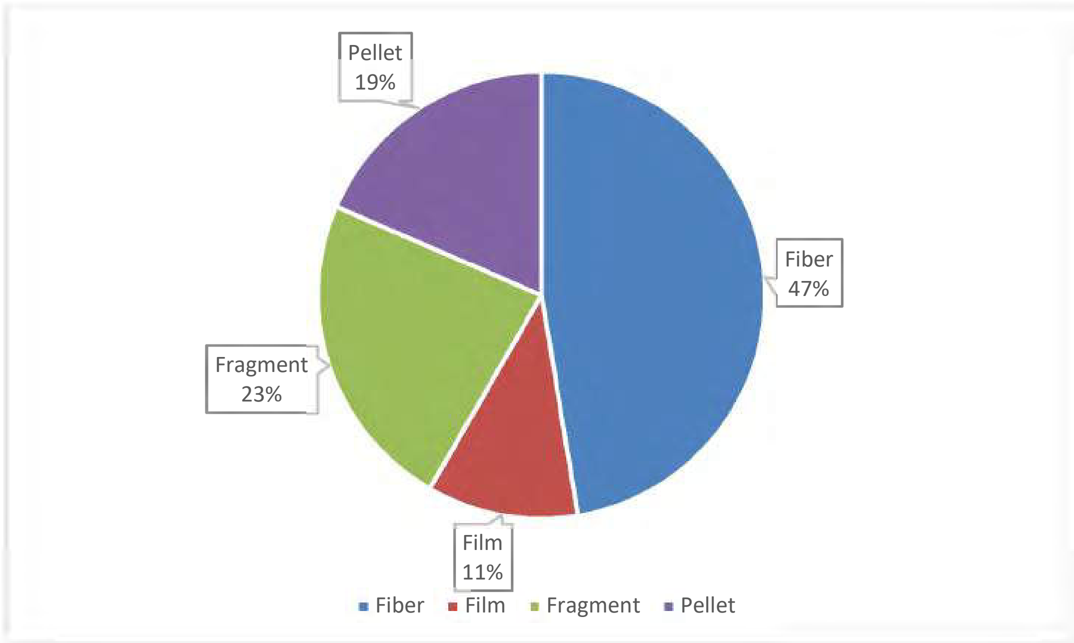


Fig 4.3 Composite data of all sites showing percentage of MPs by Shape

4.5 DEGRADATION POTENTIAL OF MICROBES FOR MICROPLASTIC DEGRADATION

In polymer degradation, bond scissions and subsequent changes in material properties and appearance are the consequence of chemical, physical and biological events. Polymer degradation is dependent on a number of conditions and involves each of these types of reactions. These categories are determined by the different causes of polymer degradation. Hydrolysis, oxidation, or both are examples of complicated reactions that are typically involved in the process of chemical degradation in the environment. These activities can be enhanced by the action of microorganisms, heat, light, or these combinations of elements. Several polymers, including non-biodegradable polymers like polyethylene (PET), polyvinyl chloride (PVC), polypropylene (PP) and polystyrene (PS), as well as biodegradable polymers like polylactic acid (PLA) and polycaprolactone) (PCL), have been subjected to research and their breakdown behavior has been documented in the scientific literature. An example of this would be PET, which accounts for around 12% of the world's solid waste. A quick enzymatic depolymerization process can contribute to the development of a circular carbon economy for PET by facilitating its conversion and valorization into other goods.

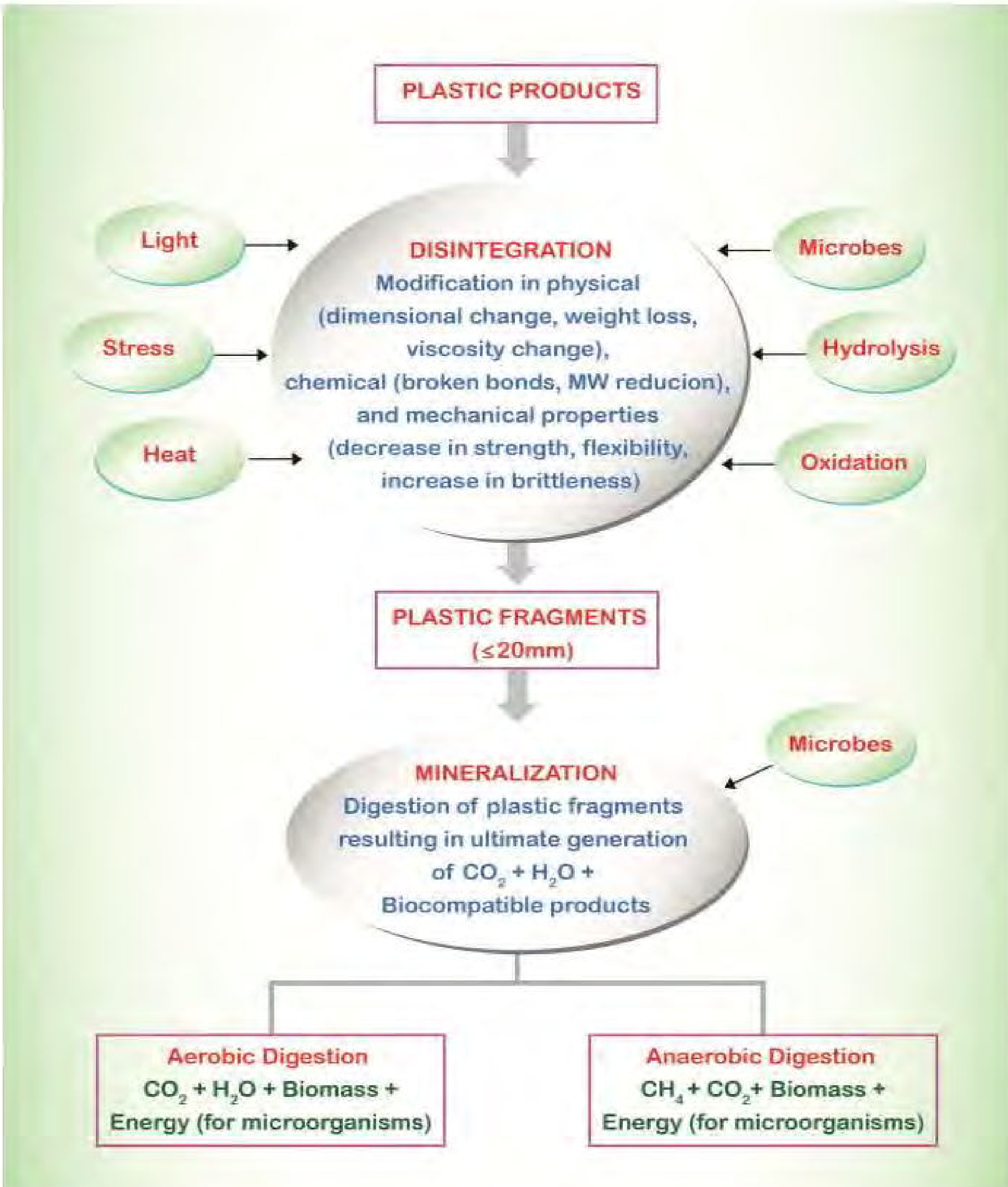


Fig. 4.4 Schematic representation of plastic degradation (Krzan et.al., 2006)

4.6 SCREENING AND ISOLATION OF PLASTIC -DEGRADING BACTERIA

A diverse array of microorganisms inhabits the marine environment (Austin, 1988). The majority of bacteria found in the ocean exhibit distinct differences from those in non-marine habitats. This discrepancy likely arises because adventitious bacteria either fail to sustain themselves in the marine environment or lose their morphological identity during acclimatization. Bacteria from the genera *Pseudomonas, Serratia, Marinococcus, Micrococcus and Staphylococcus* have been documented in seawater and soil (Austin, 1988). Along the Karachi coast, Pakistan, *Pseudomonas, Micrococcus, Methanococcus, Staphylococcus* and *Bacilli* have been identified (Seymour, 2014). The ocean is replete with various organic compounds and the bacteria present have evolved mechanisms to metabolize and detoxify numerous types of organic substances (Poli et.al., 2017).

For studying the degradation potential of different microbial species in sediment samples, first the isolation process was done as described in chapter 3. Followed by isolation, the cultures were allowed to grow on microplastics as carbon source. For this, only 3 types of plastics namely PET, PE and PVC were selected for degradation experiments as these are the most extensively used plastic types. Initially, the general growth of all colonies was observed against selected plastic types. Thereafter, only the species having considerable growth were further isolated for comparative degradation experiments. A brief site-wise tabulated summary of bacterial isolation and selection for degradation studies has been appended in table 4.6

Table 4.6: Bacterial Isolation data showing site-wise Isolates selection and species data for all sites

Location	Total No. of Isolates	No. of Isolates selected for degradation studies	Species Identified
CLF	5	3	*Pseudomonas azotoformans, Micrococcus luteus, Vibrio alginolyticus*
GDN	5	2	*Pseudomonas aeruginosa, Halomonas campaniensis*
GHR	5	1	*Bacillus subtilis*
GWD	4	1	*Halomonas campaniensis*
HKB	5	0	N/A
IBH	5	1	*Bacillus subtilis*
JWN	3	0	N/A
KDM	3	2	*Bacillus flexus, Pseudomonas aeruginosa*
KTB	4	0	N/A
MNR	5	2	*Micrococcus luteus, Halomonas campaniensis*
ORM	3	0	N/A
PDP	3	1	*Vibrio alginolyticus, Micrococcus luteus*
PSN	3	1	*Halomonas campaniensis*
PTQ	5	2	*Pseudomonas aeruginosa, Vibrio alginolyticus*
SMN	4	2	*Bacillus flexus, Pseudomonas azotoformans*

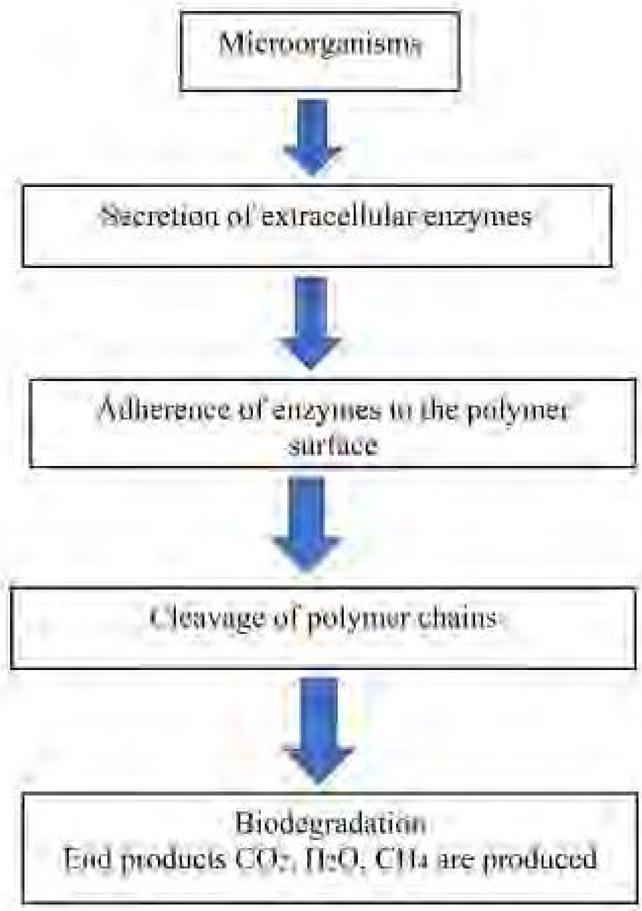


Fig. 4.5 Mechanism of enzymatic biodegradation of polymers (Alshehrei, 2017)

4.7 BACTERIAL SPECIES IDENTIFICATION

As explained earlier, bacterial species, 16S rRNA gene sequencing was performed through services of MACROGEN, Korea. Sequences of closely related strains were obtained from suitable databases by analyzing the BLAST searches and phylogenetic tree was constructed. Phylogenetic data is appended below:

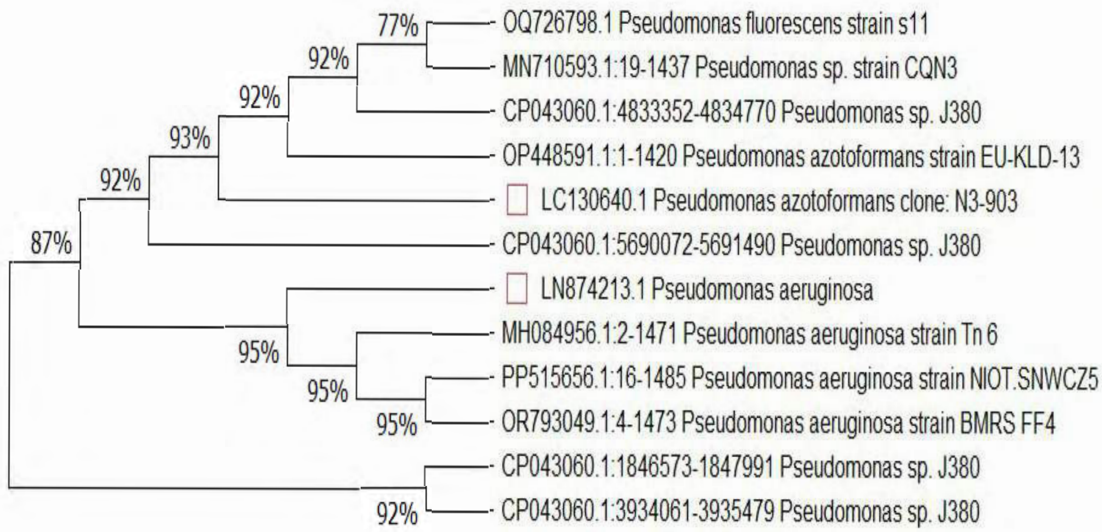


Fig. 4.6 Phylogenetic trees through neighbor joining for pseudomonas bacteria

Generally, thus, a high percentage means a closer relationship on a genetic basis.

The tree includes other Pseudomonas species, such as *Pseudomonas fluorescens* and various strains of Pseudomonas sp., showing highly varying degrees of genetic similarity with *Pseudomonas azotoformans* and *Pseudomonas aeruginosa*. The values probably express some connection between the bootstraps or how close the sequences are genetically, that is, bootstrap values or genetic similarity scores, which emphasizes tree's branch support and agreement with the overall clustering pattern.

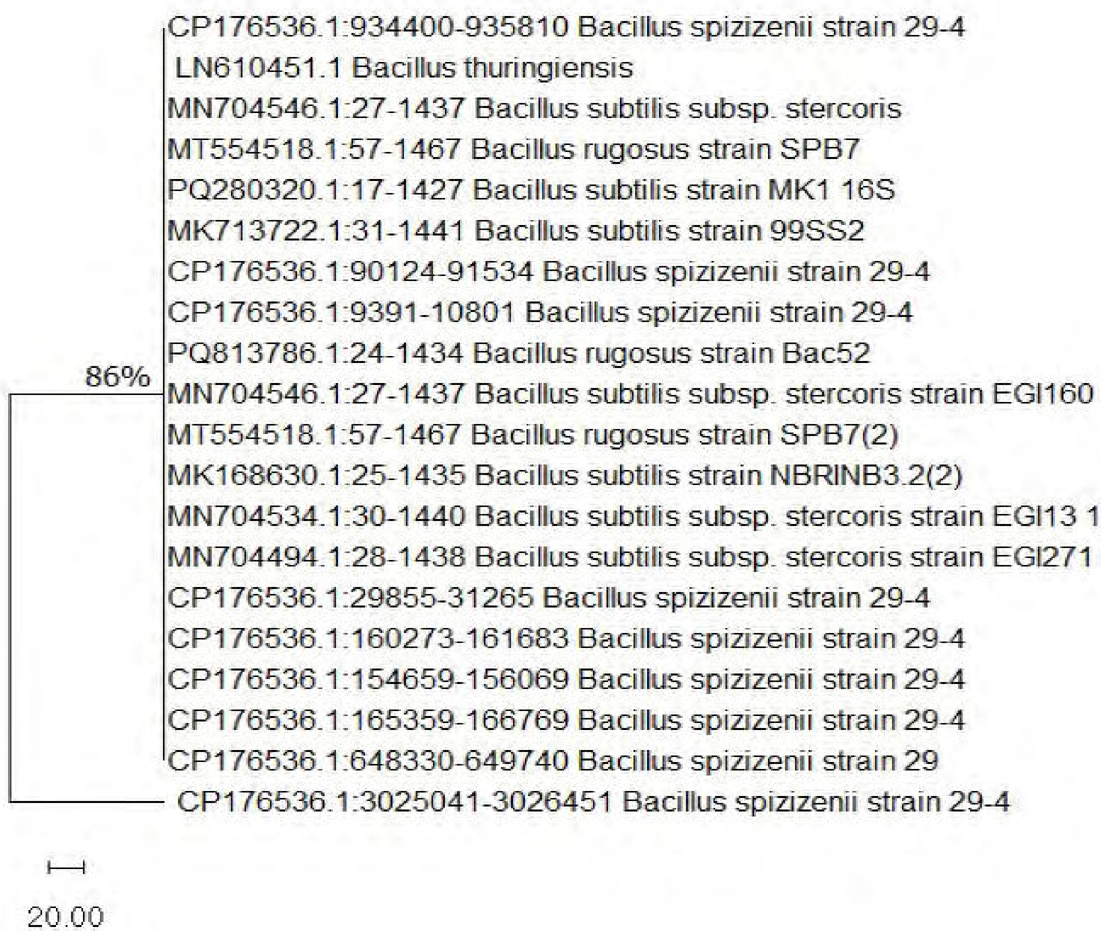


Fig. 4.7 Phylogenetic trees through neighbor joining for Bacillus bacteria

Analysis of the phylogenetic relationships among Bacillus species, such as *Bacillus subtilis* and *Bacillus flexus*, was conducted to unveil their evolutionary connections. The phylogenetic tree, shown in Figure 4.7 placed the sequences analyzed into distinct clades, indicating some genetic proximity among different Bacillus strains.

Much of the *Bacillus subtilis* strains sit together, forming a monophyletic group with a high level of bootstrap support. *Bacillus spizizenii* strain 29-4 clustered within this group as well, tightly with other *Bacillus subtilis* strains, indicating high genetic similarity of lines within the subtilis group. Also included in the analysis, *Bacillus thuringiensis* sits within a clade close to this clade, suggesting that it bears an evolutionary similarity to *B. subtilis*.

Additionally, *Bacillus rugosus* strains (SPB7 and Bac52) and *Bacillus subtilis* subsp. stercoris strains (EGI160, EGI13 and EGI271) were clearly separated into two distinct taxonomic groups on the phylogenetic tree upholding their distinct taxonomic placements. This branching has a bootstrap support value of 86%, which strengthens this notion of their evolutionary affiliations.

This analysis includes *Bacillus flexus*, which offers a comparative tool with which the divergence of Bacillus should be gauged. Regarding the placement of *Bacillus flexus* in the current phylogenetic tree, it suggests that *Bacillus flexus* may be distantly related when compared to the complex of *B. subtilis*.

To conclude, the phylogenetic tree outlines the diversity within Bacillus species and provides insights into the evolutionary relationships arising between *B. subtilis* and its subspecies when contrasted against their close cousins of more distant geographical regions old: *Bacillus rugosus* and *Bacillus flexus*. Therefore, these findings can represent a better insight into the phylogenetic positioning of Bacillus strains and what this may mean from a microbial taxonomy and functional perspective.

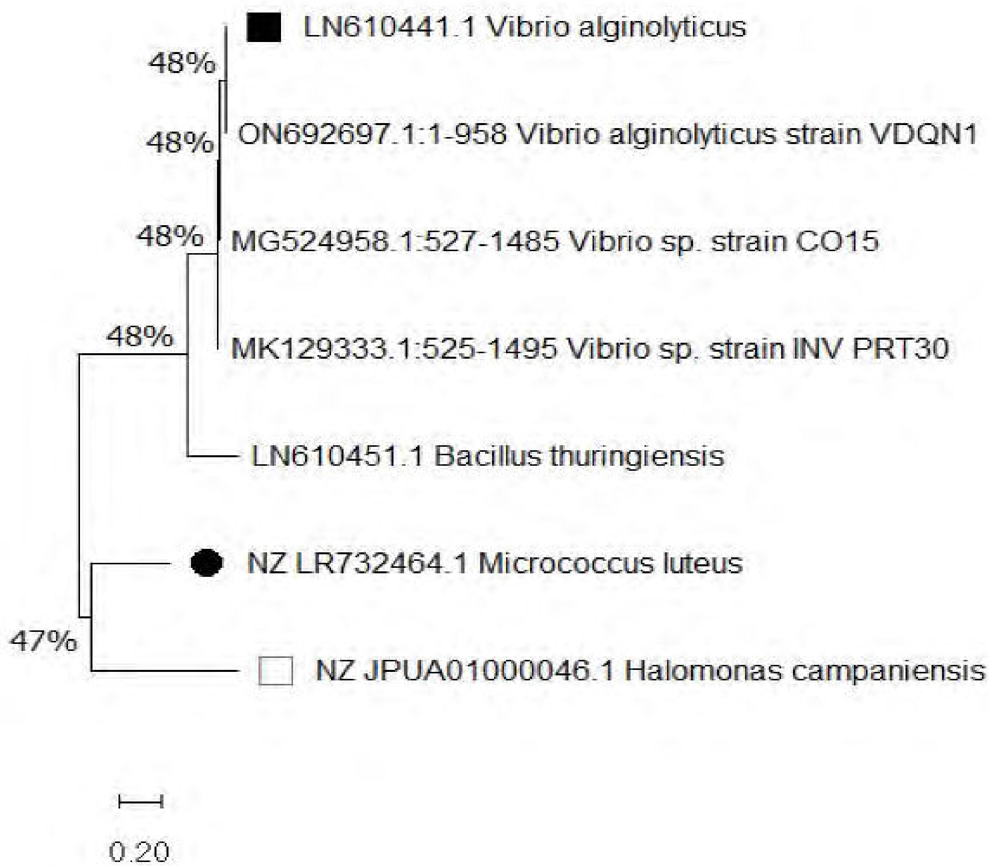


Figure 4.8 Phylogenetic trees through neighbor joining for pseudomonas bacteria

A phylogenetic tree was constructed in order to compare some selected bacterial strains namely *Vibrio alginolyticus*, *Micrococcus luteus*, *Bacillus thuringiensis* and *Halomonas campaniensis*, from which some genetic information is inferred with regard to their evolutionary relationship. This provided some new evidence regarding the phylogenetic position of the strains of *Vibrio alginolyticus*, where two, *Vibrio alginolyticus* strain VDQN1 (ON692697.1), Vibrio sp. strain CO15 (MG524958.1) and Inv PRT30 (MK129333.1), were found to have occupied a monophyletic clade strongly supported by 48% bootstrap support. This demonstrates a genetic closeness between the two groups of strains-*Vibrio alginolyticus* strains suggesting a close term common descent. Interestingly, *Bacillus thuringiensis* (LN610451.1) was positioned separately from the Vibrio groups, indicating it is of distinct evolutionary origin. This reaffirms the genetic divergence of Bacillus, apart from those of Vibrio, even if both are wide-ranging bacterial species of great industrial and ecological importance. *Micrococcus luteus* (NZ LR732464.1) branched out distinctly on a different clade indicating its phylogenetic distance from the groups of Vibrio and Bacillus. *Halomonas campaniensis* (NZ JPUA01000046.1) also branched out isolated away from the other bacterial species investigated. A bootstrap value of 47% at this node shows moderate support for the separation of these respective bacterial taxa. Overall, these analyses sketch out the genes in respect to available bacterial strains highlighted. The utility of strains of *Vibrio alginolyticus* mentioned in these analyses shows them in close phylogeny, while reflects genetic diffusion of *Bacillus thuringiensis*, *Micrococcus luteus* and *Halomonas campaniensis* having different taxonomic implications. Therefore, they add substantially to a more comprehensive understanding of the taxonomic affiliations of these bacterial strains and act as a database for similar comparative genomic studies in the future.

4.8 MICROBIAL DEGRADATION EXPERIMENTS

The degradation potential of each microbial specie for 3 plastic types was studied by performing in-vitro experiments. Table 4.7 to Table 4.9 show the degradation potential of species expressed in terms of percentage loss of plastic type applied.

Table 4.7: Degradation potential of identified microbial species expressed in terms of percentage weight loss of PET applied

Specie Name	PET			Mean
	R1	R2	R3	
Pseudomonas azotoformans	29.75	28.30	31.25	29.77
Pseudomonas aeruginosa	31.25	27.45	31.5	30.07
Micrococcus luteus	25	22	27.54	24.85
Bacillus subtilis	16.94	19.5	14.52	16.99
Vibrio alginolyticus	12.75	13.65	15.21	13.87
Halomonas campaniensis	9.85	8.94	9.75	9.51
Bacillus flexus	8.25	10.15	8.45	8.95

Table 4.8: Degradation potential of identified microbial species expressed in terms of percentage weight loss of PVC applied

Specie Name	PVC			Mean
	R1	R2	R3	
Pseudomonas azotoformans	17.55	19.45	18.45	18.48
Pseudomonas aeruginosa	20.00	19.00	18.25	19.08
Micrococcus luteus	21.00	19.00	18.00	19.33
Bacillus subtilis	21.45	20.75	19.25	20.48
Vibrio alginolyticus	21.50	22.50	23.50	22.50
Halomonas campaniensis	26.75	24.5	22.45	24.57
Bacillus flexus	29.25	27.45	25.88	27.53

Table 4.9: Degradation potential of identified microbial species expressed in terms of percentage weight loss of PE applied

Specie Name	PE			Mean
	R1	R2	R3	
Pseudomonas azotoformans	39.45	36.45	38.00	37.97
Pseudomonas aeruginosa	31.75	29.85	32.85	31.48
Micrococcus luteus	17.00	19.00	18.00	18.00
Bacillus subtilis	20.54	21.45	22.45	21.48
Vibrio alginolyticus	13.25	11.25	13.04	12.51
Halomonas campaniensis	13.75	11.45	12.55	12.58
Bacillus flexus	25	27.5	26	26.17

4.9 DATA ANALYSIS

To compare data among plastic types and microbial species, a comprehensive data analysis was preformed to extract useful and logical information. Few analyses are appended below:

4.9.1 Descriptive Statistics

Basic statistical data compromising of mean degradation percentages, standard deviations and variance for each plastic type was populated in one table for better understanding. Table 4.10 provides the mean degradation percentages along with Standard Deviation and variance against each microbial specie.

Table 4.10: A tabular representation of mean degradation percentages, standard deviations and variance for each plastic type, for all bacterial species

Microbial Species	PET Mean	PET SD	PET Variance	PVC Mean	PVC SD	PVC Variance	PE Mean	PE SD	PE Variance
Pseudomonas azotoformans	29.77	1.95	3.82	18.48	0.98	0.96	37.97	1.77	3.14
Pseudomonas aeruginosa	30.07	2.07	4.29	19.08	0.89	0.79	31.48	1.65	2.73
Micrococcus luteus	24.85	2.77	7.67	19.33	1.00	1.00	18.00	1.00	1.00
Bacillus subtilis	16.99	2.02	4.08	20.48	1.10	1.21	21.48	1.00	1.00
Vibrio alginolyticus	13.87	1.26	1.58	22.50	1.00	1.00	12.51	0.10	0.01
Halomonas campaniensis	9.51	0.45	0.20	24.57	2.14	4.57	12.58	1.19	1.42
Bacillus flexus	8.95	0.95	0.91	27.53	1.71	2.92	26.17	1.27	1.60

Data in table 4.10 implies that *Pseudomonas azotoformans* showed the highest degradation for PE (37.97%), followed by PET (29.77%) and the least for PVC (18.48%). *Bacillus flexus* showed the highest degradation for PVC (27.53%), while its activity was lower for PET. *Halomonas campaniensis* performed much better on PVC (24.57%) than on PET and PE. Variance and standard deviations indicate that *Micrococcus luteus* has the most variation in degradation percentages.

4.9.2 T-Test Comparisons between PVC, PET and PE

T-Test was applied to data for pair-wise comparisons of plastic species. Significance for each comparison was worked out and placed in Table 4.11

Table 4.11: Paired comparisons of microbial Degradation MP of types using T Test

Comparison (MP Types)	t-value	p-value	Significance
PET vs. PVC	3.22	0.008	Significant
PET vs. PE	2.98	0.012	Significant
PVC vs. PE	1.45	0.187	Not Significant

The table indicates that PET vs. PVC and PET vs. PE showed significant differences. This indicates that microbial degradation varies significantly between these plastics. PVC vs. PE does not show significant difference, meaning microbes degrade them at similar rates.

4.9.3 Correlation Matrix between PET, PVC and PE Degradation

A correlation matrix was developed to understand the relationship between degradation performances across different plastics. Table 4.12 explains correlation matrix between all three plastic types under discussion:

Table 4.12 Correlation Matrix between PET, PVC and PE

	PET	PVC	PE
PET	1.00	0.61	0.75
PVC	0.61	1.00	0.68
PE	0.75	0.68	1.00

The data implies that PET and PE have the highest correlation (0.75), meaning that microbes that degrade PET well also degrade PE efficiently. PET and PVC have a lower correlation (0.61), suggesting different microbial efficiency on these plastics. However, PVC and PE have moderate correlation (0.68).

4.10 COMPARITIVE ANALYSIS OF MPs AND MICROBIAL SPECIES

Figure 4.9 describes the increase or decrease of degradation potential for different microbial species, when MP type is changed. It is worth noting and natural that not all species can degrade all MP types. Figure 4.10 shows species wise degradation potential expressed in percentage weight loss of MPs.

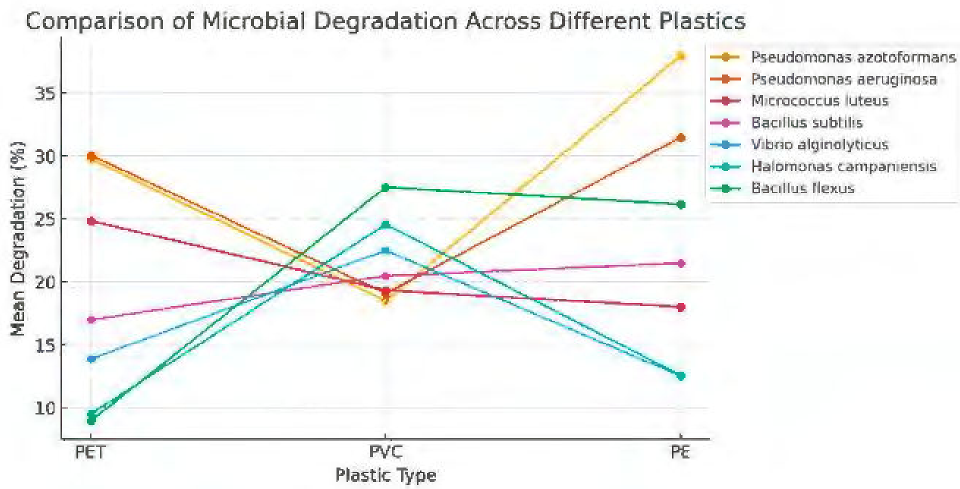


Figure 4.9 **Variation degradation potential of different Microbial species against different plastic types.**

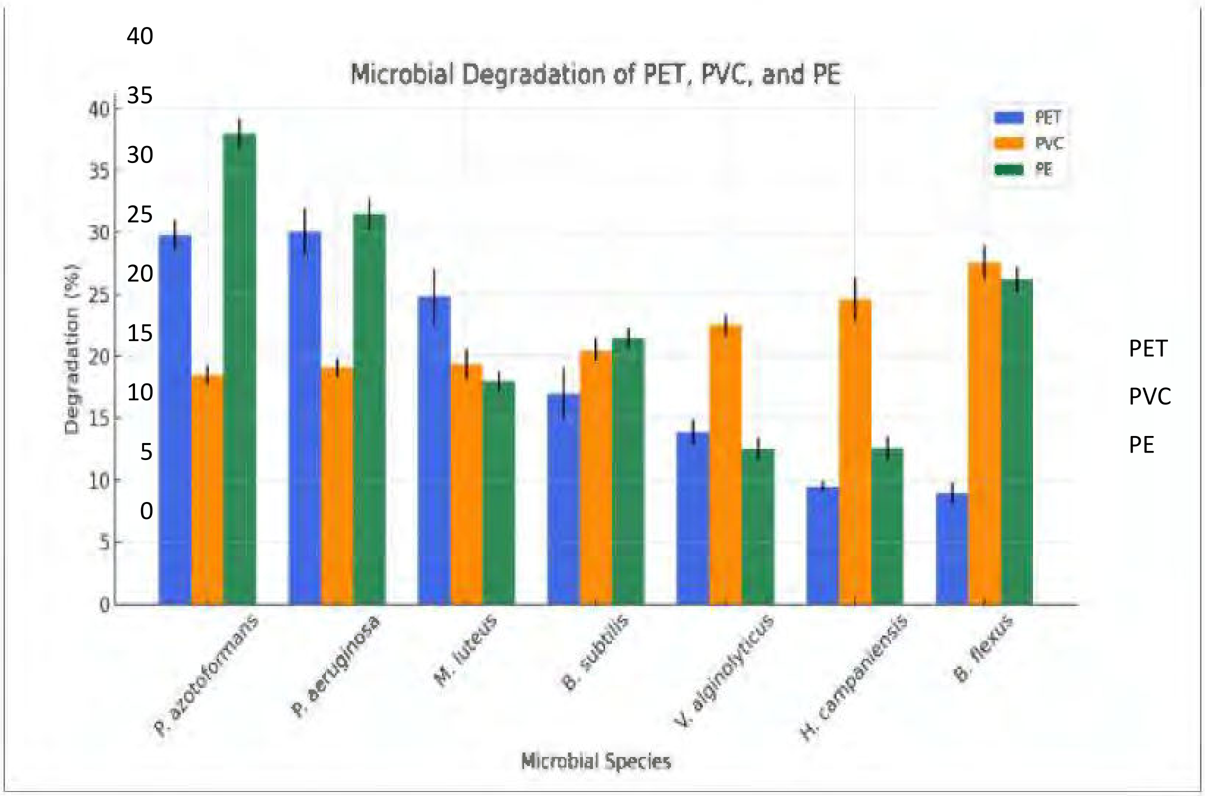


Fig 4.10 **Species wise performance of bacterial isolates for degradation of all MP types expressed in percentage of weight loss in MPs**

4.11 DISCUSSIONS

Microplastics have emerged as a major environmental issue globally due to their durability, prevalence, and possible effects on ecosystems and human health. The quantification of microplastics across many regions, including Pakistan, offers critical insights into the level of pollution and aids in the development of mitigation methods. This document presents a discourse on microplastic quantification within the global, Asian and Pakistani frameworks, substantiated by pertinent references. Geyer et al. (2017) estimated that some 8.3 billion metric tons of plastic had been manufactured worldwide, with a considerable fraction becoming microplastics in the environment.

Research in South Asia has indicated microplastic concentrations between 100 and 1,000 MPs/kg in coastal sediments. A study conducted in India identified microplastic concentrations ranging from 200 to 500 MPs/kg in coastal regions (Sarker et al., 2020). The mean result of 200 MPs/kg in Keti Bandar aligns with these data, suggesting comparable pollution levels in the area. Microplastic concentrations in coastal sediments worldwide exhibit significant variability, ranging from less than 10 MPs/kg in rural regions to over 1,000 MPs/kg in severely polluted metropolitan beaches (Eriksen et al., 2014). The microplastic concentration in Keti Bandar is less than that of severely contaminated locations such as those in China (e.g., Bohai Sea: 270–1,450 MPs/kg) but exceeds that of unspoiled ecosystems. Microplastics in sediments can degrade habitat quality for benthic creatures and disrupt ecosystem functioning. The principal sources of microplastics include industrial abrasives, personal care items, and synthetic textiles, whereas secondary sources arise from the disintegration of bigger plastic waste. Asia significantly contributes to global plastic pollution owing to its high population density, fast industrialization and insufficient waste treatment infrastructure. Lebreton et al. (2017) identified Asian rivers, including the Yangtze, Indus and

Ganges, as significant conduits for the transportation of plastic garbage to the oceans. Coastal & Marine Pollution Research in nations such as China, India, and Indonesia has documented elevated levels of microplastics in coastal waters, sediments, and biota. Zhao et al. (2015) identified microplastic concentrations between 0.27 to 1.45 particles per cubic meter in the Bohai Sea, China. Urban and industrial regions in Asia have elevated microplastic concentrations resulting from intensified human activity.

Pakistan, characterized by its extensive coastline along the Arabian Sea and numerous rivers, is confronting the issue of microplastic pollution. The data gathered from 15 locations in Pakistan underscores the degree of contamination in coastal and marine ecosystems. The data indicates considerable heterogeneity in microplastic concentrations among locations, with elevated counts in metropolitan regions such as Clifton (mean: 760 MPs/kg) and Manora (mean: 607 MPs/kg) in contrast to less urbanized sites such Kund Malir (mean: 107 MPs/kg) and Jiwani (mean: 167 MPs/kg). This pattern aligns with global trends, indicating that urban and industrial regions demonstrate elevated microplastic concentrations. The principal sources of microplastics in Pakistan consist of untreated urban wastewater, industrial effluents, and inadequate disposal of plastic trash. The Indus River, transporting plastic debris from interior regions, significantly contributes to coastal microplastic contamination. The microplastic concentrations in Pakistan are analogous to those documented in other South Asian nations. Research conducted in India has indicated microplastic concentrations between 100 and 1,000 particles per kilogram in coastal sediments. Our results accord with the trends observed in Asian nations such as China and India.

Microplastic concentrations exhibit considerable variation among the 15 sites, with urbanized and industrialized locations such as Clifton (mean: 38 MPs/50g) and Manora (mean: 30 MPs/50g) demonstrating elevated counts relative to less urbanized sites like Kund Malir (mean: 5 MPs/50g) and Jiwani (mean: 8 MPs/50g). This pattern corresponds with global trends, as densely populated and industrial areas have elevated microplastic pollution resulting from intensified human activities (Geyer et al., 2017). The MP figures in Pakistan align with research conducted in other South Asian nations. Coastal sediments in India and Bangladesh have exhibited microplastic concentrations between 100 and 1,000 MPs/kg (Sarker et al., 2020). The data from Pakistan aligns with this range, suggesting comparable pollution levels in the region. Black, white, and translucent microplastics are the most common across all sites. For example, Clifton: Black (mean: 10), White (mean: 4), Transparent (mean: 9). The prevalence of these colors indicates shared origins, including packaging materials, synthetic textiles and fishing equipment (Hidalgo-Ruz et al., 2012). Colors such as red, yellow, green, blue, and brown are seldom yet evident, signifying several origins including industrial pellets, personal care items and deteriorated plastic waste.

Marine species can ingest microplastics, resulting in physical damage, chemical exposure and possible transmission within the food web (Rochman et al., 2015). The existence of colored microplastics, especially black and translucent particles, may elevate the likelihood of ingestion owing to their similarity to natural prey. Microplastics in sediments can modify habitat quality for benthic organisms and influence ecosystem functionality (Browne et al., 2011).

Table 4.7 illustrates the degrading capacity of several microbial species based on the percentage weight loss of PET (Polyethylene Terephthalate). The microbial breakdown of PET is a viable alternative for alleviating plastic pollution. The table presents data on the efficacy of several microbial species in degrading PET, indicated by the percentage weight loss over three replicates (R1, R2, R3) and their average values. Pseudomonas species display significant degradation capabilities, with *Pseudomonas aeruginosa* and *Pseudomonas azotoformans* showing the highest degradation potential, recording mean percentage weight losses of 30.06% and 29.76%, respectively. The findings correspond with earlier research that has recognized Pseudomonas species as proficient PET degraders, attributing to their capacity to synthesize extracellular enzymes such as esterases and lipases, which decompose PET into smaller monomers (Yoshida et al., 2016; Urbanek et al., 2018). *Micrococcus luteus* exhibited moderate ability for PET degradation, with an average weight reduction of 24.85%. This species is recognized for its capacity to decompose complex polymers, and its efficacy in this investigation indicates its prospective function in PET bioremediation (Shah et al., 2013). *Bacillus subtilis* demonstrated an average weight reduction of 16.98%. Although inferior to Pseudomonas species, this degrading potential remains considerable. Bacillus species possess enzymatic capabilities, notably cutinases, which can hydrolyze PET (Ronkvist et al., 2009). *Vibrio alginolyticus, Halomonas campaniensis*, and *Bacillus flexus* exhibited comparatively diminished degradation capacities, with average weight losses of 13.87%, 9.51%, and 8.95%, respectively. These species may necessitate additional optimization or genetic modification to improve their PET-degrading capacities (Wei & Zimmermann, 2017). The elevated degradation efficiency of Pseudomonas species positions them as formidable candidates for additional investigation and implementation in PET bioremediation. Their enzymatic systems could be utilized for extensive plastic waste handling. The moderate efficacy of *Micrococcus luteus* and *Bacillus subtilis* indicates that these species may contribute to PET breakdown, especially within mixed microbial communities or under optimal circumstances. PET is generally more degradable than PVC and PE due to its ester bonds, which can be hydrolyzed by microbial enzymes like esterases and lipases.

Table 4.8 illustrates the breakdown capacity of microbial species based on the percentage weight loss of PVC (Polyvinyl Chloride), a commonly utilized yet ecologically durable plastic. The data illustrates the differential efficacy of various microbial species in decomposing PVC, with mean values derived from three replicates (R1, R2, R3). *Bacillus flexus* demonstrated the highest average PVC degradation potential (27.53%), indicating its robust enzymatic capacity to decompose PVC. This corresponds with research indicating the capacity of Bacillus species to generate extracellular enzymes that decompose complex polymers (Shah et al., 2013). *Halomonas campaniensis* (24.57%) and *Vibrio alginolyticus* (22.50%) exhibited notable PVC breakdown capability. These species are recognized for their adaptability to extreme conditions, perhaps augmenting their polymer-degrading capabilities (Urbanek et al., 2018). *Bacillus subtilis* (20.48467%) and *Micrococcus luteus* (19.34%) exhibited moderate degradation of PVC. Their performance aligns with their established enzymatic activities, such as esterases and lipases, capable of hydrolyzing synthetic polymers (Ronkvist et al., 2009). *Pseudomonas aeruginosa* (19.08%) and *Pseudomonas azotoformans* (18.48%) demonstrated comparatively diminished PVC degradation relative to other species. This contrasts with their significant PET degradation potential (Table 4.7), indicating substrate-specific enzymatic activity (Yoshida et al., 2016). *Bacillus flexus* and *Halomonas campaniensis* are viable alternatives for PVC bioremediation owing to their superior degradation efficiency. PVC is highly resistant to microbial degradation due to its chlorine content and strong carbon-chlorine bonds.

Table 4.9 illustrates the degrading capacity of microbial species, quantified by the percent weight loss of polyethylene (PE), a prevalent and ecologically persistent material. The data underscores the differential efficacy of various microbial species in the degradation of polyethylene (PE). *Pseudomonas azotoformans* exhibited the highest average potential for PE degradation at 37.97%. This aligns with research indicating that Pseudomonas species can generate extracellular enzymes, including lipases and esterases, capable of degrading hydrophobic polymers such as polyethylene

(Yoshida et al., 2016; Urbanek et al., 2018). *Pseudomonas aeruginosa* (31.48%) and *Bacillus flexus* (26.17%) demonstrated considerable polyethylene breakdown capability. These species are recognized for their enzymatic flexibility and capacity to breakdown complex polymers (Shah et al., 2013). *Bacillus subtilis* (21.48%) and *Micrococcus luteus* (18%) demonstrated considerable polyethylene breakdown. Their performance corresponds with their established enzymatic capacities, encompassing the synthesis of cutinases and other hydrolytic enzymes (Ronkvist et al., 2009). *Vibrio alginolyticus* (12.51%) and *Halomonas campaniensis* (12.58%) exhibited comparatively diminished polyethylene degradation capacity. This indicates that these species may necessitate optimization or genetic modification to improve their polyethylene-degrading capacities (Wei & Zimmermann, 2017). *Pseudomonas azotoformans* and *Pseudomonas aeruginosa* are viable options for polyethylene bioremediation owing to their superior degradation efficacy. PE is hydrophobic and has a high molecular weight, making it difficult for microbes to degrade. However, some bacteria can oxidize PE surfaces, leading to partial degradation. The percentages are approximate and depend on factors like microbial strain, environmental conditions (temperature, pH, oxygen availability) and pretreatment of microplastics (e.g., UV exposure).

4.12 CURRENT LIMITATIONS AND FUTURE CHALLENGES

In order to address the problem of microplastics, additional research and action are required in a variety of areas. These areas include the development of standard measurement methods, the understanding of sources and fate, the investigation of health impacts, the promotion of environmentally friendly alternatives, the raising of awareness and the addressing of challenges in plastic degradation research. It is possible for us to make great headway in reducing the adverse effects of microplastic pollution if we carry out exhaustive research, put into practice environmentally responsible behaviors and educate stakeholders.

4.13 STRATEGIES FOR REDUCING MPs POLLUTION AND POLICY RECOMMENDATIONS

Strategies for reducing microplastic pollution are critical in addressing the growing environmental concern surrounding the widespread presence of these tiny particles in ecosystems. One of the most effective approaches to tackling this issue is the exploration and implementation of environmentally friendly alternatives to synthetic materials that contribute to the proliferation of microplastics. A promising alternative lies in the use of natural fibers, which offer a more sustainable option compared to synthetic fibers commonly found in products like clothing, cleaning materials and industrial applications.

Natural fibers such as cotton, wool, hemp and bamboo have gained significant attention as replacements for synthetic fibers like polyester, nylon and acrylic. These synthetic materials shed microplastics during washing, contributing to the accumulation of harmful pollutants in aquatic environments. In contrast, natural fibers are biodegradable, meaning they break down more easily and do not release harmful chemicals or microplastics into the environment when they decompose.

4.14 CONCLUSIONS:

The primary objective of this study was to assess the quantity, color and classification of microplastics (MPs) that are less than 5 millimeters in size in sediment samples collected from coastal regions of Pakistan. According to our hypothesis, the level of MP contamination of sediment will be higher in areas where the sampling station is located in close proximity to populated, commercial and touristic centers.

Due to the fact that they have the potential to cause harm to ecosystems as well as to human health, microplastics are becoming an increasingly recognized environmental hazard. Microplastics are particles of plastic that are minute in size. It is possible to find them in a broad variety of habitats, such as oceans, rivers, soil and the air. They can be found in a variety of forms, such as fibers, pieces and micro beads. The negative effects of microplastics outweigh the possible benefits and they argue for the requirement of alternative materials for medical and industrial uses. On the other hand, there are some potential benefits to microplastics, such as their usage in applications in the medical and industrial fields.

The research on discovery of new potential plastic-degrading microorganisms has been a remarkable achievement and has revealed drastically significant results. This degradation of MPs through microbes is a natural and seems to be a practical solution for remediation of microplastics pollution.

In this study, bacterial strains isolated from Pakistan's coastal region were checked for their ability to biodegrade 03 plastic species PET, PVC and PE. These strains seemed to have potential for bioremediation of different plastic types.

The findings in this investigation highlighted that the genera *Bacillus*, *Pseudomonas*, *Helomonas, Micrococcus and Vibrio* and their corresponding species were significantly involved in MPs degradation. This indicates that these microorganisms can act as functional agents in reducing MPs.

As a result, studies such as this one highlight the significance of undertaking additional research, particularly when taking into consideration the design of procedures with tests conducted under genuine environmental settings. It is essential that this be done in order to ensure that, in the future, the interaction of bacteria with MPs will have significant practical and biotechnological value on a broad scale. This will be done with the intention of reducing the negative effects that these chemicals have on the environment.

The available data is significant to necessitate immediate action by the scientific community, the industry, as well as policy and civil societies, in order to limit the constant flow of plastics and their toxic additives into marine environments. Despite the fact that additional scientific research on the impacts of MPs on marine environments, the food chain and human health may still be useful. The scientific community has reached a consensus that, in the absence of quick implementation of comprehensive preventative measures, the environmental impact and the economic implications of plastics contamination will continue to aggravate the situation. It is imperative that the government increase its investments in solid infrastructures in order to permit much improved trash collection and management. The adoption of such activities would pave the way for a substantial and favorable change in our societies, which will contribute to the improvement of the health of the public environment and marine habitats.

4.15 FUTURE RESEARCH NEEDS

The natural processes involved in the spatial biodegradation of materials and their eventual disappearance from ecosystems are highly intricate. Previous studies have established that a wide range of intrinsic and extrinsic factors, such as the structural properties of polymers, molecular weight, bond types, surface characteristics, adhesiveness, thermal stability and texture, all play pivotal roles in the biodegradation pathways of plastics (Lear et.al., 2022). These factors, alongside microbial attributes, significantly influence how plastics break down in natural environments. It has become evident that environmental microorganisms are not only influenced by their inherent properties but also stabilized through various interspecific and intraspecific interactions, especially those tied to enzymatic activities. For instance, when microbes interact with nanoparticles and other abiotic factors, these elements can facilitate the immobilization of enzymes that drive biodegradation under more complex conditions. Therefore, a deeper understanding of bacterial-assisted biodegradation mechanisms holds promise for advancing green technologies and enhancing natural biodegradation processes, particularly in the context of reducing plastic pollution across ecosystems.

However, as current research stands, several gaps remain in our understanding of these processes. For example, the complexity of plastic samples themselves—especially the additional co-existing pollutants adhering to microplastics (MPs) and nanoplastics (NPs)—complicates the biodegradation pathways (Tang et.al., 2022). This suggests that future studies should account for these complexities to better understand the biodegradation process. Among the various methodologies employed to investigate plastic and polymer biodegradation, culture-based techniques are most commonly used to identify enzymes that break down plastics. This method is straightforward, making it a popular choice for many researchers, as it provides a qualitative assay of various enzymes from selected microorganisms or plastic-degrading microbes. However, it should be noted that these methods are time-consuming. Therefore, using a mixed consortium of both aerobic and anaerobic bacteria could offer a more efficient solution for MNP degradation. These consortia, which possess a greater diversity of genotypic and phenotypic characteristics as well as a wider array of enzymes, may outperform individual bacterial strains in breaking down plastic pollutants. In light of this, forming artificial consortia by selecting specific plastic-degrading bacteria, whether via a top-down approach (adding bacterial strains to an existing consortium) or a bottom-up approach (combining independently isolated strains), could be an effective strategy to enhance biodegradation efficiency (Lear et.al., 2021).

The role of technology in advancing biodegradation research is also becoming increasingly critical. Technological developments, particularly in bioinformatics, have enabled researchers to analyze emerging datasets that are invaluable in biodegradation studies. Computational tools and databases assist in identifying the enzymes involved in specific metabolic pathways and forecasting harmful chemical biodegradation processes. These advancements lay the groundwork for developing innovative approaches to plastic biodegradation (Ali et.al., 2021). However, despite the potential benefits of bioinformatics, a significant challenge remains in the lack of experimental data validation. This gap must be addressed to enable further progress in biodegradation research.

In addition to bioinformatics, genetic engineering techniques hold considerable promise for enhancing plastic biodegradation. By engineering bacteria and omnivorous enzymes capable of breaking down plastics, it may be possible to accelerate the process of plastic waste disposal. However, it is crucial to conduct thorough ecological risk assessments before implementing these methods in field applications to ensure that the interaction dynamics of modified organisms in uncontrolled environments are well understood, preventing any unintended negative outcomes. Over the next few years, advancements in metabolic engineering, bioinformatic tools, molecular genetics and systems biology could potentially offer sustainable and feasible solutions to MNP biodegradation.

Furthermore, recent innovations in enzyme immobilization techniques have opened up new opportunities for the stabilization and reuse of microbial enzymes. This approach allows enzymes to maintain their effectiveness over time, making them a promising tool for tackling plastic waste. Nanomaterials, in particular, offer several advantages over their bulk counterparts, such as higher surface areas, customizable shapes, size-related features and ease of surface modification (Mandal et.al., 2022b; Schwaminger et.al., 2021). Despite the advantages, the application of enzymes in plastic biodegradation still faces significant challenges, including structural instability, chemical sensitivity and the high costs associated with enzyme production and storage. Furthermore, to achieve high immobilization efficiencies, specialized manufacturing techniques are required. Despite these limitations, the development of "nanozymes"—nanomaterials engineered to function as artificial enzymes—has been a breakthrough. These nanozymes have shown promise in breaking down various pollutants, including dyes, pesticides and endocrine-disrupting chemicals (Lopez-Cantu et.al., 2022). Several nanozyme-based systems have demonstrated enhanced stability, adaptability and cost-effectiveness, positioning them as a potential solution for addressing plastic pollution (Cárdenas-Alcaide et.al., 2022). Although research on the use of nanozymes for the degradation of MP and NP is still in its early stages, this area of study offers exciting prospects for overcoming the limitations of natural enzymes in plastic waste degradation. To further refine these technologies, optimization strategies should be considered to ensure that experimental methods are effective, sustainable and reusable.

The widespread use of plastics has undoubtedly impacted human societies, both positively and negatively. While plastics are integral to modern life, their widespread use and subsequent disposal have led to massive environmental contamination, particularly in the form of plastic litter. The COVID-19 pandemic, with its associated surge in plastic-based medical supplies and packaging, further exacerbated the global plastic waste problem. Improper disposal of plastic waste poses a significant risk to wildlife, plants and humans, particularly as plastics degrade into smaller particles, including MPs and NPs, which accumulate in ecosystems. These particles can have unpredictable effects on environmental health and organismal interactions. Research on enzyme-based biodegradation technologies, such as immobilized enzymes, nanotechnology and consortia-based approaches, is slowly gaining traction as potential solutions to mitigate plastic pollution in the long term. However, it has become increasingly clear that no single technology is likely to be a universal solution for the biodegradation of all types of plastics. A combination of techniques, tailored to specific types of plastics, may prove to be the most effective approach, offering improvements in biodegradation speed, stability, resilience and enzyme efficiency.

This study emphasizes the importance of continuing to explore bacterial enzyme-based degradation methods and encourages further research in this area. With the growing global problem of MNP pollution, it is crucial for the scientific community, environmental organizations, governments and industry leaders to work together to find sustainable solutions. By highlighting the significant challenges in utilizing bacterial enzymes for MNP degradation, this study hopes to guide future research and foster a multidisciplinary approach to solving the plastic pollution crisis.

REFERENCES

A.A. Hussein, S.H. Khudhair, I.K. Al-Mayaly., Optimum conditions for LDPE strips biodegradation by local bacterial isolates J. Int. Environ. Appl Sci., 10 (4) (2015), pp. 399-407.

Ahmed, Nand A. Yasmin. 1988. Isolation, Characterization and Genetics of marine bacteria. In: *Marine science of Arabian Sea.* (Eds. Thompson, M.F. and N.M. Tirmizi) American Institute of Biological Science. Washington D.C. Pp.485-488.

Ahmed, Q., Ali, Q. M., Bat, L., Öztekin, A., Memon, S., Baloch, A. (2022). Preliminary Study on Abundance of Microplastic in Sediments and Water Samples Along the Coast of Pakistan (Sindh and Baluchistan)-Northern Arabian Sea. Turkish Journal of Fisheries and Aquatic Sciences, 22(SI), TRJFAS19998

Al Mamun, A., Prasetya, T. A. E., Dewi, I. R., & Ahmad, M. (2023). Microplastics in human food chains: Food becoming a threat to health safety. Science of The Total Environment, 858, 159834.

Ali, S., et al. (2021). Microplastic pollution in the coastal environment of Pakistan: A review. Environmental Science and Pollution Research, 28(4), 4076-4094.

Alomar, C., Estarellas, F., Deudero, S., (2016).Microplastics in the Mediterranean Sea:deposition in coastal shallow sediments, spatial variation and preferential grainsize. *Mar. Environ. Res.* 115, 1-10.

Andrady, A.L., (2011). Microplastics in the marine environment.*Mar. Pollut. Bull*. 62,1596-1605.

Andreeßen, C., & Steinbüchel, A. (2019). Recent developments in non-biodegradable biopolymers: Precursors, production processes and future perspectives. Applied microbiology and biotechnology, 103(1), 143-157.

Arthur, Courtney; Baker, Joel; Bamford, Holly (January 2009). "Proceedings of the International Research Workshop on the Occurrence, Effects and Fate of Microplastic Marine Debris". NOAA Technical Memorandum.

Asutin, B. 1988. *Marine Microbiology.* Cambridge University .Press, Cambridge, New York, Sydney. Pp.lS-23.

Atlas, R.M. and R. Bartha. 1981. *Microbial Ecology, fundamentals and applications.* Addison-Wesely Publishing

Ballent, A., Corcoran, P.L., Madden, O., Helm, P.A., Longstaffe, F.J., (2016). Sources andsinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach*sediments. Mar. Pollut. Bull.* 110, 383-395.

Bargagli, R., & Rota, E. (2022). Microplastic interactions and possible combined biological effects in Antarctic marine ecosystems. Animals, 13(1), 162.

Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., (2009).Accumulation andfragmentation of plastic debris in global environments.*Philos. Trans. Roy. Soc*. 364, 1985-1998

Batel, A., Linti, F., Scherer, M., Braunbeck, T. (2016). The transfer of benzo(a)pyrenefrom microplastics to Artemianauplii and further to zebra fish via trophic foodweb experiment-CYP1A induction and visual tracking of persistent organic*pollutants. Environ. Toxicol. Chem*. 35 (7), 1656-1666.

Besley, A., Vijver, M.G., Behrens, P., Bosker, T., 2017. A standardized method for sampling and extraction methods for quantifying microplastics in beach sand. Mar. Pollut. Bull. 114, 77–83.

Bhardwaj, H., Gupta, R., Tiwari, A., (2012). Microbial population associated withplastic degradation. *Sci. Rep*. 5, 272-274.

Brennecke, D., Duarte, B., Paiva, F., Cacador, I., Canning-Clode, J., (2016).Microplastics as vectors for heavy metal contamination from the marine environment.*Estuar.Coast.Shelf Sci*. 1-7. https://doi.org/10.1016/j.ecss.2015.12.003.

Browne, M. A., Crump, P., Niven, S. J., et al. (2011). Accumulation of microplastic on shorelines woldwide: Sources and sinks. Environmental Science & Technology, 45(21), 9175-9179.

Chen, D., Wang, P., Liu, S., Wang, R., Wu, Y., Zhu, A. X., & Deng, C. (2024). Global patterns of lake microplastic pollution: Insights from regional human development levels. Science of The Total Environment, 176620.

Chubarenko, I., Esiukova, E., Khatmullina, L., Lobchuk, O., Grave, A., Kileso, A., & Haseler, M. (2020). From macro to micro, from patchy to uniform: analyzing plastic contamination along and across a sandy tide-less coast. Marine Pollution Bulletin, 156, 111198.

Cruz-Salas, A. A., Alvarez-Zeferino, J. C., Ojeda-Benitez, S., Cruz-Sotelo, S. E., & Vazquez-Morillas, A. (2022). Solid waste and microplastics on the beaches of Holbox island, Mexico. Regional Studies in Marine Science, 53, 102423.

Dang TCH., Nguyen DT, Thai H, Nguyen TC, Tran TTH., Le VH, Nguyen QT (2018). Plastic degradation by thermophilic *Bacillus* sp. BCBT21 isolated from composting agricultural residual in Vietnam. Advances in Natural Sciences: Nanoscience and Nanotechnology, 9(1), 015014.

Demirkan E., Guller B.E., Sevgi T. Analysis by scanning electron microscopy of polyethylene terephthalate and nylon biodegradation abilities of *Bacillus* sp. strains isolated from soil. *J. Biol. Environ. Sci.* 2020;14:107–114.

Dey, S., Veerendra, G. T. N., Babu, P. A., Manoj, A. P., & Nagarjuna, K. (2024). Degradation of plastics waste and its effects on biological ecosystems: A scientific analysis and comprehensive review. Biomedical Materials & Devices, 2(1), 70-112.

Ding, L., Mao, R., Ma, S., Guo, X., & Zhu, L. (2020). High temperature depended on the ageing mechanism of microplastics under different environmental conditions and its effect on the distribution of organic pollutants. Water Research, 174, 115634.

Dong, H., Chen, Y., Wang, J., Zhang, Y., Zhang, P., Li, X., ... & Zhou, A. (2021). Interactions of microplastics and antibiotic resistance genes and their effects on the aquaculture environments. Journal of hazardous materials, 403, 123961.

Dris, R., Imhof, H., Sanchez, W., Gasperi, J., Galgani, F., Tassin, B., Laforsch, C., (2015).Beyond the ocean: contamination of freshwater ecosystems with (micro)*plastic particles. Environ. Chem*. 12 (5), 539-550.

Duis, K., Coors, A., (2016). Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. *Environ. Sci. Eur*. 28, 2.

Eerkes-Medrano, D., Thompson, R.C., Aldridge, D.C., (2015).Microplastics in fresh-water systems: a review of the emerging threats, identification of knowledge gaps and prioritization of research needs. *Water Res.* 75, 63-82.

Eriksen, M., Lebreton, L. C., Carson, H. S., Thiel, M., Moore, C. J., Borerro, J. C., ... & Reisser, J. (2014). Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS ONE, 9(12), e111913.

Esmaeili A, Pourbabaee AA, Alikhani HA, Shabani F, Esmaeili E (2013). Biodegradation of Low-Density Polyethylene (LDPE) by Mixed Culture of *Lysinibacillus xylanilyticus* and *Aspergillus niger* in Soil. PLOS. ONE, 8.

Fossi, M.C., Marsili, L., Baini, M., Giannetti, M., Coppola, D., Guarranti, C., Caliani, I.,Minutoli, R., Lauriano, G., Finoia, M.G., Rubegni, F., Panigada, S., Be rube , M.,Ramirez, J.U., Panti, C., (2016). Fin whales and microplastics: the MediterraneanSea and the Sea of Cortez scenarios. *Environ. Pollut.*209, 6878.

Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. Science Advances, 3(7), e1700782.

Giacomucci L., Raddadi N., Soccio M., Lotti N., Fava F. Polyvinyl chloride biodegradation by *Pseudomonas citronellolis* and *Bacillus flexus*. *New Biotechnol.* 2019; 52:35–41.

Green, D.S., (2016). Effects of microplastics on European flat oysters, Ostrea edulis andtheir associated benthic communities.*Environ. Pollut*.216, 95e10.

Grey, A. (2020). Blue carbon and edaphic influences on microbial metabolism: Bull Island's sediments and polyhydroxyalkanoates (PHAs) distribution (Doctoral dissertation, Dublin City University).

Hidalgo Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: A review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060–3075.

Hidalgo-Ruz, V., Gutow, L., Thompson, R. C., & Thiel, M. (2012). Microplastics in the marine environment: A review of the methods used for identification and quantification. Environmental Science & Technology, 46(6), 3060-3075.

Jain R., Ashish Gaur , Renuka Suravajhala , Uttra Chauhan , Manu Pant , Vishal Tripathi , Gaurav Pant Microplastic pollution: Understanding microbial degradation and strategies for pollutant reduction. Science of the Total Environment Volume 905, 20 December 2023, 167098

Keay L, Wildi BS (1970). Proteinases of the genus *Bacillus*. I. Neutral proteinases. Biotechnol. Bioeng., 12: 179-212.

Khalida Jabeen, Lei Su, Jiana Li, Dongqi Yang, Chunfu Tong, Jingli Mu, Huahong Shi, 2017, Microplastics and mesoplastics in fish from coastal and fresh waters of China, Environmental Pollution, 221:141-149

Koitabashi M, Sameshima-Yamashita Y, Watanabe T, Shinozaki Y, Kitamoto H (2016). Phylloplane fungal enzyme accelerates decomposition of biodegradable plastic film in agricultural settings. Japan Agricultural Research Quarterly, 50: 329-324.

Kourmentza, C., Plácido, J., Venetsaneas, N., Burniol-Figols, A., Varrone, C., Gavala, H. N., & Reis, M. A. (2017). Recent advances and challenges towards sustainable polyhydroxyalkanoate (PHA) production. Bioengineering, 4(2), 55.

Kumari, P., Kiran, B. R., & Mohan, S. V. (2022). Polyhydroxybutyrate production by Chlorella sorokiniana SVMIICT8 under nutrient-deprived mixotrophy. Bioresource Technology, 354, 127135.

Kyaw BM, Champakalakshmi R, Sakharkar MK, Lim CS, Sakharkar KR (2012). Biodegradation of low density polythene (LDPE) by *Pseudomonas* species. Indian Journal of Microbiology, 52: 411-419.

Lebreton, L. C., Van der Zwet, J., Damsteeg, J. W., Slat, B., Andrady, A., & Reisser, J. (2017). River plastic emissions to the world's oceans. Nature Communications, 8, 15611.

Liu, Y., Thomas, M. L., Coupland, G. T., Wang, P., Zheng, D., & McKirdy, S. J. (2023). Info-gap theory to determine cost-effective eradication of invasive species. Scientific reports, 13(1), 2744.

Lonnstedt, O.M., Eklov, P., (2016). Environmentally relevant concentrations of microplastic particles influence larval fish ecology. Ecotoxicol.Science352,6290.

Lots, F.A., Behrens, P., Vijver, M.G., Horton, A.A., Bosker, T., 2017. A large-scale investigation of microplastic contamination: abundance and characteristics of microplastics in European beach sediment. Mar. Pollut. Bull. 123, 219–226.

Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., Ren, H., (2016). Uptake and accumulation of polystyrene microplastics in zebra fish (Danio rerio) andtoxic effects in liver.*Environ. Sci. Technol*. 50, 4054e4060.

Lusher, A.L., Tirelli, V., O'Connor, I., Officer, R., (2016). Microplastics in Arctic polar waters: the first reported values of particles in surface and sub-surface samples.*Sci. Rep.* 5, 14947.

McCormick, A.R., Hoellein, T.J., London, M.G., Hittie, J., Scott, J.W., Kelly, J.J., (2016). Microplastic in surface waters of urban rivers: concentration, sources and associated bacterial assemblages. *Ecosphere* 7 (11).https://doi.org/10.1002/.1556 e01556.

Mishra, R. K., Mentha, S. S., Misra, Y., & Dwivedi, N. (2023). Emerging pollutants of severe environmental concern in water and wastewater: A comprehensive review on current developments and future research. Water-Energy Nexus.

Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., (2016). Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. *Environ. Sci. Technol.*https://doi.org/10.1021/acs.est.5b05416.

Naji, A., Nuri, M., Amiri, P., Niyogi, S., 2019. Small microplastic particles (S-MPPs) in

Nelms, S. E., Galloway, T. S., Godley, B. J., Jarvis, D. S., & Lindeque, P. K. (2018). Investigating microplastic trophic transfer in marine top predators. Environmental pollution, 238, 999-1007.

Ng, K.L., Obbard, J.P., (2006).Prevalence of microplastics in Singapore's coastal marineenvironment.*Mar. Pollut. Bull.* 52, 761-767.

Nor, N. H. M., & Obbard, J. P. (2014). Microplastics in Singapore's coastal mangrove ecosystems. Marine pollution bulletin, 79(1-2), 278-283.

Ogunola, O.S., Palanisami, T., (2016).Microplastics in the marine environment: current status, assessment methodologies, impacts and solutions. J. Pollut. *Eff. Control* 4, 161.https://doi.org/10.4172/2375-4397.1000161.

Panel Mamun Mandal, Anamika Roy, Robert Popek, Abhijit Sarkar, Micro- and nano- plastic degradation by bacterial enzymes: A solution to 'White Pollution', The Microbe Volume 3, June 2024, 100072

Pathak, V. M. (2017). Review on the current status of polymer degradation: a microbial approach. Bioresources and Bioprocessing, 4(1), 1-31.

Plastics Europe (2015), Plastics – the facts 2014/2015. An Analysis of European plastics production, demand and waste data. PlasticsEurope, Brussels, Belgium: 30 pp.

Poli, A., Finore, I., Romano, I., Gioiello, A., Lama, L., & Nicolaus, B. (2017). Microbial diversity in extreme marine habitats and their biomolecules. Microorganisms, 5(2), 25.

Rochman, C. M., Tahir, A., Williams, S. L., Baxa, D. V., Lam, R., Miller, J. T., ... & Teh, S. J. (2015). Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Scientific Reports, 5, 14340.

Ronkvist, Å. M., Xie, W., Lu, W., & Gross, R. A. (2009). Cutinase-catalyzed hydrolysis of poly(ethylene terephthalate). Macromolecules, 42(14), 5128-5138.

Ronda, A. C., Menéndez, M. C., Tombesi, N., Álvarez, M., Tomba, J. P., Silva, L. I., & Arias, A. H. (2023). Microplastic levels on sandy beaches: are the effects of tourism and coastal recreation really important?. Chemosphere, 316, 137842.

Ryan, P. G. (2019). Ingestion of plastics by marine organisms. Hazardous chemicals associated with plastics in the marine environment, 235-266.

Sarker, A., Deepo, D. M., Nandi, R., & Das, K. (2020). Microplastic pollution in the coastal waters of the Chittagong region, Bangladesh. Marine Pollution Bulletin, 160, 111633.

sediments of mangrove ecosystem on the northern coast of the Persian Gulf. Mar.Pollut. Bull. 146, 305–311.

Seymour, J. R. (2014). A sea of microbes: the diversity and activity of marine microorganisms. Microbiology Australia, 35(4), 183-187.

Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S. (2013). Biological degradation of plastics: A comprehensive review. Biotechnology Advances, 26(3), 246-265.

Shen, M., Zeng, Z., Wen, X., Ren, X., Zeng, G., Zhang, Y., & Xiao, R. (2021). Presence of microplastics in drinking water from freshwater sources: the investigation in Changsha, China. Environmental Science and Pollution Research, 28, 42313-42324.

Shim, W.J., Thompson, R.C., (2015).Microplastics in the ocean.*Arch. Environ. Contam.Toxicol*. 69, 265-268.

Siddiqui, P .J.A. 1986. Studies on detrital system on mangrove leaves. M.phil Thesis. Karachi University, Karachi, Pakistan. Pp.2-18.

Siddiqui,S.K. 1990. Biochemical and Genetics Studies on indigenous bacterial Isolates. M.Sc. Thesis. Karachi University, Karachi, Pakistan. Pp.l4-32.

Smith, M., Love, D. C., Rochman, C. M., & Neff, R. A. (2018). Microplastics in seafood and the implications for human health. Current Environmental Health Reports, 5(3), 375-386.

Sobti, R. C., Sharma, A., & Soni, S. K. (2022). Applications of Biotechnological Techniques in Mitigating Environmental Concerns. In Genomic, Proteomics and Biotechnology (pp. 249-312). CRC Press.

Sutton, R., Mason, S.A., Stanek, S.K., Willis-Norton, E., Wren, I.F., Box, C., (2016)Microplastic contamination in the San Francisco Bay, California, USA.*Mar. Pollut. Bull*. https://doi.org/10.1016/j.marpolbul.2016.05.077.

Urbanek, A. K., Rymowicz, W., & Mirończuk, A. M. (2018). Degradation of plastics and plastic-degrading bacteria in cold marine habitats. Applied Microbiology and Biotechnology, 102(18), 7669-7678.

Van Cauwenberghe, L., Devriese, L., Galgani, F., Robbens, J., Janssen, C.R., (2015).Microplastics in sediments: a review of techniques, occurrence and effects. Mar.Environ. Res. 111, 5-17.

Wagner, M., Scherer, C., Alvarez-Mun~oz, D., Brennholt, N., Bourrain, X., Buchinger, S.,Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R.,Vethaak, A.D., Winther-Nielsen, M., Reifferscheid, G., (2014). Microplastics in freshwater ecosystems: what we know and what we need to know. *Environ. Sci. Eur*. 2014 (26), 12.

Wei Cheng, Yue Zhou, Yichun Xie, Yan Li, Ren Zhou, Hui Wang, Yan Feng, Yan Wang, 2023, Combined effect of polystyrene microplastics and bisphenol A on the human embryonic stem cells-derived liver organoids: The hepatotoxicity and lipid accumulation, Science of The Total Environment, 854, 2023, 158585

Wei, R., & Zimmermann, W. (2017). Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: How far are we? Microbial Biotechnology, 10(6), 1308-1322.

Wright, S.L., Thompson, R.C., Galloway, T.S., (2013). The physical impacts of micro-plastics on marine organisms: a review. *Environ. Pollut.* 178, 483-492.

Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., ... & Oda, K. (2016). A bacterium that degrades and assimilates polyethylene terephthalate). Science, 351(6278), 1196-1199.

Yue Li, Le Tao, Qiong Wang, Fengbang Wang, Gang Li and Maoyong Song, Potential Health Impact of Microplastics: A Review of Environmental Distribution, Human Exposure and Toxic Effects, (2003), Environment & Health, 1 (4): 249-257.

Zhao, S., Zhu, L., & Li, D. (2015). Microplastic in three urban estuaries, China. Environmental Pollution, 206, 597-604.

Zhao, S., Zhu, L., Wang, T., Li, D., (2014) Suspended microplastics in the surface waterof the Yangtze Estuary System, China: first observations on occurrence, distri-*bution. Mar. Pollut. Bull*. 86, 562-568.

Zubari,R. 1986. Quantitative and Qualitative aspects of the bacterial flora of Karachi coastal water shrimp and their effect on the handling and the subsequent changes during storage. Ph.D. Thesis. Karachi University, Karachi, Pakistan. P:.29-57.

APPENDIX

Data of MPs enumeration for all 15 sites (15 Tables)

SITE NAME	Replicates	Total MP Count	
		Original Value 50g	Corresponding value 1 Kg
KETI BANDAR	R1	8	160
	R2	10	200
	R3	12	240
	Mean	10	200

SITE NAME	Replicates	Total MP Count	
		Original Value 50g	corresponding value 1 Kg
GHARO	R1	17	340
	R2	19	380
	R3	21	420
	Mean	19	380

SITE NAME	Replicates	Total MP Count	
		Original Value 50g	corresponding value 1 Kg
PORT QASIM	R1	21	420
	R2	19	380
	R3	18	360
	Mean	19	387

SITE NAME	Replicates	Total MP Count	
		Original Value 50g	corresponding value 1 Kg
KUND MALIR	R1	8	160
	R2	5	100
	R3	3	60
	Mean	5	107

SITE NAME	Replicates	Total MP Count	
		Original Value 50g	Corresponding value 1 Kg
SONMIANI	R1	10	200
	R2	11	220
	R3	13	260
	Mean	11	227

SITE NAME	Replicates	Total MP Count	
		Original Value 50g	Corresponding value 1 Kg
GADANI	R1	12	240
	R2	15	300
	R3	14	280
	Mean	14	273

SITE NAME	Replicates	Total MP Count	
		Original Value 50g	Corresponding value 1 Kg
MANORA	R1	32	640
	R2	31	620
	R3	28	560
	Mean	30	607

SITE NAME	Replicates	Total MP Count	
		Original Value 50g	Corresponding value 1 Kg
PARADISE POINT	R1	20	400
	R2	16	320
	R3	18	360
	Mean	18	360

SITE NAME	Replicates	Total MP Count	
		Original Value 50g	Corresponding value 1 Kg
HAWKE'S BAY	R1	19	380
	R2	24	480
	R3	25	500
	Mean	23	453

SITE NAME	Replicates	Total MP Count	
		Original Value 50g	Corresponding value 1 Kg
CLIFTON	R1	35	700
	R2	38	760
	R3	41	820
	Mean	38	760

SITE NAME	Replicates	Total MP Count	
		Original Value 50g	Corresponding value 1 Kg
IBRAHIM HYDERI	R1	28	560
	R2	25	500
	R3	23	460
	Mean	25	507

SITE NAME	Replicates	Total MP Count	
		Original Value 50g	Corresponding value 1 Kg
PASNI	R1	12	240
	R2	14	280
	R3	18	360
	Mean	15	293

SITE NAME	Replicates	Total MP Count	
		Original Value 50g	Corresponding value 1 Kg
GAWADAR	R1	16	320
	R2	17	340
	R3	16	320
	Mean	16	327

SITE NAME	Replicates	Total MP Count	
		Original Value 50g	Corresponding value 1 Kg
JIWANI	R1	8	160
	R2	10	200
	R3	7	140
	Mean	8	167

SITE NAME	Replicates	Total MP Count	
		Original Value 50g	Corresponding value 1 Kg
ORMARA	R1	10	200
	R2	14	280
	R3	9	180
	Mean	11	220

Data of MPs Colors for all 15 Locations (15 Tables)

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Color							
			Black	White	Red	Yellow	Green	Transparent	Blue	Brown
KETI BANDAR	R1	8	2	2	0	0	0	2	1	1
	R2	10	2	1	1	0	1	3	1	1
	R3	12	3	1	1	0	2	3	1	1
	Mean	10	2	1	1	0	1	3	1	1

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Color							
			Black	White	Red	Yellow	Green	Transparent	Blue	Brown
GHARO	R1	17	4	2	2	0	3	4	2	0
	R2	19	5	3	1	0	3	5	2	0
	R3	21	5	2	3	0	4	5	2	0
	Mean	19	5	2	2	0	3	5	2	0

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Color							
			Black	White	Red	Yellow	Green	Transparent	Blue	Brown
PORT QASIM	R1	21	6	3	0	1	3	5	2	1
	R2	19	5	1	2	1	3	4	2	1
	R3	18	5	2	0	1	3	4	2	1
	Mean	19	5	2	1	1	3	4	2	1

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Color							
			Black	White	Red	Yellow	Green	Transparent	Blue	Brown
KUND MALIR	R1	8	2	1	0	0	1	2	1	1
	R2	5	2	1	0	0	1	1	0	0
	R3	3	3	0	0	0	0	0	0	0
	Mean	5	2	1	0	1	1	1	0	0

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Color							
			Black	White	Red	Yellow	Green	Transparent	Blue	Brown
SONMIANI	R1	10	2	1	1	0	2	2	1	1
	R2	11	2	2	1	0	2	2	1	1
	R3	13	3	1	2	0	2	3	1	1
	Mean	11	2	1	1	0	2	2	1	1

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Color							
			Black	White	Red	Yellow	Green	Transparent	Blue	Brown
GADANI	R1	12	3	1	1	0	2	3	2	0
	R2	15	4	1	1	1	3	3	2	1
	R3	14	4	1	0	1	2	3	2	1
	Mean	14	4	1	1	1	2	3	2	1

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Color							
			Black	White	Red	Yellow	Green	Transparent	Blue	Brown
MANORA	R1	32	8	4	4	1	5	6	4	0
	R2	31	7	5	3	1	5	6	4	0
	R3	28	6	2	3	2	6	5	4	0
	Mean	30	7	4	3	1	5	6	4	0

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Color							
			Black	White	Red	Yellow	Green	Transparent	Blue	Brown
PARADISE POINT	R1	20	5	3	4	1	1	4	2	0
	R2	16	4	1	2	1	3	3	2	0
	R3	18	5	2	1	2	3	3	2	0
	Mean	18	5	2	2	1	2	3	2	0

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Color							
			Black	White	Red	Yellow	Green	Transparent	Blue	Brown
HAWKE'S BAY	R1	19	3	3	2	1	3	5	2	0
	R2	24	4	4	1	2	4	6	3	0
	R3	25	4	4	2	0	5	6	3	1
	Mean	23	4	4	2	1	4	6	3	0

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Color							
			Black	White	Red	Yellow	Green	Transparent	Blue	Brown
CLIFTON	R1	35	9	4	3	1	6	8	3	1
	R2	38	10	3	4	1	6	9	3	2
	R3	41	11	4	2	1	7	10	4	2
	Mean	38	10	4	3	1	6	9	3	2

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Color							
			Black	White	Red	Yellow	Green	Transparent	Blue	Brown
IBRAHIM HYDERI	R1	28	7	2	2	1	3	6	4	3
	R2	25	6	3	0	1	3	5	4	3
	R3	23	6	2	0	1	3	5	3	3
	Mean	25	6	2	1	1	3	5	4	3

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Color							
			Black	White	Red	Yellow	Green	Transparent	Blue	Brown
PASNI	R1	12	3	1	1	0	2	3	2	0
	R2	14	4	2	1	0	3	3	1	0
	R3	18	5	2	2	1	3	4	1	0
	Mean	15	4	2	1	0	3	3	1	0

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Color							
			Black	White	Red	Yellow	Green	Transparent	Blue	Brown
GAWADAR	R1	16	4	2	2	0	3	3	2	0
	R2	17	4	2	2	1	3	3	2	0
	R3	16	4	1	2	1	3	3	2	0
	Mean	16	4	2	2	1	3	3	2	0

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Color							
			Black	White	Red	Yellow	Green	Transparent	Blue	Brown
JIWANI	R1	8	1	1	0	0	2	2	1	1
	R2	10	2	1	1	0	2	2	1	1
	R3	7	1	2	0	0	1	2	1	0
	Mean	8	1	1	0	0	2	2	1	1

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Color							
			Black	White	Red	Yellow	Green	Transparent	Blue	Brown
ORMARA	R1	10	2	1	1	0	2	2	1	1
	R2	14	3	2	0	1	2	3	2	1
	R3	9	1	1	1	0	2	2	1	1
	Mean	11	2	1	1	0	2	2	1	1

Data of MPs Shapes for all 15 Locations (15 Tables)

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Shape			
			Fiber	Film	Fragment	Pellet
KETI BANDAR	R1	8	4	1	2	1
	R2	10	5	0	3	2
	R3	12	6	1	3	2
	Mean	10	5	1	3	2

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Shape			
			Fiber	Film	Fragment	Pellet
GHARO	R1	17	9	1	4	3
	R2	19	10	2	5	2
	R3	21	11	2	5	3
	Mean	19	10	2	5	3

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Shape			
			Fiber	Film	Fragment	Pellet
PORT QASIM	R1	21	10	2	4	5
	R2	19	9	2	4	4
	R3	18	8	2	4	4
	Mean	19	9	2	4	4

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Shape			
			Fiber	Film	Fragment	Pellet
KUND MALIR	R1	8	4	1	2	1
	R2	5	3	1	1	0
	R3	3	2	0	1	0
	Mean	5	3	1	1	0

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Shape			
			Fiber	Film	Fragment	Pellet
SONMIANI	R1	10	6	1	2	1
	R2	11	6	2	2	1
	R3	13	7	2	3	1
	Mean	11	6	2	2	1

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Shape			
			Fiber	Film	Fragment	Pellet
GADANI	R1	12	5	1	3	3
	R2	15	7	2	4	3
	R3	14	6	2	4	2
	Mean	14	6	2	4	3

SITE NAME	Replicates	Total MP Count	Classification by Shape			
		Original Value 50g	Fiber	Film	Fragment	Pellet
MANORA	R1	32	16	3	8	5
	R2	31	15	4	7	5
	R3	28	14	3	7	4
	Mean	30	15	3	7	5

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Shape			
			Fiber	Film	Fragment	Pellet
PARADISE POINT	R1	20	10	2	4	4
	R2	16	8	2	3	3
	R3	18	9	2	4	3
	Mean	18	9	2	4	3

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Shape			
			Fiber	Film	Fragment	Pellet
HAWKE'S BAY	R1	19	10	2	4	3
	R2	24	12	3	5	4
	R3	25	13	1	5	6
	Mean	23	12	2	5	4

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Shape			
			Fiber	Film	Fragment	Pellet
CLIFTON	R1	35	16	5	6	8
	R2	38	17	5	7	9
	R3	41	19	5	7	10
	Mean	38	17	5	7	9

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Shape			
			Fiber	Film	Fragment	Pellet
IBRAHIM HYDERI	R1	28	15	4	6	3
	R2	25	13	3	6	3
	R3	23	12	3	5	3
	Mean	25	13	3	6	3

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Shape			
			Fiber	Film	Fragment	Pellet
PASNI	R1	12	5	1	3	3
	R2	14	6	1	4	3
	R3	18	7	2	5	4
	Mean	15	6	1	4	3

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Shape			
			Fiber	Film	Fragment	Pellet
GAWADAR	R1	16	7	1	4	4
	R2	17	7	2	4	4
	R3	16	7	1	4	4
	Mean	16	7	1	4	4

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Shape			
			Fiber	Film	Fragment	Pellet
JIWANI	R1	8	3	1	2	2
	R2	10	4	0	3	3
	R3	7	3	0	2	2
	Mean	8	3	0	2	2

SITE NAME	Replicates	Total MP Count Original Value 50g	Classification by Shape			
			Fiber	Film	Fragment	Pellet
ORMARA	R1	10	4	1	2	3
	R2	14	6	1	3	4
	R3	9	4	1	2	2
	Mean	11	5	1	2	3

PICTORAL HIGHLIGHTS

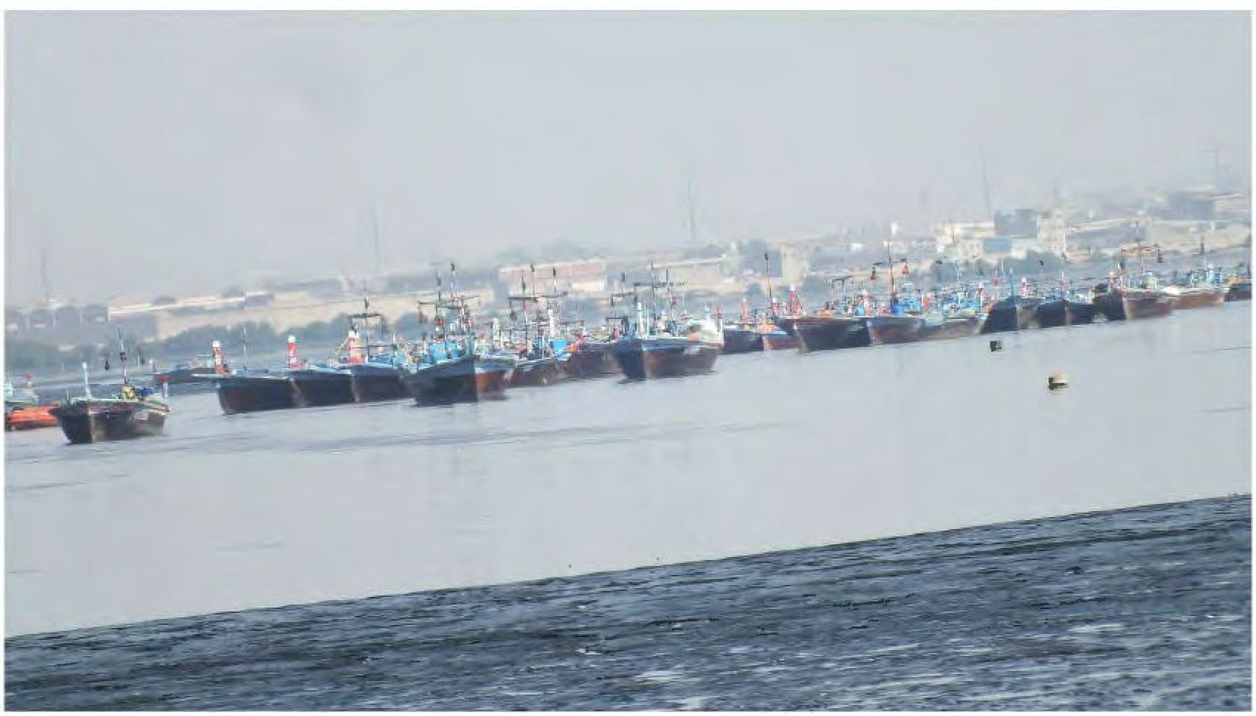
Keti Bandar – A Marshy Site

Kund Malir – A Sandy Beach

Power Plant near Port Qasim

Ship Breaking Industry at Gaddani

Approaching a sampling site at Port Qasim



A view of Harbor at Manora

Samples under processing for density separation of Microplastics

Sample Processing of Bacterial Isolate